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Wisconsin Procedures for Soil Testing, Plant Analysis and Feed & Forage Analysis

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<http://uwlab.soils.wisc.edu/lab-procedures/>Last revised: October 2013* This on-line document replaces the publication “Wisconsin Procedures for Soil Testing, Plant Analysis and Feed & Forage Analysis”, No. 6, Soil Fertility Series; last revised 1987 by E.E. Schulte, J.B. Peters and P.R. Hodgson.

The work of these individuals as well as many other current and former laboratory staff members is gratefully acknowledged.** This page requires a Javascript enabled web browser.

Introduction

The procedures outlined herein are employed in the University of Wisconsin Soil and Forage Analysis Laboratory, Marshfield, and the Soil and Plant Analysis Laboratory, Madison. Several private soil testing laboratories also follow these procedures, including all Wisconsin DATCP Certified Soil Testing Laboratories.

A laboratory test is only as good as the research upon which it is based. These procedures have been modified from the research efforts of a great many individuals, at the University of Wisconsin and

elsewhere, and adapted for routine analysis. This is a continuing process. As new information is uncovered through research, soil, plant, and forage tests must be updated to include the latest findings. Consequently, the procedures found herein are revised from time-to-time as needed to keep them current.

Development of Soil Testing Procedures

Soil test correlation: The first step in developing a soil test is to find a suitable extracting solution. This is the objective of a correlation study. A large number of the more important agricultural soils are collected. These soils are then cropped in the greenhouse, where most of the variables can be controlled. After a specified period, the assay crop is harvested; the amount of the element to be tested that is taken up by the crop is measured.

From knowledge of the chemistry of the element in the soil, several different possible extracting solutions are used to extract the element from the soil. An ideal extractant would remove the same amount of the element as is taken up by the plant. This is rarely achieved in practice, but a close correlation between plant uptake and the amount of the element extracted chemically is sought. In some cases, a regression equation that considers other soil properties may improve the prediction of plant availability of the element in question. Once a suitable extractant has been found, the effects of shaking time, solution-to-soil ratio, reagent concentration, etc. must be studied before the test can be run on a routine basis.

Soil test calibration: After a soil test procedure has been developed through greenhouse and laboratory experimentation, it is necessary to calibrate the test on a large number of sites under field conditions. The objective of soil calibration is to determine the amount of nutrient that must be added to the soil at different soil test levels of that nutrient to obtain maximum yield.

Because variables such as climate, insects, disease, drainage, etc. cannot be controlled as closely in the field as in the greenhouse, it is necessary to repeat field calibration studies three to five years before definite conclusions can be drawn.

Sampling

Correct usage of soil, plant, and forage results depends on 1) a sample representative of the area or batch from which it was taken, 2) an accurate laboratory analysis, and 3) the correct interpretation of lab results. The laboratory analysis should be the most accurate step unless gross analytical errors go undetected or poor laboratory technique is allowed. Built-in checks can minimize these possibilities. The interpretation of the lab results depends on knowledge of the relationship between the test value and plant, soil, and animal response.

The greatest source of error is usually the sample itself. Since physical samples may be extremely

heterogeneous, it is important that the sample tested be truly representative. Procedures for taking representative samples may be found in the following articles:

- [Manure Sampling Instructions](#)
- [Sampling Soils for Testing \(A2100\)](#)
- [Sampling Garden Soils and Turf Areas for Testing \(A2166\)](#)
- [Sampling for Plant Analysis](#)
- [Focus on Forage: Sampling and Evaluating Total Mixed Rations](#)
- [Sampling Soils for Preplant \(2 feet\) and Pre-sidedress \(1 foot\) Soil Nitrate Tests](#)

[Lime and Fertilizer Recommendations](#)

The interpretation of soil test results and the procedure for making lime and fertilizer recommendations are covered in detail in [‘Nutrient Application Rate Guidelines for Field, Vegetable, and Fruit Crops in Wisconsin’ \(A2809\)](#).

[Soil Analysis](#)

1. [Sample Preparation](#)
2. [Internal Check System](#)
3. [Standard Soils Protocol](#)
4. [pH & Sikora Lime Requirement](#)
5. [Available P](#)
6. [Available K \(this is the official WI method for soil K\)](#)
7. [Organic Matter \(Weight loss-on-ignition\)](#)
8. [Available Zinc](#)
9. [Available Boron](#)
10. [Available Manganese](#)
11. [Exchangeable Cations \(Ca⁺⁺, Mg⁺⁺, K⁺, Na⁺\)](#)
12. [Calculated Cation Exchange Capacity](#)
13. [Sulfate-Sulfur](#)
14. [Soluble Salts \(Electrical Conductivity\)](#)
15. [Particle Size Analysis \(Physical Analysis\)](#)
16. [Inorganic Nitrogen](#)
 - a. [Nitrate-N \(Colorimetric Method\)](#)
 - b. [Nitrate and Nitrite by Flow Injection Analysis](#)
 - c. [Ammonium-N by Flow Injection Analysis \(see above\)](#)
17. [Total Nitrogen](#)
18. [Organic Carbon](#)

19. [Total Elemental Analysis with ICP-OES and ICP-MS](#)
20. [Heavy Metals](#)
21. [Chloride](#)
22. [Lead](#)
23. [Ash](#)
24. [Phosphorus for Forest Soil](#)
25. [Mound Sand](#)

Plant Analysis

1. [Total Elemental Analysis with ICP-OES](#)
 - a. [Alternate Digestion Using Dry Ash Method](#)
2. [Total Nitrogen by Flow Injection Analysis](#)
3. [Nitrogen – Inorganic Forms \(Includes Ammonium, Nitrate, Nitrite\)](#)
4. [Chloride](#)
5. [Organic Carbon](#)
6. [Heavy Metals](#)
7. [Potato Petiole Nitrate](#)
8. [Ash](#)

Feed and Forage Analysis

1. **Wet Chemical Analysis**
 - a. [Sample Preparation & Lab Dry Matter](#)
 - b. [Total Dry Matter](#)
 - c. [Crude Protein \(CP\)](#)
 - d. [NDF \(Neutral Detergent Fiber\)](#)
 - e. [ADF \(Acid Detergent Fiber\)](#)
 - f. [Lignin](#)
 - g. [ADF_{CP}](#)
 - h. [NDF_{CP}](#)
 - i. [Ash](#)
 - j. [Fat](#)
 - k. [In Vitro Digestibility](#)
 - l. [Total Starch](#)
 - m. [Starch Digestibility; Degree of Starch Access](#)
 - n. [Major Mineral Analysis \(P, K, Ca, Mg\)](#)
 - o. [Sulfur Determination in Manure and Forage](#)

- p. [Total Elemental Analysis with ICP-OES](#)
- q. [Nitrate Nitrogen](#)
- r. [Heavy Metals](#)
- s. [Selenium](#)
- t. [Chloride](#)
- u. [Ash](#)

2. **NIR Analysis**

- a. [Sample Collection](#)
- b. [Subsampling](#)
- c. [Drying](#)
- d. [Grinding](#)
- e. [Mixing Dried and Ground Sample](#)
- f. [Packing and Scanning](#)
- g. [Equation Use](#)

i. References for Calibrations Used

- i. Alfalfa Hay: NIRS Forage and Feed Testing Consortium, June 2007 alfalfa hay calibration, file name: ah50-3. Parameters used: DM, CP, ADF, NDF, dNDF48, Ca, P, K, Mg, ash, lignin, fat, RUP.
- ii. Grass Hay: NIRS Forage and Feed Testing Consortium, June 2007 grass hay calibration, file name: gh50-2. Parameters used: DM, CP, ADF, dNDF48, NDF, Ca, P, K, Mg, Ash.
- iii. Mixed Hay: NIRS Forage and Feed Testing Consortium, June 2007 mixed hay calibration, file name: mh50-3. Parameters used: DM, CP, ADF, dNDF48, NDF, Ca, P, K, Mg, ash, fat, lignin, RUP.
- iv. Mixed Haylage: NIRS Forage and Feed Testing Consortium, June 2007 mixed haylage calibration, file name: hg50-3. Parameters used: DM, CP, ADF, dNDF48, NDF, Ca, P, K, Mg, ash, fat, lignin, ADP, RUP.
- iv. Fermented Corn Silage: NIRS Forage and Feed Testing Consortium, June 2007 fermented corn silage calibration, file name: cs50-2. Parameters used: DM, CP, ADF, dNDF48, IVTDMD, NDF, Ca, P, K, Mg, ash, fat, lignin.

h. [Sample Storage](#)

Manure Analysis

1. Manure

- a. [Sample Preparation & Lab Dry Matter](#)
- b. [All Other Methods](#)

2. Sediment Analysis

- a. Carbon
 - i. [Total Carbon](#)
 - ii. [Organic Carbon](#)
- b. [Total Elemental Analysis with ICP-OES](#)

[Greenhouse & Lime](#)

- 1. [Greenhouse Media](#)
- 2. [Lime](#)

[Individual Method References](#)

Internal Check System

To insure reliability of laboratory results, an internal check system is essential. This is in addition to any external sample exchange between laboratories.

The first sample in every tray should be a standard sample of known composition. This standard should be prepared by drying, grinding, and homogenizing a 25 to 50 lb sample. Homogenize the ground sample thoroughly and store the bulk sample in a heavy plastic bag inside a 5-gal closed container away from lab fumes.

After this standard soil has been analyzed 50 times, calculate the mean and standard deviation for each analysis. On graph paper, prepare a chart with the mean and \pm one standard deviation on the vertical axis and date of analysis on the horizontal axis. The graph over time should be a sequence of points forming a near-horizontal line within the one standard deviation, above and below the mean from the 50 analyses. Post this chart (a separate chart for each element) next to the instrument used to measure that element. The analyst records the value of the standard sample on the chart at the start of the tray and can see at a glance if the analysis is within tolerable limits. If not, the problem should be resolved before proceeding.

The mean and standard deviation of the standard sample should be recalculated after every 50 trays to determine whether the instruments are drifting or the sample itself is changing. Scrupulous adherence to this internal check program will help insure reliable data.

For matching colorimeter tubes dilute 1 mL of 3 N $\text{Na}_2\text{Cr}_2\text{O}_7$ in 10 N H_2SO_4 to 1 L and mix thoroughly. Place this solution into the colorimeter tubes to be matched and read.

December 2010

Protocol for the use of Standard Soils at the UW Soil Testing Laboratories

1. For all routine farm soil, lawn and garden and research trays, use position #1 as the location for the standard soil. The corresponding box in the tray should be inverted and left empty for all trays to assure this location is available for analyzing the standard soil.
2. Use the same soil each time for each of the assays. You can use a different soil for B vs. P/K but within each test keep using the same soil.
3. If variation exceeds 0.1 pH units for pH or Sikora buffer pH, stop and evaluate the situation. For other tests use an acceptable variation of +/- 10% of the long term mean for that parameter.
4. Save the data for a minimum of three years and have it readily available for a QA/QC audit.
4. If the supply of a standard soil is starting to be exhausted, obtain a new one and start testing it along with the original one to establish a baseline value for the new standard.

Available Phosphorus

1. Application

This procedure covers the extraction and analysis of plant available phosphorus (P) from soil.

2. Summary of Methods

Plant available phosphorus (P) is extracted from the soil with 0.03 N NH₄F in 0.025 N HCl (Bray P1 extract). This extractant primarily measures P adsorbed by Al compounds. The Al is complexed by F⁻ ions, liberating P. Lesser amounts of Fe⁻, MN⁻, and Ca-P may be extracted, along with water-soluble P. Extracted P is reacted with ammonium molybdate to form a blue phosphomolybdate compound in the presence of a reducing agent.

The concentration of P is determined colorimetrically or by UV – Vis spectrophotometer.

Potassium is extracted simultaneously with P and analyzed separately.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Color development is complete in 15 minutes but will continue at a slower rate. For this reason, samples should be read within two hours. Arsenic forms a blue molybdate complex but is usually present in very low amounts unless an arsenical pesticide has been applied in the past.

Very high soil pH interferes with phosphorus by this extraction method.

The Bray test for P is less reliable in alkaline soil containing free CaCO₃. The carbonate reacts with HCl in the Bray extract, forming CaCl₂, and the Ca⁺⁺ ions react with F⁻, precipitating CaF₂. Where alkaline soils predominate, NaHCO₃ (Olsen) is the preferred extractant.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 1.5 g of light-colored silt loam soil.
- 5.2 Erlenmeyer flasks (50-ml)
- 5.3 Pipette banks (3-ml)
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Filter paper (9-cm Whatman no. 2 or equivalent)
- 5.6 Funnel tubes (15-ml)
- 5.7 Matched colorimetric tubes (10-ml)
- 5.8 UV-Vis spectrophotometer
- 5.9 Brewer Automatic Pipetting Machine (SEPCO Model #40A)

6. Reagents

- 6.1 Stock P-A solution (1.25 N HCl, 1.5 N NH₄F): Add 54 ml of 48% HF to 700 ml of deionized water. Neutralize to pH 7.0 with NH₄OH. Add 108 ml of concentrated HCl (11.6 N) and dilute to 1 liter
- 6.2 Dilute P-A solution (0.025 N HCl, 0.03 N NH₄F): Dilute 20 ml of stock P-A solution to 1 liter with deionized water.
- 6.3 P-B solution (0.87 N HCl, 0.38% ammonium molybdate, 0.5% H₃BO₃): Dissolve 3.8 g ammonium molybdate, (NH₄)₆Mo₇O₂₄·4H₂O, in 300 ml of deionized water at about 60° C. Cool. Dissolve 5.0 g boric acid, H₃BO₃, in 500 ml of deionized water, and add 75 ml concentrated HCl (11.6 N). Then, add the molybdate solution and dilute to 1 liter with deionized water.
- 6.4 P-C powder: Thoroughly mix and grind to a fine powder 2.5 g of 1-amino-2-naphthol-4 sulfonic acid, 5.0 g sodium sulfite (Na₂SO₃), and 146 g of sodium metabisulfite (Na₂S₂O₅).
- 6.5 P-C solution: Dissolve 8 g of dry P-C powder in 50 ml of warm deionized water. Let stand overnight, if possible. A fresh reagent should be prepared every three weeks. (Upon standing, some material may crystallize out, but this is still satisfactory.)
- 6.6 Standard P solution (1000 ppm P, 500 ppm P)
- 6.7 Working standards (0, 1.0, 2.5, 5, 10, 20, 40 ppm P, prepares with same matrix as the samples.)

7. Methods

- 7.1 Place a 1.5 g scoop of soil into a 50-ml Erlenmeyer flask.
- 7.2 Add 15 ml of P-A solution with Automatic Brewer Pipette.
- 7.3 Shake the suspension on oscillating shaker for 5 minutes.
- 7.4 Filter through filter paper into a 15-ml funnel tube.
- 7.5 Pipette a 3.0-ml aliquot of filtrate with constant suction pipette apparatus and transfer to a 10-ml colorimeter tube.
- 7.6 Add 3.0 ml of P-B solution with the same pipette apparatus and mix well.
- 7.7 Add 3 drops of P-C solution, and mix immediately.
- 7.8 Read color after 15 min., but before two hr., with a photoelectric colorimeter or a UV-Vis spectrophotometer.

Note: UV – Vis spectrophotometer should be set at 645 nm.

- 7.10 Calibrate the instrument to read directly in ppm P in soil using working standards. These standard preparations are treated in the same manner as the soil extracts. (color development is complete in 15 minutes. and standards should be read within two hours.).

8. Calculations

In lieu of direct calibration of the colorimeter scale, calculate extractable P,
 $\text{ppm P in soil} = \text{ppm P in solution} \times 15 \text{ ml}/1.5 \text{ g} = \text{ppm P in solution} \times 10.$

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable P content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm P in soil. (Strictly speaking, the results should be reported as Mg P per dm^3 of soil because a known volume, rather than a weight is used. This is not a familiar unit, however. Use of a volume of soil is reasonable because it represents a volume-fraction of an acre plow layer.)

11. References

- 11.1 Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soil. *Soil Sci.* 59: 39-45
- 11.2 Munter, R.C. 1988. Laboratory factors affecting the extractability of nutrients. Pp. 8-10. *In* W.C Dahnke (ed.), *Recommended Chemical Soil Test procedures for the North Central Region.* NCR Publ. 221 (revised). ND Agr. Exp. Sta., Fargo, ND.
- 11.3 Frank, K., D, Beegle, and J. Denning. 1998. Phosphorus, pp. 21-26. *In* J.R. Brown (ed.) *Recommended Chemical Soil Test Procedures for the North Central Region.* NCR Publ. No. 221 (revised). Missouri Agr. Sta. SB 1001. Columbia, MO.

Available Potassium

1. Application

This procedure covers the extraction and analysis of plant available potassium (K) in soil.

2. Summary of Methods

Plant available K is extracted with the Bray P1 reagent, 0.03 N NH_4F , 0.025 N HCl . It is not the same procedure, but it is the same extracting solution. The NH_4^+ and H^+ ions displace exchangeable K from cation exchange sites in the soil. Water soluble K is also extracted. This procedure extracts approximately 90 % as much K as the 1 N NH_4OAc procedure.

Extracted K is analyzed using an atomic absorption spectrophotometer, ICP-OES or a flame photometer. Phosphorus is extracted simultaneously with K and analyzed separately.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Potassium is partially ionized in the air acetylene flame of AA. To suppress ionization, cesium nitrate or chloride solution can be added to give a final concentration of 1000 ppm in all solutions including the standards and blank. The purest available cesium compound must be used to avoid potassium contamination.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 1.5 g of light-colored silt loam soil.
- 5.2 Erlenmeyer flasks (50 ml).
- 5.3 Constant suction pipette apparatus (15 ml).
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Filter paper (9 cm Whatman No. 2 or equivalent).

- 5.6 Funnel tubes (15 ml)
- 5.7 Disposable plastic test tubes (13x100).

6. Reagents.

- 6.1 Stock P-A solution (1.25 N HCl, 1.5 N NH₄F): Add 54 ml of 48% HF to 700 ml of deionized water. Neutralize to pH 7.0 with NH₄OH (This makes 2 N HF). Add 108 ml of concentrated HCl (11.6 N) and dilute to 1 liter.
- 6.2 Dilute P-A solution (0.025 N HCl, 0.03 N NH₄F): Dilute 20 ml of stock P-A solution to 1 liter with deionized water.
- 6.3 Standard K stock solution: 10,000 ppm K
- 6.4 15 ppm K bulk standard solution (1.5 ml 10,000 ppm K stock solution diluted to 1 L with dilute P-A solution.)
- 6.5 Optional: 10,000 ppm cesium chloride solution (12.67g cesium chloride [ultra configuration grade] in 1 liter of 1% HNO₃).

Note: To suppress ionization of K, cesium chloride solution is added to all samples, blanks and standards to give a final concentration of 1000 ppm.

7. Methods

- 7.1 Place a 1.5-g scoop of soil into a 50-ml Erlenmeyer flask.
- 7.2 Add 15 ml of dilute P-A solution with the constant suction pipetting apparatus.
- 7.3 Shake the suspension on an oscillating shaker for 5 min.
- 7.4 Filter through filter paper into a 15-ml funnel tube.
- 7.5 Determine K in the clear filtrate using an atomic absorption spectrophotometer, ICP-OES or a flame photometer.

8. Calculations

Any necessary weight to volume dilutions are performed by computer during analysis, (in this case ppm in soil x 10).

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) - At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil - One or more standard soils of known extractable K content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as available ppm K in soil.

11. References

- 11.1 Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soil. *Soil Sci.* 59:39-45.
- 11.2 Munter, R.C. 1988. Laboratory factors affecting the extractability of nutrients. pp. 8-10. *In* W.C. Dahnke (ed.), *Recommended Chemical Soil Test Procedures for the North Central Region*. NCR Publ. 221 (revised). ND Agr. Exp. Sta., Fargo, ND.

Organic Matter

Weight Loss-on-Ignition (LOI 360°)

1. Application

This procedure is used for the routine estimation of soil organic matter by the loss of weight in a sample heated at a temperature high enough to burn organic matter but not so high as to decompose carbonates.

2. Summary of Methods

A sample of soil is dried at 105° C to remove moisture. The sample is weighed, heated at 360° C for 2 hours and weighed again after the temperature drops below 150° C.

3. Safety

Care should be exercised in handling hot samples. Be sure to cool the oven to 150° C before removing the samples from the oven. Use a good pair of tongs and grasp the sample firmly.

4. Interferences

Any material that losses moisture below 360° C is a potential source of error. Therefore, soil moisture must be removed before the base weight of the sample is taken. Also, ignited samples must not be allowed to re-absorb moisture from the air before they are weighed.

Gypsum loses water of hydration gradually. Soils containing gypsum should be heated initially at 150° C instead of 105° C. Some hydrated clays may also lose water below 360° C.

It is important that the results of this method be calibrated against organic carbon, preferably using a carbon analyzer, on soils from the area for which the test will be used.

5. Apparatus and Materials

- 5.1 Oven, or muffle furnace capable of being heated to 400° C and controlled to within $\pm 10^\circ$ C.
- 5.2 Beakers, 20 ml
- 5.3 Crucible rack, stainless steel
- 5.4 Balance accurate to ± 0.001 g in a draft free, low humidity environment
- 5.5 Soil scoop calibrated to hold 5 g of light-colored silt loam soil
- 5.6 Drying oven, 105° C

6. Reagents

An advantage of this method is that no reagents are required.

7. Methods

- 7.1 Place a 5 g scoop of soil into a tared 20-ml beaker
- 7.2 Dry for 2 hours or longer at 105° C
- 7.3 Record weight to ± 0.001 g
- 7.4 Bring oven to 360° C. Samples must then remain at 360° C for two hours.
- 7.5 Cool to < 150° C
- 7.6 Weigh to ± 0.001 g, in a draft-free environment

8. Calculations

- 8.1 Calculate percent weight loss-on-ignition (LOI)

$$\text{LOI} = \frac{(\text{wt. at } 105^{\circ}\text{C}) - (\text{wt. at } 360^{\circ}\text{C})}{\text{Wt. at } 105^{\circ}\text{C}} \times 100$$

- 8.2 Estimate % organic matter. Organic matter is estimated from LOI using regression analysis. Select soils covering the range in organic matter expected in the area serviced by the lab. Determine % organic matter using a carbon analyzer or by the Walkley-Black procedure for organic carbon. Regress OM on LOI.

9. Quality Control

- 9.1 At least one standard soil of known LOI value should be run with each batch of samples. If the result is not within the known standard deviation, corrective action is required.
- 9.2 All beakers should be re-tared monthly. Two beakers from each batch of 50 should be re-tared weekly. If the results are not within ± 0.002 g of the previous tared weight; re-tare all beakers in the batch.

10. Reporting

Data are reported as % LOI or as estimated % O.M.

11. References

- 11.1 Combs, S.M., and Nathan, M.V. 1998. Soil organic matter. Pp. 57-58. *In* J.R. Brown (Ed.), Recommended Chemical Soil Test Procedure for the North Central Region. NCR Publ. N0. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.
- 11.2 Schulte, E.E., and Hopkins, B.G. 1996. Estimation of soil organic matter by weight

loss-on-ignition. Pp.21-31. *In* F.R. Magdoff, M.A. Tabatabai, and E.A. Hanlon, Jr. (eds.), *Soil Organic Matter: Analysis and Interpretation*. Soil Sci. Soc. Am., Madison, WI.

Available Soil Zinc

1. Application

This procedure covers the extraction and analysis of plant available zinc (Zn) from soil.

2. Summary of Methods

Zinc is extracted with 0.1 N HCl. The extract is analyzed via AA spectrophotometry.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Contamination rather than interferences is a concern in Zn analysis. Some paint, rubber, and galvanized material contain this metal. Contamination is a problem because of the low concentrations of Zn in soil.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 1.5 g of light colored silt loam soil.
- 5.2 Phillips beaker (125 ml)
- 5.3 Constant suction pipette bank (15 ml)
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Funnel tube (10 ml)
- 5.6 Disposable plastic test tubes (13 x 100)
- 5.7 Filter paper (9 cm Whatman No. 2 or equivalent).
- 5.8 Atomic absorption spectrophotometer (AA), (Varian SpectrAA 220 FS with SIPS pump unit and auto sampler SPS -5)

6. Reagents

- 6.1 Extracting solution (0.1 N HCl): Dilute 8.67 ml of concentrated HCl (11.6 N) to 1 liter with deionized water. Store in plastic container or weathered glass. Avoid contact with rubber stoppers or tubing to prevent contamination.
- 6.2 1000 ppm Zn stock solution.

- 6.3 1.6 ppm Zn Bulk Standard (.4 ml 1000 ppm Zn stock solution diluted to 250 ml with 0.1 N HCl)

7. Methods

- 7.1 Transfer a 1.5 g scoop of soil to a 125 ml Phillips beaker.
7.2 Add 15 ml of extracting solution.
7.3 Shake the sample for 15 minutes on an oscillating shaker.
7.4 Filter the extract through Whatman No. 2 or equivalent filter paper into 10 ml funnel tubes.
7.5 Determine Zn in the filtered extract via AA spectrophotometry, using a bulk Zn standard containing 1.6 ppm Zn, which is diluted by the AA to make as many standards as the user specifies.

8. Calculations

$$\text{ppm Zn in soil} = \text{ppm Zn in solution} \times 10$$

9. Quality control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if the LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
9.2 Standard soil – One or more standard soils of known extractable Zn content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm available Zn in soil.

11. References

- 11.1 Whitney, D.A. 1998. Micronutrients: Zinc, iron, manganese, and copper. Pp.41-44. *In* J.R Brown (Ed.), Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publ. No. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.

Available Boron

1. Application

This method covers extraction and analysis of available boron (B) in soil, using hot, distilled water as the extractant and colorimetric analysis of the extracted B.

2. Summary of Methods

Boron is extracted with near-boiling deionized water on a heating block. Boron in the extract is reacted with curcumin to form an orange-colored complex. The concentration of B is determined colorimetrically, or by UV-Vis spectrophotometry.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

Normal precautions and common sense with heating blocks must be followed.

4. Interferences

Soluble organic matter, moisture, and sediment give positive interferences. Activated charcoal can be used to remove color from extracts of organic soils. Moisture is removed by heating an aliquot of the extract, and turbidity is avoided by flocculating colloids with CaCl_2 and by careful filtration. Samples should be filtered while still hot to prevent reabsorption of boron.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 10 g of light-colored silt loam soil
- 5.2 Folin digest tubes, 50-ml
- 5.3 Heating block capable of reaching 150° C
- 5.4 Filter paper, boron free (11 cm Whatman #40 or equivalent)
- 5.5 Pipetting device, 0.5-ml, 2-ml, and 10-ml
- 5.6 Beaker, 50-ml, polypropylene
- 5.7 Water bath or oven, 55° ± 3° C
- 5.8 Colorimeter tubes
- 5.9 Vortex stirrer or similar
- 5.10 Colorimeter or spectrophotometer

6. Reagents

- 6.1 Curcumin reagent: Dissolve 0.04 g of curcumin and 5 g oxalic acid in 100 ml of 95% ethanol. Store in freezer. Prepare fresh reagent weekly.
- 6.2 Standard B solution (100 ppm B):
- 6.3 Working B standards (0.25, 0.5, 1.0, and 2.0 ppm B)

7. Methods

- 7.1 Transfer a 10 g scoop of soil to a 50-ml folin digestion tube.
- 7.2 Add 20 ml of deionized water.
- 7.3 Place in a heating block set at 125° C, which has been preheated to approximately 85° C.
- 7.4 Bring samples to a boil and continue to boil samples for 3 to 5 minutes, stirring frequently.
- 7.5 Remove from heat, stir and filter immediately through B-free filter paper.
- 7.6 Place a 0.5-ml aliquot of the filtrate into a 50-ml polypropylene beaker.
- 7.7 Add 2-ml of curcumin reagent, and mix thoroughly.
- 7.8 Evaporate to dryness in a 55° ± 3° C oven.
- 7.9 After all visible liquid has disappeared; continue to heat for 15 min.
- 7.10 Add 10 ml of 95% ethanol, and stir to dissolve residue.
- 7.11 Transfer to a colorimeter tube.
- 7.12 Read the color at 540 nm. within 2 hours with a colorimeter or UV-Vis spectrophotometer.
- 7.13 Calibrate the instrument to read directly in ppm B in soil using the working B standards. These standard preparations are treated in the same manner as the soil extracts.

8. Calculations

(Note: intermediate dilutions are not included because standards and soil extracts are diluted alike.)

$$\text{ppm B in soil} = \text{ppm B in final solution}$$

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable B content is analyzed with each batch of samples to check for instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm B in soil.

11. References

- 11.1 Berger, K.C., and E. Truog. 1964 Soil Sci. Soc. Am. Proc. 10: 113.
- 11.2 Dible, et. Al. 1954. Anal. Chem. 26: 418
- 11.3 Watson, M.E. 1998. Boron. Pp. 45-48. *In* J.R. Brown (ed.), Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publ. No. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.

Available Soil Manganese

1. Application

This procedure covers the extraction and analysis of plant available manganese (Mn) from soil.

2. Summary of Methods

Available manganese is extracted with 0.1 N H₃PO₄. The extracted Mn is determined by (AA) atomic absorption spectrophotometry.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Ca, Fe, and Al may interfere unless masked by a manganese buffer.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 5 g of light-colored silt loam soil
- 5.2 Phillips beaker, (125 ml)
- 5.3 Pipettes (3, 25 ml)
- 5.4 Filter paper (9 cm Whatman No. 2 or equivalent)
- 5.5 Funnel tubes, (10 ml)
- 5.6 Disposable plastic test tubes (13x100)
- 5.7 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.8 Atomic absorption spectrophotometer (Varian Spectra AA 220 FS with SIPS pump unit and auto sampler SPS-5)

6. Reagents

- 6.1 Extracting solution (0.1 N H₃PO₄): Dilute 2.28 ml of 85% H₃PO₄ to 1 liter with deionized water.
- 6.2 Phosphoric acid: H₃PO₄, 85 %
- 6.3 NH₄OAc, 1 N: add 57 ml of glacial acetic acid to approximately 800 ml of deionized water in a volumetric flask. Mix thoroughly. Add 67 ml concentrated NH₄OH

slowly, in small increments, swirling flask after each increment. Mix; cool to room temperature. Adjust the pH of the solution to 7.0 with dilute NH_4OH or HOAc .

6.4 Manganese buffer (6:41-6:44)

6.4.1 Add 100 ml of 1 N NH_4OAc to a 1 liter volumetric flask, and dilute to approximately 300 ml with deionized water.

6.4.2 Add 37.2 g of Na_2EDTA , and swirl to dissolve.

6.4.3 Dissolve 0.88 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.24 g FeCl_3 , and 0.49 g $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ in approximately 100 ml of deionized water and transfer to above solution.

6.4.4 Add 35 ml of concentrated NH_4OH , dilute to 1000 ml with deionized water, and mix thoroughly.

6.5 1000 ppm Mn stock solution

6.6 8 ppm Mn Bulk Std. (4 ml 1000 ppm Mn stock solution diluted to 500 ml with 1:1 mixture of 0.1 N Phosphoric Acid: Mn Buffer solution)

7. Method

7.1 Place a 5 g scoop of soil into a 125 ml Phillips beaker.

7.2 Add 25 ml of extracting solution with a pipette.

7.3 Shake on an oscillating shaker for 15 minutes.

7.4 Filter into funnel tubes through filter paper.

7.5 Dilute 3ml of soil extract with 3 ml of manganese buffer and mix.

7.6 Determine Mn by AA using a bulk Mn standard containing 8ppm Mn, which is then diluted by the AA to make as many standards as the user specifies.

8. Calculations

8.1 $\text{ppm Mn in soil} = \text{ppm Mn in solution} \times 5$, where ppm Mn in solution is the concentration in the initial soil extract or the standard solutions before dilution.

9. Quality Control

9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.

9.2 Standard soil – One or more standard soils of known extractable Mn content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm available Mn in soil.

11. References

11.1 Hammes, J.K., and K.C. Berger. 1960. Soil Sci. 90:239-244.

- 11.2 Randall, G.W., E.E. Schulte, and R.B. Corey. 1976. Correlation of plant manganese with extractable soil manganese and soil factors. *Soil Sci. Soc. Am. J.* 40:282-287.
- 11.3 Whitney, D.A. 1998. Micronutrients: Zinc, iron, manganese, and copper. Pp. 41-44. In J.R. Brown (Ed.), *Recommended Chemical Soil Test Procedures for the North Central Region*. NCR Publ. No. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.

Exchangeable Cations (Ca^{++} , Mg^{++} , K^+ , Na^+)

1. Application

This procedure covers the extraction and analysis of exchangeable cations (Ca^{++} , Mg^{++} , K^+ , and Na^+) in soil.

2. Summary of methods

Exchangeable cations are extracted from the soil using an extracting solution (1 N NH_4OAc) at pH 7.0. The extracted solution is then analyzed by AA (atomic absorption) for the soil cations.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Ca, Mg, K and Na are partially ionized in the nitrous oxide–air acetylene or air acetylene flame of AA. To suppress ionization, cesium nitrate or chloride solution is added to give a final concentration of 1000 ppm in all solutions including the standards and blank. The purest available cesium compound must be used to avoid potassium contamination.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 1.5 g of light-colored silt loam soils.
- 5.2 Erlenmeyer flasks (50 ml).
- 5.3 Constant suction pipette apparatus (15 ml).
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Filter paper (9 cm Whatman No. 2 or equivalent).
- 5.6 Acid washed filter paper (9 cm Whatman No. 2 or equivalent).
- 5.7 Funnel tubes (15 ml)
- 5.8 Disposable plastic test tubes (13x100).
- 5.9 Atomic absorption spectrophotometer (AA), (Varian SpectrAA 220 FS with SIPS pump unit and auto sampler SPS-5).

6. Reagents

- 6.1 Extracting solution (1 N NH₄OAc; add 57 ml glacial acetic acid to 800 ml of deionized water in a volumetric flask. Mix thoroughly and slowly add 67 ml of concentrated NH₄OH. Mix and cool to room temperature. Adjust the pH of the solution to 7.0 by adding acetic acid or NH₄OH and dilute to 1 liter).
- 6.2 10,000 ppm cesium chloride solution (12.67g cesium chloride [ultra configuration grade] in 1 liter of 1% HNO₃).
- 6.3 10,000 ppm Ca stock solution
- 6.4 10,000 ppm Mg stock solution
- 6.5 10,000 ppm K stock solution
- 6.6 10,000 ppm Na stock solution
- 6.7 40 ppm Ca/Mg bulk solution (2 ml each 10,000 ppm Ca and 10,000 ppm Mg stock solution diluted to 500 ml with 1 N NH₄OAc)
- 6.8 15 ppm Exchangeable K bulk solution (.75 ml 10,000 ppm K stock solution diluted to 500 ml with 1 N NH₄OAc)
- 6.9 15 ppm Na bulk solution (.75 ml 10,000 ppm Na stock solution diluted to 500 ml with 1 N NH₄OAc)

7. Methods

- 7.1 Place a 1.5 g scoop of soil into a 50-ml Erlenmeyer flask.
- 7.2 Add 15 ml of extracting solution (1 N NH₄OAc, pH 7.0) by constant suction pipette.
- 7.3 Shake the suspension on an oscillating shaker for 15 minutes.
- 7.4 Filter through Whatman No. 2 filter paper into 15-ml funnel tubes. Acid washed filter papers should be used for Na extractions.
- 7.5 Determine Ca, Mg, K and Na in the filtered extract via AA spectrophotometry, using a bulk standard containing 40 ppm of Ca / Mg (run simultaneously); 15 ppm of K; or 15 ppm of Na respectively; which is diluted by the AA to make as many standards as the user specifies.

Note: Ca, Mg determinations are made using a nitrous oxide–acetylene flame. To suppress ionization for all of these elements, cesium chloride solution is added to all samples, blanks and standards to give a final concentration of 1000 ppm using the SIPS pump unit.

8. Calculations

Any necessary weight to volume dilutions are performed by computer during analysis, (in this case ppm in soil x 10).

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if the LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.

9.2 Standard soil – One or more standard soils of known extractable Ca, Mg, and content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm of exchangeable Ca, Mg, K and Na in soil. If the results are to be used to estimate cation exchange capacity (CEC), convert ppm of each cation to meq/100g of soil, then sum the values for the four cations:

$$\text{Estimated CEC} = \left[\frac{\text{Ca (ppm)}}{200} + \frac{\text{Mg (ppm)}}{122} + \frac{\text{K (ppm)}}{391} \right] \times \frac{(\text{soil} + \text{tare}) - \text{tare}^*}{4.25}$$

$$\left[\frac{(\text{soil} + \text{tare}) - \text{tare}^*}{4.25} \right] = \text{Soil density}$$

if soil density is not given:

$$\text{Estimated CEC} = \left[\frac{\text{Ca (ppm)}}{200} + \frac{\text{Mg (ppm)}}{122} + \frac{\text{K (ppm)}}{391} \right] \times \frac{\text{5-g scoop}}{\text{default wt}^*}$$

default wt* = 5 for texture code 2 (medium + fine) and 4 (red) soils
 wt = 6.25 for texture code 1 soils (sands)
 wt = 3.5 for texture code 3 soils (mucks)

11. References

- 11.1 Thomas, G.W. 1982. Exchangeable cations. pp. 159-165. *In* A.L. Page et al. (eds.), *Methods of Soil Analysis, Part 2*, 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- 11.2 Warncke, D., and J.R. Brown. 1982. Potassium and other basic cations. pp. 31-33. *In* J.R. Brown (ed.), *Recommended Chemical Soil Test Procedures for the North Central Region*. (Revised.) Missouri Agr. Exp. Sta. SB1001. Columbia, MO.

Available Soil Sulfate-Sulfur

1. Application

This method covers the extraction of sulfur in the form of sulfate (SO_4^{2-} -S) and turbidimetric analysis of the extracted SO_4^{2-} .

2. Summary of Methods

Sulfate-S is extracted with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ in 2 N HOAc. Sulfate in the extract is precipitated as BaSO_4 and measured turbidimetrically.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

In solutions containing small amounts of sulfate, dissolved organic matter acts as a sulfur protective colloid and causes low results. At high concentrations of sulfate, organic matter coprecipitates with barium sulfate and causes high results. The interference of organic matter can be removed by the addition of activated charcoal.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 10 g of light-colored silt loam soil.
- 5.2 Charcoal scoop calibrated to hold 0.1 g.
- 5.3 Erlenmeyer flask (50 ml).
- 5.4 25 ml filter tubes.
- 5.5 Nephelometer tubes (25 ml).
- 5.6 Constant suction pipette bank (25 ml).
- 5.7 Activated charcoal, SO_4^{2-} free, prepared as follows:
 - Boil approximately 20 g of charcoal in 200 ml of 6 N HCl for 10 min.
 - Filter under suction
 - Wash with deionized water until free of Cl^-
 - Dry at 105° C.
- 5.8 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.9 Acid washed filter paper (11 cm Whatman No. 2 or equivalent).
- 5.10 Filter paper (24 cm Whatman #2 and Whatman #8 or equivalent).

- 5.11 Filtering Apparatus: 27 cm buchner funnel, 4000 ml Erlenmeyer flask, vacuum pump.
- 5.12 Turbidimeter (HF Scientific, DRT 100B, model # 20052).
- 5.13 Nephelometer tubes (50 ml) matched.

6. Reagents

- 6.1 Extracting solution (500 ppm P in 2 N HOAc): dissolve 2.03 g of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ in about 800 ml of deionized water. Add 115 ml of glacial acetic acid and dilute to liter.
- 6.2 Gum Arabic (BaCl_2 -HOAc): dissolve 5 g of gum Arabic in about 500 ml of hot deionized water. Cool slightly, then filter with filtering apparatus. Put 24 cm filter papers into buchner funnel one at a time #2 on the bottom, and #8 on the top and wet them down with deionized water. Add 50 g of $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and 450 ml of glacial acetic acid, and dilute to 1 liter.
- 6.3 Sulfur stock solution (1000 ppm)
- 6.4 Working S standards (0, 2, 4, 6, 8, and 10 ppm S).
Dilute from sulfur stock solution (1000 ppm) as follows:
 - 0 ppm: Sulfur extracting solution (6.1).
 - 2 ppm: 0.5 ml of 1000 ppm S stock solution diluted to 250 ml with extracting solution.
 - 4 ppm: 1 ml of 1000 ppm S stock solution diluted to 250 ml with extracting solution.
 - 6 ppm: 1.5 ml of 1000 ppm S stock solution diluted to 250 ml with extracting solution.
 - 8 ppm: 2 ml of 1000 ppm S stock solution diluted to 250 ml with extracting solution.
 - 10 ppm: 2.5 ml of 1000 ppm S stock solution diluted to 250 ml with extracting solution.

7. Methods

- 7.1 Transfer a 10 g scoop of soil to a 50-ml Erlenmeyer flask.
- 7.2 Add 25 ml of extracting solution by means of constant suction pipette bank.
- 7.3 Add a 0.1 g scoop of activated charcoal.
- 7.4 Shake the sample for 15 minutes on an oscillating shaker.
- 7.5 Filter through S-free filter paper into a clean, dry 50 ml Erlenmeyer flask.
- 7.6 Transfer a 10-ml aliquot of filtrate to a 25 ml nephelometer tube.
- 7.7 Rinse pipette bank with deionized water before adding gum Arabic solution.
- 7.8 Add 10 ml of BaCl_2 -gum Arabic-HOAc solution. Dip the pipette a few centimeters below the surface of the solution in the nephelometer tube, and bubble the mixture for 5 seconds.
- 7.9 Read NTU (National Turbidity Units) on turbidimeter, with the instrument adjusted to 0 with the zero S standard.
- 7.10 Preparation of standard curve: (Prepare a new standard curve each day)
 - Take a 10 ml aliquot of each working standard and treat as with samples

(steps 7.6-7.8).

- Plot ppm S against NTU. Alternatively, regress NTU against ppm S, and use the resulting regression equation to calculate ppm S in unknown solutions.

8. Calculations

The concentration of SO_4^{2-} -S in the working standards (0, 2, 4, 6, 8, 10) is equivalent to 0, 5, 10, 15, 20, and 25 ppm of SO_4^{2-} -S in the soil when put through the following equation:

$$\text{ppm } \text{SO}_4^{2-}\text{-S in soil} = \text{ppm } \text{SO}_4^{2-}\text{-S in the solution} \times 2.5$$

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more of the standard soils of known extractable SO_4^{2-} -S content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.
- 9.3 Precautions: Pipette all of the sample extracts (step 7.6) and the working standard solutions before rinsing the pipettes in the pipette bank with water. Then pipette the BaCl_2 -gum Arabic-HOAc solution (step 7.7). Otherwise, a film of BaSO_4 will coat the inside of the pipette and will be difficult to remove, and may contaminate subsequent samples. The BaCl_2 -gum Arabic-HOAc solution should be added to all samples and standards at the same rate. That is, the delivery rate of each pipette in the bank should be the same. The rate of addition influences the size of the BaSO_4 particles that develop. Large particles will give a different nephelometer / turbidometer reading than the same amount of S in fine particles.

10. Reporting

Results are reported as ppm SO_4^{2-} -S in soil.

11. References

- 11.1 Combs, S.M., J.L. Denning, and K.D. Frank. 1998 Sulfate-Sulfur. Pp. 35-40. In J.R. Brown (Ed.), Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publ. No. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.
- 11.2 Hoefl, R.G., L.M. Walsh, and D.R. Keeney. 1973. Soil Sci. Soc. Am. Proc. 37:401-404.

11.3 Hesse, P.R. 1957. The effect of colloidal organic matter on the precipitation of barium sulfate and a modified method for determinant soluble sulfate in soils. Analyst 82:710-712

Manure Sampling Instructions

Soil Science Department, UW-Madison

Solid manure - Dairy, Beef, Swine, Poultry

Sampling while loading - *Recommended method for sampling from a stack or bedded pack.* Take at least five samples while loading several spreader loads and combine to form one composite sample. Thoroughly mix the composite sample and take an approximately one pound sub-sample using a one-gallon plastic bag. *Sampling directly from a stack or bedded pack is not recommended.*

During spreading - Spread tarp in field and catch the manure from one pass. Sample from several locations and create a composite sample. Thoroughly mix composite sample together and take a one pound sub-sample using a one-gallon plastic bag.

Dairy haul - Place a five-gallon pail under the barn cleaner 4-5 times while loading a spreader. Thoroughly mix the composite sample together and take a one pound sub-sample using a one-gallon plastic bag. Repeat sampling 2-3 times over a period of time and test separately to determine variability.

Poultry In-house - Collect ten samples from throughout the house to the depth the litter will be removed. Samples near feeders and waterers may not be indicative of the entire house and sub-samples taken near here should be proportionate to their space occupied in the whole house. Mix the samples well in a five-gallon pail and take one pound sub-sample, place it in a gallon zip-lock bag.

Stockpiled litter - Take ten sub-samples from different locations around the pile at least 18 inches below the surface. Mix in a five-gallon pail and place a one pound composite sample in a gallon zip-lock bag.

Liquid Manure - Dairy, Beef, Swine

From storage - Agitate storage facility thoroughly before sampling. Collect at least five samples from storage facility or during loading using a five-gallon pail. Place sub-sample of the composite sample in a one-quart plastic container. *Sampling a liquid manure storage facility without proper agitation (2-4 hrs. minimum) is not recommended.*

During application - Place buckets around field to catch manure from spreader or irrigation equipment. Combine and mix samples into one composite sub-sample in a one-quart plastic container.

Sample handling and storage

Solid/Semi-solid samples - Thoroughly mix composite sample and fill one-gallon plastic heavy-duty ziplock bag approximately one-half full. One method of mixing a composite sample is to pile the manure and then shovel from the outside to the inside of the pile until well mixed. Squeeze out excess air, close and seal. Store sample in freezer if not delivered to the lab immediately.

Liquid samples - Thoroughly mix composite sample and fill a one-quart plastic bottle not more than three-quarters full. Using a plunger and an up-and-down action works well for mixing liquid manure in a five-gallon pail. Store sample in freezer if not delivered to the lab immediately.

Sample Identification and Delivery

Identify the sample container with information regarding the farm, animal species and date. This information should also be included on the sample information sheet along with application method, which is important in determining first year availability of nitrogen. To obtain an information sheet please see the submission form page at <http://uwlab.soils.wisc.edu/forms.htm>.

Keep all manure samples frozen until shipped or delivered to a laboratory. Ship early in the week (Mon.-Wed.) and avoid holidays and weekends.

Soluble Salts (Electrical Conductivity)

1. Application

This procedure covers the determination of soluble salts in soil by measuring the electrical conductivity (EC) of a 1:2 soil:water suspension

2. Summary of Methods

The electrical conductivity of a soil suspension increases as the salt concentration increases. In this procedure, a suspension of soil in water (1 part soil:2 parts water) is placed in a conductivity cell, and the electrical conductivity is measured.

3. Safety

No hazardous chemicals are used in this analysis.

4. Interferences

This procedure estimates soluble salts indirectly from electrical conductivity. It does not identify which salts are present.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 10.0 g of light-colored silt loam soil.
- 5.2 Conductivity meter with an operating range between 0.001-20 dS/m.
- 5.3 50-ml beaker or 13-dram vial

6. Reagents

- 6.1 Prepare a 0.01 N KCl solution, with a conductivity of 1.412 dS/m (141.2 mhos $\times 10^{-5}$ /cm) at 25°C. Dissolve 0.746 g of oven-dried (105°C) KCl in deionized water and dilute to 1 liter.

7. Methods

- 7.1 Place a 10 g scoop of soil in beaker or vial.
- 7.2 Add 20-ml of deionized water.
Note: Any 1:2 ratio of soil:water can be used such as 5g soil with 10ml water.
- 7.3 Stir, let stand for 15 minutes.
- 7.4 Place conductivity probe into the 0.01 N KCl solution in a 50-ml beaker.
- 7.5 Set the instrument to read 1.412 dS/m.

7.6 The conductivity meter is now ready for use. Place conductivity probe into sample suspensions and read directly off display while stirring the probe in the solution.

Note: The bottom of the probe must be totally immersed in the solution for suspension to read properly.

8. Calculations

dS/m = mmhos/cm

9. Quality Control

9.1 Standard – One or more standard solutions of known electrical conductivity are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as electrical conductivity in dS/m (equivalent to mmhos/cm).

The interpretation of these results is dependent on soil texture and the soil:water ratio. The interpretation of soils prepared with a 1:2 (soil:water) ratio is listed below.

For sandy textured soils	Low	0-0.25 dS/m
	Medium	0.26-0.75 dS/m
	Excessive	>0.75 dS/m

For silty textured soils	Low	0-0.40 dS/m
	Medium	0.41-1.05 dS/m
	Excessive	>1.05 dS/m

Conversion factor: $(\text{mhos} \times 10^{-5}/\text{cm})/100 = \text{dS/m}$

11. References

11.1 Whitney, D.A. Soil Salinity. Pp. 59-60. In J.R. Brown (Ed.), Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publ. No. 221 (revised). Missouri Agr. Exp. Sta. SB 1001. Columbia, MO.

Particle Size Analysis (Hydrometer Method)

1. Application

The percentage of sand, silt and clay in the inorganic fraction of soil is measured in this procedure. The method is based on Stoke's law governing the rate of sedimentation of particles suspended in water.

2. Summary of Methods

The sample is treated with sodium hexametaphosphate to complex Ca^{++} , Al^{3+} , Fe^{3+} , and other cations that bind clay and silt particles into aggregates. Organic matter is suspended in this solution. The density of the soil suspension is determined with a hydrometer calibrated to read in grams of solids per liter after the sand settles out and again after the silt settles. Corrections are made for the density and temperature of the dispersing solution.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

The principal source of error in this procedure is the incomplete dispersion of soil clays. These clays are cemented by various chemical agents and organic matter into aggregates of larger size. Failure to effect complete dispersion results in low values for clay and high values for silt and sand. The rate of sedimentation also is affected by temperature and the density of the dispersing solution.

5. Apparatus and Materials

- 5.1 Glass cylinders, 1000-ml capacity
- 5.2 Thermometer, Fahrenheit
- 5.3 Hydrometer, Bouyoucos (Fisherbrand Model # 14-331-5c)
- 5.4 Electric mixer with dispersing cup
- 5.5 Plunger
- 5.6 Balance sensitive to $\pm 0.01\text{g}$

6. Reagents

- 6.1 Dispersing solution, 5%: Dissolve 50 g of sodium hexametaphosphate, $\text{Na}_6(\text{PO}_3)_6$ in deionized water and dilute to 1 liter.

7. Methods

- 7.1 Mix 100 ml of the 5% dispersing solution and 880 ml of deionized water in a 1000 ml cylinder. This mixture is the blank. (Note: 100 ml + 880 ml = 980 ml. This blank is not diluted to 1000 ml; the other 20 ml is the volume occupied by 50 g of soil.).
- 7.2 Weigh 25-50 g of soil and transfer to a dispersing cup. Record weight to $\pm 0.01\text{g}$.
- 7.3 Add 100-ml of 5% dispersing solution.
- 7.4 Attach dispersing cup to mixer and mix the sample for 30 – 60 sec.
- 7.5 Transfer the suspension quantitatively from the dispersing cup to a 1000 ml cylinder.
- 7.6 Fill to the 1000-ml mark with deionized water equilibrated to room temperature, or allow to stand overnight to equilibrate.
- 7.7 At the beginning of each set, record the temperature, and the hydrometer reading of the blank, using the procedure described below.
- 7.8 To determine the density insert plunger into suspension, and carefully mix for 30 sec. until a uniform suspension is obtained. Remove plunger (begin 40 second timer) and gently insert the hydrometer into the suspension.
- 7.9 Record the hydrometer reading at 40 sec. This is the amount of silt plus clay suspended. The sand has settled to the bottom of the cylinder by this time. (Repeat 7.8 – 7.9 for each sample)
- 7.10 Record the hydrometer reading again after 6 hours, 52 minutes. This is the amount of clay in suspension. The silt has settled to the bottom of the cylinder by this time.

8. Calculations

- 8.1 Temperature and density corrections:
 - add 0.2 unit to the readings of the samples for every 1° F above 67° F, and subtract 0.2 unit for every 1° F below 67° F.
 - subtract the density of the blank at each reading, from the corresponding density readings for the samples.

- 8.2 Percent clay:

$$\% \text{ clay} = \text{corrected hydrometer reading at 6 hrs, 52 min.} \times 100 / \text{wt. of sample}$$

8.3 Percent silt:

$$\% \text{ silt} = \text{corrected hydrometer reading at 40 sec.} \times 100 / \text{wt. of sample} - \% \text{ clay}$$

8.4 Percent sand:

$$\% \text{ sand} = 100\% - \% \text{ silt} - \% \text{ clay}$$

9. Quality Control

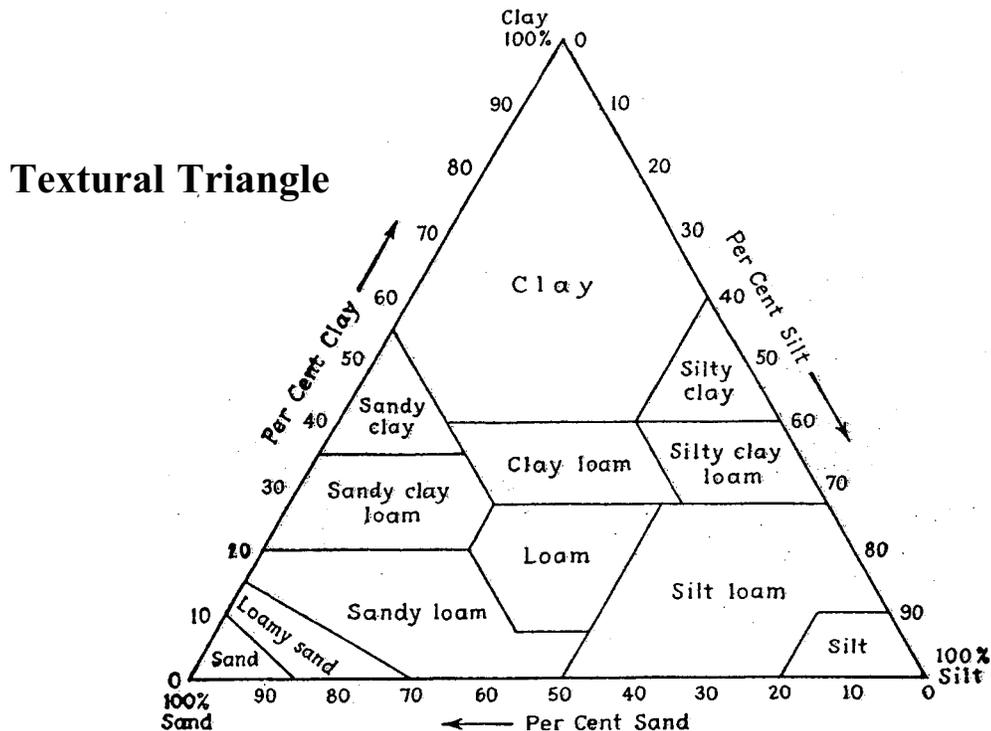
9.1 Standard soil - a standard soil of known particle size content is analyzed with each batch of samples to check for instrument calibration and procedural accuracy.

10. Reporting

Results are reported as percentages of the mineral fraction, % sand, % silt, and % clay. Soil texture is based on the USDA textural triangle. (see chart below)

11. References

11.1 Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54:464-465.



DATE: SEPT 2004

Soil Inorganic Nitrogen Nitrate Nitrogen (Colorimetric Method)

1. Application

In this procedure, nitrogen in the form of the nitrate ion ($\text{NO}_3^- \text{N}$) is extracted from the soil with water and measured colorimetrically after reaction with phenoldisulphonic acid.

2. Summary of Methods.

Water is used to extract $\text{NO}_3^- \text{N}$, using 1 part soil to 5 parts water. Colloids are precipitated with Ca^{++} , and soluble organics are removed with activated charcoal. After filtration, an aliquot of extract is reacted with phenoldisulphonic acid. The $\text{NO}_3^- \text{N}$ forms a blue-colored complex, which is analyzed with a colorimeter.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis. In this procedure, fuming sulfuric acid is used to prepare the phenoldisulphonic acid.

4. Interferences

Principles interferences are chloride and soluble organic compounds. Chloride is precipitated with Ag_2SO_4 . Colored organic compounds are co-precipitated with $\text{Cu}(\text{OH})_2$ by the addition of CuSO_4 , followed by $\text{Ca}(\text{OH})_2$.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to contain 10 g of light-colored silt loam.
- 5.2 Erlenmeyer flask, 125- ml
- 5.3 Graduate cylinder, 50-ml, 100-ml
- 5.4 Oscillating shaker
- 5.5 Measuring scoop, $\frac{1}{2}$ tsp
- 5.6 Beaker, 150-ml
- 5.7 Funnel tubes
- 5.8 Hotplate
- 5.9 Pipette, 10-ml
- 5.10 Medicine dropper, 3-ml
- 5.11 Burette, 50-ml
- 5.12 Colorimeter or spectrophotometer
- 5.13 Colorimeter tubes, matched

6. Reagents.

- 6.1 CuSO₄ solution, saturated: Add 210 g of CuSO₄·5H₂O to 100 ml of water.
- 6.2 Ag₂SO₄ solution, saturated: Add 10 g of Ag₂SO₄ to 100 ml of water.
- 6.3 Ca(OH)₂: finely ground powder
- 6.4 MgCO₃: finely ground powder
- 6.5 Activated charcoal: Heat in a muffle furnace at 500 °C for 1 hour to remove NO₃⁻.
- 6.6 Phenoldisulphonic acid: Dissolve 83 g pure phenol in 500 ml of concentrated H₂SO₄. Dissolve until clear. (Check the H₂SO₄ for NO₃⁻ contamination by dropping several crystals of phenol in several ml of the acid. The solution must remain clear.) Add a 1-pint bottle of fuming H₂SO₄. (Use the fume hood!) Place in a boiling water bath for two hours. Store in an amber bottle in a dark cabinet. **This reagent is extremely corrosive.**
- 6.7 NH₄OH, 1:1: Mix equal volumes of concentrated NH₄OH and distilled water.
- 6.8 Stock standard nitrate solution, 500 ppm N: Dissolve 3.60 g KNO₃, dried at 105 °C, in water and dilute to 1 liter with water.
- 6.9 Dilute standard nitrate solution, 20 ppm N: Dilute 20 ml of 500 ppm N to 500 ml with water.

7. Methods

- 7.1 Place a 10-g scoop of soil into a 125-ml Erlenmeyer flask.
- 7.2 Add 50 ml of water by means of a graduate cylinder.
- 7.3 Add 2 drops of Ag₂SO₄ and 3 drops of CuSO₄.
- 7.4 Shake 10 min on an oscillating shaker (or 30 min intermittently by hand).
- 7.5 Add ½ tsp of Ca(OH)₂; shake thoroughly by hand and let stand 10 minutes.
- 7.6 Decant about 30 ml of the suspension into a 150-ml beaker.
- 7.7 Add ½ tsp of MgCO₃ and swirl.
- 7.8 Add ½ tsp of activated charcoal; shake by hand and let stand 2 to 3 minutes.
- 7.9 Filter into funnel tubes.
- 7.10 Wash the 150-ml beakers employed in steps 7.6 – 7.9.
- 7.11 Pipette 10 ml of filtrate into the same 150-ml beaker, and evaporate to dryness on a hotplate. The temperature of the hotplate should not be high enough to permit spattering as the solution approaches dryness. The sample must be completely dry.
- 7.12 Cool; then add 3 ml of phenoldisulphonic acid rapidly to the residue in the beaker. Use a rapid delivery medicine dropper calibrated to deliver 3 ml. The reagent should flood the bottom of the beaker rapidly to prevent formation and loss of volatile nitrogen oxides.
- 7.13 Swirl; let stand until the residue is dissolved and the solution is clear.
- 7.14 Carefully add approximately 20 ml of distilled water.
- 7.15 Cool.
- 7.16 With a 50-ml burette in a fume hood, carefully add 1:1 NH₄OH until full yellow color develops and then 3 ml in excess (approximately 15 ml total).

- 7.17 Transfer the sample to a 100-ml graduate cylinder and dilute to 99 ml with water. Mix the solution by pouring back-and-forth from cylinder to beaker several times. (A small amount of solution will remain as a film in the beaker. Also, a graduate cylinder is calibrated “to deliver” rather than “to contain” a given volume. A 100-ml graduate cylinder will contain slightly more than 100 ml, the excess being retained as a film on the cylinder walls when the cylinder is emptied. To compensate, the cylinder is filled to only 99 ml. A volumetric flask should be used for precise work.)
- 7.18 Determine the $\text{NO}_3^- \text{N}$ using a colorimeter at 420 nm. Zero the colorimeter with a reagent blank.
- 7.19 Prepare a standard curve by evaporating the volumes of 20 ppm $\text{NO}_3^- \text{N}$ solution indicated in the table below to dryness in a 150-ml beaker, and proceed with steps 7.12 above.

$\text{NO}_3^- \text{N}$ equivalents of different volumes of standard 20 ppm $\text{NO}_3^- \text{N}$ solution.

Vol. of 20 ppm $\text{NO}_3^- \text{N}$ soln.	Final conc. of $\text{NO}_3^- \text{N}$	$\text{NO}_3^- \text{N}$ equiv- alent in soil*
ml	ppm	ppm
0	0.0	0
1	0.2	5
2	0.4	10
3	0.6	15
4	0.8	20
5	1.0	25
7	1.4	35
10	2.0	50

* $\text{NO}_3^- \text{N}$ equivalent in soil using wt. of soil and solution volumes indicated in Methods.

8. Calculations

$$\begin{aligned} \text{ppm } \text{NO}_3^- \text{N in soil} &= \text{ppm } \text{NO}_3^- \text{N in final solution} \times \frac{50 \text{ ml}}{10 \text{ g}} \times \frac{100 \text{ ml}}{10 \text{ ml}} \\ &= \text{ppm } \text{NO}_3^- \text{N in final soln.} \times 50 \end{aligned}$$

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable $\text{NO}_3^- \text{N}$ content is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm of nitrogen in the form of nitrate $\text{NO}_3^- \text{N}$ in soil.

Nitrate and Ammonium in Soil and Tissue

1. Application

In this procedure nitrogen, in the form of nitrate and nitrite ion, is extracted from soil or tissue samples and analyzed by flow injection.

2. Summary of Methods

KCl is used to extract NO_3^- -N and NH_4^- -N from the soil and tissue samples.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Apparatus and Materials

- 5.1 Weigh boat (metal or glass)
- 5.2 Erlenmeyer flasks (50-ml)
- 5.3 Pipette bank (15-ml)
- 5.4 Time-controlled, oscillating shaker.
- 5.5 Filter paper, 9-cm (Whatman No. 2 or equivalent)
- 5.6 Funnel tubes (15-ml)
- 5.7 Glass test tubes (6.2-ml)
- 5.8 Flow injection

6. Reagents

- 6.1 2 N KCl solution (1044.40 g of KCl to 7 liters of de-ionized water).

7. Methods

- 7.1 Weigh out 1.50 g of soil or .25 g of tissue into a weigh boat.
- 7.2 Transfer sample to a 50-ML Erlenmeyer flask.
- 7.3 Add 15-ml of 2 N KCl solution using constant suction pipette.
- 7.4 Shake for 15 minutes on oscillating shaker.
- 7.5 Filter immediately.

- 7.6 Pipette 5-ml of filtrate into glass test tube.
- 7.7 Analyze by flow injection.

8. Calculations

Sample concentration is calculated from a regression equation by plotting response versus standard concentration.

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm of nitrogen in the form of nitrate NO_3^- -N or ammonium NH_4^- -N in soil.

11. References

- 11.1 Lachat Instruments. 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuickChem Method 12-107-04-1-B.
- 11.2 Jaromir Ruzicka. 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. Analytical Chemistry 55: 1040A-1053A.

Nitrate and Ammonium in Soil and Tissue

1. Application

In this procedure nitrogen, in the form of nitrate and nitrite ion, is extracted from soil or tissue samples and analyzed by flow injection.

2. Summary of Methods

KCl is used to extract NO_3^- -N and NH_4^- -N from the soil and tissue samples.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Apparatus and Materials

- 5.1 Weigh boat (metal or glass)
- 5.2 Erlenmeyer flasks (50-ml)
- 5.3 Pipette bank (15-ml)
- 5.4 Time-controlled, oscillating shaker.
- 5.5 Filter paper, 9-cm (Whatman No. 2 or equivalent)
- 5.6 Funnel tubes (15-ml)
- 5.7 Glass test tubes (6.2-ml)
- 5.8 Flow injection

6. Reagents

- 6.1 2 N KCl solution (1044.40 g of KCl to 7 liters of de-ionized water).

7. Methods

- 7.1 Weigh out 1.50 g of soil or .25 g of tissue into a weigh boat.
- 7.2 Transfer sample to a 50-ML Erlenmeyer flask.
- 7.3 Add 15-ml of 2 N KCl solution using constant suction pipette.
- 7.4 Shake for 15 minutes on oscillating shaker.
- 7.5 Filter immediately.

- 7.6 Pipette 5-ml of filtrate into glass test tube.
- 7.7 Analyze by flow injection.

8. Calculations

Sample concentration is calculated from a regression equation by plotting response versus standard concentration.

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm of nitrogen in the form of nitrate NO_3^- -N or ammonium NH_4^- -N in soil.

11. References

- 11.1 Lachat Instruments. 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuickChem Method 12-107-04-1-B.
- 11.2 Jaromir Ruzicka. 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. Analytical Chemistry 55: 1040A-1053A.

Nitrogen (Total/Kjeldahl)

1. Application

This method covers the digestion of samples for Nitrogen (Total/Kjeldahl)

2. Summary of Methods

Total nitrogen (Org N + NH₄-N + NO₃-N, NO₂-N) digested with sulfuric acid, metal catalyst, salicylic acid.

Total Kjeldahl Nitrogen (Org N + NH₄-N) digested with sulfuric acid and metal catalyst.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Samples must not consume more than one fifth of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.7 to 7.0% (v/v) H₂SO₄ in the diluted digestion sample without any change in signal intensity.
- 4.2 Samples with particles remaining after digestion will require filtering prior to analysis by FIA.

5. Sample Collection, Preservation and Handling

- 5.1 Soil and plant samples are dried at 55°C, 65°C, respectively. The dried soil is then ground to pass a 12 mesh screen and plant tissue is ground to pass a 2 mm screen.
- 5.2 Water sample are stored at 4°C.

6. Apparatus and Materials

- 6.1 Scale 0.001 g
- 6.2 QuickChem 8000 Automated Ion Analyzer
- 6.3 Block Digester (Easy digest 40/20) Westco Scientific Instruments
- 6.4 75 ml digestion tubes
- 6.5 Vortex Mixer

7. Reagents (FIA 7.1-7.4) (N Digestion 7.5-7.7)

Flow Injection:

- 7.1 Buffer – Dissolve 65.0 sodium hydroxide, 50.0 g sodium potassium tartrate and 26.8 g sodium phosphate dibasic heptahydrate in deionized water (10 megohm) and dilute to 1 liter. Degas the buffer solution by passing He at 140 kPa through a helium degassing tube for one minute.
- 7.2 Color Reagent – Dissolve 150.0 g sodium salicylate and 1.0 g sodium nitroprusside in deionized water and dilute to 1 liter. Degas the solution with He.
- 7.3 Hypochlorite Solution – In a 1 L volumetric flask, add 60.0 ml regular Chlorox bleach (5.25% sodium hypochlorite), dilute to 1 L with deionized water.
- 7.4 Carrier – In a 1 L volumetric flask containing approximately 600 ml deionized water, add 70.0 ml of sulfuric acid, 30.0 g of potassium sulfate, and 2.5 g of copper sulfate. Dilute to 1 L with deionized water.

Nitrogen Digestion:

- 7.5 Conc. H₂SO₄
- 7.6 Metal Catalyst (digestion tablet – potassium sulfate 93%, cupric sulfate 7%)
- 7.7 Salicylic acid (75 g salicylic acid/2.5 L H₂SO₄) is used when including NO₃-N + NO₂-N

8. Methods

- 8.1 Weigh out 0.15-0.20 g of dried plant tissue or 0.45-0.5 g of soil into a clean, dry digestion tube. Carry a (LRB) blank through all steps of the procedure (see 10.1).
- 8.2 For Total Kjeldahl N (Org N + NH₄-N): To each tube add 1 (metal catalyst) digestion tablet and 3.5 ml of concentrated H₂SO₄.
- 8.3 For Total N (Org N + NH₄-N + NO₃-N + NO₂-N): To each tube add 1 (metal catalyst) digestion tablet and 3.5 ml of H₂SO₄ with Salicylic acid.
- 8.4 Place tubes in a block digester. Set temperature 160°C and time 1 to 20 minutes. Set temperature to 380°C and time 240 minutes.
- 8.5 Remove the samples from the block and allow 15 minutes for cooling.
- 8.6 Fill with deionized water to 50.0 ml. If samples are not run immediately, they should be covered to prevent evaporation.
- 8.7 Transfer \approx 7 ml of digested solution to FIA tubes.
- 8.8 Determine the ammonium concentration by FIA.

9. Calculations

The nitrogen content is calculated using the formula:

$$\text{ppm N} = 50/W_S X C_D \quad (\text{for soil sample})$$

$$\% \text{ N} = 50/W_S X C_D / 10,000 \quad (\text{for plant sample})$$

where W_S = Weight of sample (g)
 C_D = Concentration in the digest (mg N/l)

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB must be analyzed with each batch of samples in order to assess contamination from the laboratory environment. If LRB values exceed the method detection limit, laboratory or reagent contamination should be suspected, take correction action before continuing the analysis.
- 10.2 Laboratory Fortified Blank (LFB) – At least one LFB must be analyzed with each batch of samples. Calculate accuracy as percent recovery. If the recovery of any analyte falls outside the required control limits of 90-110%, the analyte is judged out of control, take corrective action for continuing analysis.
- 10.3 Instrument Performance Check Solution (IPC) – For all determinations, a mid-range check standard and a calibration blank must be analyzed immediately after daily instrument calibration, after every tenth sample, and at the end of the sample run. This process verifies that the instrument is within 10% of calibration. If the IPC solution indicates that the calibration is outside of present limits, take corrective action before continuing analysis.

11. Reporting

- 11.1 Data is reported as mg/l of N for soil and % N for plant tissue on a dry weight basis.
- 11.2 Detection limit = 0.01 mg/l

12. References

- 12.1 Lachat Instruments 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuikChem Method 13-107-06-2-D.
- 12.2 Jaromir Ruzicka 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. Analytical Chemistry 55: 1040A-1053A.
- 12.3 Bremner, J. M. 1965. Total nitrogen. In C. A. Black et al. (ed.) Methods of soil analysis, Part 2. Agronomy 9:1149-1178. Am. Soc. Of Agron., Inc., Madison, Wis.

DATE: 9/21/2011

Carbon (Total, Organic, and Inorganic)

1. Application

This method covers the determination of total carbon (TC), organic carbon (OC) and inorganic carbon (IC) concentrations in soil, plant tissues and manures by dry combustion using a LECO CNS-2000 analyzer.

The LECO CNS-2000 Carbon, Nitrogen and Sulfur Analyzer is a non-dispersive, infrared, microcomputer based instrument, designed to measure the total carbon, nitrogen, and sulfur content in a wide variety of materials (soil, plant tissue, fertilizers, meat products, dairy products, seeds, food, resins, and environmental wastes) in a nominal 200 mg sample weight.

2. Summary of Method

Total carbon and organic carbon contents of a sample are determined in two separate combustion conditions/profiles. The first combustion profile will maximize the recovery of TC while the second profile will minimize the decomposition of carbonate C and maximize the recovery of OC. The two main variables of these profiles are the furnace temperatures and the oxygen flow rate. The furnace temperatures are set at 1350°C and 900°C for the TC and OC profiles, respectively. IC is calculated as the difference between the TC and OC values.

Although SPAL uses 900°C for the determination of OC, temperatures between 375 and 1000°C are found in the literature. Based on this, SPAL could accommodate specific requests from clients to run OC samples at a specific temperature.

In some cases hydrochloric acid has been used for the decomposition of carbonates. However, this treatment generates Cl gases that can damage the infrared detector and requires the use of scrubbing substrates in the system to prevent damage. Therefore, if a client needs to determine organic and inorganic carbon (by difference) the first option given is the OC at 900°C, the second option is OC at a temperature defined by the client, and as a last option (and therefore more expensive) the use of hydrochloric acid (by the client or by SPAL) to remove carbonates previous to the OC determination.

3. Safety

3.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

3.2 Follow the manufacturer's recommendation for safe operation of the instrument.

3.3 Secure compressed gas cylinders and use the proper gas regulators.

3.4 Sample boats being unloaded from the furnace are extremely hot - do not handle them until they cool down.

4. Interferences

4.1 Fineness of the ground sample affects sample combustion and thus analysis results. All samples should be ground to pass an 18 mesh sieve (1-mm) or finer.

4.2 Sample boats will be contaminated with inorganic carbonates (IC) following the analysis for organic carbon. To remove the carbonates place the contaminated boats in a muffle furnace at 1000°C for one hour or run the boats as blanks at 1350°C in the Leco CNS-2000

5. Sample Collection, Preservation and Handling

5.1 Soil and plant samples are dried at 55°C and 65°C, respectively. The dried soil sample is then ground to pass a 12 mesh screen and the plant tissue is ground to pass a 2 mm screen.

5.2 Acid-digested or acid-treated samples should not be run on the Leco CNS-2000

5.3 The Leco COM-CAT combustion accelerator can be used to insure complete combustion when large samples are used or when total sulfur determination is required

6. Apparatus and Materials

6.1 Scale 0.0001 g

6.2 Leco CNS-2000 Carbon, Nitrogen and Sulfur Analyzer

6.3 Autoloader Assembly with 49-position sample rack

6.4 Printer

6.5 Sample spatula

7. Reagents

7.1 COM-CAT combustion catalyst (Tungsten Tri-oxide, Leco 501-426)

7.2 Anhydron (Anhydrous Mg perchlorate, Leco 501-171)

7.3 Lecosorb (Sodium hydroxide, Leco 502-174)

7.4 Sulfamethazine (Leco 502-304), EDTA (Leco 502-092) or soil standards (Leco 502-309, 502-308)

7.5 Glass wool

7.6 UHP helium gas

7.7 UHP oxygen gas

7.8 Compressed air (low water content)

8. Methods

8.1 Operate instrument according to manufacturer's instructions. The following are generalized instructions:

8.1.1 Turn furnace on (or take off standby).

8.1.2 Turn gas regulators to desired flow rate (40 psi).

8.1.3 Select the appropriate method for either TC or OC.

8.1.4 Wait until the furnace has stabilized at the set temperature.

8.1.5 Test for leaks in the helium lines, ballast tank and combustion system.

8.1.6 Define the standard by entering the appropriate carbon content of the pure primary standard.

8.1.7 Include ten blanks and three dried (or desiccated pure) primary standards at the beginning of each run to calculate the calibration factor for determining carbon (to correct for drift).

8.1.8 Weigh out 0.15 to 2.0g of dried soil or plant tissue, respectively, in a clean sample ceramic crucible (boat). 1.0g of COM-CAT accelerator can be added to the boats before the sample is weighed. Weights are automatically transferred to the microprocessor by pressing the print button on the scale pad.

8.1.9 Transfer the weighed samples to the 49-position sample rack and load the rack onto the autoloader.

8.1.10 Run the samples.

9. Calculations

The inorganic carbon content is calculated as the difference between total carbon and organic carbon as follows:

$$\% \text{ IC} = \% \text{ TC} - \% \text{ OC}$$

10. Quality Control

10.1 The method's analysis range (lower limit is based on 3x standard deviation of the blank) is 0.02 - 200 mg carbon. Analysis precision is RSD 0.4%.

10.2 At least 10 blanks must be analyzed daily before each run and the blank with the value closest to zero should be selected and used for blank correction. Three to five standards should be analyzed and the one with the value closest to the real value of the standard should be used to correct for drift in the calibration curve.

10.3 At least one standard of the same material as the samples should be run with every ten unknowns and at the end of each run to verify calibration.

11. Reporting

11.1 Data is reported as %C for soil and plant tissue on a dry weight basis.

11.2 The detection limit is 0.020 mg C.

12. References

12.1 Leco Corporation. 2002. CNS-2000 elemental analyzer. CNS-2000 instruction manual.

12.2 Organic Carbon Dry Combustion method, Leco CN-2000, FP 2000, or CNS-2000 (JD Schwab, Personal communications).

12.3 Islam, K.R. and R.R. Weil. 1998. A rapid microwave method for colorimetric measurement of soil organic carbon. *Commu- soil-sci-plant-anal.* 29 (15/16):2269-2284.

12.4 Matejovic, I. 1997. Determination of carbon and nitrogen in samples of various soils by dry combustion. *Commu-soil- sci-plant-anal.* 28(17/18):1499-1511.

12.5 Leco Corporation 2003. Organic application note. Carbon, nitrogen and sulfur testing in soil/plant tissue.

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Soil and Sediment Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of soil and sediment samples for the analysis of *leachable* components (major, minor, and trace elements or total minerals, heavy metals, and micro-nutrients) by ICP-OES (TJA Iris Advanced ICP-OES) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus ICP-MS).

- 1.1 Soil and sediment samples contain major (Si, Al, Fe, Ti, Mn, Ca, Mg and Na), minor, and trace components. Alternatively, soil and sediment samples contain fraction one or structural components which are held within aluminum-silicate minerals and fraction two components which are held in soil and sediment by other mechanisms (precipitated, replaced, absorbed, complexed, exchanged, etc).
- 1.2 If a soil/sediment sample is totally dissolved, such as with a mixture of hydrofluoric acid (HF) and other acids, the measured components include both fraction one and fraction two components and the measured concentrations are “total

concentrations” of a sample. These concentrations are comparable to the concentrations obtained by other methods such as XRF methods and NAA methods.

- 1.3 The exclusive analysis of fraction two components has more applications than the analysis of total concentrations in agricultural or environmental areas, since fraction one components are “inert” while fraction two components are “active” and “available” in agricultural or environmental processes.
 - 1.3.1 Fraction two components are supposed to be “all-leached” out by treating samples with concentrated acids (except HF acid) at a high temperature and the measured concentrations are “total leachable concentrations.” These leachable concentrations are often referred as “total concentrations” or “total minerals,” although these “total concentrations” are conceptually not true total concentrations at all.

- 1.3.2 The total leachable concentrations are not directly comparable to XRF or NAA results, since the XRF or NAA concentrations are true total concentrations. However, this is highly element-dependent and may be sample-dependent. For example, the leachable concentration of silicon is far less than the total concentration of silicon, but the leachable concentration of mercury is usually close to (>95%) the total concentration of mercury in samples.
- 1.4 The total (leachable) components seem simple and well defined conceptually but the analysis of these leachable components is actually defined operationally. The measured results could be widely variable if a given sample is processed (leached) with different procedures or conditions. The results of leachable concentrations in soil or sediment samples should be interpreted carefully, keeping these considerations in mind.
- 1.5 There are unlimited versions of procedures available in literature for the process of soil and sediment samples, considering the numerous combinations of sample weight, acid type, acid amount, acid concentration, digestion time duration, digestion temperature, digestion pressure, and equipments. Since the measured results could be variable if a given sample is processed with different/alternative procedures or conditions, *a procedure without alternative steps* is preferred, developed, and used at this laboratory to achieve the greatest consistency in analyzing

different types of samples and/or samples at different times. In general, results obtained by a consistent method are comparable mutually.

2. Summary of method

- 2.1 A dried and ground sample (0.5 gram) and 5 mL of concentrated nitric acid are added into a 50-mL Folin digestion tube. The mixture is heated at 120-130 °C for 14-16 hours and then is treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is further 1:1 diluted for the analysis of major and minor components by ICP-OES and further 1:9 diluted for the analysis of minor and trace components by ICP-MS.
- 2.2 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.

4. Interference

- 4.1 This method covers the analysis of over 30 elements by ICP-OES and ICP-MS. Even a general discussion of interferences is lengthy but not necessarily relevant to a specific element/isotope. The analysis of

metals and non-metals by ICP-OES and ICP-MS has been established and there is an enormous amount of literature available relevant to this subject. Reading the published articles is recommended.

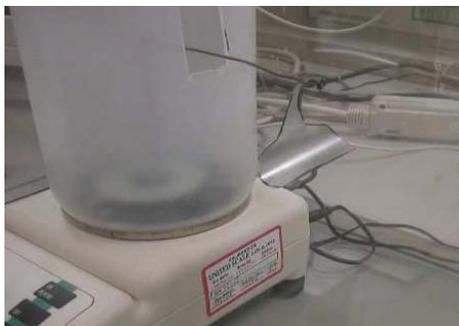
- 4.2 In this method, the solution for ICP-OES analysis contains < 500 ppm of dissolved solid and the solution for ICP-MS analysis contains <100 ppm of dissolved solid. The major components are Fe, Al, K, Ca, Mg and Mn. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences in general are not expected, although some specific element/isotope may be interfered.

5. Sample collection, preservation and handling

A representative sample of soil/sediment is dried and ground. A five-gram vial or equivalent is used to hold a sub sample for airtight storage.

6. Apparatus and device

- 6.1 Analytical balance (accurate to 1 milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



- 6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

- 6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate, Model: HP 53014C).



- 6.4 Ten-mL universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

- 6.5 ICP-OES: TJA Iris Advantage ICP-OES.
- 6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat #2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”
- 6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).
- 6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes, Cat #14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water for several times. The tubes are air-dried before use.

7. Reagents

- 7.1 Concentrated nitric acid (> 68%) (e.g. TraceMetal grade. Fisher A509-212).
- 7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).
- 7.3 Single-element and multi-element primary standard solutions.

8. Pre-digestion

- 8.1 Dry samples at 60 °C for two days. Large stones/rocks or plant materials are removed. Grind the samples (Calcareous samples may be ground to very fine powders). Small-size samples are wrapped in

plastic film and broken or ground to avoid contamination of normal grounding. Extremely small size samples are used “as-is.” Store in a five-gram vial or other appropriate container for airtight storage. Note: Samples may be dried at 60 °C or at 110 °C. The water content could be different.

- 8.2 Weigh 0.50±0.01 g of the sample (unknown samples, in-house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried digestion tubes (Finely ground calcareous sample powders: 0.25 gram, sandy samples: 1.00 gram). Make one to three digestion blanks.
- 8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an IRS (internal reference standard) for the analysis by ICP-OES. Spike 0.2 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.
- 8.4 Carefully add drops of 20–30% (v/v) nitric acid to moisten the samples. This is especially important for calcareous samples to prevent them from foaming over.
- 8.5 After the samples have been moistened with the diluted nitric acid, add 5 mL of concentrated nitric acid. Soak at room temperature for 2-3 hours.

Note: A digestion with perchloric acid should be avoided for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and

several other isotopes using quadrupole ICP-MS.

9. Hot plate digestion

9.1 Place all of the digestion tubes in a block heater. Cover the tubes with plastic film to retard water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7). Turn the power on.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this could cause higher blank concentrations for several elements.

9.3 The temperature will ramp up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is very important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the hydrogen peroxide is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add hydrogen peroxide (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Leave overnight to let particles settle down. After this digestion (1st dilution), nominal dilution factor = (50 mL/0.5 gram) = 100. Y = 8 ppm. Rh = 40 ppb.

Note: A typical digestion time table at SPAL – start heating in the afternoon (3 pm), heat overnight with plastic film cover, take the cover off in the early morning (7 am) the next day, and add hydrogen peroxide afterwards.

Note: Samples may not be heated above 130-140°C. Localized overheating may cause a sample to boil over and be lost.

Note: Soil/sediment samples may contain MnO₂. Hydrogen peroxide reacts with MnO₂ quickly. Hydrogen peroxide also reacts with some other components quickly in a hot nitric acid medium. Therefore, add hydrogen peroxide only after the sample tubes have been cooled.

Note: After a soil sample is digested with concentrated acid (without HF) at a high temperature, the majority of the sample remains as a solid and 5-10% of the sample is leached into solution (this ratio

is much higher for calcareous soil samples). If a sample is digested at a dilution factor (DF) of 100 (e.g. 0.5 gram of soil sample is digested and diluted to 50 mL) the solution does not contain 1% of the total dissolved solid (TDS) but contains <0.1% of the TDS. This kind of solution can generally be directly introduced to ICP-OES or ICP-MS. However, most components may still be significantly higher than “optimum” concentration ranges. In SPAL, the solution is analyzed by ICP-OES with a further 1:1 dilution for major and most minor elements. With the SPAL’s specific model of the ICP-MS instrument (VG PlasmaQuad PQ2 Turbo Plus ICP-MS), this kind of solution is analyzed with a further 1:9 dilution for minor and trace elements. One may argue that why not to use less amount of soil at the start so that the second dilution or any further dilution is avoided. Firstly, as it is pointed out in section 1 (Application), any “alternative” steps should be avoided as much as possible in order to achieve a consistent analysis. The leaching efficiency would be different if the acid to soil ratio is changed. Secondly, larger-size sample is more “representative” than smaller-size sample for samples such as soil or sediment which is usually fairly “inhomogeneous.” Thirdly, the size of half a gram of sample is widely used in other procedures. The size of a sample of course can be changed if the consistency is not an issue in some special projects.

10. Measurement by ICP-OES

10.1. Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube. Mix. After this 2nd dilution (for ICP-OES), nominal dilution factor = $(6 \text{ mL}/3 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 200$. Y = 4 ppm.

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using up-side down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2. Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3. Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Add sample solutions (1 mL) to the 14-mL Falcon tubes containing 9 mL of 2% nitric acid. Mix well. After this dilution (2nd for ICP-MS), total dilution factor = $(10 \text{ mL}/1 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 1,000$. Rh = 4 ppb.

11.1.2 Depending on sample matrix and analyte concentration, the sample may be diluted in other ratios.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Digest blanks are also measured with other samples.

11.2.3 In the menu, select “soil” and edit it if needed.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 1,000, after this procedure is followed exactly. Otherwise, adjust the DF accordingly.

Scenario 1: 10 mg/kg (10 ppm or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.2 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 40 ppb) is further diluted by 1:9 (2nd DF = 10) to contain 10 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 1000). This 2nd solution is measured against a standard containing 1 ppb of X and 4 ppb of Rh and the measured result is 10 ppb. After applying the overall dilution factor of 1000, the concentration of X in the solid material is $10 \text{ ppb} \times \text{DF } 1000 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario 2: Element X in the 2nd solution (X = 10 ppb and Rh = 4 ppb) is still much higher than the standard (X = 1 ppb and Rh = 4

ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 10 \times 5 = 5000$) to contain 2 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (2 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (1 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 10 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$. Option 2: At step 13.2, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 2 ppb against a standard of 1 ppb X with 4 ppb Rh. Now the total DF is 5000 and X in the solid sample is $2 \text{ ppb} \times 5000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure. Some basics are given in “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS.”

– End –

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Soil and Sediment Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of soil and sediment samples for the analysis of *leachable* components (major, minor, and trace elements or total minerals, heavy metals, and micro-nutrients) by ICP-OES (TJA Iris Advanced ICP-OES) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus ICP-MS).

- 1.1 Soil and sediment samples contain major (Si, Al, Fe, Ti, Mn, Ca, Mg and Na), minor, and trace components. Alternatively, soil and sediment samples contain fraction one or structural components which are held within aluminum-silicate minerals and fraction two components which are held in soil and sediment by other mechanisms (precipitated, replaced, absorbed, complexed, exchanged, etc).
- 1.2 If a soil/sediment sample is totally dissolved, such as with a mixture of hydrofluoric acid (HF) and other acids, the measured components include both fraction one and fraction two components and the measured concentrations are “total

concentrations” of a sample. These concentrations are comparable to the concentrations obtained by other methods such as XRF methods and NAA methods.

- 1.3 The exclusive analysis of fraction two components has more applications than the analysis of total concentrations in agricultural or environmental areas, since fraction one components are “inert” while fraction two components are “active” and “available” in agricultural or environmental processes.
 - 1.3.1 Fraction two components are supposed to be “all-leached” out by treating samples with concentrated acids (except HF acid) at a high temperature and the measured concentrations are “total leachable concentrations.” These leachable concentrations are often referred as “total concentrations” or “total minerals,” although these “total concentrations” are conceptually not true total concentrations at all.

1.3.2 The total leachable concentrations are not directly comparable to XRF or NAA results, since the XRF or NAA concentrations are true total concentrations. However, this is highly element-dependent and may be sample-dependent. For example, the leachable concentration of silicon is far less than the total concentration of silicon, but the leachable concentration of mercury is usually close to (>95%) the total concentration of mercury in samples.

1.4 The total (leachable) components seem simple and well defined conceptually but the analysis of these leachable components is actually defined operationally. The measured results could be widely variable if a given sample is processed (leached) with different procedures or conditions. The results of leachable concentrations in soil or sediment samples should be interpreted carefully, keeping these considerations in mind.

1.5 There are unlimited versions of procedures available in literature for the process of soil and sediment samples, considering the numerous combinations of sample weight, acid type, acid amount, acid concentration, digestion time duration, digestion temperature, digestion pressure, and equipments. Since the measured results could be variable if a given sample is processed with different/alternative procedures or conditions, *a procedure without alternative steps* is preferred, developed, and used at this laboratory to achieve the greatest consistency in analyzing

different types of samples and/or samples at different times. In general, results obtained by a consistent method are comparable mutually.

2. Summary of method

2.1 A dried and ground sample (0.5 gram) and 5 mL of concentrated nitric acid are added into a 50-mL Folin digestion tube. The mixture is heated at 120-130 °C for 14-16 hours and then is treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is further 1:1 diluted for the analysis of major and minor components by ICP-OES and further 1:9 diluted for the analysis of minor and trace components by ICP-MS.

2.2 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.

4. Interference

4.1 This method covers the analysis of over 30 elements by ICP-OES and ICP-MS. Even a general discussion of interferences is lengthy but not necessarily relevant to a specific element/isotope. The analysis of

metals and non-metals by ICP-OES and ICP-MS has been established and there is an enormous amount of literature available relevant to this subject. Reading the published articles is recommended.

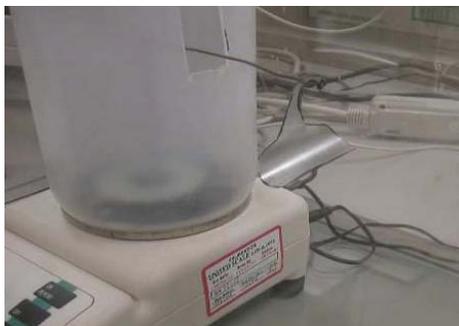
- 4.2 In this method, the solution for ICP-OES analysis contains < 500 ppm of dissolved solid and the solution for ICP-MS analysis contains <100 ppm of dissolved solid. The major components are Fe, Al, K, Ca, Mg and Mn. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences in general are not expected, although some specific element/isotope may be interfered.

5. Sample collection, preservation and handling

A representative sample of soil/sediment is dried and ground. A five-gram vial or equivalent is used to hold a sub sample for airtight storage.

6. Apparatus and device

- 6.1 Analytical balance (accurate to 1 milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



- 6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.
- 6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate, Model: HP 53014C).



- 6.4 Ten-mL universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

- 6.5 ICP-OES: TJA Iris Advantage ICP-OES.
- 6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat #2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”
- 6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).
- 6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes, Cat #14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water for several times. The tubes are air-dried before use.

7. Reagents

- 7.1 Concentrated nitric acid (> 68%) (e.g. TraceMetal grade. Fisher A509-212).
- 7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).
- 7.3 Single-element and multi-element primary standard solutions.

8. Pre-digestion

- 8.1 Dry samples at 60 °C for two days. Large stones/rocks or plant materials are removed. Grind the samples (Calcareous samples may be ground to very fine powders). Small-size samples are wrapped in

plastic film and broken or ground to avoid contamination of normal grounding. Extremely small size samples are used “as-is.” Store in a five-gram vial or other appropriate container for airtight storage. Note: Samples may be dried at 60 °C or at 110 °C. The water content could be different.

- 8.2 Weigh 0.50±0.01 g of the sample (unknown samples, in-house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried digestion tubes (Finely ground calcareous sample powders: 0.25 gram, sandy samples: 1.00 gram). Make one to three digestion blanks.
- 8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an IRS (internal reference standard) for the analysis by ICP-OES. Spike 0.2 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.
- 8.4 Carefully add drops of 20–30% (v/v) nitric acid to moisten the samples. This is especially important for calcareous samples to prevent them from foaming over.
- 8.5 After the samples have been moistened with the diluted nitric acid, add 5 mL of concentrated nitric acid. Soak at room temperature for 2-3 hours.

Note: A digestion with perchloric acid should be avoided for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and

several other isotopes using quadrupole ICP-MS.

9. Hot plate digestion

9.1 Place all of the digestion tubes in a block heater. Cover the tubes with plastic film to retard water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7). Turn the power on.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this could cause higher blank concentrations for several elements.

9.3 The temperature will ramp up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is very important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the hydrogen peroxide is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add hydrogen peroxide (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Leave overnight to let particles settle down. After this digestion (1st dilution), nominal dilution factor = (50 mL/0.5 gram) = 100. Y = 8 ppm. Rh = 40 ppb.

Note: A typical digestion time table at SPAL – start heating in the afternoon (3 pm), heat overnight with plastic film cover, take the cover off in the early morning (7 am) the next day, and add hydrogen peroxide afterwards.

Note: Samples may not be heated above 130-140°C. Localized overheating may cause a sample to boil over and be lost.

Note: Soil/sediment samples may contain MnO₂. Hydrogen peroxide reacts with MnO₂ quickly. Hydrogen peroxide also reacts with some other components quickly in a hot nitric acid medium. Therefore, add hydrogen peroxide only after the sample tubes have been cooled.

Note: After a soil sample is digested with concentrated acid (without HF) at a high temperature, the majority of the sample remains as a solid and 5-10% of the sample is leached into solution (this ratio

is much higher for calcareous soil samples). If a sample is digested at a dilution factor (DF) of 100 (e.g. 0.5 gram of soil sample is digested and diluted to 50 mL) the solution does not contain 1% of the total dissolved solid (TDS) but contains <0.1% of the TDS. This kind of solution can generally be directly introduced to ICP-OES or ICP-MS. However, most components may still be significantly higher than “optimum” concentration ranges. In SPAL, the solution is analyzed by ICP-OES with a further 1:1 dilution for major and most minor elements. With the SPAL’s specific model of the ICP-MS instrument (VG PlasmaQuad PQ2 Turbo Plus ICP-MS), this kind of solution is analyzed with a further 1:9 dilution for minor and trace elements. One may argue that why not to use less amount of soil at the start so that the second dilution or any further dilution is avoided. Firstly, as it is pointed out in section 1 (Application), any “alternative” steps should be avoided as much as possible in order to achieve a consistent analysis. The leaching efficiency would be different if the acid to soil ratio is changed. Secondly, larger-size sample is more “representative” than smaller-size sample for samples such as soil or sediment which is usually fairly “inhomogeneous.” Thirdly, the size of half a gram of sample is widely used in other procedures. The size of a sample of course can be changed if the consistency is not an issue in some special projects.

10. Measurement by ICP-OES

10.1. Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube. Mix. After this 2nd dilution (for ICP-OES), nominal dilution factor = $(6 \text{ mL}/3 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 200$. Y = 4 ppm.

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using up-side down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2. Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3. Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Add sample solutions (1 mL) to the 14-mL Falcon tubes containing 9 mL of 2% nitric acid. Mix well. After this dilution (2nd for ICP-MS), total dilution factor = $(10 \text{ mL}/1 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 1,000$. Rh = 4 ppb.

11.1.2 Depending on sample matrix and analyte concentration, the sample may be diluted in other ratios.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Digest blanks are also measured with other samples.

11.2.3 In the menu, select “soil” and edit it if needed.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 1,000, after this procedure is followed exactly. Otherwise, adjust the DF accordingly.

Scenario 1: 10 mg/kg (10 ppm or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.2 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 40 ppb) is further diluted by 1:9 (2nd DF = 10) to contain 10 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 1000). This 2nd solution is measured against a standard containing 1 ppb of X and 4 ppb of Rh and the measured result is 10 ppb. After applying the overall dilution factor of 1000, the concentration of X in the solid material is $10 \text{ ppb} \times \text{DF } 1000 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario 2: Element X in the 2nd solution (X = 10 ppb and Rh = 4 ppb) is still much higher than the standard (X = 1 ppb and Rh = 4

ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 10 \times 5 = 5000$) to contain 2 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (2 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (1 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 10 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$. Option 2: At step 13.2, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 2 ppb against a standard of 1 ppb X with 4 ppb Rh. Now the total DF is 5000 and X in the solid sample is $2 \text{ ppb} \times 5000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure. Some basics are given in “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS.”

– End –

Chloride (Soil, Tissue, and Water)

1. Application

This automated method is applicable to soil, tissue, waters (drinking, surface and saline waters), and domestic and industrial wastes.

2. Summary of Methods

Chloride is determined by an automatic chloride titrator (Digital chloridometer) by coulometric-amperometric titration with silver ions. In the chloride titrator, a constant direct current is passed between a pair of silver electrodes, causing release of silver ions into the titration solution at a constant rate. The silver ions react with chloride to precipitate ($\text{Ag}^+ + \text{Cl}^- \implies \text{AgCl}$). The end-point is after the increasing concentration of free silver ions cause a rising current to flow through a pair of silver indicator electrodes connected to a Meter-Relay. At a preset increment of indicator current the relay is actuated, stopping a timer, which runs concurrently with generation of silver ion. The amount of chloride precipitated is proportional to the elapsed time.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Chloride from skin may contaminate samples, rubber gloves are necessary during sample handling.
- 4.2 Washed filters must be used when filtering samples for chlorides. Unwashed filters contain varying amounts of chloride and will affect results.
- 4.3 Iodine, bromide, ferricyanide and ferric iron cause high results and must be removed. Chromate and dichromate should be reduced to chromic state or removed where contamination is minor. Some contaminants can be destroyed by adding nitric acid.

5. Sample Collection, Preservation and Handling

Soil and tissue samples are oven-dried at 55°C and ground to pass a 2-mm screen and stored in paper boxes (soil sample) or plastic bottles (for plant tissues). Water samples are stored at 4°C until analysis. Water samples should be completed within 28 days of the date of sampling.

6. Apparatus and Materials

- 6.1 Digital Chloridometer (LabConCo model # 442-5000)
- 6.2 Erlenmeyer flasks (125 ml)
- 6.3 Funnel tubes (15 ml)
- 6.4 Acid washed filter paper (9 cm Whatman No. 2 or equivalent).
- 6.5 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 6.6 Glass vials (5 ml)
- 6.7 Disposable examination gloves
- 6.8 Balance – capable of 0.01 g

7. Reagents

- 7.1 Deionized water
- 7.2 Concentrated standard solution 0.1 N HNO₃ 10% CH₃COOH solution
- 7.3 1:1 glacial acetic acid / deionized water
- 7.4 Nitric-acid reagent (for tissue only) (0.1 N HNO₃ and 10% glacial acetic acid): To 900 ml of water, add 6.4 ml of concentrated nitric acid and 100 ml of glacial acetic acid. Volumes are approximate. (Acids should be reagent grade). Mix thoroughly.
- 7.5 Gelatin reagent (to 100ml of hot deionized water add 0.62 g of gelatin mixture (LabConCo gelatin reagent #442-5064), mix until dissolved. Reagent is good for six months if kept refrigerated)
- 7.6 Standard Chloride solution (0.0141 N NaCl). Dissolve 0.8241 g pre-dried (140°C) NaCl in deionized water, dilute to 1 liter (1 ml = 0.5 mg Cl)
- 7.7 Reference solution (Environmental Resource Associates) Lot No. 99101

8. Methods

- 8.1 Weigh out 0.25-0.50 g (plant tissue), 0.4-0.5 g (for sandy soil) or 0.2-0.3 g (for silt, clay or muck soil) into a 125 Erlenmeyer flask. (For analysis of water, skip to step 8.4)
- 8.2 Add 10 ml of deionized water, plus 1 drop of 1:1 glacial acetic acid mixture to facilitate filtering.
- 8.3 Shake the suspension on an oscillating shaker, at 160 excursions per minute for 20 minutes, then filter. (Water samples high in suspended solids need filtering prior to analysis).
- 8.4 Transfer 3 ml of samples to glass vials, add 1 ml of (7.2) concentrated standard solution and 4 drops of (7.5) gelatin reagent.

8.5 Place glass vial on chloridometer, press start, when timer stops, record meg/L reading.

9. Calculations

Typical conditions for measuring low concentration of chloride over more than a hundredfold range are as follows:

ANTICIPATED CONCENTRATIONS* mEq/L

		0.05-0.9	0.9-5	5-30**
Conditions	Blank	1.8-3.2	33-177	177-1065ppm
mL sample	0	3.0	0.5	0.1
mL .4 N HNO ₃				
40% CH ₃ COOH	1.0	1.0	1.0	1.0
Drops gelatin	4	4	4	4
mL distilled H ₂ O	3	0	2.5	3.0
Total volume	4.0	4.0	4.0	4.1
Display reading	-	15-270	45-250	50-300
Correction factor	--	1/300	1/50	1/10

* Assumes using **LOW** range in all cases.

** Concentrations in this range can use the serial titration procedure and the standard acid solution.

CORRECTION FORMULA FOR CONVERTING DISPLAY UNITS

For solid samples: mEqCl/L to %NaCl:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{\text{original sample weight, grams}}$$

This assumes a known weight of a solid sample has been mixed in a known final volume of solution.

For liquid samples:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{(\text{sample volume, liters})(\text{density g/L})}$$

mEq Cl/L to mg NaCl/L: mg NaCl/L = (reading x 58.5)

mEq Cl/L to ppm Cl: ppm Cl = (reading) x (35.5)

10. Quality Control

10.1 Standard reference material from Lab Chem Inc. (LC13010-1) is analyzed with each run as well as reagent blanks.

11. Reporting

11.1 Samples are reported in ppm Cl \pm 0.5

12. Reference

Adapted from Standard methods for the Examination of Water and Wastewater, 15th Edition, 1980, (pgs 273-275). Method 407C

Soil Lead

1. Application

This procedure covers the extraction and analysis of lead in soil.

2. Summary of Methods

Lead is extracted from the soil with 1.0 N nitric acid. The extractant is then analyzed by atomic absorption (AA).

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Soil samples containing high levels of calcium carbonate tend to produce a gas, upon reacting with the nitric acid. Nitric acid should be added to samples slowly and with caution, while watching for reaction with this type of sample.

5. Apparatus and Materials

- 5.1 Balance accurate to 0.01 g.
- 5.2 125-ml pyrex flasks
- 5.3 Bottle top pipettor able to dispense 25-ml (x2), or Graduated cylinder (50-ml).
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Filter paper, 9-cm. (Whatman No. 2 or equivalent).
- 5.6 Funnel tubes (15-ml).
- 5.7 Disposable plastic test tubes (13x100).
- 5.8 Atomic absorption spectrophotometer (AA) equipped with Pb hollow cathode lamp, (Varian SpectrAA 220 FS with SIPS pump unit and auto sampler SPS -5).

6. Reagents

- 6.1 Extracting solution (1.0 N nitric acid).
- 6.2 1000 ppm Pb stock solution.
- 6.3 20 ppm Pb Bulk Standard (5 ml 1000 ppm Pb stock solution diluted to 250 ml with 1 N HNO₃)

7. Methods

- 7.1 Weigh out 5.00 g of soil.
- 7.2 Place soil into 125-ml pyrex flask.
- 7.3 Dispense 50-ml of extracting solution slowly into each flask containing the soil.
- 7.4 Place flasks onto shaker and secure. Shake on oscillating shaker for 1 hour. If samples contain calcium carbonate, swirl briefly by hand before placing on shaker.
- 7.5 Remove samples from shaker and filter through Whatman No. 2 filter paper.
- 7.6 Transfer filtrate to 8-ml plastic test tubes.
- 7.7 Analyze using Atomic absorption spectrophotometer (AA) equipped with Pb hollow cathode lamp at wavelength 217.0nm.

8. Calculations

$$\frac{\text{ml of extraction solution}}{\text{grams of soil}} \times \text{Conc.} = \text{ppm Lead in Soil}$$

Calculations are performed by the Atomic absorption spectrophotometer (AA).

9. Quality Control

- 9.1 A NIST standard soil of known lead concentration, as well as a procedural blank are analyzed with each batch of samples.

10. Reporting

- 10.1 Results are reported as ppm lead in soil.

11. References

- 11.1 R.L. Chaney and S.B. Sterrett. 1984. The potential for heavy metal exposure from urban gardens and soils. In J.R. Preer (ed.) Proc. Symp. Heavy Metals in Urban Gardens. Div. Dist. Columbia Ext. Serv., Washington D.C. p. 37-84.
- 11.2 R.L. Chaney. 1983. Potential effects of waste constituents on the food chain. In J.F. Parr, P.B. Marsh, J.M. Kila (eds). Land Treatment of Hazardous Wastes. P. 152-240.

Ash

1. Application

This method covers the determination of ash from soil, tissue and waste samples.

2. Summary of Methods

Ash is determined by use of a muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

3. Safety

All chemical compounds should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

None

5. Sample Collection, Preservation and Handling

Samples are dried at 55°C

6. Apparatus and Materials

- 6.1 Muffle furnace
- 6.2 High temperature crucibles
- 6.3 Balance capable of reading to 0.001 g

7. Reagents

None

8. Methods

- 8.1 Record weight of high temperature crucible to 0.001 g
- 8.2 Weigh out 1 – 5 grams of soil, tissue, or waste into crucible and record weight of sample and crucible.
- 8.3 Place in muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$. Sample must remain at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

8.4 Remove samples from muffle furnace, cool and re-weigh to 0.001 g.

9. Calculations

Before ashing record:

$$\text{crucible weight} + (\text{crucible weight} + \text{sample weight}) = \text{sample weight}$$

After ashing record:

$$\text{crucible weight} + \text{sample weight}$$

$$\% \text{ ash} = \frac{\text{sample weight ash} - \text{crucible weight}}{\text{sample weight dry} - \text{crucible weight}} \times 100$$

10. Quality Control

10.1 Standard laboratory soil # 4

11. Reporting

11.1 Samples are reported in $\% \pm 0.1$

12. Reference

Standard Methods (for the examination of water and wastewater). 15th Edition, 1980 Pg. 97 (209G). Volatile and Fixed Matter in Nonfilterable Residue and in Solid and Semisolid Samples.

Phosphorus for Forest Soil

1. Application

This procedure covers the extraction and analysis of plant available phosphorus (P) in forest soil.

2. Summary of Methods

Plant available phosphorus (P) is extracted from the soil with 0.002 N sulfuric acid. The extracted P is reacted with a sulfuric-molybdate to form a blue phosphomolybdate compound in the presence of a reducing agent.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Color development is complete in 15 minutes but will continue at a slow rate. For this reason, samples should be read within two hours. Arsenic forms a blue molybdate complex but is usually present in very low amounts unless a special arsenical pesticide has been applied in the past.

Very high soil pH interferes with phosphorus by this extraction method.

5. Apparatus and Materials

- 5.1 Soil scoop calibrated to hold 1.5 g of forest soil.
- 5.2 Erlenmeyer flasks (500 ml)
- 5.3 Pipette banks
- 5.4 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 5.5 Filter paper (9 cm Whatman No. 2 or equivalent).
- 5.6 Funnel tubes (15 ml).
- 5.7 Matched colorimetric tubes (10 ml).
- 5.8 UV-Vis spectrophotometer

6. Reagents

- 6.1 N/10 sulfuric acid
- 6.2 Sulfuric acid extracting solution: Dissolve 3 g of $(\text{NH}_4)_2\text{SO}_4$ in approximately 0.5 L of deionized water. Add 20 ml of N/10 sulfuric acid and dilute to 1 L with deionized water.
- 6.3 Standard phosphate solution (Weigh out 0.4394 g of KH_2PO_4 , bring to 1 liter with extracting solution. This solution contains 100 ppm of phosphorus. Prepare a second stock solution by taking 50 ml of 100 ppm Phosphorus and diluting to 1 liter with extracting solution; this solution contains 5 ppm of phosphorus. Pipette 4, 8, 12, 16, and 20 ml of the second stock solution into 100-ml volumetric flasks and bring to volume with extracting solution. These solutions contain 0.2, 0.4, 0.6, 0.8, and 1.0 ppm of phosphorus.)
- 6.4 Sulfuric-molybdate solution (Dissolve 8.5716 g ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4 \text{H}_2\text{O}$, in 500 ml of deionized water. Dissolve 0.1959 g of antimony potassium tartrate, $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2} \text{H}_2\text{O}$, in the above solution. Slowly add 100 ml of H_2SO_4 and mix well.) Let cool and dilute to 1 liter. Store in a brown polyethylene or Pyrex bottle in a refrigerator.
- 6.5 Ascorbic acid solution (Dissolve 0.528 g of ascorbic acid in 30 ml of deionized water. Prepare fresh each day.)
- 6.6 Color reagent (Add 30 ml of ascorbic acid solution to 70 ml of sulfuric-molybdate stock solution. This solution should be made fresh daily.)

7. Methods

- 7.1 Place a 1.5 g scoop of soil into a 500-ml Erlenmeyer flask.
- 7.2 Add 150 ml of 0.002 N sulfuric acid.
- 7.3 Shake the suspension on shaker for 30 min.
- 7.4 After shaking, immediately filter suspension through filter paper. Filtrate should be discarded until clear.
- 7.5 Place 5 ml of filtrate into a clear 10-ml tube and add 1 ml of sulfuric-molybdate and ascorbic acid solution.
- 7.6 Shake tube and wait 15 minutes.
- 7.7 Transfer solution to a colorimeter tube.
- 7.8 Percent transmittance is determined by a spectrophotometer set at 882 m μ or by a filter colorimeter at 660 m μ , and concentration of P is determined using a standard curve.

8. Calculations

In lieu of direct calibration of the colorimetric scale, calculate extractable P, ppm P in soil = ppm P in solution x 150 ml/1.5 g = ppm P in solution x 100.

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable forest P content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm P in soil. (Strictly speaking, the results should be reported as Mg P per dm³ of soil because a known volume, rather than a weight is used. This is not a familiar unit however. Use of a volume of soil is reasonable because it represents a volume-fraction of an acre plow layer.)

11. References

- 11.1 Truog, 1930. Journal Am. Soc. Agr. 22 (pgs 874-882)
- 11.2 Murphy and Riley, 1962.
- 11.3 Wilde, S.A., R.B. Corey, J.G. Iyer and G.K. Voigt. 1979. Soil and Plant Analysis for Tree Culture. Oxford IBH, Bombay and New Delhi. Reprinted 1985.

Mound Sand

1. Application

This procedure covers the determination of mound sand quality.

2. Summary of Methods

The sample of dried mound sand is passed through a nest of 3/8 inch, 4, 8, 16, 30, 50, 100, 200 mesh sieves. The % passing each sieve is recorded.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

None

5. Apparatus and Materials

- 5.1 Shaker (Ro-tap^R Model CL340)
- 5.2 Sieves ASTM 3/8 inch, 4, 8, 16, 30, 50, 100, 200
- 5.3 Balance (capable of 0.1 g)

6. Reagents

None

7. Methods

- 7.1 Weigh a 100.0 gram sample of dried mound sand
- 7.2 Transfer to the top of the nest of sieves (3/8 inch, 4, 8, 16, 30, 50, 100, 200)
- 7.3 Place the nest of sieves on the Ro-tap^R shaker and shake for 10 minutes.
- 7.4 Weigh and record the sample weight on each sieve and bottom pan.

8. Calculations

8.1 Calculate and record the % passing each sieve. Starting at the 200 sieve and work up to the 3/8 inch sieve. See reference 11.

9. Quality Control

9.1 A reference (House Blend) mound sand is analyzed and compared to the average \pm 10%

10. Reporting

10.1 Results reported to the nearest 1%

11. References

11.1 ASTM C33, Section 5.2

Sieve Size or Number	ASTM C 33 Specifications Percent Passing
3/8 Inch	100
No. 4	95 - 100
No. 8	80 - 100
No. 16	50 - 85
No. 30	25 - 60
No. 50	5 - 30
No. 100	0 - 10
No. 200	

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Plant Tissue Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of plant tissue samples and the analysis of major, minor and trace elements (total minerals, heavy metals and micro-nutrients) in these samples by ICP-OES (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometry) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus Inductively Coupled Plasma Mass Spectrometry).

- 1.1 Plant tissue samples (including some other types of samples such as cheese, manure, lichens, etc) mainly consist of carbon, hydrogen, oxygen and nitrogen. Other abundant components are K, Mg, Ca, P, S and Na. These six elements account for less than 10% of a sample on a dry weight basis.
- 1.2 An open-vessel acid digestion with $\text{HNO}_3 + \text{H}_2\text{O}_2$ (or $\text{HNO}_3 + \text{HClO}_4$ in some applications) in a hot plate could be incomplete for some samples. In other words, the digestion without HF acid is incomplete for some siliceous materials, although the sources of siliceous materials may be variable.

Elements closely associated with siliceous materials such as Al, Fe and Na may not be completely released from solid material to solution. Therefore, the results by ICP-OES or ICP-MS may not be directly comparable to the results by XRF methods or NAA methods. However, this problem might be significant only in limited cases.

- 1.3 When a sample is digested at a dilution factor of 100 (e.g. 0.5 g to 50 mL digestion and dilution), the amount of total dissolved solids is close to or less than 0.1% (1,000 mg/liter or 1,000 ppm), although the concentrations of K and Ca in digested sample solutions can be higher than 500 ppm respectively. The sample solution is directly analyzed by ICP-OES, but may be further diluted for ICP-MS.

2. Summary of method

- 2.1 Half a gram of dried sample (or equivalent) and five mL of concentrated nitric acid are added to a 50-mL Folin digestion tube. The mixture is heated to 120-130 °C for 14-

16 hours and is then treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is analyzed by ICP-OES for major and minor components, and further 1:1 diluted and analyzed by ICP-MS for minor and trace components.

- 2.2 Alternatively, samples like cheese or manure may be directly digested without having been dried. The results are either reported “as is” or reported based on dry weight after the water contents are obtained from sub-samples.
- 2.3 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

- 3.1 All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.
- 3.2 The use of perchloric acid for a sample digestion must be conducted in a hood designed specifically for perchloric acid. The user must be aware of the dangers involved using perchloric acid, such as the explosive nature of anhydrous perchloric acid and its extreme corrosive nature.

4. Interference

- 4.1 This method covers the analysis of over 30 elements in different kinds of samples by ICP-OES and ICP-MS. A

general discussion of interference is lengthy but not necessarily relevant to a specific element, which is especially true if the sample matrix is not specifically defined. An enormous amount of literature is available to the analysis of metals and non-metals by ICP-OES and by ICP-MS. Reading the published articles is recommended.

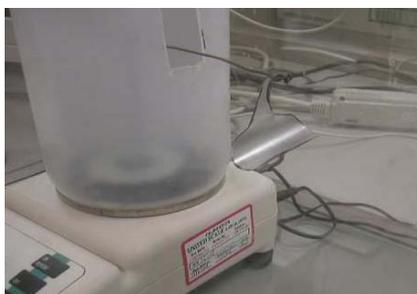
- 4.2 In this method, the solution contains less than 1,000 ppm of dissolved solids for ICP-OES and ICP-MS analysis. The major components are K, Mg, Ca, P, S and Na. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences are not expected, although some specific elements and or isotopes may be interfered.

5. Sample Collection, Preservation and Handling

A representative sample of plant tissue is dried and ground. A 5-gram vial or equivalent is used to hold a sub sample in airtight storage. Wet samples may be kept frozen.

6. Apparatus and Device

- 6.1 Analytical balance (accurate to one milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate. Model: HP 53014C).



6.4 Ten universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

6.5 ICP-OES: TJA Iris Advantage ICP-OES.

6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat # 2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”

6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).

6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes. Cat # 14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water. The tubes are air-dried before use.

7. Reagents

7.1 Concentrated nitric acid (e.g. TraceMetal grade. Fisher A509-212).

7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).

8. Pre-Digestion

8.1 Dry samples at 60 °C for two days. Grind in a stainless steel Wiley mill. Store in a 5-gram vial or equivalent for airtight storage. Process other wet samples “as is” without drying under certain limitations. The water content is obtained from a sub-sample.

Note: Samples may be dried at 60 °C or at 110 °C. The water contents could be different.

8.2 Weigh 0.50 ± 0.01 g of dry sample, or 1.0 ± 0.02 g of wet sample, or 5 mL of liquid sample (unknown samples, in-

house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried Folin digestion tubes. Make one to three digestion blanks.

Note: Depending on sample availability, the sample size can be scaled down. The relative significance of “contamination” from lab-wares and from reagents may increase if the sample size is very “small.” TEFLON digestion tubes may be used instead of glass digestion tubes.

8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an internal reference standard (IRS) for the analysis by ICP-OES. Spike 0.04 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.

8.4 Add 5 mL of concentrated nitric acid. Soak the samples at room temperature for 2-3 hours.

Note: Perchloric acid may be used in some special applications but should be avoided as much as possible for safety concerns. Samples digested with HClO_4 are not good for the analysis of V, Cr, As, ^{77}Se , Rb and several other isotopes by using quadrupole ICP-MS.

9. Hot Plate Digestion

9.1 Place all of the Folin tubes in the block heater. Cover the tubes with plastic film to retard the water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this

could cause higher digestion blank concentrations for several elements.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7).

9.3 The temperature ramps up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove the film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H_2O_2 are not good for Sn analysis if the H_2O_2 is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add H_2O_2 (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Nominal dilution factor = 100. Y = 8 ppm. Rh = 8 ppb.

10. Measurement by ICP-OES

10.1 Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Transfer sample solutions from 50-mL tubes to 8-mL tubes.

10.1.3 For samples with extremely high analytes, the samples may be further diluted. Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube (2nd dilution. Nominal dilution factor = 200. Y = 4 ppm). Mix

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might be an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using upside down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the nominal dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2 Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3 Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Set 14-mL Falcon tubes in the ICP-MS autosampler racks. Transfer the sample solutions to the Falcon tubes.

11.1.2 Adjust the volume to 5 mL. Add 5 mL of 2% nitric acid. Mix well. The nominal dilution factor is 200 and the IRS is 4 ppb of Rh.

11.1.3 Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. If the concentrations of target elements are expected to be relatively high, the samples are further diluted, either by 2+8 dilution or 1+9 dilution. Otherwise, a sample solution may be directly analyzed without any further dilution (i.e. 10+0 dilution). During the data processing in later stage, the nominal dilution factor is always 200, whether the dilution is 1+9, 2+8, 5+5 or 10+0.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Edit the menu depending on specific samples or analytical requests.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 200, after this procedure is followed exactly, although the actual dilution could be variable as presented above in 11.1.3. Otherwise, adjust the DF accordingly.

Scenario one: 10 ppm (or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.04 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 8 ppb) is further diluted by 5:5 (2nd DF = 2) to contain 50 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 200). This 2nd solution is measured against a standard containing 10 ppb of X and 4 ppb of Rh and the measured result is 50 ppb. After applying the overall dilution factor of 200, the concentration of X in the solid material is $50 \text{ ppb} \times \text{DF } 200 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario two: Element X in the 2nd solution (X = 50 ppb and Rh = 4 ppb) is still much higher than the standard (X = 10 ppb and Rh = 4 ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 2 \times 5 = 1000$) to contain 10 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (10 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (10 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 50 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $50 \text{ ppb} \times 200 = 10 \text{ ppm}$. Option 2: At step 13.1, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 10 ppb against a standard of 10 ppb X with 4 ppb Rh. Now the total DF is 1000 and X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

12.1 It should be kept in mind that ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure.

12.2 Some QA/QC practices are presented in “Elemental analysis of solution samples with ICP-OES” and in “Elemental analysis of solution samples with ICP-MS.” In addition, an

in-house standard, alfalfa, is included with each batch of plant tissue samples. Digestion blank is measured and the values are kept in a database.

– End –

Nitrogen (Total/Kjeldahl)

1. Application

This method covers the digestion of samples for Nitrogen (Total/Kjeldahl)

2. Summary of Methods

Total nitrogen (Org N + NH₄-N + NO₃-N, NO₂-N) digested with sulfuric acid, metal catalyst, salicylic acid.

Total Kjeldahl Nitrogen (Org N + NH₄-N) digested with sulfuric acid and metal catalyst.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Samples must not consume more than one fifth of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.7 to 7.0% (v/v) H₂SO₄ in the diluted digestion sample without any change in signal intensity.
- 4.2 Samples with particles remaining after digestion will require filtering prior to analysis by FIA.

5. Sample Collection, Preservation and Handling

- 5.1 Soil and plant samples are dried at 55°C, 65°C, respectively. The dried soil is then ground to pass a 12 mesh screen and plant tissue is ground to pass a 2 mm screen.
- 5.2 Water sample are stored at 4°C.

6. Apparatus and Materials

- 6.1 Scale 0.001 g
- 6.2 QuickChem 8000 Automated Ion Analyzer
- 6.3 Block Digester (Easy digest 40/20) Westco Scientific Instruments
- 6.4 75 ml digestion tubes
- 6.5 Vortex Mixer

7. Reagents (FIA 7.1-7.4) (N Digestion 7.5-7.7)

Flow Injection:

- 7.1 Buffer – Dissolve 65.0 sodium hydroxide, 50.0 g sodium potassium tartrate and 26.8 g sodium phosphate dibasic heptahydrate in deionized water (10 megohm) and dilute to 1 liter. Degas the buffer solution by passing He at 140 kPa through a helium degassing tube for one minute.
- 7.2 Color Reagent – Dissolve 150.0 g sodium salicylate and 1.0 g sodium nitroprusside in deionized water and dilute to 1 liter. Degas the solution with He.
- 7.3 Hypochlorite Solution – In a 1 L volumetric flask, add 60.0 ml regular Chlorox bleach (5.25% sodium hypochlorite), dilute to 1 L with deionized water.
- 7.4 Carrier – In a 1 L volumetric flask containing approximately 600 ml deionized water, add 70.0 ml of sulfuric acid, 30.0 g of potassium sulfate, and 2.5 g of copper sulfate. Dilute to 1 L with deionized water.

Nitrogen Digestion:

- 7.5 Conc. H₂SO₄
- 7.6 Metal Catalyst (digestion tablet – potassium sulfate 93%, cupric sulfate 7%)
- 7.7 Salicylic acid (75 g salicylic acid/2.5 L H₂SO₄) is used when including NO₃-N + NO₂-N

8. Methods

- 8.1 Weigh out 0.15-0.20 g of dried plant tissue or 0.45-0.5 g of soil into a clean, dry digestion tube. Carry a (LRB) blank through all steps of the procedure (see 10.1).
- 8.2 For Total Kjeldahl N (Org N + NH₄-N): To each tube add 1 (metal catalyst) digestion tablet and 3.5 ml of concentrated H₂SO₄.
- 8.3 For Total N (Org N + NH₄-N + NO₃-N + NO₂-N): To each tube add 1 (metal catalyst) digestion tablet and 3.5 ml of H₂SO₄ with Salicylic acid.
- 8.4 Place tubes in a block digester. Set temperature 160°C and time 1 to 20 minutes. Set temperature to 380°C and time 240 minutes.
- 8.5 Remove the samples from the block and allow 15 minutes for cooling.
- 8.6 Fill with deionized water to 50.0 ml. If samples are not run immediately, they should be covered to prevent evaporation.
- 8.7 Transfer \approx 7 ml of digested solution to FIA tubes.
- 8.8 Determine the ammonium concentration by FIA.

9. Calculations

The nitrogen content is calculated using the formula:

$$\text{ppm N} = 50/W_S X C_D \quad (\text{for soil sample})$$

$$\% \text{ N} = 50/W_S X C_D / 10,000 \quad (\text{for plant sample})$$

where W_S = Weight of sample (g)
 C_D = Concentration in the digest (mg N/l)

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB must be analyzed with each batch of samples in order to assess contamination from the laboratory environment. If LRB values exceed the method detection limit, laboratory or reagent contamination should be suspected, take correction action before continuing the analysis.
- 10.2 Laboratory Fortified Blank (LFB) – At least one LFB must be analyzed with each batch of samples. Calculate accuracy as percent recovery. If the recovery of any analyte falls outside the required control limits of 90-110%, the analyte is judged out of control, take corrective action for continuing analysis.
- 10.3 Instrument Performance Check Solution (IPC) – For all determinations, a mid-range check standard and a calibration blank must be analyzed immediately after daily instrument calibration, after every tenth sample, and at the end of the sample run. This process verifies that the instrument is within 10% of calibration. If the IPC solution indicates that the calibration is outside of present limits, take corrective action before continuing analysis.

11. Reporting

- 11.1 Data is reported as mg/l of N for soil and % N for plant tissue on a dry weight basis.
- 11.2 Detection limit = 0.01 mg/l

12. References

- 12.1 Lachat Instruments 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuikChem Method 13-107-06-2-D.
- 12.2 Jaromir Ruzicka 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. *Analytical Chemistry* 55: 1040A-1053A.
- 12.3 Bremner, J. M. 1965. Total nitrogen. In C. A. Black et al. (ed.) *Methods of soil analysis, Part 2*. Agronomy 9:1149-1178. Am. Soc. Of Agron., Inc., Madison, Wis.

Nitrate and Ammonium in Soil and Tissue

1. Application

In this procedure nitrogen, in the form of nitrate and nitrite ion, is extracted from soil or tissue samples and analyzed by flow injection.

2. Summary of Methods

KCl is used to extract NO_3^- -N and NH_4^- -N from the soil and tissue samples.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Apparatus and Materials

- 5.1 Weigh boat (metal or glass)
- 5.2 Erlenmeyer flasks (50-ml)
- 5.3 Pipette bank (15-ml)
- 5.4 Time-controlled, oscillating shaker.
- 5.5 Filter paper, 9-cm (Whatman No. 2 or equivalent)
- 5.6 Funnel tubes (15-ml)
- 5.7 Glass test tubes (6.2-ml)
- 5.8 Flow injection

6. Reagents

- 6.1 2 N KCl solution (1044.40 g of KCl to 7 liters of de-ionized water).

7. Methods

- 7.1 Weigh out 1.50 g of soil or .25 g of tissue into a weigh boat.
- 7.2 Transfer sample to a 50-ML Erlenmeyer flask.
- 7.3 Add 15-ml of 2 N KCl solution using constant suction pipette.
- 7.4 Shake for 15 minutes on oscillating shaker.
- 7.5 Filter immediately.

- 7.6 Pipette 5-ml of filtrate into glass test tube.
- 7.7 Analyze by flow injection.

8. Calculations

Sample concentration is calculated from a regression equation by plotting response versus standard concentration.

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results are reported as ppm of nitrogen in the form of nitrate NO_3^- -N or ammonium NH_4^- -N in soil.

11. References

- 11.1 Lachat Instruments. 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuickChem Method 12-107-04-1-B.
- 11.2 Jaromir Ruzicka. 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. Analytical Chemistry 55: 1040A-1053A.

Chloride (Soil, Tissue, and Water)

1. Application

This automated method is applicable to soil, tissue, waters (drinking, surface and saline waters), and domestic and industrial wastes.

2. Summary of Methods

Chloride is determined by an automatic chloride titrator (Digital chloridometer) by coulometric-amperometric titration with silver ions. In the chloride titrator, a constant direct current is passed between a pair of silver electrodes, causing release of silver ions into the titration solution at a constant rate. The silver ions react with chloride to precipitate ($\text{Ag}^+ + \text{Cl}^- \implies \text{AgCl}$). The end-point is after the increasing concentration of free silver ions cause a rising current to flow through a pair of silver indicator electrodes connected to a Meter-Relay. At a preset increment of indicator current the relay is actuated, stopping a timer, which runs concurrently with generation of silver ion. The amount of chloride precipitated is proportional to the elapsed time.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Chloride from skin may contaminate samples, rubber gloves are necessary during sample handling.
- 4.2 Washed filters must be used when filtering samples for chlorides. Unwashed filters contain varying amounts of chloride and will affect results.
- 4.3 Iodine, bromide, ferricyanide and ferric iron cause high results and must be removed. Chromate and dichromate should be reduced to chromic state or removed where contamination is minor. Some contaminants can be destroyed by adding nitric acid.

5. Sample Collection, Preservation and Handling

Soil and tissue samples are oven-dried at 55°C and ground to pass a 2-mm screen and stored in paper boxes (soil sample) or plastic bottles (for plant tissues). Water samples are stored at 4°C until analysis. Water samples should be completed within 28 days of the date of sampling.

6. Apparatus and Materials

- 6.1 Digital Chloridometer (LabConCo model # 442-5000)
- 6.2 Erlenmeyer flasks (125 ml)
- 6.3 Funnel tubes (15 ml)
- 6.4 Acid washed filter paper (9 cm Whatman No. 2 or equivalent).
- 6.5 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 6.6 Glass vials (5 ml)
- 6.7 Disposable examination gloves
- 6.8 Balance – capable of 0.01 g

7. Reagents

- 7.1 Deionized water
- 7.2 Concentrated standard solution 0.1 N HNO₃ 10% CH₃COOH solution
- 7.3 1:1 glacial acetic acid / deionized water
- 7.4 Nitric-acid reagent (for tissue only) (0.1 N HNO₃ and 10% glacial acetic acid): To 900 ml of water, add 6.4 ml of concentrated nitric acid and 100 ml of glacial acetic acid. Volumes are approximate. (Acids should be reagent grade). Mix thoroughly.
- 7.5 Gelatin reagent (to 100ml of hot deionized water add 0.62 g of gelatin mixture (LabConCo gelatin reagent #442-5064), mix until dissolved. Reagent is good for six months if kept refrigerated)
- 7.6 Standard Chloride solution (0.0141 N NaCl). Dissolve 0.8241 g pre-dried (140°C) NaCl in deionized water, dilute to 1 liter (1 ml = 0.5 mg Cl)
- 7.7 Reference solution (Environmental Resource Associates) Lot No. 99101

8. Methods

- 8.1 Weigh out 0.25-0.50 g (plant tissue), 0.4-0.5 g (for sandy soil) or 0.2-0.3 g (for silt, clay or muck soil) into a 125 Erlenmeyer flask. (For analysis of water, skip to step 8.4)
- 8.2 Add 10 ml of deionized water, plus 1 drop of 1:1 glacial acetic acid mixture to facilitate filtering.
- 8.3 Shake the suspension on an oscillating shaker, at 160 excursions per minute for 20 minutes, then filter. (Water samples high in suspended solids need filtering prior to analysis).
- 8.4 Transfer 3 ml of samples to glass vials, add 1 ml of (7.2) concentrated standard solution and 4 drops of (7.5) gelatin reagent.

8.5 Place glass vial on chloridometer, press start, when timer stops, record meg/L reading.

9. Calculations

Typical conditions for measuring low concentration of chloride over more than a hundredfold range are as follows:

ANTICIPATED CONCENTRATIONS* mEq/L

		0.05-0.9	0.9-5	5-30**
Conditions	Blank	1.8-3.2	33-177	177-1065ppm
mL sample	0	3.0	0.5	0.1
mL .4 N HNO ₃				
40% CH ₃ COOH	1.0	1.0	1.0	1.0
Drops gelatin	4	4	4	4
mL distilled H ₂ O	3	0	2.5	3.0
Total volume	4.0	4.0	4.0	4.1
Display reading	-	15-270	45-250	50-300
Correction factor	--	1/300	1/50	1/10

* Assumes using **LOW** range in all cases.

** Concentrations in this range can use the serial titration procedure and the standard acid solution.

CORRECTION FORMULA FOR CONVERTING DISPLAY UNITS

For solid samples: mEqCl/L to %NaCl:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{\text{original sample weight, grams}}$$

This assumes a known weight of a solid sample has been mixed in a known final volume of solution.

For liquid samples:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{(\text{sample volume, liters})(\text{density g/L})}$$

mEq Cl/L to mg NaCl/L: mg NaCl/L = (reading x 58.5)

mEq Cl/L to ppm Cl: ppm Cl = (reading) x (35.5)

10. Quality Control

10.1 Standard reference material from Lab Chem Inc. (LC13010-1) is analyzed with each run as well as reagent blanks.

11. Reporting

11.1 Samples are reported in ppm Cl \pm 0.5

12. Reference

Adapted from Standard methods for the Examination of Water and Wastewater, 15th Edition, 1980, (pgs 273-275). Method 407C

DATE: 9/21/2011

Carbon (Total, Organic, and Inorganic)

1. Application

This method covers the determination of total carbon (TC), organic carbon (OC) and inorganic carbon (IC) concentrations in soil, plant tissues and manures by dry combustion using a LECO CNS-2000 analyzer.

The LECO CNS-2000 Carbon, Nitrogen and Sulfur Analyzer is a non-dispersive, infrared, microcomputer based instrument, designed to measure the total carbon, nitrogen, and sulfur content in a wide variety of materials (soil, plant tissue, fertilizers, meat products, dairy products, seeds, food, resins, and environmental wastes) in a nominal 200 mg sample weight.

2. Summary of Method

Total carbon and organic carbon contents of a sample are determined in two separate combustion conditions/profiles. The first combustion profile will maximize the recovery of TC while the second profile will minimize the decomposition of carbonate C and maximize the recovery of OC. The two main variables of these profiles are the furnace temperatures and the oxygen flow rate. The furnace temperatures are set at 1350°C and 900°C for the TC and OC profiles, respectively. IC is calculated as the difference between the TC and OC values.

Although SPAL uses 900°C for the determination of OC, temperatures between 375 and 1000°C are found in the literature. Based on this, SPAL could accommodate specific requests from clients to run OC samples at a specific temperature.

In some cases hydrochloric acid has been used for the decomposition of carbonates. However, this treatment generates Cl gases that can damage the infrared detector and requires the use of scrubbing substrates in the system to prevent damage. Therefore, if a client needs to determine organic and inorganic carbon (by difference) the first option given is the OC at 900°C, the second option is OC at a temperature defined by the client, and as a last option (and therefore more expensive) the use of hydrochloric acid (by the client or by SPAL) to remove carbonates previous to the OC determination.

3. Safety

3.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

3.2 Follow the manufacturer's recommendation for safe operation of the instrument.

3.3 Secure compressed gas cylinders and use the proper gas regulators.

3.4 Sample boats being unloaded from the furnace are extremely hot - do not handle them until they cool down.

4. Interferences

4.1 Fineness of the ground sample affects sample combustion and thus analysis results. All samples should be ground to pass an 18 mesh sieve (1-mm) or finer.

4.2 Sample boats will be contaminated with inorganic carbonates (IC) following the analysis for organic carbon. To remove the carbonates place the contaminated boats in a muffle furnace at 1000°C for one hour or run the boats as blanks at 1350°C in the Leco CNS-2000

5. Sample Collection, Preservation and Handling

5.1 Soil and plant samples are dried at 55°C and 65°C, respectively. The dried soil sample is then ground to pass a 12 mesh screen and the plant tissue is ground to pass a 2 mm screen.

5.2 Acid-digested or acid-treated samples should not be run on the Leco CNS-2000

5.3 The Leco COM-CAT combustion accelerator can be used to insure complete combustion when large samples are used or when total sulfur determination is required

6. Apparatus and Materials

6.1 Scale 0.0001 g

6.2 Leco CNS-2000 Carbon, Nitrogen and Sulfur Analyzer

6.3 Autoloader Assembly with 49-position sample rack

6.4 Printer

6.5 Sample spatula

7. Reagents

7.1 COM-CAT combustion catalyst (Tungsten Tri-oxide, Leco 501-426)

7.2 Anhydron (Anhydrous Mg perchlorate, Leco 501-171)

7.3 Lecosorb (Sodium hydroxide, Leco 502-174)

7.4 Sulfamethazine (Leco 502-304), EDTA (Leco 502-092) or soil standards (Leco 502-309, 502-308)

7.5 Glass wool

7.6 UHP helium gas

7.7 UHP oxygen gas

7.8 Compressed air (low water content)

8. Methods

8.1 Operate instrument according to manufacturer's instructions. The following are generalized instructions:

8.1.1 Turn furnace on (or take off standby).

8.1.2 Turn gas regulators to desired flow rate (40 psi).

8.1.3 Select the appropriate method for either TC or OC.

8.1.4 Wait until the furnace has stabilized at the set temperature.

8.1.5 Test for leaks in the helium lines, ballast tank and combustion system.

8.1.6 Define the standard by entering the appropriate carbon content of the pure primary standard.

8.1.7 Include ten blanks and three dried (or desiccated pure) primary standards at the beginning of each run to calculate the calibration factor for determining carbon (to correct for drift).

8.1.8 Weigh out 0.15 to 2.0g of dried soil or plant tissue, respectively, in a clean sample ceramic crucible (boat). 1.0g of COM-CAT accelerator can be added to the boats before the sample is weighed. Weights are automatically transferred to the microprocessor by pressing the print button on the scale pad.

8.1.9 Transfer the weighed samples to the 49-position sample rack and load the rack onto the autoloader.

8.1.10 Run the samples.

9. Calculations

The inorganic carbon content is calculated as the difference between total carbon and organic carbon as follows:

$$\% \text{ IC} = \% \text{ TC} - \% \text{ OC}$$

10. Quality Control

10.1 The method's analysis range (lower limit is based on 3x standard deviation of the blank) is 0.02 - 200 mg carbon. Analysis precision is RSD 0.4%.

10.2 At least 10 blanks must be analyzed daily before each run and the blank with the value closest to zero should be selected and used for blank correction. Three to five standards should be analyzed and the one with the value closest to the real value of the standard should be used to correct for drift in the calibration curve.

10.3 At least one standard of the same material as the samples should be run with every ten unknowns and at the end of each run to verify calibration.

11. Reporting

11.1 Data is reported as %C for soil and plant tissue on a dry weight basis.

11.2 The detection limit is 0.020 mg C.

12. References

12.1 Leco Corporation. 2002. CNS-2000 elemental analyzer. CNS-2000 instruction manual.

12.2 Organic Carbon Dry Combustion method, Leco CN-2000, FP 2000, or CNS-2000 (JD Schwab, Personal communications).

12.3 Islam, K.R. and R.R. Weil. 1998. A rapid microwave method for colorimetric measurement of soil organic carbon. *Commu- soil-sci-plant-anal.* 29 (15/16):2269-2284.

12.4 Matejovic, I. 1997. Determination of carbon and nitrogen in samples of various soils by dry combustion. *Commu-soil- sci-plant-anal.* 28(17/18):1499-1511.

12.5 Leco Corporation 2003. Organic application note. Carbon, nitrogen and sulfur testing in soil/plant tissue.

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Soil and Sediment Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of soil and sediment samples for the analysis of *leachable* components (major, minor, and trace elements or total minerals, heavy metals, and micro-nutrients) by ICP-OES (TJA Iris Advanced ICP-OES) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus ICP-MS).

- 1.1 Soil and sediment samples contain major (Si, Al, Fe, Ti, Mn, Ca, Mg and Na), minor, and trace components. Alternatively, soil and sediment samples contain fraction one or structural components which are held within aluminum-silicate minerals and fraction two components which are held in soil and sediment by other mechanisms (precipitated, replaced, absorbed, complexed, exchanged, etc).
- 1.2 If a soil/sediment sample is totally dissolved, such as with a mixture of hydrofluoric acid (HF) and other acids, the measured components include both fraction one and fraction two components and the measured concentrations are “total

concentrations” of a sample. These concentrations are comparable to the concentrations obtained by other methods such as XRF methods and NAA methods.

- 1.3 The exclusive analysis of fraction two components has more applications than the analysis of total concentrations in agricultural or environmental areas, since fraction one components are “inert” while fraction two components are “active” and “available” in agricultural or environmental processes.
 - 1.3.1 Fraction two components are supposed to be “all-leached” out by treating samples with concentrated acids (except HF acid) at a high temperature and the measured concentrations are “total leachable concentrations.” These leachable concentrations are often referred as “total concentrations” or “total minerals,” although these “total concentrations” are conceptually not true total concentrations at all.

1.3.2 The total leachable concentrations are not directly comparable to XRF or NAA results, since the XRF or NAA concentrations are true total concentrations. However, this is highly element-dependent and may be sample-dependent. For example, the leachable concentration of silicon is far less than the total concentration of silicon, but the leachable concentration of mercury is usually close to (>95%) the total concentration of mercury in samples.

1.4 The total (leachable) components seem simple and well defined conceptually but the analysis of these leachable components is actually defined operationally. The measured results could be widely variable if a given sample is processed (leached) with different procedures or conditions. The results of leachable concentrations in soil or sediment samples should be interpreted carefully, keeping these considerations in mind.

1.5 There are unlimited versions of procedures available in literature for the process of soil and sediment samples, considering the numerous combinations of sample weight, acid type, acid amount, acid concentration, digestion time duration, digestion temperature, digestion pressure, and equipments. Since the measured results could be variable if a given sample is processed with different/alternative procedures or conditions, *a procedure without alternative steps* is preferred, developed, and used at this laboratory to achieve the greatest consistency in analyzing

different types of samples and/or samples at different times. In general, results obtained by a consistent method are comparable mutually.

2. Summary of method

2.1 A dried and ground sample (0.5 gram) and 5 mL of concentrated nitric acid are added into a 50-mL Folin digestion tube. The mixture is heated at 120-130 °C for 14-16 hours and then is treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is further 1:1 diluted for the analysis of major and minor components by ICP-OES and further 1:9 diluted for the analysis of minor and trace components by ICP-MS.

2.2 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.

4. Interference

4.1 This method covers the analysis of over 30 elements by ICP-OES and ICP-MS. Even a general discussion of interferences is lengthy but not necessarily relevant to a specific element/isotope. The analysis of

metals and non-metals by ICP-OES and ICP-MS has been established and there is an enormous amount of literature available relevant to this subject. Reading the published articles is recommended.

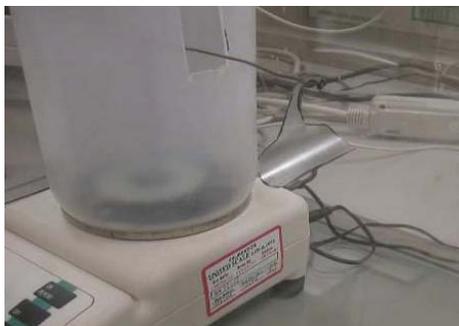
- 4.2 In this method, the solution for ICP-OES analysis contains < 500 ppm of dissolved solid and the solution for ICP-MS analysis contains <100 ppm of dissolved solid. The major components are Fe, Al, K, Ca, Mg and Mn. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences in general are not expected, although some specific element/isotope may be interfered.

5. Sample collection, preservation and handling

A representative sample of soil/sediment is dried and ground. A five-gram vial or equivalent is used to hold a sub sample for airtight storage.

6. Apparatus and device

- 6.1 Analytical balance (accurate to 1 milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



- 6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

- 6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate, Model: HP 53014C).



- 6.4 Ten-mL universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

- 6.5 ICP-OES: TJA Iris Advantage ICP-OES.
- 6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat #2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”
- 6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).
- 6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes, Cat #14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water for several times. The tubes are air-dried before use.

7. Reagents

- 7.1 Concentrated nitric acid (> 68%) (e.g. TraceMetal grade. Fisher A509-212).
- 7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).
- 7.3 Single-element and multi-element primary standard solutions.

8. Pre-digestion

- 8.1 Dry samples at 60 °C for two days. Large stones/rocks or plant materials are removed. Grind the samples (Calcareous samples may be ground to very fine powders). Small-size samples are wrapped in

plastic film and broken or ground to avoid contamination of normal grounding. Extremely small size samples are used “as-is.” Store in a five-gram vial or other appropriate container for airtight storage. Note: Samples may be dried at 60 °C or at 110 °C. The water content could be different.

- 8.2 Weigh 0.50±0.01 g of the sample (unknown samples, in-house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried digestion tubes (Finely ground calcareous sample powders: 0.25 gram, sandy samples: 1.00 gram). Make one to three digestion blanks.
- 8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an IRS (internal reference standard) for the analysis by ICP-OES. Spike 0.2 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.
- 8.4 Carefully add drops of 20–30% (v/v) nitric acid to moisten the samples. This is especially important for calcareous samples to prevent them from foaming over.
- 8.5 After the samples have been moistened with the diluted nitric acid, add 5 mL of concentrated nitric acid. Soak at room temperature for 2-3 hours.

Note: A digestion with perchloric acid should be avoided for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and

several other isotopes using quadrupole ICP-MS.

9. Hot plate digestion

9.1 Place all of the digestion tubes in a block heater. Cover the tubes with plastic film to retard water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7). Turn the power on.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this could cause higher blank concentrations for several elements.

9.3 The temperature will ramp up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is very important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the hydrogen peroxide is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add hydrogen peroxide (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Leave overnight to let particles settle down. After this digestion (1st dilution), nominal dilution factor = (50 mL/0.5 gram) = 100. Y = 8 ppm. Rh = 40 ppb.

Note: A typical digestion time table at SPAL – start heating in the afternoon (3 pm), heat overnight with plastic film cover, take the cover off in the early morning (7 am) the next day, and add hydrogen peroxide afterwards.

Note: Samples may not be heated above 130-140°C. Localized overheating may cause a sample to boil over and be lost.

Note: Soil/sediment samples may contain MnO₂. Hydrogen peroxide reacts with MnO₂ quickly. Hydrogen peroxide also reacts with some other components quickly in a hot nitric acid medium. Therefore, add hydrogen peroxide only after the sample tubes have been cooled.

Note: After a soil sample is digested with concentrated acid (without HF) at a high temperature, the majority of the sample remains as a solid and 5-10% of the sample is leached into solution (this ratio

is much higher for calcareous soil samples). If a sample is digested at a dilution factor (DF) of 100 (e.g. 0.5 gram of soil sample is digested and diluted to 50 mL) the solution does not contain 1% of the total dissolved solid (TDS) but contains <0.1% of the TDS. This kind of solution can generally be directly introduced to ICP-OES or ICP-MS. However, most components may still be significantly higher than “optimum” concentration ranges. In SPAL, the solution is analyzed by ICP-OES with a further 1:1 dilution for major and most minor elements. With the SPAL’s specific model of the ICP-MS instrument (VG PlasmaQuad PQ2 Turbo Plus ICP-MS), this kind of solution is analyzed with a further 1:9 dilution for minor and trace elements. One may argue that why not to use less amount of soil at the start so that the second dilution or any further dilution is avoided. Firstly, as it is pointed out in section 1 (Application), any “alternative” steps should be avoided as much as possible in order to achieve a consistent analysis. The leaching efficiency would be different if the acid to soil ratio is changed. Secondly, larger-size sample is more “representative” than smaller-size sample for samples such as soil or sediment which is usually fairly “inhomogeneous.” Thirdly, the size of half a gram of sample is widely used in other procedures. The size of a sample of course can be changed if the consistency is not an issue in some special projects.

10. Measurement by ICP-OES

10.1. Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube. Mix. After this 2nd dilution (for ICP-OES), nominal dilution factor = $(6 \text{ mL}/3 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 200$. Y = 4 ppm.

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using up-side down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2. Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3. Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Add sample solutions (1 mL) to the 14-mL Falcon tubes containing 9 mL of 2% nitric acid. Mix well. After this dilution (2nd for ICP-MS), total dilution factor = $(10 \text{ mL}/1 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 1,000$. Rh = 4 ppb.

11.1.2 Depending on sample matrix and analyte concentration, the sample may be diluted in other ratios.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Digest blanks are also measured with other samples.

11.2.3 In the menu, select “soil” and edit it if needed.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 1,000, after this procedure is followed exactly. Otherwise, adjust the DF accordingly.

Scenario 1: 10 mg/kg (10 ppm or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.2 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 40 ppb) is further diluted by 1:9 (2nd DF = 10) to contain 10 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 1000). This 2nd solution is measured against a standard containing 1 ppb of X and 4 ppb of Rh and the measured result is 10 ppb. After applying the overall dilution factor of 1000, the concentration of X in the solid material is $10 \text{ ppb} \times \text{DF } 1000 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario 2: Element X in the 2nd solution (X = 10 ppb and Rh = 4 ppb) is still much higher than the standard (X = 1 ppb and Rh = 4

ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 10 \times 5 = 5000$) to contain 2 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (2 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (1 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 10 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$. Option 2: At step 13.2, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 2 ppb against a standard of 1 ppb X with 4 ppb Rh. Now the total DF is 5000 and X in the solid sample is $2 \text{ ppb} \times 5000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure. Some basics are given in “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS.”

– End –

Potato Petioles Nitrate-Nitrogen

1. Application

In this procedure, nitrogen in the form of nitrate ($\text{NO}_3\text{-N}$) is extracted from potato leaf petioles with 2% acetic acid (at room temperature) and analyzed by flow injection.

2. Summary of Methods

Inorganic nitrate in plant tissue are readily water soluble and is extracted by water from samples that have been oven dried and finely ground. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Results are reported on a dry weight basis.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Efficiency of nitrate reduction by the cadmium reduction tube can be adversely affected by interferences in plant extracts. A dilution ratio of no less than 1:10 should be used when nitrate is measured in extracts of oven-dried samples (0.1 g to 25 ml 2% acetic acid).
- 4.2 Build-up of suspended matter in the reduction column will restrict sample flow. Since $\text{NO}_3\text{-N}$ is soluble, the sample may be pre-filtered.
- 4.3 Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this effect.
- 4.4 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium in the reduction column. This interference can be eliminated by pre-extracting the sample with an organic solvent (e.g. methanol).

5. Sample Collection, Preservation, and Handling

The youngest fully-expanded leaves of potato plants, usually the 4th or 5th leaf from the apex, are to be sampled. Leaflets should be stripped from the petioles by hand. Petioles are to be dried at 50-55°C and ground in a Wiley Mill and sieved through a 12 mesh screen. When stripping could not be done immediately after sampling, leaflets should be kept cool during transit to the laboratory where they can be stored at 4°C.

6. Apparatus and Materials

- 6.1 Weigh paper
- 6.2 Erlenmeyer flasks (125ml)
- 6.3 Pipette bank (15ml)
- 6.4 Time-controlled, oscillating shaker
- 6.5 Filter paper, 9cm (Whatman No. 2 or equivalent)
- 6.6 Funnel tubes (15ml)
- 6.7 Glass test tubes (6.2ml)
- 6.8 Flow injection analyzer (Lachat QuikChem 8000)

7. Reagents

- 7.1 2% acetic acid solution (40 ml acetic acid, bring to 2L with distilled water)
- 7.2 15 M sodium hydroxide
- 7.3 Ammonium chloride buffer, pH = 8.5
- 7.4 Sulfanilamide color reagent
- 7.5 Potassium nitrate standards

8. Methods

- 8.1 Weigh out 0.10 g of dried leaf petiole sample into weigh paper.
- 8.2 Transfer sample to a 125ml Erlenmeyer flask.
- 8.3 Add 25 ml of 2% acetic acid.
- 8.4 Shake for 15 minutes on oscillating shaker.
- 8.5 Filter immediately into funnel tubes.
- 8.6 Pour filtrate into glass test tubes.
- 8.7 Analysis using flow injection analyzer.
- 8.8 Dilute sample with DI water as necessary.

9. Calculations

- 9.1 Sample concentration (mg/L) is calculated from a regression equation by plotting response versus standard concentration. Final nitrate content of sample is calculated as follows:
- 9.2 Nitrate content (mg/L) =
$$\frac{\text{measure value (mg/L)} \times \text{sample volume (ml)}}{\text{weight of sample (g)}}$$
- 9.3 Original nitrite in the sample is assumed to be negligible and not included in the calculations.

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 10.2 Potato Petioles Standard – One or more standards of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

11. Reporting

Results are reported as ppm (mg/L) nitrogen in the form of nitrate NO_3^- -N.

12. References

- 12.1 AOAC 1980. Methods of Analysis. Nitrogen. W. Horwitz (ed.). Association of Official Analytical Chemists. Washington DC.
- 12.2 Lachat Instruments 2000. Determination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis. QuickChem Method 10-107-04-1-A.
- 12.3 MacKown and Weik 2004. Comparison of Laboratory and Quick-test Methods for Forage Nitrate. Crop Sci. 44:218-226.

Ash

1. Application

This method covers the determination of ash from soil, tissue and waste samples.

2. Summary of Methods

Ash is determined by use of a muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

3. Safety

All chemical compounds should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

None

5. Sample Collection, Preservation and Handling

Samples are dried at 55°C

6. Apparatus and Materials

- 6.1 Muffle furnace
- 6.2 High temperature crucibles
- 6.3 Balance capable of reading to 0.001 g

7. Reagents

None

8. Methods

- 8.1 Record weight of high temperature crucible to 0.001 g
- 8.2 Weigh out 1 – 5 grams of soil, tissue, or waste into crucible and record weight of sample and crucible.
- 8.3 Place in muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$. Sample must remain at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

8.4 Remove samples from muffle furnace, cool and re-weigh to 0.001 g.

9. Calculations

Before ashing record:

$$\text{crucible weight} + (\text{crucible weight} + \text{sample weight}) = \text{sample weight}$$

After ashing record:

$$\text{crucible weight} + \text{sample weight}$$

$$\% \text{ ash} = \frac{\text{sample weight ash} - \text{crucible weight}}{\text{sample weight dry} - \text{crucible weight}} \times 100$$

10. Quality Control

10.1 Standard laboratory soil # 4

11. Reporting

11.1 Samples are reported in $\% \pm 0.1$

12. Reference

Standard Methods (for the examination of water and wastewater). 15th Edition, 1980 Pg. 97 (209G). Volatile and Fixed Matter in Nonfilterable Residue and in Solid and Semisolid Samples.

Sample Preparation & Lab Dry Matter for Feed and Forage

1. Application

All forage samples received at the lab are thoroughly mixed, sub-sampled, weighed, dried, and ground prior to analysis. The mixing and sub-sampling operations should ensure a homogeneous mixture for analysis. Depending on the analyses requested, some fresh sample will be saved.

2. Summary of Methods

3. Safety

Basic precautions regarding mechanical equipment and electric motors must be followed. All electrical equipment is properly grounded and installed and maintained by qualified electricians. Dust masks, safety glasses and ear protection plugs should be used when grinding forages.

4. Interferences

5. Sample Collection, Preservation, and Handling

Forage samples are typically received in a fresh, heterogeneous state, unsuitable for most analysis. The amount of sample needed for analysis is generally 130-150 grams. Many samples are larger than this as received. These samples must be sub-sampled to ensure the most representative sample as possible of appropriate volume. This is normally accomplished by placing the entire sample in a large plastic tub and thoroughly mixing the contents prior to sub-sampling for dry matter analysis.

6. Apparatus and Materials

- 6.1 Cabinet-type, forced-air drying oven at 55 C, ± 3 C
- 6.2 Analytical electronic balance, accurate to 0.1 mg
- 6.3 Aluminum pan, approximately 20 cm diameter, 5 cm deep
- 6.4 Bucket or bin and large counter for mixing and sub-sampling
- 6.5 Wiley mill, 4 mm size
- 6.6 Cyclone grinder, 1 mm size
- 6.7 Forage sample trays accommodating five rows of ten sample cups
- 6.8 Forage sample cups with covers, plastic, 6 cm in diameter, 8 cm deep

7. Reagents

None applicable.

8. Methods

- 8.1 Record tare weight of aluminum pan.
- 8.2 Thoroughly mix the sample in a bucket.
- 8.3 Sub-sample about 130-150 grams in the aluminum pan. Record initial weight of sample plus pan.
- 8.4 Take special notes of the following forage types and/or analyses:
 - 8.4.1 For large sample volumes such as TMR's spread the sample out on a counter to thoroughly mix the sample. Divide the TMR sample into four sections. Put one section in an aluminum pan to weigh and save one other section in a bag. Place that portion of the sample in a freezer to save until after all analyzes are complete. The remaining sections can be discarded.
 - 8.4.2 For any sample requiring the Degree of Starch Access (DSA), mold & yeast, or mycotoxin analysis, spread the sample out on a counter after thoroughly mixing the sample. Divide the sample into sections, depending on how much sample has been submitted. Put one section in an aluminum pan to weigh and save one other section in a bag. Place the sample in a freezer until further analysis can be completed on an undried and unground.
- 8.5 Place pans in drying oven for 24-48 hours.
- 8.6 Weigh pans back to record dry weight of sample plus pan.
- 8.7 Grind sample thru 4 mm Wiley mill, followed by the 1 mm Cyclone grinder, and place in sample cup and cap sample before placing in a numbered sample tray.

9. Calculations

Percent Lab Dry Matter (% DM):

$$\% \text{ Lab DM} = \{(\text{Dry Weight of Sample and Container} - \text{Tare Weight of Container}) / (\text{Initial Weight of Sample and Container} - \text{Tare Weight of Container})\} \times 100$$

Percent Lab Moisture:

$$\% \text{ Lab Moisture} = 100 - \% \text{ DM}$$

10. Quality Control

11. Reporting

Results are reported as % Lab Dry Matter on an as received basis.

12. References

Total Dry Matter by Oven Drying for 3 hr at 105 C

1. Application

This procedure is applicable for the determination of dry matter on ground air-dry or partially dried ($\geq 85\%$ dry matter) forages with low volatile acid content. This method is often used following lab dry matter determination using method 1.

2. Summary of Methods

Moisture is evaporated from the sample by oven drying. Dry matter is determined gravimetrically as the residue remaining after drying.

3. Safety

Basic precautions regarding mechanical equipment, electric motors, and glassware must be followed. All electrical equipment is properly grounded and installed and maintained by qualified electricians.

4. Interferences

Samples dried by this procedure are not appropriate for subsequent fiber, lignin, or acid detergent insoluble nitrogen analysis. Volatile acids and alcohols may be lost from fermented samples when using this method. This procedure is recommended for developing forage dry matter calibration for NIR.

5. Sample Collection, Preservation, and Handling

All samples are dried at 105 C in a cabinet-type forced air dryer for 3 hrs.

6. Apparatus and Materials

- 6.1 Forced-air drying oven at 105 C, ± 3 C. Oven should be equipped with a wire rod shelf to allow the circulation of air. It should be vented and operated with vents open.
- 6.2 Analytical electronic balance, accurate to 0.001 g.
- 6.3 Aluminum dish (pan), ≥ 50 mm diameter, ≤ 40 mm deep.
- 6.4 Desiccator

7. Reagents

None.

8. Methods

- 8.1 Dry aluminum dish at $105\text{ C} \pm 3\text{ C}$ for at least 2 hours.
- 8.2 Remove dishes to desiccator. Immediately cover desiccator and allow dishes to cool to room temperature. Do not allow dishes to remain in desiccator more than 2-3 hours.
- 8.3 Weigh dishes to nearest 0.001 g, removing one at a time from desiccator and keeping desiccator closed between dish removals.
- 8.4 Add approximately 2 g ground sample to each dish. Record weight of dish and sample to nearest 0.001 g.
- 8.5 Shake dish gently to uniformly distribute the sample and expose the maximum area for drying.
- 8.6 Insert samples into preheated oven at 105 C and dry for 3 hours after oven has returned to temperature.
- 8.7 Move samples to desiccator, seal desiccator and allow to cool to room temperature. Do not allow sample to remain in desiccator for more than 2-3 hours.
- 8.8 Weigh dish and dried sample, recording weight to nearest 0.001 g.

For NIR calibration replace step 8.4 above with:

- 8.4.1 Load NIR sample cup placing one scoop of dried and ground sample on each third of the glass surface to ensure that portions of different sub-samples are scanned. Overfill the sample cup and scrape off any excess. Press back into holder until tight and level.
- 8.4.2 Scan sample in NIR instrument and store spectra.
- 8.4.3 Immediately remove sample from NIR instrument and weigh 2 g forage from sample cup to aluminum dishes. Record weight of dish and sample to nearest 0.001 g and proceed with steps 8.5 thru 8.8 above.

Notes:

- Time and temperature must be adhered to closely.
- Samples should be placed in drying oven so that air can circulate freely. Containers should not touch.
- Slide the desiccator lid open. Do not place the lid on the countertop with the grease side down. The grease will pick up dirt, preventing formation of a seal.
- Seals should be kept clean and well greased and the lid should always slide easily on or off. If the lid “grabs,” it is time to remove the old grease and apply fresh lubricant.
- If a lid can be directly lifted off the desiccator, either the desiccator was not properly sealed or, more likely, it needs fresh lubricant.
- Rubber stoppers in the lid should always be pliable.
- Sample dishes should not be packed excessively tight in the desiccator. Air movement is necessary to cool sample dishes. Dishes should not touch each other.
- Open a loaded desiccator very slowly after samples have cooled. A vacuum forms during cooling and abrupt opening results in turbulence which can blow samples out of uncovered containers.

- Desiccator lid should be slid open for the removal of each container and closed during weighing. Leaving the lid open allows samples to absorb moisture.
- Desiccant should be checked and dried periodically. Replace at least twice annually. Use of desiccant with color indicator for moisture is recommended.

9. Calculations

Percent Total Dry Matter (Total DM):

$\% \text{ Total DM} = \{(\text{Dry Weight of Sample and Dish} - \text{Tare Weight of Dish}) / (\text{Initial Weight of Sample and Dish} - \text{Tare Weight of Dish})\} \times 100$

Percent Total Moisture:

$\% \text{ Total Moisture} = 100 - \% \text{ Total DM}$

10. Quality Control

Include at least one set of duplicates in each run if single determinations are being made. An acceptable average standard deviation among replicated analyses for moisture or dry matter is about ± 0.10 %DM, which results in a warning limit (2s) of ± 0.20 and a control limit (3s) of ± 0.30 . Plot the results of the duplicated analyses on an R-control chart and examine the chart for trends. Results outside the 95 percent confidence limits warn of possible problems with the analytical system. Results outside the 99 percent confidence limits indicate loss of control, and results of the run should be discarded. If more than five or six points in succession fall on one side or the other of the 50 percent line, it is a strong indication that something has changed and is cause for investigation.

11. Reporting

Results are reported as % Total Dry Matter and % Total Moisture.

12. References

NFTA, 2001. Moisture task force report.

Crude Protein Determination in Feed and Forages Macro-Kjeldahl Method

1. Application

This procedure is applicable for the determination of nitrogen (N) in forage. Crude protein is derived through a calculation using this nitrogen value.

2. Summary of Methods

The Kjeldahl method is the standard method of nitrogen determination. The procedure consists of three basic steps: 1) digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia; 2) distillation of the ammonia into a trapping solution; and 3) quantification of the ammonia by titration with a standard solution.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Reagent proportions, heat input and digestion time are critical factors and any variations to these could result in improper analysis.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm mill.

6. Apparatus and Materials

- 6.1 #1 Whatman filter papers
- 6.2 Analytical balance, sensitive to 0.1 mg
- 6.3 Kjeldahl flasks, 800 ml
- 6.4 Kjeldahl packets (see reagents)
- 6.5 Boiling chips, selenized
- 6.6 Kjeldahl digestion unit with fume removal manifold
- 6.7 Kjeldahl rack

- 6.8 Rubber stoppers
- 6.9 Erlenmeyer flasks, 500 ml
- 6.10 Burettes

7. Reagents

7.1 Sodium Hydroxide

- 7.1.1 Add 5lbs each of sodium hydroxide flakes to two plastic 4000 ml beakers. Use a scale under a hood. Gently add 3500 ml distilled water, avoid splashing. Stir flakes until completely dissolved; approximately 5 minutes. Heat is emitted in this step so place in sink filled with cold water to level of the solution in beakers. Let beakers cool in water approximately 5 hours.
- 7.1.2 After 5 hours bring solution in each beaker to 4000 ml with distilled water. Add both beakers of solution to a 15 L carboy. Rinse beakers with 100 ml distilled water and add to carboy. Bring volume to total of 10 liters.
- 7.1.3 In a 4000 ml glass beaker, add 240 g sodium thiosulfate to approximately 2500 ml distilled water. Stir until dissolved or put on a stir plate. Bring to 3000 ml volume with distilled water. Add to carboy and rinse the beaker with 2000 ml distilled water. Total volume of 15 liters in carboy.

7.2 14 L Boric Acid

7.3 Boric Acid Indicator

- 7.3.1 0.40000g Bromocresol Green
- 7.3.2 0.0800g Methyl Red indicator
- 7.3.3 480 ml 95% Ethanol

7.4 0.1 N Sulfuric Acid

7.5 Kjeldahl Packets, per pack

- 7.5.1 10g potassium sulfate (K_2SO_4), anhydrous
- 7.5.2 0.3g copper sulfate ($CuSO_4$), anhydrous
- 7.5.3 0.1g pumice

7.6 Mossy zinc

8. Methods

Digestion:

- 8.1 Weigh out approximately 1g forage samples on #1 Whatman filter paper circles. Record weight to nearest 0.1 mg. Fold filter paper around forage and put in Kjeldahl flasks.
- 8.2 Run one blank with each set. An laboratory standard forage sample should also be run to gauge acceptability of the run.
- 8.3 Put one Kjeldahl packet and 2-3 boiling chips in flask with sample.

- 8.4 Add 30 ml sulfuric acid.
- 8.5 Turn on water aspirator of the Kjeldahl unit. Also turn on ventilation fan for fume extraction.
- 8.6 Digest on Kjeldahl unit for 2 hours with burners on high. Turn flasks a half turn after one half hour of digesting.
- 8.7 After 2 hours, turn off burners and let flasks sit for 5-10 minutes. Remove flasks from burners and cap immediately with rubber stoppers and place on Kjeldahl rack. Turn off water aspirator.
- 8.8 Let flasks cool for at least 30 minutes.

Distillation:

- 8.9 Put 50 ml of boric acid in 500 ml Erlenmeyer flasks for each sample being run. Put these flasks on the Kjeldahl unit for distillation.
- 8.10 Make sure the sodium hydroxide and water burettes are filled and turn on water for condensing. Turn burners on high.
- 8.11 In the following order, add 250 ml distilled water, 3-4 chips of mossy zinc, and 100 ml sodium hydroxide slowly. Immediately attach to the condenser.
- 8.12 Turn off burners and move flasks off the condenser tubes when the liquid reaches 200 ml. Distillation takes about 30 minutes. Shut off water and wash Kjeldahl flasks when all done.

Titration:

- 8.13 Titrate the liquid in the Erlenmeyer flask using 0.1N sulfuric acid until the blue liquid returns to a purplish pink color. Be careful not to add too much sulfuric acid. Read ml of sulfuric acid used on burette and record number.

9. Calculations

- 9.1 % Crude Protein = $\{(ml\ titrated - blank)(.8756)/(sample\ wt\ in\ grams)(\% \text{ lab DM})\} * 100$
- 9.2 % Nitrogen = $\{(ml\ titrated - blank)(.1401)/(sample\ wt\ in\ grams)(\% \text{ lab DM})\} * 100$

10. Quality Control

A reagent blank and at least one sample of in-house standard are run as check of the correctness of the procedure. If digestion is not complete, make appropriate adjustments.

11. Reporting

Results are reported as crude protein (CP) as a % of Dry Matter.

12. References

- 12.1 Protein (Crude) Determination in Animal Feed: Copper Catalyst Kjeldahl Method. (984.13) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
- 12.2 Protein (Crude) Determination in Animal Feed: CuSO₄/TiO₂ Mixed Catalyst Kjeldahl Method. (988.05) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.

Neutral Detergent Fiber (NDF)

1. Application

This procedure is applicable for the determination of neutral detergent fiber in all types of forages and feeds.

2. Summary of Methods

A neutral detergent solution is used to dissolve the easily digested pectins and plant cell contents (proteins, sugars, and lipids); leaving a fibrous residue (aNDF) that is primarily cell wall components of plants (cellulose, hemicellulose, and lignin). Detergent is used to solubilize the proteins and sodium sulfite also helps remove some nitrogenous matter; EDTA is used to chelate calcium and remove pectins at boiling temperatures; triethylene glycol helps to remove some non-fibrous matter from concentrate feeds; and heat-stable amylase is used to remove starch. Two additions of amylase (one during refluxing and one during filtration) have been observed to aid aNDF analyses and minimize filtering difficulties. Heat-stable amylases are used in hot solutions to inactivate potential contaminating enzymes that might degrade fibrous constituents.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. A subsample is then dried at 105°C for 3 hours to determine laboratory DM content.

6. Apparatus and Materials

- 6.1 Refluxing apparatus, condenser connections should be made from neoprene rubber or ground glass.
- 6.2 600 ml Berzelius beakers
- 6.3 Analytical electronic balance, accurate to 0.1 mg

- 6.4 Sintered glass crucibles (Gooch), use tall form, coarse porosity, plate 40mm in diameter, large enough to hold 40-50 ml liquid
- 6.5 Suction manifold of 6 crucible capacity, with trap in line and valve to break vacuum
- 6.6 Drying ovens set at 105° C

7. Reagents

- 7.1 NDF solution – Using a 2 L volumetric flask, add 10 L distilled water to a 20 L glass carboy. Add the following ingredients:
 - 7.1.1 334.98 g disodium dihydrogen ethylene diamine tetraacetate (also known as ethylenedinitrilo – tetraacetic acid or EDTA)
 - 7.1.2 82.08 g disodium hydrogen phosphate
 - 7.1.3 540 g lauryl sulfate, USP grade
 - 7.1.4 122.58 g sodium borate decahydrate
 - 7.1.5 180 ml ethylene glycol monoethyl ether (purified grade)
 - 7.1.6 Place on magnetic stir plate. Stir while adding the remaining 8 L distilled water. Let the solution stir overnight.
 - 7.1.7 Check pH of the solution after it is well mixed. It should range within 6.9-7.1. If needed, adjust with concentrated HCl or NaOH as necessary.
- 7.2 Acetone, reagent grade
- 7.3 Amylase solution
 - 7.3.1 1 ml amylase to 40 ml distilled water

8. Methods

Sample processing:

- 8.1 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.
- 8.2 Dry 50 ml glass crucibles overnight at 105°C and hot weigh, recording weight to nearest 0.1 mg.
- 8.3 Thoroughly mix sample and weigh out approximately 0.5 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

- 8.4 Pour approximately 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while you weigh out samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.5 Thoroughly mix sample and then weigh 0.5 g into plastic weigh pan. Run an in-house standard to gauge run acceptability.
- 8.6 Add 0.5 g of sodium sulfite to each sample in pans.
- 8.7 When solution is gently boiling (it takes approximately 15 minutes to reach boiling) pour sample from pan into burette, rinsing pan with a squeeze bottle of NDF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 50 ml.
- 8.8 After solution returns to boiling (note time, needs to reflux 60 minutes), add 2 ml amylase solution and rinse down sides of burette with squeeze bottle of NDF solution.
- 8.9 Reflux for 60 minutes.

Filtration:

- 8.10 Hot weight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.
- 8.11 Put crucibles on vacuum unit below each burette. Turn on vacuum and constant hot water supply.
- 8.12 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, power will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.13 Plugging on forage samples:
 - 8.13.1 Continue running hot water on outside of crucible.
 - 8.13.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
 - 8.13.3 Add acetone to crucible until it slowly filters out. Keep adding acetone until it eventually filters.
 - 8.13.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).
- 8.14 Plugging on corn or starchy samples:
 - 8.14.1 Add 2 ml amylase directly to crucible.

Rinsing:

- 8.15 After all samples are evacuated from burettes and filtered turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.16 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.16 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.

- 8.17 Put samples with crucibles on small muffin tin and put into a 105°C oven overnight.
8.18 Weigh samples with crucibles the following day.

9. Calculations

- 9.1
$$\text{NDF} = \left\{ \frac{(\text{Crucible Weight} + \text{Fiber}) - \text{Crucible Weight w/o Fiber}}{\text{Sample Weight} \times \text{lab DM as decimal}} \right\} \times 100$$

10. Quality Control

An in-house standard is run to gauge run acceptability.

11. Reporting

Results are reported as % NDF on a dry matter basis.

12. References

- 12.1 Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Agricultural Research Service. Handbook number 379 as modified by D.R. Mertens (1992, Personal Communication).
- 12.2 Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Science* 74:3583-3597.
- 12.3 Mertens, D.R. 1992. Critical conditions in determining detergent fiber. Proceedings of NFTA Forage Analysis Workshop. Denver, CO. p C1-C8.

Acid Detergent Fiber Procedure (ADF)

1. Application

This procedure is applicable for the determination of acid detergent fiber (ADF) in all types of forages.

2. Summary of Methods

An acidified quaternary detergent solution is used to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). ADF is determined gravimetrically as the residue remaining after extraction.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. A subsample is then dried at 105°C for 3 hours to determine laboratory DM content.

6. Apparatus and Materials

- 6.1 Refluxing apparatus, condenser connections should be made from neoprene rubber or ground glass.
- 6.2 600 ml Berzelius beakers
- 6.3 Sintered glass crucibles (Gooch), use tall form, coarse porosity, plate 40mm in diameter, large enough to hold 40-50 ml liquid
- 6.4 Analytical electronic balance, accurate to 0.1 mg
- 6.5 Suction manifold of 6 crucible capacity with trap in line and valve to break vacuum
- 6.6 Drying ovens set at 105° C

7. Reagents

7.1 ADF Solution

7.1.1 10.0 L Distilled Water

7.1.2 360 g Hexadecyltrimethylammonium Bromide

7.1.3 500 ml sulfuric acid technical

7.1.4 Bring to 18.0 L with 6.0 L Distilled Water

7.1.5 Standardize ADF Solution:

- a. Pipette 10 ml ADF Solution into 150 ml beaker.
- b. Mix 1.0 g of Phenolphthaline to 100 ml of 95% ethanol. Mix thoroughly and add 5 drops of indicator to beaker of ADF solution.
- c. Titrate using 1 N sodium hydroxide. Solution should begin “clear” and end of the reaction should be “very light pink.”
- d. Adjust solution to desired titration of 10.0 ml If NaOH is below 10.0 ml add 10 ml sulfuric acid technical for every 0.1 ml below or 100 ml distilled water for every 0.1 ml above.

7.2 Acetone, reagent grade - Use grade of acetone that is free of color and will leave no residue upon evaporation.

8. Methods

Sample processing:

8.1 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.

8.2 Dry 50 ml glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.

8.3 Thoroughly mix sample and weigh out approximately 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

8.4 Pour approximately 95 ml ADF solution in digestion burette on Fiber rack. Start heating the solution while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.

8.5 When solution is gently boiling, approximately 15 minutes, pour sample from weigh pan into burette, rinse pan with a squeeze bottle of ADF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 100 ml.

8.6 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.

8.7 Reflux for 60 minutes.

Filtration:

- 8.8 Hot weight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.
- 8.9 Put crucibles on vacuum unit below each burette. Turn on vacuum and constant hot water supply, in excess of 95°C.
- 8.10 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, capacity will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.11 Plugging on forage samples:
 - 8.11.1 Continue running hot water on outside of crucible.
 - 8.11.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
 - 8.11.3 Add acetone to crucible until it slowly filters out.
 - 8.11.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).

Rinsing:

- 8.12 After all samples are evacuated from burettes and filtered turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.13 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.14 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
- 8.15 Put samples with crucibles on small muffin tin and put into 105° C oven overnight.
- 8.16 Weigh hot samples with crucibles the following day, recording to nearest 0.1 mg.

9. Calculations

- 9.1 $\%ADF = \{((\text{Crucible Weight} + \text{Fiber}) - \text{Crucible Weight w/o Fiber}) / (\text{Sample Weight} \times \text{lab DM as decimal})\} \times 100$

10. Quality Control

An in-house standard is run to gauge run acceptability.

11. Reporting

Results are reported as % ADF on a dry matter basis.

12. References

12.1 Fiber (Acid Detergent) and Lignin in Animal Feed. (973.18) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.

Acid Detergent Lignin Procedure (ADL)

1. Application

This procedure is applicable for the determination of acid detergent lignin (ADL) in all types of forages.

2. Summary of Methods

An acidified quaternary detergent solution is used to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). ADL is determined gravimetrically as the residue remaining upon ignition after 72% H₂SO₄ treatment.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. A subsample is then dried at 105°C for 3 hours to determine laboratory DM content.

6. Apparatus and Materials

- 6.1 Refluxing apparatus, condenser connections should be made from neoprene rubber or ground glass.
- 6.2 600 ml Berzelius beakers
- 6.3 Sintered glass crucibles (Gooch), use tall form, coarse porosity, plate 40mm in diameter, large enough to hold 40-50 ml liquid
- 6.4 Analytical electronic balance, accurate to 0.1 mg
- 6.5 Suction manifold of 6 crucible capacity with trap in line and valve to break vacuum
- 6.6 Drying ovens set at 100° C
- 6.7 50 ml beakers
- 6.8 Desiccator
- 6.9 Muffle furnace at 500°C

7. Reagents

7.1 ADF Solution

7.1.1 10.0 L Distilled Water

7.1.2 360 g Hexadecyltrimethylammonium Bromide

7.1.3 500 ml sulfuric acid technical

7.1.4 Bring to 18.0 L with 6.0 L Distilled Water

7.1.5 Standardize ADF Solution:

- a. Pipette 10 ml ADF Solution into 150 ml beaker.
- b. Mix 1.0 g of Phenolphthaline to 100 ml of 95% ethanol. Mix thoroughly and add 5 drops of indicator to beaker of ADF solution.
- c. Titrate using 1 N sodium hydroxide. Solution should begin “clear” and end of the reaction should be “very light pink.”
- d. Adjust solution to desired titration of 10.0 ml If NaOH is below 10.0 ml add 10 ml sulfuric acid technical for every 0.1 ml below or 100 ml distilled water for every 0.1 ml above.

7.2 Acetone, reagent grade - Use grade of acetone that is free of color and will leave no residue upon evaporation.

7.3 Sulfuric Acid (72%) on a per weight basis

7.3.1 63.9 ml of concentrated H₂SO₄, specific gravity of 1.84

7.3.2 36.1 ml of distilled water

8. Methods

Sample processing:

8.1 Sample should be oven dried at 55°C to ≥85% dry matter, then ground to pass a 1mm forage mill.

8.2 Dry 50 ml glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.

8.3 Thoroughly mix sample and weigh out approximately 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

8.4 Pour approximately 95 ml ADF solution in digestion burette on Fiber rack. Start heating the solution while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.

8.5 When solution is gently boiling, approximately 15 minutes, pour sample from pan into burette, rinse pan with a squeeze bottle of ADF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 100 ml.

- 8.6 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.
- 8.7 Reflux for 60 minutes.

Filtration:

- 8.8 Hot weigh six glass crucibles, or metal crucibles with Dacron, before filtration.
- 8.9 Put crucibles on vacuum unit below each burette. Turn on vacuum and hot water, in excess of 95°C.
- 8.10 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, power will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.11 Plugging on forage samples:
 - 8.11.1 Continue running hot water on outside of crucible.
 - 8.11.2 Use rubber policeman to break up fiber mat on bottom of crucible.
 - 8.11.3 Add acetone to crucible until it slowly filters out.
 - 8.11.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).

Rinsing:

- 8.12 After all samples are evacuated from burettes and filtered turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.13 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.14 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
- 8.15 Put samples with crucibles on small muffin tin and put into 105° C oven overnight.
- 8.16 Weigh hot samples with crucibles the following day, recording to nearest 0.1 mg.

Isolation of Acid Insoluble Lignin:

- 8.17 Place crucible in a 50 ml beaker for support and cover the contents of the crucible with cooled (15°C) 72% H₂SO₄ and stir with a glass rod to a smooth paste, breaking all lumps.
- 8.18 Fill crucible about halfway with acid and stir. Let glass rod remain in crucible; refill with 72% H₂SO₄ and stir at hourly intervals as acid drains away. Keep crucible at about 20-30°C.
- 8.19 After 3 hours, filter off as much acid as possible with vacuum and wash contents with hot water until free from acid. Rinse and remove stirring rod.
- 8.20 Dry crucible at 105°C for 8 hours or overnight and cool in a desiccator for at least 1 hour. Weigh crucible to the nearest 0.1 mg.
- 8.21 Ignite crucible in a muffle furnace at 500°C for 2 hours. While still hot, place crucible in desiccator, cool to constant temperature and weigh to 0.1 mg.

9. Calculations

- 9.1 $\%ADF = \{((\text{Crucible Weight} + \text{Fiber}) - \text{Crucible Weight w/o Fiber}) / (\text{Sample Weight} \times \text{lab DM as decimal})\} \times 100$
- 9.2 $\%ADL = (\text{Crucible Weight after Acid Soak} - \text{Crucible Weight after Ignition}) / (\text{Sample Weight} \times \text{lab DM as decimal})$

10. Quality Control

An in-house standard is run to gauge run acceptability.

11. Reporting

Results are reported as % ADF and % ADL on a dry matter basis.

12. References

- 12.1 Fiber (Acid Detergent) and Lignin in Animal Feed. (973.18) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.

Acid Detergent Insoluble Nitrogen (ADIN) and Acid Detergent Fiber Crude Protein (ADF-CP); also known as ADP or ICP (Insoluble CP)

1. Application

This procedure is applicable for the determination of acid detergent fiber insoluble nitrogen in all types of forages. Acid detergent insoluble nitrogen (ADIN) is the nitrogen remaining in the acid detergent fiber residue and, while some occurs naturally in all plant material, is generally considered to be an estimate of heat damage occurring during storage or processing. Nitrogen in excessively heated samples is usually unavailable to animals.

2. Summary of Methods

ADIN is determined as the nitrogen in ADF residue. The two options used to determine ADIN differ in the amount of the ADF residue that is analyzed for nitrogen. If the total ADF residue is collected on filter paper and analyzed for nitrogen, ADIN (% DM basis) is determined by measuring the nitrogen (corrected for a filter paper blank) in the total ADF residue and dividing by the original dry sample weight. The other option involves ADF residues from fritted glass (Gooch) crucibles. It is difficult, if not impossible to collect all ADF residues from fritted glass (Gooch) crucibles, therefore only a sub-sample of the total ADF residue is analyzed for nitrogen. When only a part of the ADF residue is analyzed, by sampling the ADF residue from a fritted glass crucible (or from filter paper), the nitrogen content of the ADF residue must be determined by dividing the nitrogen in the ADF sample by the ADF sample weight. Then ADIN (% DM basis) is calculated by multiplying the nitrogen content of the ADF by the ADF content in the dry matter. When sampling ADF residues from fritted glass crucibles, be careful not to scrape glass particles into the partial ADF residue that is analyzed for nitrogen. Acid detergent fiber crude protein (ADF-CP) is ADIN expressed as crude protein on a dry matter basis.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hrs. After drying the sample is ground to pass through a 1 mm forage mill.

6. Apparatus and Materials

- 6.1 See procedure “Kjeldahl Nitrogen and Crude Protein in Forages” for nitrogen determination method.
- 6.2 See procedure “Acid Detergent Fiber (ADF)” for acid detergent fiber.
- 6.3 Filter paper, acid hardened, #4 Whatman or equivalent.

7. Reagents

- 7.1 See procedure “Kjeldahl Nitrogen and Crude Protein in Forages” for nitrogen determination method.
- 7.2 See procedure “Acid Detergent Fiber (ADF)” for acid detergent fiber method.

8. Methods

Option A: Determination of ADIN using total ADF residue (filter paper)

- 8.1 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.
- 8.2 Dry at least 6 filter papers overnight at 100°C to determine average filter paper DM content. Weigh filter papers to be used to collect ADF residues to nearest 0.1 mg.
- 8.3 Thoroughly mix sample and weigh out approximately 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

- 8.4 Pour approximately 95 ml ADF solution in a digestion burette on fiber rack. Start solution heat while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.5 When solution is gently boiling, approximately 15 minutes, pour sample from pan into burette, rinse pan with a squeeze bottle of ADF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 100 ml.
- 8.6 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.
- 8.7 Reflux for 60 minutes.

Filtration:

- 8.8 Hot weigh filter paper before filtration.
- 8.9 Put filter paper on funnel on vacuum unit below each burette. Turn on vacuum and hot water, in excess of 95°C.
- 8.10 Open vacuum under 4-6 funnels at a time. If not enough are open the filter paper may tear due to too much vacuum. Open stop cock on burette to drain into paper, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.11 Plugging on forage samples:
 - 8.11.1 Create more suction by slowly closing a few more vacuum ports.
 - 8.11.2 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50g).

Rinsing:

- 8.12 After all samples are evacuated from burettes and filtered, turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.13 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.14 Suction off acetone, rinsing down sides of the filter paper with acetone to finish the rinsing portion.
- 8.15 Fold filter paper to retain sample, dry a minimum of 3 hours at 105° C in an oven.
- 8.16 Weigh hot the samples with filter paper, recording to nearest 0.1 mg.
- 8.17 Insert filter paper and sample into Kjeldahl flasks, add 5 ml additional acid to digest the filter paper and determine nitrogen by “Kjeldahl Nitrogen and Crude Protein in Forages” procedure.

Option B: Determination of ADIN using partial ADF residue (from fritted glass crucibles)

- 8.18 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.
- 8.19 Dry 50 ml fritted glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.
- 8.20 Thoroughly mix sample and weigh out approximately 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

- 8.21 Pour approximately 95 ml ADF solution in digestion burette on fiber rack. Start solution heat while weighing out samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.22 When solution is gently boiling, approximately 15 minutes, pour sample from pan into burette, rinse pan with a squeeze bottle of ADF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 100 ml.
- 8.23 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.
- 8.24 Reflux for 60 minutes.

Filtration:

- 8.25 Hot weight eight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.
- 8.26 Put crucibles on vacuum unit below each burette. Turn on vacuum and hot water, in excess of 95°C.
- 8.27 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, power will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.28 Plugging on forage samples:
 - 8.28.1 Continue running hot water on outside of crucible.
 - 8.28.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
 - 8.28.3 Add acetone to crucible until it slowly filters out.
 - 8.28.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).
- 8.29 Plugging on corn or starchy samples:
 - 8.29.1 Add 2 ml amylase directly to crucible.

Rinsing:

- 8.30 After all samples are evacuated from burettes and filtered, turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.31 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.32 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
- 8.33 Dry a minimum of 3 hours at 100° C in an oven. Weigh hot, samples with crucibles, recording to nearest 0.1 mg.
- 8.34 Sample a portion of the ADF residue from fritted glass crucible using a Teflon or plastic policeman into Kjeldahl flasks. Do not scrape so hard as to dislodge glass from the fritted disk.
- 8.35 Weigh partial ADF residue, recording weight to nearest 0.1 mg.

- 8.36 Determine nitrogen content of the ADF residue sub-sample using the “Kjeldahl Nitrogen and Crude Protein in Forages” procedure.

9. Calculations

- 9.1 Option A: Percent Acid Detergent Insoluble Nitrogen (ADIN), DM basis using total ADF residue (filter paper):
- 9.1.1 $\% \text{ ADIN (DM basis)} = \{(\text{ml titrated} - \text{blank})(.8756)/(\text{sample wt in grams})(\% \text{ lab DM})\} * 100$
 - 9.1.2 Acid Detergent Insoluble Nitrogen (as percent of total nitrogen), also called ADIN to N ratio. $\% \text{ ADIN (of total N)} = \{[\% \text{ ADIN (DM basis)}] / [\% \text{ N (DM basis)}]\} * 100$
 - 9.1.3 Percent Acid Detergent Fiber Crude Protein (ADF-CP), DM basis.
 $\% \text{ ADF-CP (DM basis)} = \% \text{ ADIN (DM basis)} * 6.25$
- 9.2 Option B: Percent Acid Detergent Insoluble Nitrogen (ADIN), DM basis using particle ADF residues (from fritted glass crucibles or filter paper):
- 9.2.1 $\% \text{ ADIN (DM basis)} = [\% \text{ N of ADF residue} * \% \text{ ADF (DM basis)}] / 100$
 - 9.2.2 Acid Detergent Insoluble Nitrogen (as percent of total nitrogen), also called ADIN to N ratio. $\% \text{ ADIN (of total N)} = \{[\% \text{ ADIN (DM basis)}] / [\% \text{ N (DM basis)}]\} * 100$
 - 9.2.3 Percent Acid Detergent Fiber Crude Protein (ADF-CP), DM basis.
 $\% \text{ ADF-CP (DM basis)} = \% \text{ ADIN (DM basis)} * 6.25$

10. Quality Control

Samples are typically run in duplicate due to increased risk of filter paper tearing when using option A.

11. Reporting

Results are reported as % ADF-CP on a dry matter basis.

12. References

- 12.1 Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Agricultural Research Service. Agriculture Handbook No. 379.
- 12.2 Goering, H.K., C.H. Gordon, R.W. Hemken, D.R. Waldo, P.J. Van Soest, and L.W. Smith. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. Journal of Dairy Science. 55(9): 1275-1280.

Neutral Detergent Insoluble Nitrogen (NDIN) and Neutral Detergent Fiber Crude Protein (NDF-CP)

1. Application

This procedure is applicable for the determination of neutral detergent fiber insoluble nitrogen in all types of forages. Neutral detergent insoluble nitrogen (NDIN) is the nitrogen remaining in the neutral detergent fiber residue and, while some occurs naturally in all plant material, is generally considered to be an estimate of heat damage occurring during storage or processing. Nitrogen in excessively heated samples is usually unavailable to animals.

2. Summary of Methods

NDIN is determined as the nitrogen in NDF residue. The two options used to determine NDIN differ in the amount of the NDF residue that is analyzed for nitrogen. If the total NDF residue is collected on filter paper and analyzed for nitrogen, NDIN (% DM basis) is determined by measuring the nitrogen (corrected for a filter paper blank) in the total NDF residue and dividing by the original dry sample weight. The other option involves NDF residues from fritted glass (Gooch) crucibles. It is difficult, if not impossible to collect all NDF residues from fritted glass (Gooch) crucibles, therefore only a sub-sample of the total NDF residue is analyzed for nitrogen. When only a part of the NDF residue is analyzed, by sampling the NDF residue from a fritted glass crucible (or from filter paper), the nitrogen content of the NDF residue must be determined by dividing the nitrogen in the NDF sample by the NDF sample weight. Then NDIN (% DM basis) is calculated by multiplying the nitrogen content of NDF by the NDF content in the dry matter. When sampling NDF residues from fritted glass crucibles, be careful not to scrape glass particles into the partial NDF residue that is analyzed for nitrogen. Neutral detergent fiber crude protein (NDF-CP) is NDIN expressed as crude protein on a dry matter basis.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill.

6. Apparatus and Materials

- 6.1 See procedure “Kjeldahl Nitrogen and Crude Protein in Forages” for nitrogen determination method.
- 6.2 See procedure “Neutral Detergent Fiber (NDF)” for neutral detergent fiber method.
- 6.3 Filter paper, acid hardened, #4 Whatman or equivalent.

7. Reagents

- 7.1 See procedure “Kjeldahl Nitrogen and Crude Protein in Forages” for nitrogen determination method.
- 7.2 See procedure “Neutral Detergent Fiber (NDF)” for neutral detergent fiber method.

8. Methods

Option A: Determination of NDIN using total NDF residue (filter paper)

- 8.1 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.
- 8.2 Dry at least 6 filter papers overnight at 100°C to determine average filter paper DM content. Weigh filter papers to be used to collect NDF residues to nearest 0.1 mg.
- 8.3 Thoroughly mix sample and weigh out 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

- 8.4 Pour approximately 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.5 Thoroughly mix sample and then weigh 0.5 g into plastic weigh pan. Run an in-house standard to gauge run acceptability.
- 8.6 Add 0.5 g of sodium sulfite to each sample in pans.
- 8.7 When solution is gently boiling (it takes approximately 15 minutes to reach boiling) pour sample from pan into burette, rinsing pan with a squeeze bottle of NDF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 50 ml.

- 8.8 After solution returns to boiling (note time, needs to reflux 60 minutes), add 2 ml amylase solution and rinse down sides of burette with squeeze bottle of NDF solution.
- 8.9 Reflux for 60 minutes.

Filtration:

- 8.10 Hot weigh filter paper before filtration.
- 8.11 Put filter paper on the funnel on the vacuum unit below each burette. Turn on vacuum and hot water.
- 8.12 Open vacuum under 4-6 funnels at a time. If not enough are open the filter paper may tear. Open stop cock on burette to drain into paper, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.13 Plugging on forage samples:
 - 8.13.1 Create more suction by slowly closing a few more vacuum ports.
 - 8.13.2 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).

Rinsing:

- 8.14 After all samples are evacuated from burettes and filtered, turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.
- 8.16 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.17 Suction off acetone, rinsing down the side of the filter paper with acetone to finish the rinsing portion.
- 8.18 Fold filter paper to retain sample, dry a minimum of 3 hours at 105° C in an oven.
- 8.19 Weigh hot, samples with filter paper, recording to nearest 0.1 mg.
- 8.20 Insert filter paper and sample into Kjeldahl flasks, add 5 ml additional acid to digest the filter paper and determine nitrogen by “Kjeldahl Nitrogen and Crude Protein in Forages” procedure.

Option B: Determination of NDIN using partial NDF residue (from fritted glass crucibles)

- 8.21 Sample should be oven dried at 55°C to $\geq 85\%$ dry matter, then ground to pass a 1mm forage mill.
- 8.22 Dry 50 ml fritted glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.
- 8.23 Thoroughly mix sample and weigh out 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers.

The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

- 8.24 Pour approximately 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.25 Thoroughly mix sample and then weigh 0.5 g into plastic weigh pan. Run an in-house standard to gauge run acceptability.
- 8.26 Add 0.5 g of sodium sulfite to each sample in pans.
- 8.27 When solution is gently boiling (it takes approximately 15 minutes to reach boiling) pour sample from pan into burette, rinsing pan with a squeeze bottle of NDF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 50 ml.
- 8.28 After solution returns to boiling (note time, needs to reflux 60 minutes), add 2 ml amylase solution and rinse down sides of burette with squeeze bottle of NDF solution.
- 8.29 Reflux for 60 minutes.

Filtration:

- 8.30 Hot weight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.
- 8.31 Put crucibles on vacuum unit below each burette. Turn on vacuum and hot water.
- 8.32 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, power will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.
- 8.33 Plugging on forage samples:
 - 8.33.1 Continue running hot water on outside of crucible.
 - 8.33.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
 - 8.33.3 Add acetone to crucible until it slowly filters out. Keep adding acetone until it eventually filters.
 - 8.33.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).
- 8.34 Plugging on corn or starchy samples:
 - 8.34.1 Add 2 ml amylase directly to crucible.

Rinsing:

- 8.35 After all samples are evacuated from burettes and filtered, turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.

- 8.36 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.
- 8.37 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
- 8.38 Dry a minimum of 3 hours at 100° C in an oven. Weigh hot, samples with crucibles, recording to nearest 0.1 mg.
- 8.39 Sample a portion of the NDF residue from fritted glass crucible using a Teflon or plastic policeman into Kjeldahl flasks. Do not scrape so hard as to dislodge glass from the fritted disk.
- 8.40 Weigh partial NDF residue, recording weight to nearest 0.1 mg.
- 8.41 Determine nitrogen content of the NDF residue sub-sample using the “Kjeldahl Nitrogen and Crude Protein in Forages” procedure.

9. Calculations

- 9.1 Option A: Percent Neutral Detergent Insoluble Nitrogen (NDIN), DM basis using total NDF residue (filter paper):
 - 9.1.1 $\% \text{NDIN (DM basis)} = \{(\text{ml titrated} - \text{blank})(.8756) / (\text{sample wt in grams})(\% \text{ lab DM})\} * 100$
 - 9.1.2 Neutral Detergent Insoluble Nitrogen (as percent of total nitrogen), also called NDIN to N ratio. $\% \text{NDIN (of total N)} = \{[\% \text{NDIN (DM basis)}] / [\% \text{N (DM basis)}]\} * 100$
 - 9.1.3 Percent Neutral Detergent Fiber Crude Protein (NDF-CP), DM basis.
 $\% \text{NDF-CP (DM basis)} = \% \text{NDIN (DM basis)} * 6.25$
- 9.2 Option B: Percent Neutral Detergent Insoluble Nitrogen (NDIN), DM basis using particle NDF residues (from fritted glass crucibles or filter paper):
 - 9.2.1 $\% \text{NDIN (DM basis)} = [\% \text{N of NDF residue} * \% \text{NDF (DM basis)}] / 100$
 - 9.2.2 Neutral Detergent Insoluble Nitrogen (as percent of total nitrogen), also called NDIN to N ratio. $\% \text{NDIN (of total N)} = \{[\% \text{NDIN (DM basis)}] / [\% \text{N (DM basis)}]\} * 100$
 - 9.2.3 Percent Neutral Detergent Fiber Crude Protein (NDF-CP), DM basis.
 $\% \text{NDF-CP (DM basis)} = \% \text{NDIN (DM basis)} * 6.25$

10. Quality Control

Samples are typically run in duplicate due to increased risk of filter paper tearing when using option A.

11. Reporting

Results are reported as % NDF-CP on a dry matter basis.

12. References

- 12.1 Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Agricultural Research Service. Handbook number 379 as modified by D.R. Mertens (1992, Personal Communication).
- 12.2 Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Science* 74:3583-3597.
- 12.3 Mertens, D.R. 1992. Critical conditions in determining detergent fiber. Proceedings of NFTA Forage Analysis Workshop. Denver, CO. p C1-C8.



Plant Tissue

Insect or disease issues should be sent to the [Plant Disease Diagnostics Clinic](#).

Analyses Offered

Routine Tissue: \$25 Submission form [Front](#) | [Back](#) | [Routine Sampling Instructions](#)

Includes tissue N, P, K, Ca, Mg, S, Zn, Mn, B, Cu, and Fe. Also Includes 1 routine soil analysis if sample provided. Optional soil tests available for extra are: Calcium + Magnesium: \$3 | Boron: \$3 | Zinc: \$3 | Manganese: \$3 | Sulfur-Sulfate: \$3

Total N only: \$10

Potato petiole nitrate: \$10 [Submission Form](#) | [Potato Petiole Sampling Instructions](#)

Corn stalk nitrate: \$10 [Submission Form](#) | [Corn Stalk Nitrate Sampling Instructions](#).

Sampling

Correct sample collection is critical for plant analysis as plant nutrient composition varies with age, the

portion of the plant sampled, and many other factors. Mistakes or carelessness in selecting, collecting, handling, preparing, or shipping plant tissue for analysis can result in unreliable data, which may lead to incorrect interpretations and recommendations. Standards, against which the sample is evaluated, have been selected to represent the plant part and time of sampling that best define the relationship between nutrient composition and plant growth. Deviation from the prescribed protocol severely limits this interpretations capability. It is, therefore, critical to follow a standard sampling procedure.

Analysis and Reporting

The report will show the concentration of N, P, K, Ca, Mg, S, Zn, Mn, B, Cu, and Fe in the plant sample. If a soil was submitted with the plant sample, soil analyses for pH, organic matter, P, K, and any special soil test results will also be reported. For those plant materials where calibration data are not available, these analytical results will be provided without interpretation. Plant analysis results are interpreted by sufficiency range.

Total Ash in Forages

1. Application

This procedure is applicable for the determination of ash in all types of dried, ground forages and feeds.

2. Summary of Methods

A dried, ground sample is ignited in a furnace at 500°C to oxidize all organic matter. Ash is determined by weighing the resulting inorganic residue.

3. Safety

Basic precautions regarding mechanical equipment, electric motors, and glassware must be followed. All electrical equipment is properly grounded and installed and maintained by qualified electricians.

4. Interferences

This procedure is not applicable for ash determination in liquid feeds or feeds high in sugar content.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. A subsample is then dried at 105°C for 3 hours to determine laboratory DM content.

6. Apparatus and Materials

- 6.1 30 ml porcelain crucibles, low wide form, numbered with furnace-proof ink
- 6.2 Muffle furnace with pyrometric controller
- 6.3 Analytical balance, sensitive to 0.1 mg
- 6.4 Desiccator, with vented lid
- 6.5 Drying oven

7. Reagents

None

8. Methods

- 8.1 Remove crucibles, which have been dried for at least 2 hours at 105°C from oven, and move to desiccator. Cool and record weight of crucibles to the nearest 0.1 mg (W_1).
- 8.2 Weigh 1.5 to 2.0 g of sample into the crucible, recording weight of crucible and sample to the nearest 0.1 mg (W_2).
- 8.3 Ash in furnace at 500°C for 2 hours after the furnace reaches temperature.
- 8.4 Allow crucibles to cool in furnace to less than 200°C and place crucibles in desiccator with vented top. Cool and weigh crucible and ash to the nearest 0.1 mg (W_3).

9. Calculations

- 9.1 % Ash (DM basis) = $[(W_3 - W_1) * 100] / [(W_2 - W_1) * \text{lab DM} / 100]$
 W_1 = tare weight of crucible in grams
 W_2 = weight of crucible and sample in grams
 W_3 = weight of crucible and ash in grams

10. Quality Control

An in-house standard is run to gauge run acceptability.

11. Reporting

Results are reported as % ash on a dry matter basis.

12. References

- 12.1 Ash of Animal Feed. (942.05) Official Methods of Analysis. 1990. Association of Official Analytical Chemists, 15th Edition.

Fat by Acid Hydrolysis

1. Application

This procedure is applicable for the determination of crude fat in dried forages and mixed feeds. It is not applicable for oilseeds, baked and/or expanded products (pet foods), liquid feeds, sugar products, and feeds containing dairy products.

2. Summary of Methods

A dried, ground sample is extracted with diethyl ether which dissolves fats, oils, pigments, and other fat soluble substances. The ether is then evaporated from the fat solution. The resulting residue is weighed and referred to as ether extract or crude fat. Both the ether and the samples must be free of moisture to avoid co-extraction of water-soluble components in the sample such as carbohydrates, urea, lactic acid, glycerol, etc. If water-soluble components are present in large amounts in the sample, they are washed out of the sample prior to drying. Low temperatures are used to evaporate the ether and remove residual moisture to prevent oxidation of the fat.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

This procedure is extremely sensitive to variations in technique. Use tongs in handling beakers, and wear gloves throughout the procedure. Keep the beakers in a desiccator when not in use. Be sure the tubes are well cooled before doing ether extraction.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm Wiley mill.

6. Apparatus and Materials

- 6.1 50 ml screw-top test tubes
- 6.2 Automatic dispenser
- 6.3 Water Bath set to 75.5°C

- 6.4 Orbital Shaker
- 6.5 Pasteur pipettes
- 6.6 Cotton plugs
- 6.7 Long stem funnels
- 6.8 150 ml beakers
- 6.9 Hot plate
- 6.10 Dessicator
- 6.11 Laboratory Oven (135°C)

7. Reagents

- 7.1 Ethyl Alcohol – 95%
- 7.2 Anhydrous Ethyl Ether
- 7.3 Petroleum Ether
- 7.4 Hydrochloric Acid – 25:11 (Acid:Water) dilution

8. Methods

- 8.1 Grind the dried sample through a 1mm sieve.
- 8.2 Weigh 1 gram ground sample into a 50 ml screw-top test tube.
- 8.3 Wet sample with 1 ml of ethanol, saturating it.
- 8.4 Add 5 ml HCl.
- 8.5 Place in preheated water bath (75.5°C) for 40 minutes. Shake occasionally.
- 8.6 Remove and allow cooling to room temp.
- 8.7 Add 5 ml ethanol and mix.
- 8.8 Add 12 ml anhydrous ether – orbital shake for 1 minute.
- 8.9 Add 12 ml petroleum ether – orbital shake for 1 minute.
- 8.10 Let ether and residue separate. (With feces samples, a portion of the ether layer may get trapped under a mat of particulate matter in the tube. Vortexing samples may aid in effecting a better separation.
- 8.11 Pull off top layer into a dried and tared 150 ml beaker via a Pasteur pipette, pouring through a filter paper in a long stem funnel.
- 8.12 Repeat steps 7-10 with 8 ml portions of ethers three more times.
- 8.13 Evaporate ether and any water contained in beaker. Evaporation on a hot plate at low temperature works best. Approximately 1 hour is required.
- 8.14 Place beakers in a 135°C oven for 10 minutes.
- 8.15 Transfer to desiccator and allow cooling to room temperature.
- 8.16 Weigh beaker plus fat to +/-0.01g.

9. Calculations

- 9.1 % Fat = ((Weight of beaker and fat – tared beaker weight) / Sample weight)) * 100

10. Quality Control

An in house standard is run with each batch to assure method accuracy.

11. Reporting

Results are reported as % of Dry Matter.

12. References

- 12.1 AOAC, 1980 Official Methods of Analyses. p.132.
- 12.2 Budde, E. F. 1952 J. Assoc. Off. Agric. Chem. 35.799.

In Vitro Digestibility

1. Application

This procedure allows the determination of true digestibility and digestion kinetics of forages and other feeds based upon the measurement of undigested cell wall constituents as neutral detergent fiber using rumen fluid in an in vitro system.

2. Summary of Methods

Dried and ground samples are digested in an oxygen limiting environment using dairy rumen fluid, buffer, mineral and reducing solutions. Residual dry matter is determined and an NDF is run on residue if needed.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

This method is very sensitive to diet and temperature changes to donor animals.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill.

6. Apparatus and Materials

- 6.1 125 ml Erlenmeyer flask
- 6.2 Stoppers, with 2 inlet valves
- 6.3 Water Bath set to 39°C
- 6.4 Canulated Cow
- 6.5 Automatic dispenser; 40 ml
- 6.6 Carbon Dioxide Tank
- 6.7 Auto dispensing syringe; 2ml and 10 ml
- 6.8 Rubber policemen
- 6.9 Incubation oven
- 6.10 Thermos

- 6.11 Rumen Fluid Pump
- 6.12 2 pails
- 6.13 Blender
- 6.14 Cheesecloth
- 6.15 2000 ml side arm flask

7. Reagents

- 7.1 In Vitro Media (pH 6.8) – makes 800 ml, adjust as needed for larger or smaller sample batches
 - 7.1.1 2.0 g trypticase
 - 7.1.2 400 ml distilled water
 - 7.1.3 0.1 ml micromineral solution; see below
 - 7.1.4 200 ml buffer solution; see below
 - 7.1.5 200 ml macromineral solution; see below
 - 7.1.6 1.0 ml resazurin (0.1% solution in distilled water; keep refrigerated)
- 7.2 Micromineral Solution
 - 7.2.1 13.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
 - 7.2.2 10.0 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$
 - 7.2.3 1.0 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
 - 7.2.4 8.0 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
 - 7.2.5 Bring to 100 ml with distilled water
- 7.3 Buffer Solution
 - 7.3.1 18.0 L Distilled Water
 - 7.3.2 72.0 g Ammonium Bicarbonate
 - 7.3.3 630.0 g Sodium Bicarbonate
- 7.4 Macromineral Solution
 - 7.4.1 18.0 L Distilled Water
 - 7.4.2 102.6 g Na_2HPO_4 , anhydrous
 - 7.4.3 111.6 g KH_2PO_4 , anhydrous
 - 7.4.4 10.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
- 7.5 Reducing Solution
 - 7.5.1 0.625 g cysteine hydrochloride
 - 7.5.2 95 ml distilled water
 - 7.5.3 0.625 g sodium sulfide nonahydrate

8. Methods

Sample processing:

- 8.1 Thoroughly mix sample and weigh out 0.5 gram of sample into 125 ml Erlenmeyer flask.
- 8.2 Concurrently weigh out a 2.0 gram sample for lab dry matter determination at 105°C for 3 hrs. This will allow further calculations to be made on a dry matter basis.
- 8.3 In vitro blanks have no sample added to them, although all other reagents and rumen inoculum is added.

- 8.4 Run a neutral detergent fiber (NDF) on the original sample, to be used in calculating the NDFD after the in vitro is complete. (See NDF procedure).
- 8.5 Turn on water bath and allow the temperature to reach approximately 39°C. Be certain that the water level in the bath is above the 50 ml mark on the flasks so that the flask contents are maintained at 39°C.

Media preparation:

- 8.6 Calculate the amount of media and reducing solution needed based upon the number of samples being analyzed.
- 8.7 Add 40 ml of media per sample using automatic dispenser. Be careful not to splash sample out of flask or up on the sides.
- 8.8 Put stoppers in flasks and place in water bath, connect tubing, which will allow carbon dioxide to flow into flasks.

Reducing:

- 8.9 When all tubing and flasks are connected, turn on carbon dioxide. Adjust pressure to approximately 15-20 psi. Turn on gas flow with needle valve enough to feel gas escaping from all the flasks through inlet valve. The water manometer will cease bubbling. If “icing” of diaphragm and brass hose occurs, decrease gas flow rate by adjusting needle valve.
- 8.10 While flasks are being gassed for approximately 10 to 15 minutes, prepare the reducing solution.
- 8.11 Inject 2 ml of reducing solution into each flask with a syringe. Place rubber policeman onto each inlet valve as the solution is added.
- 8.12 When all flasks have had reducing solution added and are closed with a policeman, adjust CO₂ flow rate so that bubbling through water manometer is minimal.
- 8.13 Wait for media reduction to occur as evidenced by change in color from blue to pink to colorless (oxidized to reduced).

Collection and Preparation of Inoculum:

During processing of the rumen fluid these steps should be done as quickly as possible and effort should be taken to keep the fluid under CO₂ whenever possible.

- 8.15 Earlier in the day, place all equipment that will come in contact with rumen fluid in incubation ovens at approximately 39°C.
- 8.16 Put thermos and pump used for rumen fluid, in pails of hot water (to keep at body temperature). Fill thermos to the top with rumen fluid and seal immediately. Quickly return capped thermos to the warm water. Take immediately to the lab, noting collection time.
- 8.17 Discard approximately one-half of solids on top of fluid layer and pour contents into blender. Measure pH of rumen fluid and then blend for 60 seconds.
- 8.18 Filter through 4 layers of cheesecloth into 2000 ml side arm flask using a funnel.

Inoculating Flasks:

Time is of the essence with this procedure. The total time from collection to inoculation should not exceed 20-25 minutes.

- 8.19 While keeping rumen fluid stirring and under CO₂ quickly add 10 ml of fluid to each flask through the inlet valve using an auto dispensing syringe. Replace rubber policeman onto each inlet valve after inoculate is added. Swirl all flasks after inoculum addition, avoiding splashes up the sides.
- 8.20 Check all stoppers and policemen to be certain they are tight, and adjust CO₂ flow rate if necessary. Remember this system keeps all flasks under constant CO₂ pressure and only minimal bubbling of gas in the water manometer is necessary. If it takes excessive CO₂ pressure to bubble the manometer, check for loose stoppers, loose rubber policemen, or loose tubing causing a leak.
- 8.20 Keep track of incubation times. Record the time at the start of the digestion and when the flasks should be taken out, depending on the time point requested, and post it right on the water bath.

Fermentation Times:

- 8.21 Take flasks out of water bath at the time point you are testing (i.e. 48 hours) and rinse down the sides with distilled water to submerge all particles in liquid, stopper, and freeze immediately.
- 8.22 Alternatively, NDF on the residue can be tested immediately (see NDF procedure).

9. Calculations

- 9.1 Residue Weight = ((Crucible Weight + Fiber) – blank) – Crucible Weight w/o Fiber
- 9.2 Sample Weight Absolute = (Sample Weight * % Lab Dry Matter)
- 9.3 Indigestible Dry Matter % = Residue Weight / Sample Weight Absolute
- 9.4 IVTDM = 1 - (Indigestible Dry Matter %)
- 9.5 IVNDFD = 1 – [(Indigestible Dry Matter % * 100) / % NDF]

10. Quality Control

At least one laboratory reagent blank (LRB) is analyzed with each batch of samples to gauge run acceptability.

11. Reporting

Results are reported as % NDFD on a dry matter basis.

12. References

- 12.1 Goering, H. K., and P. J. Van Soest. 1975. Forage Fiber Analyses. Agr. Handbook No. 379. Agricultural Research Service. USDA.
- 12.2 Mertens, D. R. 1992. Critical conditions in determining detergent fibers. Page C-1 in Proc. Natl. Forage Testing Assoc. Forage Anal. Workshop, Denver, CO. Natl. Forage Testing Assoc., Omaha, NE.

Total Starch in Forages and Grains

1. Application

This procedure covers the determination of starch in biomass samples. The percent starch content is used in conjunction with other assays to determine the total composition of biomass samples.

2. Summary of Methods

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

This procedure is suitable for air-dried biomass samples, as well as for samples that have been oven dried at a temperature of 45°C or less. The assay results will be biased slightly low for samples dried at 105°C. If sample availability is limited, it may be necessary to run this analysis on a 105°C dried sample but the results must be flagged as being biased low. The assay is also suitable for wet samples if the particle size is known to be small and if the moisture content of the sample can be estimated accurately enough to predict the amount of sample needed to give 0.5 g of solids. Interferences by free glucose and cellobiose present in samples are not a problem because both glucose and cellobiose are destroyed during the NaOH solubilization step.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm forage mill. A sub-sample is then dried at 105°C for 3 hours to determine laboratory dry matter content.

6. Apparatus and Materials

- 6.1 Analytical balance, accurate to 0.1 mg.
- 6.2 YSI 2700 Select Biochemistry Analyzer – equipped with a YSI 2365 dextrose membrane and YSI 2357 buffer and calibrated with YSI 2776 2.5 g/L calibrator solution.
- 6.3 Hot plate or water bath set at 90°C ± 2°C.

- 6.4 Graduate cylinders of appropriate sizes.
- 6.5 100 ml, 500 ml, and 1000 ml volumetric flasks, class A.
- 6.6 5 and 10 ml pipets or adjustable pipettor.
- 6.7 Timer.
- 6.8 Water bath set at $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- 6.9 Erlenmeyer flasks, 125 ml.
- 6.10 Test tubes, 10 ml.

7. Reagents

- 7.1 Glucose calibration verification standards, such as YSI 2.0 and 9.0 mg/ml glucose standards.
- 7.2 Amyloglucosidase (suggested source, Sigma A-3042).
- 7.3 Corn Starch (Spectrum S1552)
- 7.4 Methanol, ACS reagent grade.
- 7.5 2N NaOH
 - 7.5.1 Weigh 40 g of sodium hydroxide pellets into a 500 ml volumetric flask.
 - 7.5.2 Add 300 ml of reagent grade water and mix.
 - 7.5.3 Cool, dilute to volume and mix.
- 7.6 2N HCl
 - 7.6.1 Measure 82.4 ml of concentrated hydrochloric acid and transfer to a 500 ml volumetric flask.
 - 7.6.2 Let cool, dilute to volume with reagent grade distilled water and mix.
- 7.7 Acetate buffer (pH 4.2)
 - 7.7.1 Weigh 9.1 g of sodium acetate into 500 ml volumetric flask.
 - 7.7.2 Add about 300 ml of reagent grade distilled water and mix until all solid is dissolved.
 - 7.7.3 Add 22.3 ml (23.4 g) of glacial acetic acid.
 - 7.7.4 Dilute to volume with distilled water and mix.
- 7.8 Amyloglucosidase working solution
 - 7.8.1 Prepare a fresh working solution of the enzyme such that it contains 60 units of activity per milliliter.
 - 7.8.2 If using the Sigma A-3042 amyloglucosidase, dilute the solution one hundred-fold into cold reagent grade water. Prepare daily and store in the refrigerator.
- 7.9 25% TCA – dissolve 50.0 g trichloroacetic acid in 200 ml reagent grade water.
- 7.10 Phosphate buffer
 - 7.10.1 Dissolve 40 g NaH_2PO_4 and 10 g Na_2HPO_4 in reagent grade water
 - 7.10.2 Bring to volume in a 1000 ml volumetric flask

8. Methods

- 8.1 Determine the total dry matter content of each sample. Record the total dry matter value as T_{final} .
- 8.2 Weigh out approximately 0.500 g of sample to the nearest 0.0001 g and transfer to a 125 ml Erlenmeyer flask. Record as W_{sample} , the initial sample weight.

- 8.3 Weigh a 0.500 g portion of pure corn starch to the nearest 0.0001 g and transfer to an Erlenmeyer flask. Record the weight as W_{standard} , the initial standard reference material weight. As with the unknown samples, the total dry matter content, T_{final} , of the standard reference material must also be determined.
- 8.4 Add 25 ml of reagent grade water to each flask. Swirl to ensure the sample is wetted and evenly dispersed. Note: A few drops of methanol may be used to pre-wet the sample which will aid in its dispersion once the water is added.
- 8.5 Add 10 ml of 2N NaOH to the solution in each flask. Place flasks on a heating unit or in a water bath preheated to 90°C. Heat for 20 minutes, swirling periodically to wet any sample that may be clinging to the side of the flask. A glass stirring rod may be needed to break up clumps of material.
- 8.6 Add 10 ml of 2N HCl to each flask and swirl to mix. Cool the flasks to below 50°C, which takes about 20 minutes.
- 8.7 Add 10 ml of acetate buffer to each flask and swirl to mix.
- 8.8 Add 5.0 ml amyloglucosidase working solution to each flask. Mix well and place the flasks in a 40°C water bath for 60 minutes.
- 8.9 After 60 minutes incubation, remove the flasks from the water bath. Immediately add 5 ml of 25% TCA to each flask to stop hydrolysis.
- 8.10 Pipette 6.5 ml of hydrolyzate into a 10 ml test tube. Add 3.5 ml phosphate buffer to each tube.
- 8.11 Since the enzyme solution may contain free glucose, an enzyme blank must be run in parallel with the samples. Dilute duplicate 5.0 ml portions of the amyloglucosidase working solution to 100 ml with reagent grade water in a volumetric flask. These enzyme blanks will be analyzed in the same manner as the sample, with the averaged results used to correct the glucose contents of the samples.
- 8.12 The sample itself may contain free glucose, which normally would be analyzed as starch. However in this procedure the glucose, and also cellobiose, is destroyed in the NaOH solubilization step. Therefore no correction for free glucose is needed when calculated the total starch content of a sample.
- 8.13 Set up and calibrate the YSI as described in the manufacturer's manual using the dextrose membrane, YSI 2357 system buffer, and YSI 2776 2.5 g/L calibrator solution. Program the instrument to auto calibrate every fourth sample or every fifteen minutes, set the sample size to 25 μ L, and use the following probe parameters:
 - 8.13.1 Chemistry – dextrose
 - 8.13.2 Units – g/L
 - 8.13.3 Calibrator – 2.50 g/L
 - 8.13.4 End point – 30 seconds
 - 8.13.5 Cal Station # - 1
- 8.14 Verify the calibration of the YSI using the glucose calibration verification standards before starting the run. Re-verify the calibration periodically during the analysis and at the end of the run.
- 8.15 Measure the glucose levels in the enzyme blanks and in all the samples. The validated linear range of the instrument is 0 – 9.0 g/L dextrose. If the value

reported exceeds the validated range, the hydrolyzate must be diluted appropriately and re-run.

9. Calculations

- 9.1 Calculate the amount of starch recovered from each analysis of the amylopectin standard reference material as follows (on a 105°C dry weight basis) and then average the recoveries:
- 9.1.1 $\% \text{ Standard recovered} = \left[\left\{ \left(\text{YSI}_{\text{standard, g/L}} - \text{YSI}_{\text{enzyme blank, g/L}} \right) * \text{total volume, L} \right\} / \left\{ \text{standard weight, g, } W_{\text{standard}} * (\% \text{ total solids, } T_{\text{final}} / 100) \right\} \right] * 0.9 * 100\%$
- 9.1.2 Note: Amylopectin recoveries of 93 to 95% have routinely been achieved with this protocol. Recoveries less than 90% indicate the data generated for the batch of samples should be rejected and the analysis repeated.
- 9.2 Calculate the amount of starch present in each sample, on a 105°C dry weight basis:
- 9.2.1 $\% \text{ Starch} = \left[\left\{ \left(\text{YSI}_{\text{sample, g/L}} - \text{YSI}_{\text{subenzyme blank, g/L}} \right) * \text{total volume, L} \right\} / \left\{ \text{standard weight, g, } W_{\text{standard}} * (\% \text{ total dry matter, } T_{\text{final}} / 100) \right\} \right] * 0.9 * 100\%$
- 9.2.2 Note: The factor 0.9 converts grams of glucose to grams of the anhydrosuger (starch, in this case). The factor can be calculated by dividing the molecular weight of glucose less one molecule of water (180-18) by the molecular weight of glucose.
- 9.3 The calculated percent starch in each sample can be correct for assay losses using the percent recovery of the standard reference material, amylopectin, as follows:
- 9.3.1 $\% \text{ Starch, corrected} = (\% \text{ starch} / \text{average } \% \text{ standard recovered}) * 100\%$

10. Quality Control

A standard reference material, corn starch, is run in parallel with each batch of samples.

11. Reporting

Report the percent starch present in the sample, to two decimal places, on a 105°C dry weight basis. If duplicate samples are run, report the average.

12. References

- 12.1 NREL Ethanol Project Laboratory Analytical Procedure #001, “Standard Method for the Determination of Total Solids in Biomass.”
- 12.2 Solvay Enzymes. 1996. “Fungal Glucoamylase for Starch Hydrolysis.” Diazyme L-200 Technical Notes.
- 12.3 YSI Incorporated. 12994. “Determination of Cook in Extruded Cereal Products.” Application Note #319.

Starch Digestibility; Degree of Starch Access

1. Application

This procedure covers the determination of starch digestibility in biomass samples.

2. Summary of Methods

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

Half of each sample is dried at 55°C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. The other half of each wet sample is retained for starch recovery (DSA).

6. Apparatus and Materials

- 6.1 Analytical balance, accurate to 0.1 mg.
- 6.2 LAB-Line Multi-Unit heaters or similar hot plates.
- 6.3 Graduate cylinders of appropriate sizes.
- 6.4 100 ml, 500 ml, and 1000 ml volumetric flasks, class A.
- 6.5 Timer.
- 6.6 1000 ml beakers.
- 6.7 Thermometer.

7. Reagents

Prepare all reagents as per normal starch assay, as listed below; noting larger volumes of reagents will be used.

- 7.1 Glucose calibration verification standards, such as YSI 2.0 and 9.0 mg/ml glucose standards.
- 7.2 Amyloglucosidase (suggested source, Sigma A-3042).
- 7.3 Corn Starch (Spectrum S1552).
- 7.4 Methanol, ACS reagent grade.
- 7.5 Phosphate buffer (pH 6.5, for solvent)

- 7.5.1 Dissolve 14 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in reagent grade water and bring to volume in a 1000 ml volumetric flask.
- 7.5.2 Determine pH and add 15M NaOH drop wise until pH reaches 6.5.
- 7.6 Acetate buffer (pH 4.2)
 - 7.6.1 Weigh 9.1 g of sodium acetate into 500 ml volumetric flask.
 - 7.6.2 Add about 300 ml of reagent grade distilled water and mix until all solid is dissolved.
 - 7.6.3 Add 22.3 ml (23.4 g) of glacial acetic acid.
 - 7.6.4 Dilute to volume with distilled water and mix.
- 7.7 Amyloglucosidase working solution
 - 7.7.1 Use the Sigma A-3042 amyloglucosidase containing 11,500 units of activity per milliliter.
 - 7.7.2 Mix 2.5 ml of A-3042 amyloglucosidase with 475 ml of reagent grade distilled water in a 500 ml beaker to achieve 60 units of activity per milliliter of enzyme solution.
 - 7.7.3 Prepare daily and store in the refrigerator.
- 7.8 25% TCA – dissolve 50.0 g trichloroacetic acid in 200 ml reagent grade water.
- 7.9 Phosphate buffer
 - 7.9.1 Dissolve 40 g NaH_2PO_4 and 10 g Na_2HPO_4 in reagent grade water
 - 7.9.2 Bring to volume in a 1000 ml volumetric flask
- 7.10 α -Amylase, heat stable – use Sigma A-3306. In a 50 ml beaker mix 2 ml of A-3306 with 18 ml of reagent grade water to obtain 20 ml of working solution.

8. Methods

- 8.1 Determine the lab dry matter and total dry matter content of each sample using the “Sample Preparation & Lab Dry Matter” and “Total Dry Matter” procedures.
- 8.2 Determine the total starch content using the “Total Starch in Forages” procedure.
- 8.3 Weigh out approximately 20.0 g of wet corn silage, TMR, or small grain silage sample to the nearest 0.001 g and transfer to a 1000 ml beaker. Alternatively, weigh out approximately 4.0 g of dry corn, HM corn, grains, or other byproducts to the nearest 0.001 g and transfer to a 1000 ml beaker. Record as W_{sample} , the initial sample weight.
- 8.4 A standard reference corn starch is run in parallel with each batch of samples and to monitor starch recovery of the assay. Weigh 2.5 g of corn starch to the nearest 0.001 g and transfer to a 1000 ml beaker. Record the weight as W_{standard} , the initial standard reference material weight. As with unknown samples, absolute DM content of the corn starch must also be determined (105°C for 3 hours).
- 8.5 Add 150 ml of reagent grade water to each beaker. Swirl vigorously to ensure the sample is wetted and evenly dispersed. Note: A few drops of methanol may be used to pre-wet the sample, which will aid in its dispersion once the water is added.
- 8.6 Add 200 ml phosphate buffer (pH 6.5) to each beaker. Loosely cover and place beaker on a heating unit on setting 70.
- 8.7 After 5 minutes increase setting to 90. Heat for approximately 25 minutes, swirling frequently to wet any sample that may be clinging to the side of the beaker and to avoid clumping of sample in the middle/bottom of the beaker. Monitor the

temperatures of beaker solutions with a thermometer being careful not to allow samples to reach temperatures > 95°C. Samples will start boiling at this point.

- 8.8 Remove from heat.
- 8.9 Add 100 ml of reagent grade water to each beaker and swirl to mix.
- 8.10 Add 3 ml of heat-stable amylase working solution to each beaker when temperatures are near 80°C. Stir gently on stir plate while allowing beaker solutions to cool to below 50°C (approximately 25 minutes.) Use thermometer to monitor beaker solution temperatures.
- 8.11 Add 100 ml of acetate buffer to each beaker and continue stirring on stir plate to mix.
- 8.12 Add 50 ml amyloglucosidase working solution to each beaker when temperatures are < 50°C. Mix well and continue stirring at approximately 40°C for 60 minutes.
- 8.13 While samples are incubating at 40°C, set up a 10 ml test tube for each sample. Pipette 0.5 ml of TCA into each tube.
- 8.14 After 60 minutes of mixing, beaker solutions should be near room temperature.
- 8.15 Pipette 6 ml of beaker solution hydrolyzate into 10 ml test tubes containing TCA.
- 8.16 Pipette 3.5 ml of phosphate buffer into each test tube and mix well.
- 8.17 Set up and calibrate the YSI as described in the manufacturer's manual using the dextrose membrane, YSI 2357 system buffer, and YSI 2776 2.5 g/L calibrator solution. Program the instrument to auto calibrate every fourth sample or every fifteen minutes, set the sample size to 25µL, and use the following probe parameters:
 - 8.17.1 Chemistry – dextrose
 - 8.17.2 Units – g/L
 - 8.17.3 Calibrator – 2.50 g/L
 - 8.17.4 End point – 30 seconds
 - 8.17.5 Cal Station # - 1
- 8.18 Verify the calibration of the YSI using the glucose calibration verification standards before starting the run. Re-verify the calibration periodically during the analysis and at the end of the run.
- 8.19 Measure the glucose levels in the enzyme blanks and in all the samples. The validated linear range of the instrument is 0 – 9.0 g/L dextrose. If the value reported exceeds the validated range, the hydrolyzate must be diluted appropriately and re-run.

9. Calculations

- 9.1 Calculate the amount of starch recovered from analysis of the reference corn starch as follows, 105°C dry weight basis:

$$9.1.1 \quad \% \text{ Standard recovered} = \left[\left\{ \left(\text{YSI}_{\text{standard, g/L}} * \text{total volume, L} \right) / \left\{ \text{standard weight, g, } W_{\text{standard}} * \left(\% \text{ total solids, } T_{\text{final}} / 100 \right) \right\} \right\} * 0.9 * 100\% \right]$$

- 9.1.2 Note: Corn starch recoveries of 93 to 95% have routinely been achieved with this protocol. Recoveries less than 90% indicate the data generated for the batch of samples should be rejected and the analysis repeated.
- 9.2 Calculate the amount of starch recovered in un-dried, un-ground silages, grains, TMR's, etc., on a 105°C dry weight basis:
- 9.2.1 $\% \text{ Starch} = [(YSI_{\text{sample}}, \text{ g/L} * \text{total volume, L}) / \{\text{standard weight, g, } W_{\text{standard}} * (\% \text{ dry matter} / 100)\}] * 0.9 * 100\%$
- 9.2.2 Note 1: The factor 0.9 converts grams of glucose to grams of the anhydrosuger (starch, in this case). The factor can be calculated by dividing the molecular weight of glucose less one molecule of water (180-18) by the molecular weight of glucose.
- 9.2.3 Note 2: Because the sample is un-dried and un-ground, $\% \text{ dry matter} = (\text{Lab dry matter} * \text{Total dry matter})$
- 9.3 Calculate the starch recovery percent ($\text{Starch}_{\text{rp}}$, % of starch) present in the sample, to two decimal places, on a 105°C dry weight basis by the following formula:
- 9.3.1 $\text{Starch}_{\text{rp}}, \% \text{ Starch} = (\% \text{ starch recovered, \% of dry matter} / \text{total starch, \% of dry matter}) * 100\%$

10. Quality Control

A standard reference corn starch is run in parallel with each batch of samples and to monitor starch recovery of the assay. Starch recovery of corn starch should be > 93.0 percent. The recovery of reference corn starch is not used to adjust DSA starch determinations and is only used to monitor assay recover. When reference starch recoveries are low entire starch procedures should be evaluated and re-run.

11. Reporting

Report as Starch Digestibility (DSA), percent of starch present in the sample, to two decimal places, on a 105°C dry weight basis. If duplicate samples are run, report the average.

<u>Starch Digestibility (DSA), % of Starch</u>	<u>Reference</u>
> 96.0	Very High
96.0-93.0	High
93.0-90.0	Medium
< 90.0	Low

12. References

- 12.1 Blasel, H., P.C. Hoffman, and R.D. Shaver. 2005. "Degree of starch access: An enzymatic method to determine starch degradation potential of corn grain and corn silage." Anim. Feed Sci. and Technol.
- 12.2 NREL Ethanol Project Laboratory Analytical Procedure #001, "Standard Method for the Determination of Total Solids in Biomass."

- 12.3 Solvay Enzymes. 1996. "Fungal glucoamylase for Starch Hydrolysis." Diazyme L-200 Technical Notes.
- 12.4 YSI Incorporated. 12994. "Determination of Cook in Extruded Cereal Products." Application Note #319.

DATE: JUNE 2007

Minerals in Feed, Forage and Manure Samples

Dry Ashing Method

1. Application

In this procedure the minerals in feed, forage and manure samples are analyzed for phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca).

2. Summary of Methods

An Atomic Absorption Unit (AA) is used to determine levels of K, Ca and Mg, while a spectrophotometer is used for P.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm sieve.

6. Apparatus and Materials

- 6.1 Glass beakers (50ml)
- 6.2 Muffle furnace at 500°C
- 6.3 Glass test tubes (20ml)
- 6.4 Erlenmeyer flasks (50ml)
- 6.5 Pipette bank (3ml and 10ml)
- 6.6 Pipette (1ml)
- 6.7 Atomic Absorption Unit (AA)
- 6.8 Spectrophotometer

7. Reagents

- 7.1 0.33 N Magnesium Acetate [Mg(OAc)₂]
 - 7.1.1 Dissolve 35.7 g of Mg(C₂H₃O₂) · 4H₂O in about 600 ml of distilled water.

- 7.1.2 Dilute to 1 liter.
- 7.2 Hydrochloric Acid (HCl)
 - 7.2.1 Dilute 85 ml concentrated HCl to 1 liter distilled water
- 7.3 Vanadomolybdate (HNO₃)
 - 7.3.1 Dissolve 2.50 g of NH₄VO₃ in 600 ml of hot distilled water. Cool and add 500 ml of concentrated HNO₃.
 - 7.3.2 Dissolve 50 g of (NH₄)₆MO₇O₂₄ · 4H₂O in 800 ml of distilled water.
 - 7.3.3 Add to vanadomolybdate solution and dilute to 2 liters.
- 7.4 La Stock
 - 7.4.1 Weigh out 23.456 g La₂O₃, slowly add 100 ml of concentrated HCl to dissolve (perform under hood, as there is fuming)
 - 7.4.2 Add 0.1525 g NaCl and transfer quantitatively to 2 liter volumetric flask, diluting with distilled water up to volume.

8. Methods

Sample Preparation

- 8.1 Forages: Mix sample thoroughly, weigh one 0.50 g sub-sample from each sample into a small beaker.
- 8.2 Manures: Mix sample thoroughly, weigh one 0.25 g sub-sample from each sample into a small beaker. Note: when a 0.25 g sample is used readings on the AA and spectrophotometer are multiplied by two to adjust for the smaller initial sample weight. Other figures are recorded as is, unless a dilution is required.
- 8.3 Grains and High Fat Samples: Mix sample thoroughly and weigh two 0.50 g sub-samples from each sample into a small beaker. In one of the sub-samples (for phosphorus analysis) add 10 ml Magnesium Acetate. Put on hot plate, heat and evaporate to dryness. The other sub-sample (for Ca, Mg, and K analysis) is run as a regular forage sample, see below.

Step 1 for P, K, Mg, Ca

- 8.4 In muffle furnace, ash the forage and evaporated grain samples for 2 hours at 500°C.
- 8.5 After ashing, take out samples and let cool.
- 8.6 Add 10 ml HCl to small beakers, let stand 15 minutes.

Step 2 for P

- 8.7 Pipette a 1 ml aliquot from small beakers into small test tubes.
- 8.8 Add 3 ml Vanadomolybdate to each test tube and aliquot.
- 8.9 Add 10 ml distilled water to test tubes. Let color develop for at least 15-20 minutes, before reading on Spectrophotometer.

Step 2 for K, Mg, Ca

- 8.10 Add 20 ml distilled water to small beakers.
- 8.11 Pipette a 1 ml aliquot from beakers into small Erlenmeyer flasks.
- 8.12 Add 10 ml La Stock to Erlenmeyer flasks.
- 8.13 Add 20 ml distilled water to Erlenmeyer flasks.

Determination of mineral content

- 8.14 Small test tubes are used for reading P on the Spectrophotometer.
- 8.15 Erlenmeyer flasks are used for K, Ca, Mg readings on the Atomic Absorption Unit.

- 8.16 Remaining aliquot in small beakers may be used for sulfur analysis, see procedure “Sulfur Determination for Manures and Forage.”

9. Calculations

- 9.1 Sample absorption is taken from spectrophotometer and AA.
9.2 Results are reported on a dry matter basis.
9.3 Manure phosphorus results are adjusted x2 when using a 0.25 g sample size.

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
10.2 Standard – One or more standards of known mineral content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

11. Reporting

Results are reported as a percent of dry matter basis.

12. References

- 12.1 Wisconsin Procedures for Soil Testing, Plant Analysis and Feed & Forage Analysis, No. 6, Soil Fertility Series; last revised 1987 by E.E. Schulte, J.B. Peters and P.R. Hodgson.

Sulfur Determination in Manure and Forage

1. Application

This procedure measures the concentration of sulfur in feed, forage and manure samples.

2. Summary of Methods

A turbidimeter is used to determine levels of sulfur in these materials.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

The turbidimetric procedure is sensitive to variations in temperature and humidity.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm sieve.

6. Apparatus and Materials

- 6.1 See procedure "Minerals in Feed, Forage and Manure Samples."
- 6.2 Funnel tubes (25ml)
- 6.3 Colorimeter tubes (20ml)
- 6.4 Turbidimeter

7. Reagents

- 7.1 See procedure "Minerals in Feed, Forage and Manure Samples" for partial list of reagents.
- 7.2 Sulfur Extract [$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$]
 - 7.2.1 Dissolve 38.6 g of Calcium Phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$] in approximately 15 liters of distilled water.
 - 7.2.2 Add 2185 ml of Glacial Acetic Acid and dilute to 19 liters.
- 7.3 Sulfur Developer [$\text{BaGl}_2\text{-HOAC}$]

- 7.3.1 Dissolve 47.5 g of Gum Arabic in approximately 4.5 liters of hot distilled water.
- 7.3.2 Filter through suction funnel with filter paper #1, twice.
- 7.3.3 Once clear add 475 g of $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$.
- 7.3.4 Add 4275 ml of Glacial Acetic Acid and dilute to 9.5 liters.

8. Methods

- 8.1 See procedure “Minerals in Feed, Forage and Manure Samples.” This procedure picks up after “Step 2 for K, Mg, Ca,” using the aliquot remaining in the small beakers.
- 8.2 Pipette a 2 ml aliquot from small beakers into 25 ml funnel tubes. Add 18 ml sulfur extract solution.
- 8.3 Pipette 10 ml from funnel tubes into the colorimeter tubes.
- 8.4 Add 10 ml of sulfur developer solution to colorimeter tubes using an automatic pipetter.
- 8.5 Let side for 10-15 minutes (until the bubbles have settled.)
- 8.6 Read using turbidimeter. Set the blank in the meter and check the standard, adjusting to correct reading if needed.
- 8.7 Continue with reading the samples.

9. Calculations

- 9.1 Forage % Sulfur = reading on turbidimeter * 2 * 0.01288
- 9.2 Manure % Sulfur = reading on turbidimeter * 2 * 0.01288 * 2

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 10.2 Standard – One or more standards of known mineral content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

11. Reporting

Results are reported as a percent of dry matter basis.

12. References

- 12.1 Wisconsin Procedures for Soil Testing, Plant Analysis and Feed & Forage Analysis, No. 6, Soil Fertility Series; last revised 1987 by E.E. Schulte, J.B. Peters and P.R. Hodgson.

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Plant Tissue Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of plant tissue samples and the analysis of major, minor and trace elements (total minerals, heavy metals and micro-nutrients) in these samples by ICP-OES (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometry) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus Inductively Coupled Plasma Mass Spectrometry).

- 1.1 Plant tissue samples (including some other types of samples such as cheese, manure, lichens, etc) mainly consist of carbon, hydrogen, oxygen and nitrogen. Other abundant components are K, Mg, Ca, P, S and Na. These six elements account for less than 10% of a sample on a dry weight basis.
- 1.2 An open-vessel acid digestion with $\text{HNO}_3 + \text{H}_2\text{O}_2$ (or $\text{HNO}_3 + \text{HClO}_4$ in some applications) in a hot plate could be incomplete for some samples. In other words, the digestion without HF acid is incomplete for some siliceous materials, although the sources of siliceous materials may be variable.

Elements closely associated with siliceous materials such as Al, Fe and Na may not be completely released from solid material to solution. Therefore, the results by ICP-OES or ICP-MS may not be directly comparable to the results by XRF methods or NAA methods. However, this problem might be significant only in limited cases.

- 1.3 When a sample is digested at a dilution factor of 100 (e.g. 0.5 g to 50 mL digestion and dilution), the amount of total dissolved solids is close to or less than 0.1% (1,000 mg/liter or 1,000 ppm), although the concentrations of K and Ca in digested sample solutions can be higher than 500 ppm respectively. The sample solution is directly analyzed by ICP-OES, but may be further diluted for ICP-MS.

2. Summary of method

- 2.1 Half a gram of dried sample (or equivalent) and five mL of concentrated nitric acid are added to a 50-mL Folin digestion tube. The mixture is heated to 120-130 °C for 14-

16 hours and is then treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is analyzed by ICP-OES for major and minor components, and further 1:1 diluted and analyzed by ICP-MS for minor and trace components.

- 2.2 Alternatively, samples like cheese or manure may be directly digested without having been dried. The results are either reported “as is” or reported based on dry weight after the water contents are obtained from sub-samples.
- 2.3 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

- 3.1 All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.
- 3.2 The use of perchloric acid for a sample digestion must be conducted in a hood designed specifically for perchloric acid. The user must be aware of the dangers involved using perchloric acid, such as the explosive nature of anhydrous perchloric acid and its extreme corrosive nature.

4. Interference

- 4.1 This method covers the analysis of over 30 elements in different kinds of samples by ICP-OES and ICP-MS. A

general discussion of interference is lengthy but not necessarily relevant to a specific element, which is especially true if the sample matrix is not specifically defined. An enormous amount of literature is available to the analysis of metals and non-metals by ICP-OES and by ICP-MS. Reading the published articles is recommended.

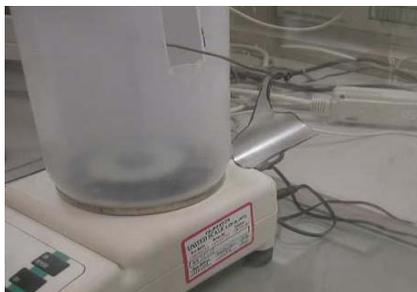
- 4.2 In this method, the solution contains less than 1,000 ppm of dissolved solids for ICP-OES and ICP-MS analysis. The major components are K, Mg, Ca, P, S and Na. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences are not expected, although some specific elements and or isotopes may be interfered.

5. Sample Collection, Preservation and Handling

A representative sample of plant tissue is dried and ground. A 5-gram vial or equivalent is used to hold a sub sample in airtight storage. Wet samples may be kept frozen.

6. Apparatus and Device

- 6.1 Analytical balance (accurate to one milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate. Model: HP 53014C).



6.4 Ten universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

6.5 ICP-OES: TJA Iris Advantage ICP-OES.

6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat # 2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”

6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).

6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes. Cat # 14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water. The tubes are air-dried before use.

7. Reagents

7.1 Concentrated nitric acid (e.g. TraceMetal grade. Fisher A509-212).

7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).

8. Pre-Digestion

8.1 Dry samples at 60 °C for two days. Grind in a stainless steel Wiley mill. Store in a 5-gram vial or equivalent for airtight storage. Process other wet samples “as is” without drying under certain limitations. The water content is obtained from a sub-sample.

Note: Samples may be dried at 60 °C or at 110 °C. The water contents could be different.

8.2 Weigh 0.50 ± 0.01 g of dry sample, or 1.0 ± 0.02 g of wet sample, or 5 mL of liquid sample (unknown samples, in-

house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried Folin digestion tubes. Make one to three digestion blanks.

Note: Depending on sample availability, the sample size can be scaled down. The relative significance of “contamination” from lab-wares and from reagents may increase if the sample size is very “small.” TEFLON digestion tubes may be used instead of glass digestion tubes.

8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an internal reference standard (IRS) for the analysis by ICP-OES. Spike 0.04 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.

8.4 Add 5 mL of concentrated nitric acid. Soak the samples at room temperature for 2-3 hours.

Note: Perchloric acid may be used in some special applications but should be avoided as much as possible for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and several other isotopes by using quadrupole ICP-MS.

9. Hot Plate Digestion

9.1 Place all of the Folin tubes in the block heater. Cover the tubes with plastic film to retard the water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this

could cause higher digestion blank concentrations for several elements.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7).

9.3 The temperature ramps up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove the film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the H₂O₂ is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add H₂O₂ (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Nominal dilution factor = 100. Y = 8 ppm. Rh = 8 ppb.

10. Measurement by ICP-OES

10.1 Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Transfer sample solutions from 50-mL tubes to 8-mL tubes.

10.1.3 For samples with extremely high analytes, the samples may be further diluted. Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube (2nd dilution. Nominal dilution factor = 200. Y = 4 ppm). Mix

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might be an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using upside down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the nominal dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2 Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3 Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Set 14-mL Falcon tubes in the ICP-MS autosampler racks. Transfer the sample solutions to the Falcon tubes.

11.1.2 Adjust the volume to 5 mL. Add 5 mL of 2% nitric acid. Mix well. The nominal dilution factor is 200 and the IRS is 4 ppb of Rh.

11.1.3 Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. If the concentrations of target elements are expected to be relatively high, the samples are further diluted, either by 2+8 dilution or 1+9 dilution. Otherwise, a sample solution may be directly analyzed without any further dilution (i.e. 10+0 dilution). During the data processing in later stage, the nominal dilution factor is always 200, whether the dilution is 1+9, 2+8, 5+5 or 10+0.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Edit the menu depending on specific samples or analytical requests.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 200, after this procedure is followed exactly, although the actual dilution could be variable as presented above in 11.1.3. Otherwise, adjust the DF accordingly.

Scenario one: 10 ppm (or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.04 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 8 ppb) is further diluted by 5:5 (2nd DF = 2) to contain 50 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 200). This 2nd solution is measured against a standard containing 10 ppb of X and 4 ppb of Rh and the measured result is 50 ppb. After applying the overall dilution factor of 200, the concentration of X in the solid material is $50 \text{ ppb} \times \text{DF } 200 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario two: Element X in the 2nd solution (X = 50 ppb and Rh = 4 ppb) is still much higher than the standard (X = 10 ppb and Rh = 4 ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 2 \times 5 = 1000$) to contain 10 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (10 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (10 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 50 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $50 \text{ ppb} \times 200 = 10 \text{ ppm}$. Option 2: At step 13.1, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 10 ppb against a standard of 10 ppb X with 4 ppb Rh. Now the total DF is 1000 and X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

12.1 It should be kept in mind that ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure.

12.2 Some QA/QC practices are presented in “Elemental analysis of solution samples with ICP-OES” and in “Elemental analysis of solution samples with ICP-MS.” In addition, an

in-house standard, alfalfa, is included with each batch of plant tissue samples. Digestion blank is measured and the values are kept in a database.

– End –

Nitrate-Nitrogen in Forage and Plant Tissues

1. Application

In this procedure nitrogen, in the form of the nitrate and nitrite ion, is extracted from forage or tissue samples and analyzed by flow injection technology.

2. Summary of Methods

A 2% solution of acetic acid is used to extract NO_3^- -N from the forage and tissue samples.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55°C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm Wiley mill.

6. Apparatus and Materials

- 6.1 Weighing paper
- 6.2 Erlenmeyer flasks (50ml)
- 6.3 Pipette bank (15ml)
- 6.4 Time-controlled, oscillating shaker
- 6.5 Filter paper, 9cm (Whatman No. 2 or equivalent)
- 6.6 Funnel tubes (15ml)
- 6.7 Glass test tubes (6.2ml)
- 6.8 Flow injection unit

7. Reagents

- 7.1 2% acetic acid solution (40 ml acetic acid, bring to 2L with distilled water)

8. Methods

- 8.1 Grind the dried sample through a 1 mm sieve.
- 8.2 Weigh 0.10 g of forage or plant tissue onto weighing paper.
- 8.3 Transfer sample to a 125 ml Erlenmeyer flask.
- 8.4 Add 25 ml 2% acetic acid solution using a constant suction pipette.
- 8.5 Shake for 15 minutes on oscillating shaker.
- 8.6 Filter immediately through filter paper atop funnel tubes.
- 8.7 Transfer filtrate into glass test tubes.
- 8.8 Analyze by flow injection technology.

9. Calculations

- 9.1 Sample concentration is calculated from a regression equation by plotting response versus standard concentration.

10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 10.2 Standard – One or more standards of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

11. Reporting

Results are reported as ppm of nitrogen (DM basis) in the form of nitrate NO_3^- -N.

12. References

- 12.1 Lachat Instruments. 1992. Nitrate/Nitrite, Nitrite in 2% Acetic Acid Plant Extracts. QuickChem Method 13-107-04-1-A.
- 12.2 Jaromir Ruzicka. 1983. Flow Injection Analysis – From Test Tube to Integrated Microconduits. Analytical Chemistry 55: 1040A-1053A.

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Plant Tissue Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of plant tissue samples and the analysis of major, minor and trace elements (total minerals, heavy metals and micro-nutrients) in these samples by ICP-OES (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometry) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus Inductively Coupled Plasma Mass Spectrometry).

- 1.1 Plant tissue samples (including some other types of samples such as cheese, manure, lichens, etc) mainly consist of carbon, hydrogen, oxygen and nitrogen. Other abundant components are K, Mg, Ca, P, S and Na. These six elements account for less than 10% of a sample on a dry weight basis.
- 1.2 An open-vessel acid digestion with $\text{HNO}_3 + \text{H}_2\text{O}_2$ (or $\text{HNO}_3 + \text{HClO}_4$ in some applications) in a hot plate could be incomplete for some samples. In other words, the digestion without HF acid is incomplete for some siliceous materials, although the sources of siliceous materials may be variable.

Elements closely associated with siliceous materials such as Al, Fe and Na may not be completely released from solid material to solution. Therefore, the results by ICP-OES or ICP-MS may not be directly comparable to the results by XRF methods or NAA methods. However, this problem might be significant only in limited cases.

- 1.3 When a sample is digested at a dilution factor of 100 (e.g. 0.5 g to 50 mL digestion and dilution), the amount of total dissolved solids is close to or less than 0.1% (1,000 mg/liter or 1,000 ppm), although the concentrations of K and Ca in digested sample solutions can be higher than 500 ppm respectively. The sample solution is directly analyzed by ICP-OES, but may be further diluted for ICP-MS.

2. Summary of method

- 2.1 Half a gram of dried sample (or equivalent) and five mL of concentrated nitric acid are added to a 50-mL Folin digestion tube. The mixture is heated to 120-130 °C for 14-

16 hours and is then treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is analyzed by ICP-OES for major and minor components, and further 1:1 diluted and analyzed by ICP-MS for minor and trace components.

- 2.2 Alternatively, samples like cheese or manure may be directly digested without having been dried. The results are either reported “as is” or reported based on dry weight after the water contents are obtained from sub-samples.
- 2.3 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

- 3.1 All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.
- 3.2 The use of perchloric acid for a sample digestion must be conducted in a hood designed specifically for perchloric acid. The user must be aware of the dangers involved using perchloric acid, such as the explosive nature of anhydrous perchloric acid and its extreme corrosive nature.

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- 4.1 This method covers the analysis of over 30 elements in different kinds of samples by ICP-OES and ICP-MS. A

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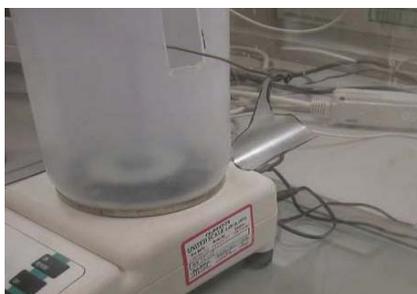
- 4.2 In this method, the solution contains less than 1,000 ppm of dissolved solids for ICP-OES and ICP-MS analysis. The major components are K, Mg, Ca, P, S and Na. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences are not expected, although some specific elements and or isotopes may be interfered.

5. Sample Collection, Preservation and Handling

A representative sample of plant tissue is dried and ground. A 5-gram vial or equivalent is used to hold a sub sample in airtight storage. Wet samples may be kept frozen.

6. Apparatus and Device

- 6.1 Analytical balance (accurate to one milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate. Model: HP 53014C).



6.4 Ten universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

6.5 ICP-OES: TJA Iris Advantage ICP-OES.

6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat # 2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”

6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).

6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes. Cat # 14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water. The tubes are air-dried before use.

7. Reagents

7.1 Concentrated nitric acid (e.g. TraceMetal grade. Fisher A509-212).

7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).

8. Pre-Digestion

8.1 Dry samples at 60 °C for two days. Grind in a stainless steel Wiley mill. Store in a 5-gram vial or equivalent for airtight storage. Process other wet samples “as is” without drying under certain limitations. The water content is obtained from a sub-sample.

Note: Samples may be dried at 60 °C or at 110 °C. The water contents could be different.

8.2 Weigh 0.50 ± 0.01 g of dry sample, or 1.0 ± 0.02 g of wet sample, or 5 mL of liquid sample (unknown samples, in-

house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried Folin digestion tubes. Make one to three digestion blanks.

Note: Depending on sample availability, the sample size can be scaled down. The relative significance of “contamination” from lab-wares and from reagents may increase if the sample size is very “small.” TEFLON digestion tubes may be used instead of glass digestion tubes.

8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an internal reference standard (IRS) for the analysis by ICP-OES. Spike 0.04 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.

8.4 Add 5 mL of concentrated nitric acid. Soak the samples at room temperature for 2-3 hours.

Note: Perchloric acid may be used in some special applications but should be avoided as much as possible for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and several other isotopes by using quadrupole ICP-MS.

9. Hot Plate Digestion

9.1 Place all of the Folin tubes in the block heater. Cover the tubes with plastic film to retard the water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this

could cause higher digestion blank concentrations for several elements.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7).

9.3 The temperature ramps up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove the film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the H₂O₂ is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add H₂O₂ (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Nominal dilution factor = 100. Y = 8 ppm. Rh = 8 ppb.

10. Measurement by ICP-OES

10.1 Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Transfer sample solutions from 50-mL tubes to 8-mL tubes.

10.1.3 For samples with extremely high analytes, the samples may be further diluted. Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube (2nd dilution. Nominal dilution factor = 200. Y = 4 ppm). Mix

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might be an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using upside down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the nominal dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2 Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3 Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Set 14-mL Falcon tubes in the ICP-MS autosampler racks. Transfer the sample solutions to the Falcon tubes.

11.1.2 Adjust the volume to 5 mL. Add 5 mL of 2% nitric acid. Mix well. The nominal dilution factor is 200 and the IRS is 4 ppb of Rh.

11.1.3 Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. If the concentrations of target elements are expected to be relatively high, the samples are further diluted, either by 2+8 dilution or 1+9 dilution. Otherwise, a sample solution may be directly analyzed without any further dilution (i.e. 10+0 dilution). During the data processing in later stage, the nominal dilution factor is always 200, whether the dilution is 1+9, 2+8, 5+5 or 10+0.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Edit the menu depending on specific samples or analytical requests.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 200, after this procedure is followed exactly, although the actual dilution could be variable as presented above in 11.1.3. Otherwise, adjust the DF accordingly.

Scenario one: 10 ppm (or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.04 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 8 ppb) is further diluted by 5:5 (2nd DF = 2) to contain 50 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 200). This 2nd solution is measured against a standard containing 10 ppb of X and 4 ppb of Rh and the measured result is 50 ppb. After applying the overall dilution factor of 200, the concentration of X in the solid material is $50 \text{ ppb} \times \text{DF } 200 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario two: Element X in the 2nd solution (X = 50 ppb and Rh = 4 ppb) is still much higher than the standard (X = 10 ppb and Rh = 4 ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 2 \times 5 = 1000$) to contain 10 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (10 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (10 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 50 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $50 \text{ ppb} \times 200 = 10 \text{ ppm}$. Option 2: At step 13.1, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 10 ppb against a standard of 10 ppb X with 4 ppb Rh. Now the total DF is 1000 and X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

12.1 It should be kept in mind that ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure.

12.2 Some QA/QC practices are presented in “Elemental analysis of solution samples with ICP-OES” and in “Elemental analysis of solution samples with ICP-MS.” In addition, an

in-house standard, alfalfa, is included with each batch of plant tissue samples. Digestion blank is measured and the values are kept in a database.

– End –



Forage

Applications: Livestock feeding program analysis.

Price: See [submission form](#).

Forms & Instructions: [Submission Form](#) | [Sampling Hay and Silage \(A2309\)](#) | [TMR Sampling Instructions](#)

Why test?

Reliable feed and forage analysis can improve your livestock feeding program. Determining the feed value of forage allows you to balance rations more accurately based on nutritive value.

Sampling

The key to successful forage analysis is taking a good, representative forage sample on the farm. Laboratory analysis can determine the quality of a submitted forage sample, but this will not help you balance rations if the submitted sample does not represent forage actually being fed to your animals. The largest error in forage analysis is improper sampling methods on the farm. You need to take a representative sample of forage from every hay or silage lot. These samples will reflect the variation in forage quality that occurs across your fields and during harvesting.

The following sampling procedures are recommended:

- Submit samples of at least 1/2 pound of material. Haylage or silage samples can be sealed in plastic bags and stored in a freezer until time of delivery to the lab is convenient. Do not mail frozen samples late in the week or just before holidays to avoid having them delayed at the USPS or UPS transfer locations.
- Samples from baled hay must be taken with a core sampler to be meaningful. A minimum of 12 cores of hay should be taken from the ends of random bales or at random areas in the mow.
- Samples of cob corn should be ground in order to obtain a good representative sample.
- Samples of chopped forages can best be collected when filling the silo. Take three to five handfuls at random from several loads about midway through the harvest of each field that differs from the next field.
- Good representative samples from stored silage are difficult to obtain. A fairly representative sample may be obtained by digging down two or three feet in several places and taking a handful of silage from each location. If the silage stored was fairly uniform and is being fed, collect several handfuls from the morning and evening feedings for the sample.

Analysis and Reporting

It is our mission to teach and demonstrate the latest research base in forage testing. Research based feed and forage analysis and reporting systems means we make every attempt to provide the dairy and livestock industry with the “real facts” concerning feed and forage testing. We use summative technology developed by researchers to more precisely predict feed energy contents and offer a UW-Recommended evaluation system which we believe is the best research based system to use. We do not microwave dry our samples prior to analytical evaluation because research has demonstrated the feed and forage protein fractions can be altered from the intense heat associated with microwave drying.

We promote wet chemistry mineral analytical systems because research suggest NIRS may not offer enough precision in today’s nutrient management programs. We promote and use NIRS technology where its utility has been fully researched. Finally, our Feed & Forage Testing Reports are designed to be educational by fully listing proper nutritional terms, abbreviations, units and the analytical methods used to determine them.

View a sample [Legume/Grass Silage report](#) or [sample UW Grain Evaluation report](#).

Additional References & Resources

- [Guide to Understanding Forage Tests](#)

- [Focus on Forage: Sampling and Evaluating Total Mixed Rations](#)
- [Wisconsin Forage Database](#)
- [White Paper from the NIRS Forage and Feed Testing Consortium](#)

Standard Operation Procedure

Elemental Analysis of Solution Samples with Inductively Coupled Plasma Optical Emission Spectrometry

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University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005, revised December 2005

1. Application

This method covers the analysis of major and minor elements in solution samples by ICP-OES (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometry).

2. Summary of method

2.1 Principle: *An aqueous sample* is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone (8,000–10,000°C). The analytes are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These emissions are separated based on their respective wavelengths and their intensities are measured (spectrometry). The intensities are proportional to the concentrations of analytes in the aqueous sample. The quantification is *an external multi-point linear standardization* by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are

prepared from single- and multi-element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant (such as the concentration of ferrous iron or ferric iron), only *total elemental concentration* is analyzed by ICP-OES.

2.2 Brief procedure: Five working standard solutions are prepared from independent primary standard solutions. The newly prepared working standard solutions are confirmed against old working standard solutions and against other independent primary standard solutions. In daily operation, the ICP-OES instrument is started, brought to operation conditions and let stabilized. The sample introduction system is checked and the wavelengths are tuned. The instrument is standardized with the five working standard solutions (multi-point linear fitting). Samples are measured with standardization blanks, other kinds of blanks, drift control samples, and quality control samples. After a batch of samples are measured, the data are downloaded to an Excel spreadsheet. The data are corrected in terms of standardization blanks, other relevant

blanks, drift correction, and dilution factor application. The results are normalized to the internal reference standard if an internal reference standard is used. For several elements, a method of “wavelength switch” is used for analytes at different concentration ranges or in different matrixes

3. Safety

All relevant laboratory safety procedures are followed.

4. Interference

This method covers the analysis of over 20 elements in different kinds of samples by ICP-OES. A general discussion of interference is lengthy but not necessarily relevant to a specific element, which is especially true if the sample matrix is not specifically defined. Reading the published articles is recommended. An enormous amount of literature is available for the analysis of metals and non-metals by ICP-OES.

5. Sample collection and preservation

Containers (bottles, vials, etc) typically are soaked in 10% nitric acid overnight and rinsed with de-ionized water for several times before use. Solution samples typically are acidified with nitric acid at a ratio of 1–5 mL of concentrated nitric acid to one liter of sample. Extra cautions need to be exercised in preventing contamination and preserving samples for some specific analyses.

6. Apparatus and device

6.1 ICP-OES: Thermo Jarrell Ash IRIS Advantage Inductively Coupled

Plasma Optical Emission Spectrometry.

6.2 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat #2110 by Perfezor Scientific) for the ICP-OES auto-sampler are used “as is.”

7. Reagents

7.1 Concentrated nitric acid (68–71% w/w. e.g. TraceMetal grade. Cat # A509-212 by Fisher Scientific).

7.2 CCV-1 multi-element primary standard set (CPI International).

7.3 ICV-2 multi-element primary standard set (SPEX).

7.4 Single-element primary standard solutions (SPEX).

8. Measurement by ICP-OES

8.1 ICP-OES working standard

8.1.1 OES_1 is a solution of 3–5% (v/v) nitric acid prepared from the concentrated nitric acid, serving as a calibration blank.

8.1.2 OES_2 is made by diluting 5 mL of Solution-A in CCV-1 primary standard to 500 mL with 3–5% nitric acid. In addition, sulfur (S) and titanium (Ti) from single-element primary standards are added, since CCV-1 does not contain these elements. OES_2 contains 27 elements, typically at 2 ppm (ppm = $\mu\text{g/mL}$ = mg/L).

8.1.3 OES_3 is made by diluting 5 mL of ICV-2 primary standard to 500 mL with 3–5% nitric acid. Phosphorus (P), sulfur (S), titanium (Ti) and antimony (Sb) from single-element primary standards are added. OES_3 contains 25 elements ranging from 1–20 ppm.

8.1.4 OES_4 contains 19 elements, typically at 100 ppm. This is made by diluting

single-element primary standards in a 500 mL volumetric flask with 3–5% nitric acid.

8.1.5 OES_5 contains 9 elements, particularly for elements at high concentration ranges. This is made by diluting single-element primary standards in a 500 mL volumetric flask with 3–5% nitric acid.

8.1.6 The details of the standards are listed in [Table 1: Multi-element working standards for ICP-OES](#).

Note: The analysis by ICP-OES is flexible and is easily expanded to other elements. These working standards may not cover the concentration ranges of several elements in samples. Therefore, these working standards may be augmented with some additional standard solutions.

8.2 Preparing sample solutions

8.2.1 Transfer “routine” samples to 8-mL polystyrene test tubes directly.

8.2.2 Prepare “none-routine” samples in some other methods, depending on the requested analyses, sample matrix, analyte concentrations, etc. For example, low-volume or “over-concentrated” samples are diluted before analysis. Turbid samples are left to stand overnight so that particles settle down to the bottom, or the samples are centrifugated so that particles are separated from the samples.

8.2.3 Yttrium (Y) may be used as an internal reference standard. After a given amount of sample (weight or volume) is spiked with a given amount of yttrium, the concentration ratio of (analyte/ytterium) is later used for quantification. Any further dilution

does not change the concentration ratio (see [Appendix 1: Internal reference standard](#)).

8.3 ICP-OES measurement

The detailed list of start and shut-down steps is given in [Appendix 2: Standard start and shut down operation procedure for Jarrell Ash IRIS Advantage ICP-OES](#). Other instrument conditions are listed in [Table 2: ICP-OES instrument conditions](#).

9. Data processing after ICP-OES analysis

9.1 After the instrument is standardized, the software generates concentration values already. However, these values are further processed.

9.2 Download all of the concentration data into an in-house Microsoft Excel spreadsheet “SPAL” program.

9.3 The results of all QC water measurements are arranged together. Based on these results, the drift (with time) per element is calculated. This drift is corrected for each sample, assuming the drift is linear within two bracketing QC water measurements.

9.4 If applicable, the results are corrected based on an internal reference standard (IRS). Yttrium is used as an IRS.

9.5 The results of the measurement blanks are averaged. This averaged blank is subtracted from all of the other samples.

9.6 If relevant, use the digestion blank(s) to correct the digest blank for digested samples, or use the appropriate blank(s) for some other kind of blank correction.

9.7 Check the analyte results against their respective detection limits.

- 9.8 Apply dilution factors if appropriate.
- 9.9 Generate out-going reports.

10. Quality assurance (QA) and quality control (QC)

An ICP-OES instrument is used for broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure. Presented here are some basic operations. The details are presented in [Appendix 3: Strategies and implementation of quality assurance \(QA\) and quality control \(QC\) in the elemental analysis of solution samples with inductively coupled plasma optical emission spectrometry](#)

- 10.1 The in-house ICP-OES working standards are made from primary standards of several independent sources. These working solutions are confirmed by using some other independent primary standards. A new set of working standards are checked and confirmed against a previous set of working standards. Where applicable, the working standard solutions are confirmed by using ICP-MS.
- 10.2 In-house quality control waters (msQC water at ppb level and QC water at ppm level) are analyzed each time after the instrument is standardized. The results of these measurements are confirmed against the expected values. The expected values are compiled from the side-by-side analysis of this msQC water with NIST 1643d water, the historical results of this msQC water by the ICP-OES and ICP-MS analysis, and the historical results of

this msQC water and QC water by the ICP-OES analysis.

- 10.3 The result of the QC water serves as a primary checking point against events such as clogged nebulizer, power abruption, low-argon gas supply, auto-sampler failure, etc. The samples after these events are re-analyzed.
- 10.4 Samples are diluted to different ratios and measured. The results are used to evaluate matrix effects and (calibration) dynamic ranges.
- 10.5 Samples are analyzed by using the calibration of internal standard addition.
- 10.6 Identical samples are analyzed multiple times within one-day's acquisition sequence and are analyzed in different-day's acquisitions. The results are used to evaluate the repeatability of the analysis.
- 10.7 The ICP-OES analytical results are confirmed by the analysis of ICP-MS.
- 10.8 Some basic performance or data are listed in [Table 3: The analysis by ICP-OES](#). The RSD (relative standard deviation) is generally better than 5% if an analyte concentration is about 10 times higher than the relevant limit of detection.
- 10.9 It should be reminded that the time in setting/evaluating the QA/QC criteria is well spent only when the sample matrix is defined, the instrument and its condition are defined, and the target element and concentration range are defined.

– End –

Table 1: Multi-element working standards for ICP-OES

Primary standards			Element	Working standards				
CPI	SPEX	SPEX		OES_1	OES_2	OES_3	OES_4	OES_5
CCV-1	ICV-2	single-element			from CCV1	from ICV2		
ppm	ppm	ppm		ppm	ppm	ppm	ppm	ppm
	100	1000	Ag	0		1	2	
200	1000	10000	Al	0	2	10	100	
200	500		As	0	2	5		
200		1000	B	0	2		10	
100	1000		Ba	0	1	10		
100	100		Be	0	1	1		
200		1000	Bi	0	1 (note)		2	
200	2000	10000	Ca	0	2	20	100	400
100	100		Cd	0	1	1		
100	500		Co	0	1	5		
50	200		Cr	0	0.5	2		
200	200	1000	Cu	0	2	2	20	
200	1000	10000	Fe	0	2	10	100	
500	2000	10000	K	0	5	20	100	500
200		1000	Li	0	2		10	
200	2000	10000	Mg	0	2	20	100	300
100	100	10000	Mn	0	1	1	4	40
200		1000	Mo	0	1 (note)		10	
	2000	10000	Na	0		20	100	
200	500		Ni	0	2	5		
500		10000	P	0	5	20	100	400
200	500		Pb	0	2	5		
		10000	S	0	2	20	100	400
		1000	Sb	0		2	5	
200	500		Se	0	2	5		
		1000	Si	0			5	10
200		1000	Sr	0	2			4
		1000	Ti	0	2	5		
200	500		Tl	0	2	5		
100	500		V	0	1	5		
		1000	Y	0			5	10
100	100	1000	Zn	0	1	1	20	

STD_1: zero concentration. Calibration blank.

STD_2: 5 mL of CPI CCV-1 diluted to 500 mL. Sulfur and titanium were spiked.

STD_3: 5 mL of SPEX ICV-2 diluted to 500 mL. Antimony, phosphorus, sulfur, and titanium were spiked.

STD_4, and STD_5: from 1,000 or 10,000 ppm single element primary standards.

Note: Mo and Bi in CPI is 100 ppm (not 200 ppm as claimed).

Table 2: ICP-OES Instrument Conditions

ICP-OES	TJA IRIS Advantage ICP-OES
Plasma forward power Plasma height	1150 W Yttrium bullet halfway between coil top and torch top
Coolant gas flow rate Auxiliary gas flow rate Nebulizer Nebulizer pressure	Instrument default Low Glass Expansion Sea Spray 26 psi
Spray chamber Pump rate Tubing Sample uptake rate Sample uptake time Acquisition Wash solution Wash time	Glass Expansion Tracey Cyclonic 100 rpm Orange-orange 1.2 mL/min 30 s 30 s low wave length range, 10 s high wave length range. Twice De-ionized water or very diluted nitric acid (< 1% v/v) 15 s

Element			Background		Tune	Element			Background		Tune
	Wavelength	Order	Left	Right	ppm		Wavelength	Order	Left	Right	ppm
Ag	reserved for future use					Mn	257.61	130	3		5
Al	308.22	109	3	14	50	Mn	293.93	114	1	15	
As	189.04	177	1		30	Mo	202.03	166	4		15
B	249.68	135	1		10	Na	589.59	57	3	14	5
B	249.77	134	3			Ni	231.60	145		15	10
Ba	455.40	74	1	15	4	Ni	232.00	145		12	
Be	reserved for future use					P	177.50	188	3		30
Bi	reserved for future use					Pb	220.35	152	1	15	20
Ca	183.80	182	1	15	40	S	182.03	184	4		50
Ca	184.01	182	1	15		Sb	reserved for future use				
Cd	228.80	147		13	10	Se	196.09	171	4	15	40
Co	228.62	147		15	10	Si	251.61	134	4	13	5
Cr	267.72	126	4	13	10	Sr	407.77	82	1	15	2
Cu	324.75	103	4		10	Ti	334.94	100	3		5
Fe	238.20	141	4	15	30	Tl	reserved for future use				
Fe	271.44	124	1			V	310.23	108	2		4
K	766.49	44	1	15	40	Y	319.56	105	3	13	10
Li	670.78	50	2	13	2	Zn	213.86	157	3		10
Mg	285.21	117	4		10						
Mg	285.21	118	2	14							

Table 3: The Analysis by ICP-OES

	LOD	2% nitric	Bovine serum			Human urine			Soft water			Hard water		
	ppm		ppm	sd	rsd	ppm	sd	rsd	ppm	sd	rsd	ppm	sd	rsd
Al	0.05	<LOD	<1.8			<0.5	0.2		<LD	0.03		<LD	0.01	
As	0.03	<LOD	<0.9			<0.3	0.1		<LD	0.005		<LD	0.012	
B	0.03		(for high Fe samples such as soil samples)											
B	0.002	<LOD	0.16	0.04	22	2.06	0.01	0.3	0.007	0.001	12	0.005	0.002	53
Ba	0.0002	<LOD	0.120	0.001	1	<0.002	0.001		<LD	0.000		0.014	0.000	1.9
Ca	0.01	<LOD	97	2	2	184	3	1	0.12	0.08	71	66	0.2	0.3
Cd	0.004	<LOD	<0.12			<0.04	0.01		<LD	0.001		<LD	0.014	
Co	0.003	<LOD	<0.09			<0.03	0.01		0.003	0.001	45	<LD	0.001	
Cr	0.001	<LOD	<0.06			<0.01	0.01		<LD	0.000		<LD	0.001	
Cu	0.005	<LOD	0.90	0.04	5	0.07	0.01	16	0.030	0.004	14	0.028	0.002	5
Fe	0.001	<LOD	2.02	0.06	3	0.02	0.01	33	0.041	0.002	6	0.136	0.003	2
K	0.03	<LOD	186	3	2	1918	3	0.2	<LD	0.04		0.94	0.04	4
Li	0.0005	<LOD	<0.03			0.011	0.002	14	0.002	0.000	9	0.002	0.000	17
Mg	0.007	<LOD	20.8	0.4	2	107	0	0.3	1.91	0.02	0.9	35.7	0.1	0.3
Mn	0.0003	<LOD	<0.03			<0.003	0.001		0.003	0.000	5	0.009	0.001	9
Mo	0.005	<LOD	0.34	0.05	14	0.18	0.27	151	<LD	0.002		<LD	0.005	
Na	0.006	<LOD	3026	48	2	2651	4	0.2	133	1	0.5	2.29	0.04	1.6
Ni	0.004	<LOD	<0.09			<0.03	0.02		<LD	0.004		<LD	0.002	
Ni	0.02		(for high Fe samples such as soil samples)											
P	0.05	<LOD	125	2	2	790	6	0.7	<LD	0.03		<LD	0.02	
Pb	0.02	<LOD	<0.6			<0.2	0.1		<LD	0.004		<LD	0.004	
S	0.05	<LOD	779	11	1	653	3	0.5	6.05	0.06	1	5.87	0.09	2
Se	0.04	<LOD	<1.2			2	3	160	0.04	0.04	99	<LD	0.010	
Si	0.007	<LOD	1.39	0.20	14	13.9	0.0	0.2	6.95	0.02	0.3	7.16	0.01	0.2
Sr	0.0001	<LOD	0.09	0.00	2	0.212	0.000	0.2	<LD	0.000		0.056	0.000	0.5
Ti	0.002	<LOD	<0.06			0.015	0.003	23	<LD	0.001		<LD	0.000	
V	0.004	<LOD	<0.12			<0.03	0.02		<LD	0.002		<LD	0.001	
Y	0.01	<LOD				<0.08	0.02		0.023	0.004	16	<LD	0.004	
Zn	0.001	<LOD	1.12	0.08	7	0.61	0.25	41	0.01	0.02	239	0.002	0.001	58

Note

LOD: Limit of the detection = 3 times of the standard deviation of the repeated analysis of 1% nitric acid. The samples were measured and the results were averaged (n = 6).
 sd (ppm) = 1 sd. rsd (%) = 100 X (sd/average).

The rsd was greater than 10% when the measured concentrations were close to the detection limits.

2% nitric: 2% (v/v) nitric acid.

Bovine serum: diluted by 30 times. The dilution factor is applied already.

Human urine: diluted by 10 times. The dilution factor is applied already.

Soft water: Madison City tap water after residential water softener.

Hard water: Madison City tap water.

Appendix 1: Internal Reference Standard

1. Internal reference standard (IRS)

An instrumental analysis is a process of comparing an unknown sample's signal with a standard's signal. If the signal of Cu in a milk sample is 100 and the signal of 10 ppm Cu in a 1% nitric acid is 100, the milk sample contains 10 ppm of Cu. Because of matrix effects (sample viscosity, presence of other inorganic or organic material, etc.), these two signals are usually different from each other. If both samples are spiked with equal amounts of yttrium (Y) as the internal reference standard (IRS), the signal ratios of Cu/Y of both samples are less affected by matrix effects. The reliable comparison between an unknown sample and a standard is greatly improved.

Using an internal standard is a common practice in ICP-OES and in ICP-MS applications. An internal standard is used to correct between sample variations – such as the differences in sample matrix, as mentioned above. It is used to correct short-term and long-term instrument drift with time caused by plasma fluctuation and other instrumental fluctuations. An important advantage is to correct the volume inaccuracy during sample preparation, as given below in section 4: A practical use of internal reference standard – correction for volume inaccuracy.

2. Disadvantages of IRS technique

Any signal is measured with some degree of uncertainty. In direct signal comparison, it is the process of comparing an unknown's $(\text{signal} \pm \text{uncertainty})$ with the standard's $(\text{signal} \pm \text{uncertainty})$. In the IRS technique, it is the process of comparing the ratio of $(\text{signal} \pm \text{uncertainty})/(\text{signal} \pm \text{uncertainty})$ of

an unknown sample with the ratio of $(\text{signal} \pm \text{uncertainty})/(\text{signal} \pm \text{uncertainty})$ of the standard. In principle, a larger margin of error is introduced by using the IRS technique since more uncertainties are involved in the calculation. In practice, this problem is less important with respect to the benefit of correcting matrix effect and of correcting instrument drift by using IRS.

3. Precautions in using IRS

There are precautions to take when selecting an internal standard. Its original or natural amount in a sample should be so low that it can be ignored with respect to the spiked amount (e.g. original amount <1% of spiked amount). If 0.04 mL of 10,000 ppm of yttrium is spiked to 0.5 gram of sample, and is subsequently diluted to 50 mL, this is equivalent to 800 ppm of yttrium in solid sample ($0.04 \times 10000/0.5 = 800$ ppm) or 8 ppm in solution. Plant tissue samples and soil samples contain less than 10 mg/kg of yttrium. Therefore, the natural yttrium in a plant sample or a soil sample is insignificant with respect to the spiked yttrium.

An internal standard should also be so selected that it does not interfere with targeted elements. This has to be confirmed for each element at the given concentrations of the IRS (e.g. 8 ppm of yttrium in a solution) and at the expected concentrations of other elements.

The fundamental requirement in using IRS is that the signals of a target element and the IRS element (e.g. yttrium) are enhanced or suppressed, equally, so that the signal ratios (of target element/reference element) are always the same no matter how other conditions change. In a multi-element method, this is usually true but not always. The worst scenario is that a target element

and the reference element behave in opposite directions. For example, from 1% nitric acid to milk, the signal of element X is enhanced by 10% but the signal of reference R is suppressed by 10%. Without the IRS technique, the error of X might be +10%. With the IRS technique, the error could be +20%. In this case, an IRS should not be used at all. Therefore, a method with IRS could be more deceiving than a plain method without IRS.

Similarly, instrument drift (with time) is usually element-specific. The drift correction based on an IRS alone could also be deceiving, as pointed out above, if a target element and the reference element drift in opposite directions. Alternatively, the drift (with time) might be corrected by inserting a drift control sample containing all of the target elements between unknown samples.

4. A practical use of internal reference standard – correction for volume inaccuracy

After a sample is spiked with an IRS (e.g. 0.04 mL of 10,000 ppm of yttrium to 0.5 gram of sample), the concentration ratios of target elements to the reference element are fixed, no matter whether the sample is later diluted by 10 times or by 100 times. Since the concentration ratios (actually the signal ratios) are used in later data processing, the volume accuracy of a container is irrelevant in the subsequent dilutions and a sample could be freely diluted.

This “free dilution” without strict volume accuracy requirement has several practical advantages. Some QA/QC protocols require the use of Class A volumetric flasks (although in many cases of environmental or agricultural analysis, rarely that is required).

Plastic lab-wares are preferred in metal analysis, especially in trace analysis or when hydrofluoric acid is used. Class A glass volumetric flasks are generally available but Class A plastic volumetric flasks are scarce. Another advantage is that an analyst could make the subsequent dilutions quickly and easily without introducing any unintentional “volumetric” error. And lastly, if one target element in a particular sample is over a standardization range, that sample is “freely” diluted and re-analyzed for that element without paying too much attention to volume accuracy or to dilution factor. For example, given a solution of 150 ppm of zinc and 8 ppm of yttrium, the zinc concentration is over a standardization range of 40 ppm. This solution is easily diluted by 10 times to contain 15 ppm of zinc and 0.8 ppm of yttrium. The calculation, since it is based on concentration ratio (actually signal ratio), treats this sample as if the sample were not diluted.

As one may deduce from the above example, a sample is diluted “freely” in theory but “limited” in practice. In other words, a concentration ratio is always the same no matter how much a sample is diluted, but a signal ratio is not and depends on the overall sample matrix and instrument response. Therefore, all of the samples should be diluted to the same extent as much as possible so that a signal ratio is still proportional to a concentration ratio consistently for all of the samples and standards.

– End –

Appendix 2: Standard Start and Shut down Operation Procedure for Jarrell Ash IRIS Advantage ICP-OES

1 Per first day of a month

- 1.1 Replace all peristaltic pump tubings.
- 1.2 Refill water to the argon gas humidifier.
- 1.3 Refill the washing water.

2 Daily start procedure

- 2.1 Check the liquid nitrogen and the liquid argon. Order if needed.
- 2.2 Check the CID temperature (-90°C).
- 2.3 Confirm that the exhaust fan is on.
- 2.4 Lock peristaltic pump platens down.
- 2.5 Drain the spray chamber if needed.
- 2.6 Ignite the plasma. Confirm that the waste is being pumped out.
- 2.7 If the plasma is not lit, most likely either there is a leak of air into the sample introduction system, or the purge was insufficient. Find the leak or increase the purge time. Try one or two more times. If the plasma is still not lit, find the cause and fix it. Options are: hard reset, reboot the computer, and reboot the instrument and the computer.
- 2.8 Warm up for at least 30 minutes.
- 2.9 Per first day of a month: Record the operation power level (LCD on the right panel).
- 2.10 Per first day of a month: select the mercury lamp as the source, acquire at several wavelengths, and record the intensities.

3 Daily operation procedure

- 3.1 Aspire a 1,000 ppm yttrium solution to the system, check and confirm that the yttrium bullet is half way between the

coil top and the torch top. The bullet position is adjusted by setting the nebulizer pressure. If the pressure is significantly out of “normal” range, find the cause and fix it.

- 3.2 Use an in-house tune solution (containing 1–50 ppm of multi-elements), carry out “Auto peak adjustment.” Still use the same tune solution and measure as one sample. Check the analyte peaks of this measurement with the peaks of previous measurements. The peaks may shift left or right, but the peak height should be about the same for the identical tune solution. When the method is switched to a different one, either carry out auto peak adjustment or run one sample to confirm the peak position.
- 3.3 Aspire 10–30% nitric acid for one to two minutes to clean the system. Set up the ICP-OES working standards, measure, and carry out the standardization. Check and confirm the standardization result by checking the slopes, intercepts, and correlation coefficients. If the slopes deviate from “normal” values significantly, find the cause and fix it.
- 3.4 Measure a 3–5% nitric acid solution for two times as the measurement blanks. Measure one msQC water (quality control water at low concentration – ppb level). Measure one QC water (quality control water at high concentration – ppm level). Check these results against established results.
- 3.5 Confirm the autosampler’s alignment before using it. Set the sample rack(s) into place. Start the whole acquisition sequence.
- 3.6 The QC water (at ppm level) is measured after every 20 samples.

4 Daily shutdown procedure

- 4.1 Thoroughly rinse the sample introduction system by aspirating de-ionized water for 5–10 minutes.
- 4.2 Shutdown the instrument (i.e. extinguish the plasma and shut down the RF generator).
- 4.3 Release the peristaltic pump.

– End –

Appendix 3: Strategies and Implementation of Quality Assurance (QA) and Quality Control (QC) in the Elemental Analysis of Solution Samples with Inductively Coupled Plasma Optical Emission Spectrometry

1. Introduction

There are different ways in developing an analytical method and implement QA/QC strategies in using an instrument with multi-element capability such as an ICP-OES. This appendix, in loosely-connected sections, provides the relevant information and rationale for the development of “Elemental analysis of solution samples with ICP-OES.”

2. Selection of elements

A multi-element method ideally includes as many elements as possible. However, neither is this necessary nor practical in reality. Two main factors are considered in selecting or deselecting elements – requested analysis and instrument capability.

- 2.1 Primary elements – Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, and Zn (21 elements). These are frequently requested elements for the agricultural, environmental, biomedical, and other applications.
- 2.2 Secondary elements – Ag, Ba, Be, Bi, Sb, Si, Sr, Ti, Tl, V, and Y (11 elements). These elements are requested less frequently for analysis. However, the instrument potentially provides good performance in terms of detection limit, linear range, and stability for the following elements: Ag, Ba, Be, Si, Sr, Ti, V, and Y. These

elements are readily available in working standard solutions (see section Working standards): Ag, Ba, Be, Bi, Sr, Tl, and V. The inclusion of these secondary elements does not prolong the analysis time of the primary elements, since the TJA IRIS ICP-OES runs simultaneously on all elements.

- 2.3 Other elements are deselected. Some elements do not have sensitive wavelengths (e.g. Cs), do not have less-interfered wavelengths (e.g. rare earth elements), are never requested for analysis (e.g. Ru, Sc, and Te), are not readily available in standard solutions (e.g. Rb, Sn, and Zr), and/or are not compatible with other elements (e.g. iodine). The SPAL’s ICP-MS instrument (VG PQ2T Plus ICP-MS) provides a much better analysis for most of these elements in terms of detection limit and interference reduction (e.g. Cs, Ge, and U) than the analysis by ICP-OES.
- 2.4 Selected elements in alphabetical order – Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sr, Ti, Tl, V, Y, and Zn (32 elements).
- 2.5 Selected elements in mass order – Li, Be, B, Na, Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Y, Mo, Ag, Cd, Sb, Ba, Tl, Pb, and Bi.

3. Working standards

There are several potential ways in making multi-element working standard solutions by using standards from single-element primary standards, multi-element primary standards, custom-style primary standards, and/or a combination of single-element and multi-element primary standards. The criteria are easy, error-proof and economic.

In making 32-element working standards from single-element primary standards, an analyst needs to transfer different amounts of standards from approximately 40 bottles of standard solutions (some primary standards at 1,000 ppm and some at 10,000 ppm) to 4–6 volumetric flasks. This process is very time consuming and is prone to mistakes. In addition, it is difficult to control and/or trace the quality of 40 bottles of primary standards. The primary standards are expensive and yet only a tiny portion of these standards may be used. Often, a good portion of the standards still remains long after their expiration dates.

Multi-element primary standards are available commercially. However, these solutions usually do not have the desired element combinations and/or the desired concentration ranges for a specific analysis. For example, digested plant solutions contain high concentrations of P, K, Ca, Mg and S (> 100 ppm), but low concentrations of B, Cu and Zn (< 1 ppm).

Custom-designed primary standards are available commercially. Desired combination of elements and desired concentration ranges are specified by customers. However, the cost of these standards is simply too high.

A practical, economical, and reliable approach is to make low-concentration working standards from multi-element primary standards (covering about 30 elements) and to make high-concentration working standards from single-element primary standards (about 10 elements). Two multi-element primary standards are purchased from two different sources: Solution-A in the set of CCV-1 from CPI International and ICV-2 from SPEX. Other single-element primary standards are

purchased from SPEX. From these primary standard solutions, five working standard solutions are made: OES_1, OES_2, OES_3, OES_4, and OES_5.

The primary standards Solution-A in CCV-1 (for OES_2) and ICV-2 (for OES_3) are from different sources and, therefore, are expected to be independent. The two working standards (OES_2 and OES_3) are prepared with a very minimum operation (i.e. very low chance to introduce operational errors). Therefore, these two standards are planned to be used to confirm each other's claimed concentrations.

4. Calibration strategy choice – 1: Single point (actually one blank point and one high concentration point) linear fitting or multi-point linear fitting

A calibration curve, in most cases, is the shape of an over-stretched “S” – from very low concentrations to very high concentrations. An instrument response is generally considered as being linear from zero to a certain point of concentration and non-linear from that point on. Linear or non-linear is relative. There is no sudden jump from a linear response to a non-linear response. A response viewed by one analyst as linear may be viewed as non-linear by another analyst. The linear response of ICP-OES is generally accepted to be over 5 orders of magnitude from sub-ppb to ppm levels.

In principle, one point (i.e. one zero and one high) linear fitting is no worse than multi-point linear fittings in the linear range. Two points define a line. Several points also define a line and do not necessarily define a “superior” line. In reality, operational errors and instrumental errors, whether systematic or random, are unavoidable. A multi-point

linear fitting would catch the majority of these errors. From this standpoint, a multi-point linear fitting is superior to a single-point linear fitting. This concept is implemented in making the working standards for the primary elements. For example, the linear fitting of cadmium (Cd) contains 3 points: one blank point (OES_1) and two 1-ppm points (OES_2 and OES_3).

5. Calibration strategy choice – 2: Curve fitting, linear fitting, or wavelength switching in a wide concentration range (i.e. Assumed to be non-linear range)

Within a linear response range, a multi-point linear fitting is no better than a one-point linear fitting in theory. However, a multi-point linear fitting is more reliable, in reality, due to operational and instrumental errors. In a non-linear response range, a multi-point linear fitting is no better than a one-point linear fitting, either in theory or in reality. Instead, the harm created by a multi-point linear fitting may be more extreme because it is prejudged that the result by a multi-point fitting is better than a one-point fitting.

Would a multi-point curve fitting be better than anything else? This is not absolutely true. Mathematically, there are no formulas which define a straight line at one end and a curve at the other end. Approximation is unavoidable and errors are introduced. These kinds of errors are less intuitive and usually more difficult to catch than errors introduced during a linear fitting.

The TJA IRIS ICP-OES is a simultaneous instrument—the analysis time is independent of the number of wavelengths. Therefore, two wavelengths per element are selected for elements with wide concentration ranges (such as Ca [0–400 ppm], Fe [0–100 ppm],

Mg [0–300 ppm], and Mn [0–40 ppm]). One wavelength is for the low concentration range and the other for the high concentration range. All are linear fittings. The blank point (OES_1) is excluded from the linear fitting in the high concentration ranges. An external calibration, by its nature, is a comparison process. Standards at low concentration ranges are not necessary for analyses at very high concentration ranges.

6. Calibration strategy choice – 3: Standardization or calibration

The TJA IRIS ICP-OES instrument is prepared in two ways: standardization and calibration (“Standardization” as defined in the TJA IRIS ICP-OES manual is a linear fitting “calibration” as we normally say. “Calibration” as defined in the TJA IRIS ICP-OES manual is actually a way of curve fitting “calibration”).

- 6.1 Standardization: Linear fitting based on a set of working standards. The standardization is carried out daily or each time before sample analysis with a whole set of standards.
- 6.2 Advantage of standardization: Quantification is based on a linear relationship. The calculation is simple. This relationship is direct and easy to diagnose in case something is inaccurate. Standardization can be based on just two points.
- 6.3 Disadvantage of standardization: Instrument response is usually not linear at very low concentration ranges or at very high concentration ranges. During each sample analysis, the whole set of standards (whether being two or ten) also have to be analyzed.
- 6.4 Calibration: Curve fitting based on a set of working standards (minimum – 4 standards). The curve is adjusted

- (normalized) daily or before each sample analysis with a “low-end” standard and a “high-end” standard.
- 6.5 Advantage of calibration – Better instrument response in a wide concentration range than a linear fitting. Only two “Norm” standards are needed for each sample analysis.
 - 6.6 Disadvantage of calibration – A curve fitting is less intuitive and makes it difficult to diagnose a problem. The calibration is less adaptive to a significant instrument change. Some examples include: auto-peak adjustment where the peak of an element can be shifted either to left or to right, nebulizer change, and plasma power change.
 - 6.7 “Standardization” is selected over “calibration” by primarily considering that a “calibration” is less tolerant to instrument condition changes and less intuitive. However, these two terms are not distinguished in other sections of this appendix.

7. Validation of the working standard

It is a widely accepted practice to use NIST SRMs (standard reference materials) to validate various kinds of laboratory operations. However, NIST SRMs are not readily available to validate these working standards due to the unique concentration ranges and element combinations. Alternatively, several different approaches are used.

- 7.1 First of all, these multi-element working standards are purposely made from primary standards of independent sources. An agreement between these primary standards themselves is an indication that the claimed concentrations are valid.

- 7.2 When a new set of working standard solutions are made, the new set of standards are measured against the old set of standards. One example is given in [Table Appendix 3-1: Validation of working standard - comparison of new set with old set](#). This agreement is better than 100±3% in most cases.
- 7.3 After the instrument is standardized with these working standards, an independent set of primary standard solutions, CLMS-1, CLMS-2 and CLMS-4 (all from SPEX), which contain 10 ppm of most elements, are measured either “as is” or diluted by 10 times. The results are given in [Table Appendix 3-2: Validation of working standard - based on independent SPEX primary standards](#). For most elements, the agreement is better than 100±5%. However, the recoveries of some elements are significantly out of the above range. MS1 contains 17 elements, MS2 contains 29 elements and MS4 contains 12 elements. A certain extent of inter-element interference is possible but this is not fully examined. The five OES working standard solutions are designed to cover high concentrations ranges for some elements such as K, Fe, Al and S. The concentrations of these elements in MS1, MS2, and MS4 solutions, especially after dilution, may not be in the optimum calibration ranges of these OES working standard solutions. SPEX MS-4 contains trace amount of HF acid. This may explain the extraordinarily high concentrations of Si (measured 19 ppm vs. claimed 10 ppm), since the sample introduction system is not HF-resistant.
- 7.4 Eight other independent standard solutions from four different sources

are measured based on these five OES working standards. These eight solutions are diluted by 10 or 20 times and then analyzed. The recovery ratios (the measured concentrations to the claimed concentrations of these eight independent standard solutions) are better than $100\pm 5\%$ for most of the elements (see [Table Appendix 3-3: Validation of working standard - based on other eight primary standard solutions](#)). The exceptions might be caused by potential inter-element interferences and/or improper dilutions.

7.5 The consistency between these working standards and other standard solutions is also confirmed by using ICP-MS for several elements where applicable. The mechanisms of inter-element interferences of ICP-OES are different from those of ICP-MS. For example, the concentration of molybdenum in Solution-A of CCV-1 (from CPI international) is found to be 100 ppm rather than as the claimed concentration of 200 ppm, based on several different analyses. Solution-A of CCV-1 is used to make the OES_2 working standard.

8. Analytical merit – limit of detection (LOD), calibration blank (cBlk) and blank correction

8.1 A solution of 3–5% nitric acid is consecutively measured 12 times in one day. The average and the standard deviation (SD) of these measurements are calculated. The limit of detection (LOD) is three times the standard deviation ($LOD = 3 \times SD$). Six sets of LODs are so collected on six different days. The average of these six sets of

LODs is taken as the instrument's LOD and listed in [Table Appendix 3-4: Analytical merits](#).

8.2 There have been extensive and endless discussions about the way of obtaining and the way of applying the limit of detection (LOD). There are also discussions about other “confusing” terms, such as: instrument detection limit, method detection limit, and limit of quantification, etc. Overall, the LOD and other terms depend on their respective definitions and depend on the conditions under which a sample is measured. The LOD and other terms should be used as general reference but not an absolute judge point.

8.3 An analogy to limit of detection is the achievable speed by a race car. Every effort is made to achieve the maximum speed of a race car. The achievable speed of a race car does provide a lot of information but does not provide all of information. If some one thinks about three types of vehicles (race car, regular car and all-terrain vehicles) in three types of fields (car-race field, regular highway and off-road), the maximally achievable speed by a race car tells virtually very little, or is not considered as critical at all.

8.4 A quick suggestion is that a researcher may need to interpret a value cautiously if the value is close to the limit of detection. It is advised to design a research to avoid such an uncertain situation. Alternatively, a different instrumentation or different analytical methods may better be used. For example, the SPAL's ICP-MS service is recommended for the analysis of minor and trace elements (the heavy metal list) in plant tissue samples. Hydride generation technique is better for arsenic and selenium in

most types of samples rather than conventional nebulization (ICP-OES or ICP-MS).

9. Calibration blank (cBlk) and blank correction

- 9.1 A solution of 3–5% nitric acid is measured every time after the instrument is standardized. All of these measurements (in one year, about 140 sets of data) are pooled, the average (Avg) and three times the standard deviation (3sd) are calculated and also listed in [Table Appendix 3-4: Analytical merits](#).
- 9.2 The 3sd values are apparently higher than the respective LODs of each element. This makes sense since the 3sd values are calculated from the pooled measurements of blank samples over a long period of time while the LOD values are based on samples measured within a very short period of time. This 3sd values in one way may be viewed as the realistic detection limits achievable in any day's analysis.
- 9.3 The average values of the blank sample (cBlk, 3–5 % nitric acid) are close to zero, which makes sense since these values should be zero statistically. However, the measured values of the cBlk sample on any particular day could be fairly away from zero. The equivalent concentrations may be negative or positive simply based on the mathematics of the calculation.
- 9.4 These equivalent concentrations of the cBlk sample, whether positive or negative, are corrected from all of the other samples. This correction is important for elements at low concentration levels (ppb levels), such as: iron or copper in water samples,

but it is undetectable for elements at high concentration levels (ppm levels), such as: calcium or magnesium in hard water samples.

- 9.5 On the other hand, this blank correction in effect does not change the analytical conclusion, when the equivalent concentrations of the cBlk sample are significantly less than their respective LODs (as listed in [Table Appendix 3-4: Analytical merits](#)), although the two procedures (with and without correction) are conceptually different.

10. In-house quality control water (msQC water at ppb levels and QC water at ppm levels)

Several NIST waters might be used as quality control solutions for trace analysis at ppb levels, but are not applicable to major and minor analysis at ppm levels. It is also expensive to include NIST waters in day-to-day analysis. Other kinds of quality control solutions are available commercially with similar problems: not matching and/or not economical.

- 10.1 An in-house quality control (QC) water is made by adding standard solutions to a 20-liter glass jar, diluting with 3–5% nitric acid, and sub-bottling it in 2.5-liter glass bottles. These 2.5-liter glass bottles are reagent bottles which were used for Trace Metal Grade nitric acid (Fisher). No more leaching-out of metals is expected from these bottles by 3 – 5% nitric acid. The 3–5 % nitric acid prevents absorption and precipitation of metals. The metals are at 5–40 ppm concentration levels and an interaction between these metals is not expected. Therefore, this QC water is expected to be stable for years.

- 10.2 An in-house quality control (msQC) water is made similarly but at ppb levels.
- 10.3 The in-house msQC and QC waters are used to confirm the instrument standardization in day-to-day analysis. The QC water is also used to correct instrument's within-day drift.

11. Long term consistency (day to day analysis)

After the TJA IRIS ICP-OES is standardized, the msQC water and the QC water are analyzed. The results of msQC water (from July 2004 to December 2005) are shown in [Figure Appendix 3-1: Analysis of elements at ppb level](#). The results of QC water (from June 2003 to December 2005) are shown in [Figure Appendix 3-2: Analysis of elements at ppm level](#). The respective averages and standard deviations are listed in [Table Appendix 3-4: Analytical merits](#).

- 11.1 The msQC water contains minor and trace elements at ppb levels. The data quality of each element needs to be evaluated after considering what the LOD is, how high the concentration is or how big the difference is between the concentration and the LOD, and how the msQC water is stored. Some examples are given below.
 - 11.1.1 The vertical scales of the plots of Al, Cu are the same in [Figure Appendix 3-1](#). Both average concentrations of Al and Cu are about 90 ppb. By looking at the respective plots, it can be concluded immediately that the data quality of Cu is much better than that of Al. This is because the concentration of Al in the msQC water is about as twice as Al's LOD, but the concentration of Cu is about 20 times higher than Cu's LOD.

- 11.1.2 Other examples may be seen from the elements of Ba, Co, Cr, Li, Mo, Ni, Sr, Ti, V and Y where the vertical scales are the same. Generally, the magnitude of standard deviation (SD, listed in [Table Appendix 3-4](#)) of data points is positively related to the magnitude of the respective LOD for each element.
 - 11.1.3 Relative standard deviation (RSD), another term for quality evaluation, is the ratio of the standard deviation to the average concentration of an element. The RSD generally decreases with the increasing concentration of an element.
 - 11.1.4 The concentrations are apparently different before and after the msQC water in two sub-bottles were measured, respectively, for Ca, Fe, Li, Na and especially Si. This is because the msQC water contains trace amount of HF acid. These elements are released from the glass bottles with time.
- 11.2 The QC water contains most elements at ppm levels. As such, the detection limit is usually not the main factor in controlling data quality. As such, the data quality is consistent across different elements for a long period of time (several years).
 - 11.2.1 In this period of time, the working standard solutions are renewed for three times. The whole laboratory is relocated to a different place. The QC water is analyzed from different sub-bottles. The consistent results as shown in [Figure Appendix 3-2](#) demonstrate the successful practice of this QA/QC implementation.
 - 11.2.2 On the other hand, the concentrations of Al, Ca, Na, and Si increase significantly with time or vary

significantly in different sub-bottles. This is quite contrary to an initial thought that these concentrations would be stable for years. It is expected that a solution of 3–5% of nitric acid would not release metals from the glass bottle. However, several solutions which were used for making the QC water contain HF acid and it might be this HF acid that releases these Al, Ca, Na, and Si elements from the glass bottle.

- 11.2.3 Since the QC water contains elements at ppm level and more standard solutions (and more HF acid) are used than the solutions used for making the msQC water, the release of these elements from the glass bottles is more significant than that of the msQC water.
- 11.2.4 The release of Al from the bottles of msQC water is not obviously observed. This might be either because the release is insignificant with respect to the release of Al in the QC water bottles, or because the release of Al is blurred by the huge scattering of the data points as seen from [Figure Appendix 3-1](#).
- 11.3 The evaluation on the data quality of a multi-element analysis is not easy or straightforward. Many factors have to be considered simultaneously. Each and every element has to be considered and evaluated individually. A cross-board expectation of data quality for all of elements is just simply impractical.
- 11.4 The result presented in [Figures Appendix 3-1 and 3-2](#) provide a visual estimation of data quality for different elements at different concentration

levels. The numerical values are provided in [Table Appendix 3-4](#).

12. Short term consistency (within day analysis)

Immediately after the instrument is standardized, the QC water is measured. This QC water is also measured at every 20 sample intervals. The ratios of subsequent concentrations to the first concentration in a time sequence indicate how much the instrument drifts over time.

- 12.1 This TJA IRIS ICP-OES instrument is typically used for 5–10 hours each time after standardization. Most of the time (>80–90%), the ratios are between 0.95 and 1.05, or the drift is less than 5%. In the first example ([Table Appendix 3-5: Short term stability as seen from the drift of QC water](#)), the instrument is standardized and used for 17 hours. The ratios are roughly one.
- 12.2 The drift sometimes can be quite significant, as seen from the second example in [Table Appendix 3-5](#). This second example also indicates that the drift is element-dependent, changes in opposite directions (up or down), and varies at different rates.
- 12.3 This element-dependent drift makes the drift-correction a challenge. Using an internal reference standard (a common practice in ICP-OES) would not help much in correcting this type of drift. Actually, the correction based on an internal reference standard makes the drift-introduced error even larger, if the internal reference standard and the target element drift in opposite directions.
- 12.4 Assuming that the drift is linear within a short period of time between two check points (i.e. between two QC

water analyses), the drift of other samples/elements could be corrected based on the drift of the QC water. After the instrument's output is loaded into an Excel spreadsheet, an in-house computer program is used to carry out this correction. Listed in **Table Appendix 3-6: Results of short-term drift correction** are some examples. Typically in one day, the tenth sample (i.e. the mid-point sample between the first QC measurement and the second QC measurement) is measured again at the end of the day's analysis (i.e. just before the last QC measurement). As seen from **Table Appendix 3-6**, the results of duplicate analyses are repeatable.

- 12.5 The nature of this drift correction is equivalent to the normalization of the instrument with the QC water after every 20 samples.

13. Ending

The data quality is judged in terms of accuracy and precision. The accuracy and precision of the SPAL's analysis is generally better than 100 ± 5 %. However, there are exceptions.

In a single-element method, experimental conditions are optimized to the best conditions for that specific element in a specific matrix. In a multi-element method, it is impossible to select the best conditions for all of the elements in various kinds of matrix, since one best condition for one element could be a worst condition for another element. A multi-element method virtually is a multi-compromising method. Therefore, there are differences between data quality obtained via a single-element method and data quality obtained via a multi-element method. And there are

differences in data quality between results of elements obtained by one analytical method.

The selection of a dilution factor is a very good example here. Samples contain components in a large concentration range from trace, minor, to major. With a limited sample preparation (e.g. one dilution factor for all), some components fall into the best concentration range for an analytical method, while other component's concentrations are either too high or too low. Consequently, the data qualities are different for different elements.

What are the criteria in accepting/rejecting an analytical result? It depends. The data quality of 0.02 ± 0.01 ppm for selenium may be as good as the data quality of 2000 ± 60 ppm for sulfur, although the relative standard deviation (RSD) of Se's result (50%) is way higher than that of S (3%). It is improper to compare the data quality of trace elements (such as Se) with the data quality of major elements (such as S). The best criteria are to consult the research literature. If the common RSD for Se found in literature is about 50% for results at 0.02 ppm level obtained by using an ICP-OES method in a multi-element analysis, the quality of the result (0.02 ± 0.01 ppm Se) could be judged as accurate and be well accepted.

Although an analyst may be proud of his/her performance in obtaining such "accurate" data, a client may not be happy because s/he needs to know whether the concentration of Se is 0.01 ppm or 0.03 ppm. In that case, a different analytical procedure has to be sought. For example, the SPAL offers the analysis of Se with hydride generation stable isotope dilution – ICP-MS, which offers a detection limit of 0.001 ppm of Se in plant

tissue. The detection limit, offered by the ICP-OES method, is 4–7 ppm.

As discussed in section *11. Long term consistency*, the interpretation and application of multi-element analyses is complicated and requires the consideration of many factors/parameters: sample type, sample preparation, element, concentration range, detection limit, contamination, and instrument performance to that specific element. The time, effort, and resources for a project are wisely spent only after these factors/parameters are well considered and controlled beforehand.

– End –

Table Appendix 3-1: Validation of working standard - comparison of new set of standard with old set of standard.

	Intended concentration				Measured concentration				Measured concentration				Ratio of new to old						
	All units are ppm				Set made on 06/24/2003				Set made on 05/27/2004										
	oes2	oes3	oes4	oes5	oes2	oes3	oes4	oes5	oes2	oes3	oes4	oes5	oes2	oes3	oes4	oes5			
Al	2	10	100		2	11	98		2	10	99		97	97	101				
As	2	5			2	5			2	5			102	101					
B	2		10		2		10		2		10		102		102				
Ba	1	10			na = not added				1	10									
Ca	2	20	100	400	2	20	103	406	2	20	104	402	100	99	102	99			
Cd	1	1			1	1			1	1			101	100					
Co	1	5			1	5			1	5			100	101					
Cr	0.5	2			0.5	2			0.5	2			101	101					
Cu	2	2	20		2	2	20		2	2	20		107	103	101				
Fe	2	10	100		2	10	100		2	11	101		102	109	101				
K	5	20	100	500	5	20	99	498	5	20	101	500	106	100	102	100			
Li	2		10		2		10		2		10		102		96				
Mg	2	20	100	300	2	20	107	298	2	19	108	296	101	92	101	100			
Mn	1	1	4	40	1	1	4	40	1	1	4	40	104	104	103	100			
Mo	1		10		1		10		1		10		103		102				
Na		20	100			20	97			21	99			106	103				
Ni	2	5			2	5			2	5			100	101					
P	5	20	100	400	5	21	106	405	5	22	107	403	103	103	101	99			
Pb	2	5			2	5			2	5			98	99					
S	2	20	100	400	2	20	101	406	2	20	101	405	102	102	100	100			
Se	2	5			2	5			2	5			101	100					
Si			5	10	na = not added						5	10							
Sr	2			4								2							na
Ti	2	5										2					5		
V	1	5										1					5		
Y			5	10														5	10
Zn	1	1	20		1	1	20		1	1	20	0	103	104	103				
	All units are ppm				Set made on 05/27/2004				Set made on 06/14/2005				Ratio of new to old						
Al	2	10	100		2	10	99		2	10	97		103	99	98				
As	2	5			2	5			2	5			99	100					
B	2		10		2		10		2		10		100	103	99				
Ba	1	10			1	10			1	10			100	100					
Ca	2	20	100	400	2	20	104	402	2	20	104	400	99	100	99	99			
Cd	1	1			1	1			1	1			100	99					
Co	1	5			1	5			1	5			100	100					
Cr	0.5	2			0.5	2			0.5	2			100	100					
Cu	2	2	20		2	2	20		2	2	20		101	100	99				
Fe	2	10	100		2	11	101		2	11	99		99	98	98				
K	5	20	100	500	5	20	101	500	5	20	100	492	100	100	99	98			
Li	2		10		2		10		2		10		100		98				
Mg	2	20	100	300	2	19	108	296	2	19	107	292	100	100	98	99			
Mn	1	1	4	40	1	1	4	40	1	1	4	40	100	100	97	99			
Mo	1		10		1		10		1		10		99		99				
Na		20	100			21	99			21	98			100	99				
Ni	2	5			2	5			2	5			100	99					
P	5	20	100	400	5	22	107	403	5	22	106	397	100	100	99	99			
Pb	2	5			2	5			2	5			100	100					
S	2	20	100	400	2	20	101	405	2	20	100	399	95	100	99	99			
Se	2	5			2	5			2	5			99	99					
Si			5	10	na = not added						5	10							
Sr	2			4								2							4
Ti	2	5										2					5		
V	1	5										1					5		
Y			5	10														5	10
Zn	1	1	20		1	1	20	0	1	1	20	0	100	100	99				

Table Appendix 3-2: Validation of working standard - based on independent SPEX primary standards

	Claimed SPEX CLMS1, CLMS2 and CLMS4 concentration. All units are ppm.			Measured "as is" based on the set made on 05/27/2004. Data are the average of three measurements on three days.			Measured after 10 times dilution based on the set made on 06/14/2005. One measurement.		
	ms1	ms2	ms3	ms1	ms2	ms3	ms1	ms2	ms4
Al		10			11.2			10.7	
As		10			10.9			10.1	
B			10			10.5			9.8
Ba		10						10.5	
Ca		10			10.5			10.1	
Cd		10			10.1			10.0	
Co		10			10.4			10.2	
Cr		10			10.5			10.4	
Cu		10			10.5			10.2	
Fe		10			11.8			10.7	
K		10			10.1			9.1	
Li		10			10.0			9.7	
Mg		10			10.7			9.8	
Mn		10			11.0			10.4	
Mo			10			10.9			10.0
Na		10			10.5			10.2	
Ni		10			11.1			10.5	
P			10			10.7			9.6
Pb		10			10.7			10.2	
S			10			9.9			8.8
Se		10			10.9			9.7	
Si			10			18.6			12.3
Sr		10						10.7	
Ti			10			10.8			9.9
V		10						10.1	
Y	10						10.5		
Zn		10			10.7			10.4	

Table Appendix 3-3: Validation of working standard - based on other eight primary standard solutions

	Claimed concentrations in the 8 standards. All ppm								Measured based on the set made on 05/27/2004 after 10 times of dilution. All recovery ratio %							
	1A	3	7	240	A	B	ES	CPI	1A	3	7	240	A	B	ES	CPI
Al			100	20	500	600	650	200			111	114	102	104	104	98
As	100	20	100	20				200	102	101	97	105				98
B	20			20				200	100			100				102
Ba	10			20	5	4		100	103			103	103	105		102
Ca	100			20	350	260	80	200	98			101	98	99	98	100
Cd	20		100	20				100	97		101	103				101
Co			100	20				100			99	97				101
Cr			100	20		15		50			98	99		105		103
Cu	20		100	20				200	99		103	96				101
Fe			100	20	200	400	350	200			98	102	99	99	98	101
K		100	100	100	200	200	150	500		103	100	102	102	103	102	101
Li		20		20				200		103		103				100
Mg			100	20	65	120	100	200			102	105	103	105	104	102
Mn	20	20	100	20		6	4	100	102	102	99	101		102	101	102
Mo		20		20				200		92		97				97
Na		20	100	20	70	55	200			106	106	103	104	101	106	
Ni		20	100	20				200		95	94	94				98
P		100		100	10	10	5	500		100		97	96	93	86	96
Pb			100	20				200			97	96				100
S		100								104						
Se	50			20				200	97			96				98
Si				100	3000	3000	3000					92	104	104	104	
Sr				20				200				102				100
Ti				20				200				98				98
V				20				100				102				102
Y			600									96				
Zn			100	20	1	5		100				98	99	87	98	100
	Measured based on the set made on 06/14/2005 after 20 times of dilution. All recovery ratio %															
	1A	3	7	240	A	B	ES	CPI								
Al			92	103	98	97	97	106								
As	104	105	109	103				109								
B	99			105				103								
Ba	99			107	100	99		101								
Ca	103			99	105	104	106	105								
Cd	105		104	102				103								
Co			104	106				103								
Cr			106	105		100		101								
Cu	99		98	109				102								
Fe			109	103	105	105	106	109								
K		95	96	101	98	97	97	99								
Li		93		99				95								
Mg			101	100	100	99	99	103								
Mn	104	104	107	107		103	104	108								
Mo		101		102				104								
Na		98	99	107	99	102	98									
Ni		106	106	106				103								
P		104		105	105	101	96	108								
Pb			106	106				104								
S		94														
Se	98			99				105								
Si				108	94	94	95									
Sr				106				92								
Ti																
V				104				100								
Y			106													
Zn			107	107	115	106		106								

Table Appendix 3-4: Analytical merits

	LOD	cBlk		msQC water		QC water	
	ppm	avg	3sd	avg	sd	avg	sd
Al	0.05	-0.0015	0.07	0.090	0.017	14.8	
As	0.03	-0.0002	0.05	0.061	0.012	4.7	0.1
B	0.03	-0.0005	0.04	(for high Fe samples such as soil samples)			
B	0.002	-0.0024	0.007	0.175	0.002	5.5	0.1
Ba	0.0002	-0.0002	0.0003	0.046	0.001	3.0	0.0
Ca	0.01	0.0002	0.02	0.810		18.8	
Cd	0.004	-0.0001	0.006	0.112	0.003	4.5	0.1
Co	0.003	-0.0003	0.005	0.017	0.002	4.9	0.1
Cr	0.001	0.0000	0.002	0.042	0.001	4.4	0.1
Cu	0.005	-0.0009	0.009	0.095	0.003	5.7	0.1
Fe	0.001	0.0002	0.007	0.227		6.3	0.1
K	0.03	-0.0106	0.05	0.137	0.011	17.8	0.3
Li	0.0005	0.0001	0.0008	0.030		4.4	0.2
Mg	0.007	-0.0004	0.014	0.264	0.015	35.1	0.6
Mn	0.0003	-0.0003	0.0008	0.051	0.001	11.2	0.2
Mo	0.005	-0.0034	0.021	0.022	0.003	5.0	0.1
Na	0.006	-0.0070	0.015	0.299		15.2	
Ni	0.004	-0.0014	0.010	0.018	0.002	6.1	0.1
Ni	0.02	-0.0002	0.12	(for high Fe samples such as soil samples)			
P	0.05	-0.0056	0.07	3.080	0.093	20.1	0.5
Pb	0.02	-0.0005	0.02	0.068	0.007	5.2	0.1
S	0.05	-0.0147	0.19	4.611	0.103	19.4	0.4
Se	0.04	-0.0031	0.08	0.059	0.009	5.6	0.2
Si	0.007	-0.0019	0.011	2.856		35.2	
Sr	0.0001	0.0000	0.0003	0.044	0.001	4.7	0.1
Ti	0.002	0.0001	0.003	0.026	0.001		
V	0.004	-0.0010	0.008	0.037	0.002		
Y	0.009	-0.0006	0.014	0.029	0.003		
Zn	0.001	-0.0010	0.003	0.052	0.002	10.9	0.2

Table Appendix 3-5: Short term (within-day) stability as seen from the drift of QC water

Example 1: Stability in 17 hours (360 samples)																		
Time	10:39	11:39	12:40	13:39	14:41	15:46	16:46	17:47	18:48	19:48	20:50	21:51	22:53	23:55	0:56	1:55	2:55	3:27
Al	1.00	1.00	1.02	0.99	1.00	0.99	0.99	1.00	1.01	0.99	0.98	0.99	0.98	0.98	0.99	1.00	0.98	0.99
As	1.00	0.99	1.00	0.99	0.99	0.97	0.98	0.98	0.96	0.93	0.93	0.93	0.93	0.90	0.93	0.92	0.91	0.91
B	1.00	1.01	1.01	1.00	1.02	1.01	1.00	1.02	1.00	1.02	1.00	1.00	1.00	1.01	1.01	1.02	1.02	1.02
Ba	1.00	1.00	1.01	0.99	0.98	0.98	0.98	0.99	1.00	0.97	0.97	0.98	0.96	0.96	0.96	0.97	0.96	0.96
Ca	1.00	1.01	1.02	1.01	1.02	1.02	1.00	1.01	0.98	0.98	0.97	0.98	0.97	0.96	0.97	0.99	0.98	0.98
Cd	1.00	0.99	1.01	0.99	1.01	1.00	0.99	1.00	0.99	0.99	0.98	0.98	0.98	0.98	0.99	1.00	1.00	1.00
Co	1.00	1.01	1.01	1.01	1.01	1.01	1.00	1.01	0.99	0.99	0.98	0.99	0.98	0.97	0.98	0.99	0.99	0.99
Cr	1.00	1.01	1.02	1.01	1.02	1.02	1.01	1.02	1.00	1.00	0.99	0.99	0.99	0.99	0.99	1.01	1.00	1.01
Cu	1.00	1.00	1.00	0.98	0.98	0.97	0.97	0.98	0.99	0.97	0.97	0.97	0.96	0.96	0.96	0.97	0.96	0.96
Fe	1.00	1.02	1.02	1.01	1.03	1.02	1.01	1.02	1.00	1.00	0.99	0.99	0.99	0.99	1.00	1.01	1.01	1.01
K	1.00	0.99	1.01	0.98	0.98	0.97	0.97	0.99	1.00	0.98	0.97	0.98	0.97	0.97	0.97	0.99	0.98	0.99
Li	1.00	0.99	1.00	0.97	0.96	0.97	0.97	0.99	1.00	0.99	0.99	0.99	0.98	0.98	0.98	0.99	0.98	0.99
Mg	1.00	0.99	1.00	0.97	0.96	0.96	0.96	0.97	0.98	0.95	0.96	0.96	0.94	0.93	0.94	0.95	0.93	0.93
Mn	1.00	1.00	1.01	0.98	0.97	0.97	0.96	0.97	0.98	0.94	0.94	0.94	0.93	0.91	0.93	0.93	0.92	0.92
Mo	1.00	0.99	1.02	0.98	0.99	0.98	0.98	0.99	0.97	0.95	0.94	0.95	0.94	0.94	0.94	0.95	0.94	0.94
Na	1.00	1.00	1.02	1.00	1.00	0.99	0.99	1.01	1.02	1.02	1.00	1.01	1.01	1.01	1.01	1.03	1.03	1.03
Ni	1.00	1.00	1.01	1.00	1.00	1.00	0.98	0.99	0.98	0.96	0.96	0.96	0.95	0.95	0.95	0.96	0.96	0.96
P	1.00	1.02	1.03	1.00	1.02	0.98	0.97	0.97	0.93	0.94	0.93	0.94	0.93	0.91	0.92	0.93	0.92	0.92
Pb	1.00	0.99	1.02	1.00	1.01	1.00	0.99	1.00	0.97	0.97	0.97	0.97	0.96	0.95	0.97	0.98	0.98	0.96
S	1.00	0.99	1.02	0.99	1.00	0.98	0.97	0.98	0.96	0.96	0.96	0.97	0.94	0.94	0.95	0.97	0.98	0.96
Se	1.00	1.08	1.02	1.06	1.09	1.08	1.07	1.08	1.04	1.02	1.01	1.02	1.01	1.01	0.99	1.00	1.00	1.02
Si	1.00	1.00	1.02	1.00	1.01	1.01	1.00	1.01	1.00	1.00	0.99	1.00	0.99	0.99	1.00	1.01	1.01	1.01
Ti	1.00	1.00	1.01	0.98	0.97	0.97	0.96	0.97	0.98	0.95	0.95	0.96	0.94	0.93	0.94	0.94	0.93	0.93
V	1.00	1.00	1.01	0.99	0.98	0.98	0.98	0.99	0.99	0.97	0.97	0.97	0.96	0.94	0.95	0.97	0.95	0.95
Y	1.00	1.02	1.03	1.02	1.03	1.01	1.02	1.03	1.02	1.02	1.00	1.01	1.00	1.01	1.00	1.03	1.02	1.02
Zn	1.00	0.99	1.01	0.99	1.00	0.99	0.99	0.99	0.98	0.98	0.97	0.97	0.95	0.94	0.95	0.98	0.95	0.95

Example 2: Stability in 3 hours				
Time	10:31	11:33	12:33	13:27
Al	1.00	0.99	1.01	1.03
As	1.00	1.03	1.06	1.13
B	1.00	0.99	1.01	1.04
Ba	1.00	0.98	1.00	1.02
Ca	1.00	1.04	1.08	1.17
Cd	1.00	1.02	1.04	1.09
Co	1.00	1.00	1.03	1.08
Cr	1.00	0.99	1.02	1.07
Cu	1.00	0.99	1.00	1.02
Fe	1.00	1.01	1.03	1.09
K	1.00	0.99	1.00	1.02
Li	1.00	0.97	0.96	0.96
Mg	1.00	1.00	1.02	1.05
Mn	1.00	1.01	1.03	1.08
Mo	1.00	1.03	1.05	1.10
Na	1.00	0.97	0.98	0.99
Ni	1.00	1.02	1.04	1.10
P	1.00	1.06	1.09	1.17
Pb	1.00	1.02	1.05	1.13
S	1.00	1.04	1.05	1.11
Se	1.00	1.00	1.03	1.09
Si	1.00	0.99	1.01	1.05
Ti	1.00	1.00	1.02	1.06
V	1.00	0.99	1.01	1.05
Y	1.00	1.00	1.03	1.09
Zn	1.00	1.01	1.03	1.08

The QC water is analyzed right after standardization and after every 20 samples. The concentration ratios of the subsequent analyses to the first analysis are listed.

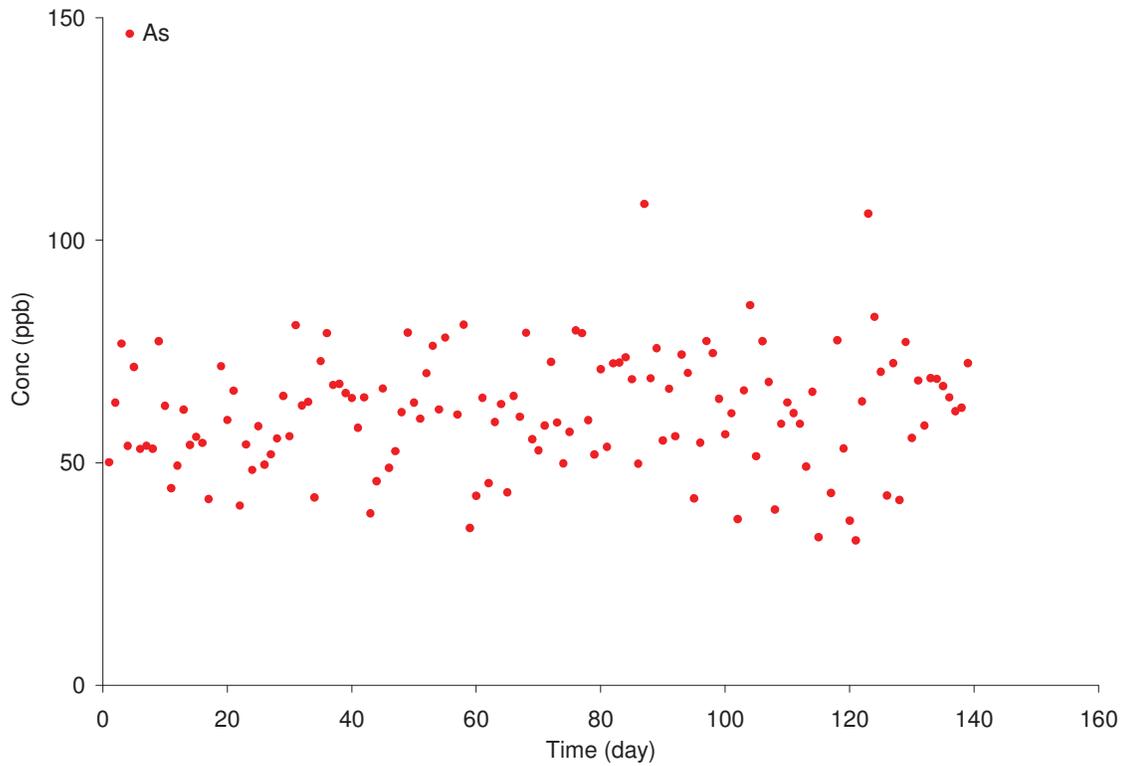
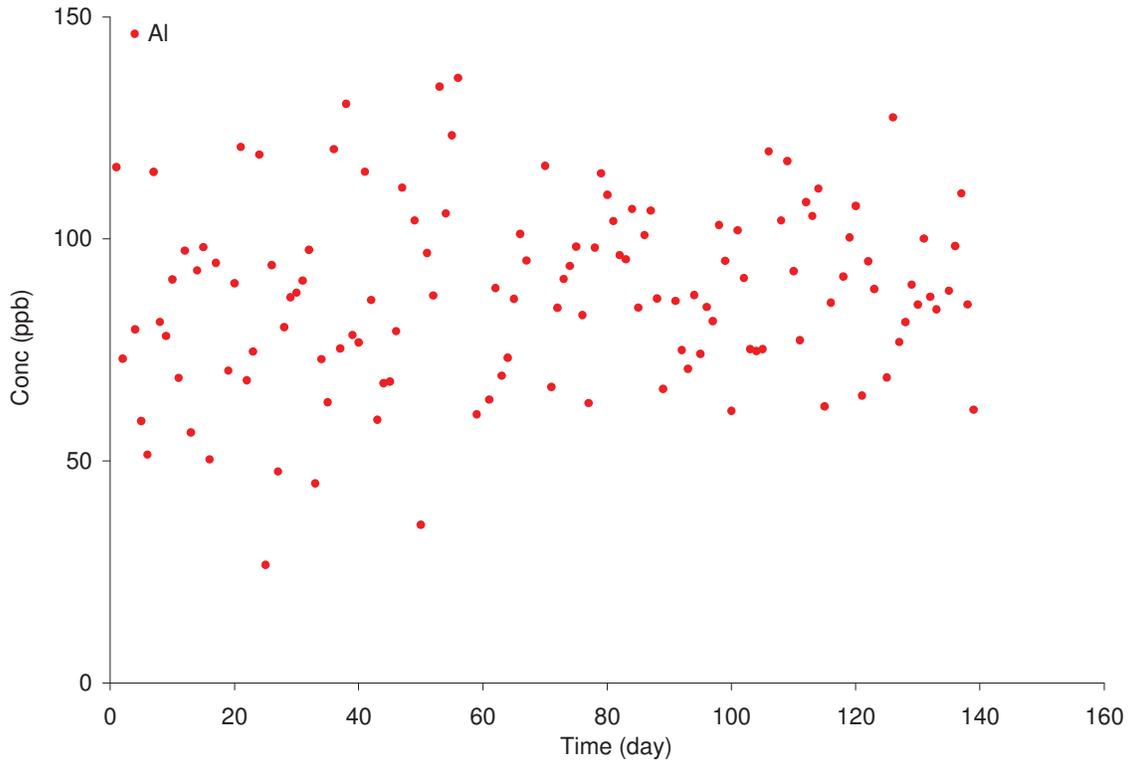
Table Appendix 3-6: Results of short-term drift correction

In a day, a sample is randomly selected for this short-term repeability check purpose.
 The sample is measured twice within one day (within one standardization).
 The drift is corrected based on the result of QC water.

	water 1		water 2		soil		plant 1		plant 2		plant 3		plant 4	
	1st and 2nd measuring time (hour:minute)													
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	13:24	15:09	10:34	15:13	8:53	14:41	13:29	13:32	12:19	12:22	11:07	11:10	9:27	13:13
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Al	155	155	1.5	1.4	11837	11538	287	275	182	177	1007	1018	23	28
B	0.31	0.29	0.15	0.15	39	40	0.74	0.68	5.7	5.5	3.6	4.0	52	53
Ca	97	99	14	14	30207	33813	365	361	490	488	528	508	22158	21538
Cu	0.029	0.038	0.17	0.18	18	17	0.80	0.94	0.61	0.80	3.1	2.7	5.5	5.1
Fe	124	123	1.95	1.96	13969	14242	370	371	164	164	1009	1019	80	81
K	26	26	495	506	2165	2042	1526	1508	1064	1068	3685	3659	20958	23008
Mg	75	74	10	10	22247	21184	190	190	170	171	403	398	4250	4489
Mn	3.9	3.9	1.3	1.3	457	463	14	14	30	30	23	23	75	76
Na	9.5	9.3	1.7	1.7	197	182	38	37	18	18	50	54	62	69
P	4.9	5.3	34	34	685	753	394	393	347	348	1475	1495	2229	2233
S	9.7	9.9	21	21	419	457	440	437	353	344	1389	1404	1237	1229
Zn	1.1	1.2	0.47	0.47	51	54	13	13	13	18	58	59	104	109

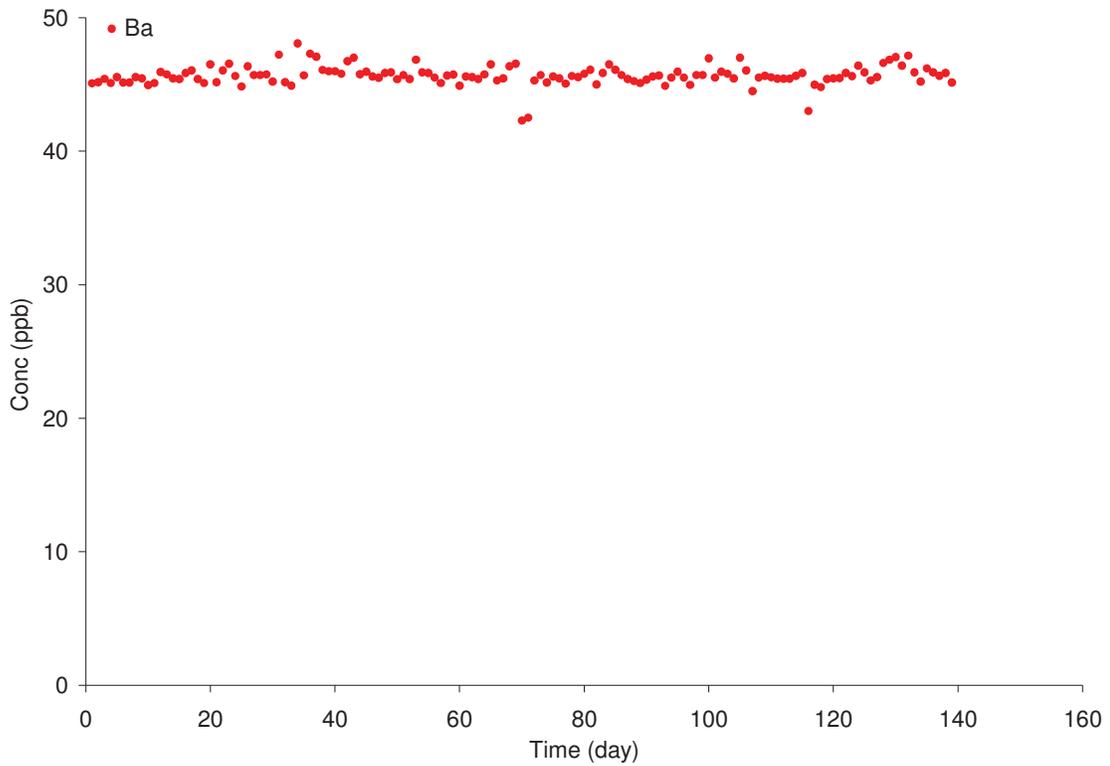
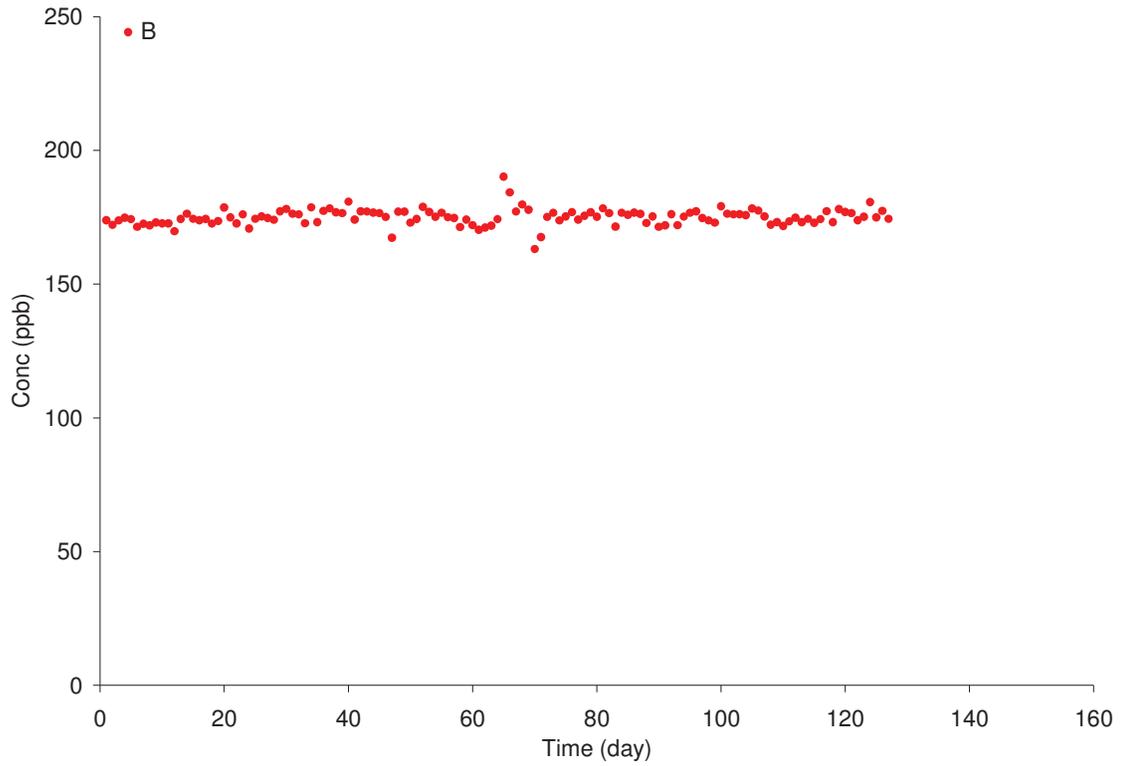
	plant 5		plant 6		plant 7		plant 8		plant 9		plant 10		plant 11	
	1st and 2nd measuring time (hour:minute)													
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	8:26	11:02	9:07	14:53	8:52	14:18	10:54	13:58	9:56	13:09	8:20	12:35	11:23	14:42
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Al	5.7	6.4	69	69	39	38	106	117	4.6	< 3	64	84	40	44
B	35	36	7.7	7.5	37	37	61	63	22	23	7.5	8.3	9.9	9.3
Ca	16083	16132	1978	1964	10561	10486	12177	12403	14703	13922	7136	7111	5741	5295
Cu	9.6	10.4	12	12	3.0	3.4	2.5	2.5	3.9	3.3	14	14	17	18
Fe	112	113	23	32	121	120	347	350	10	10	136	148	101	93
K	21521	23353	9827	9847	5509	5505	4475	4574	18871	20005	14248	14058	28464	28736
Mg	4254	4528	1944	1946	3299	3307	3321	3361	4867	4933	7887	7811	4595	4565
Mn	50	50	25	25	357	358	647	645	6.4	6.7	48	48	60	58
Na	52	57	107	107	14	14	61	66	352	377	101	142	55	59
P	2904	2935	2182	2194	1694	1688	1924	1961	4069	3953	4271	4256	4611	4364
S	1649	1696	1014	1016	1121	1065	1316	1336	4690	4555	2390	2367	1855	1771
Zn	45	50	24	29	35	36	36	37	147	146	30	31	82	82

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES



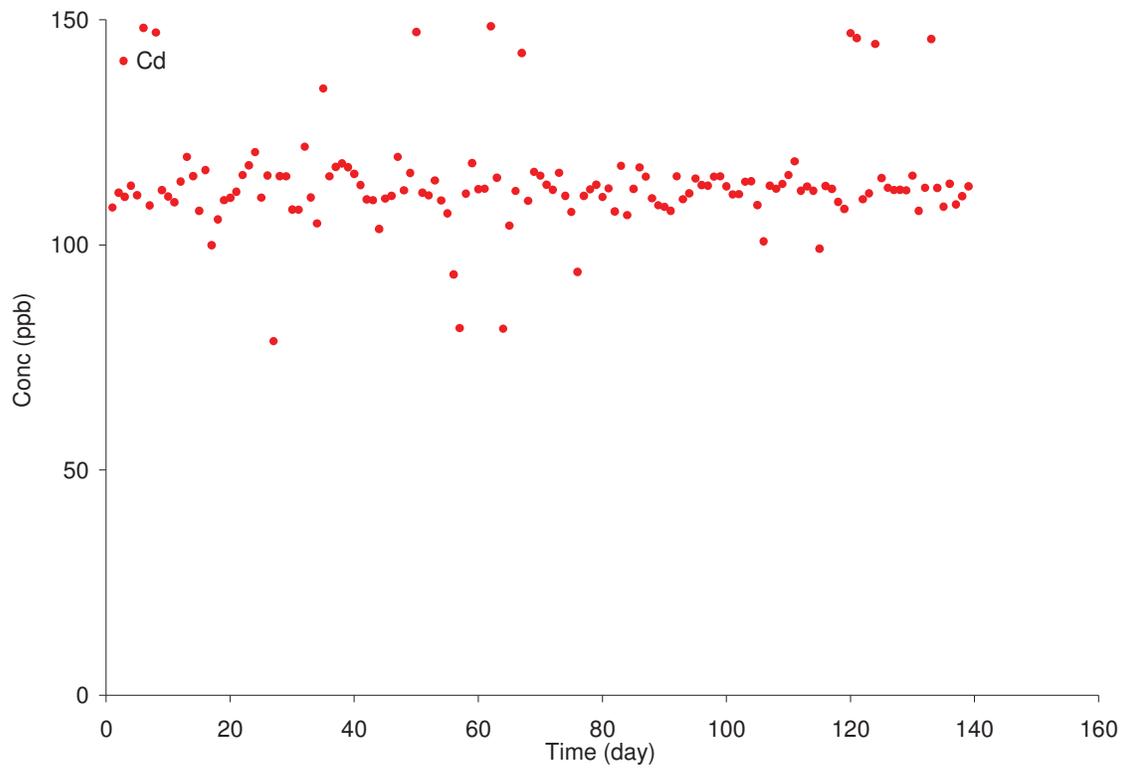
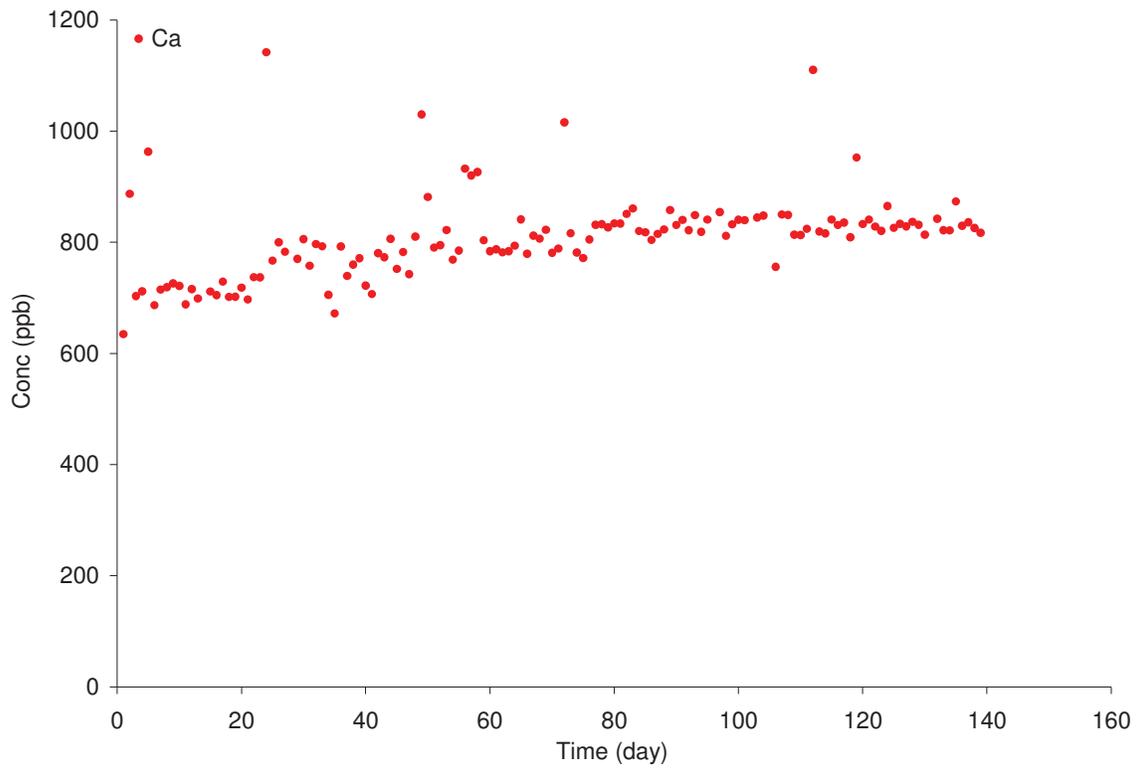
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



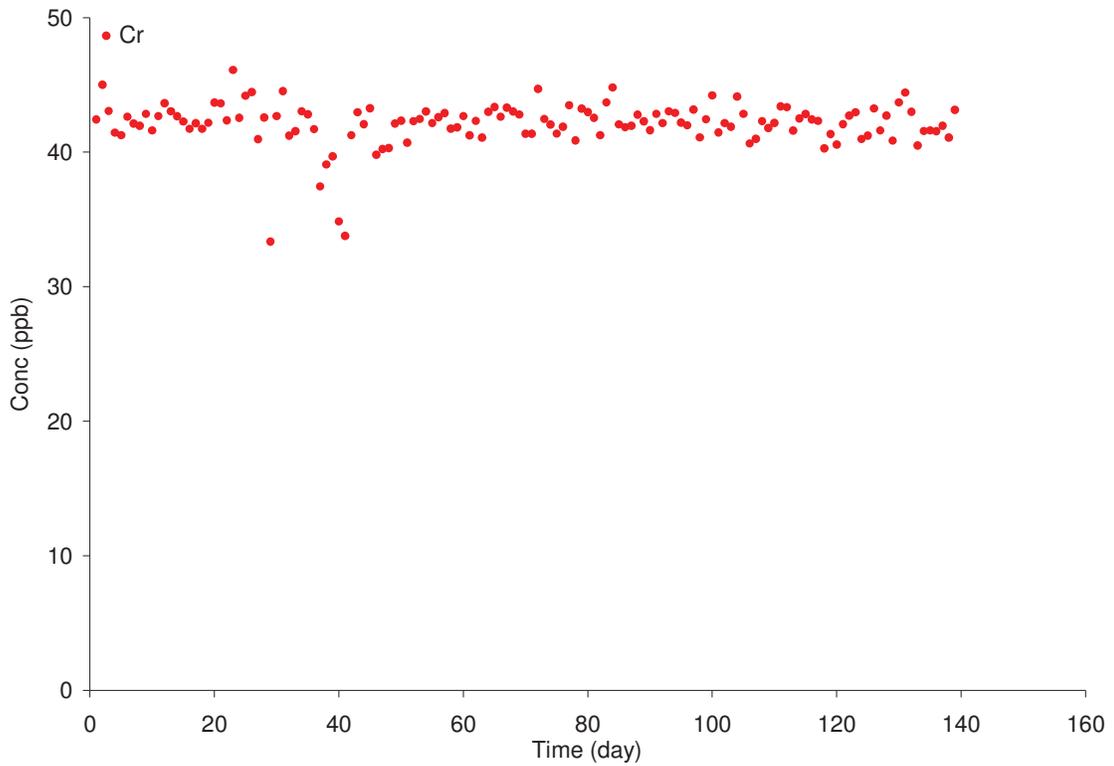
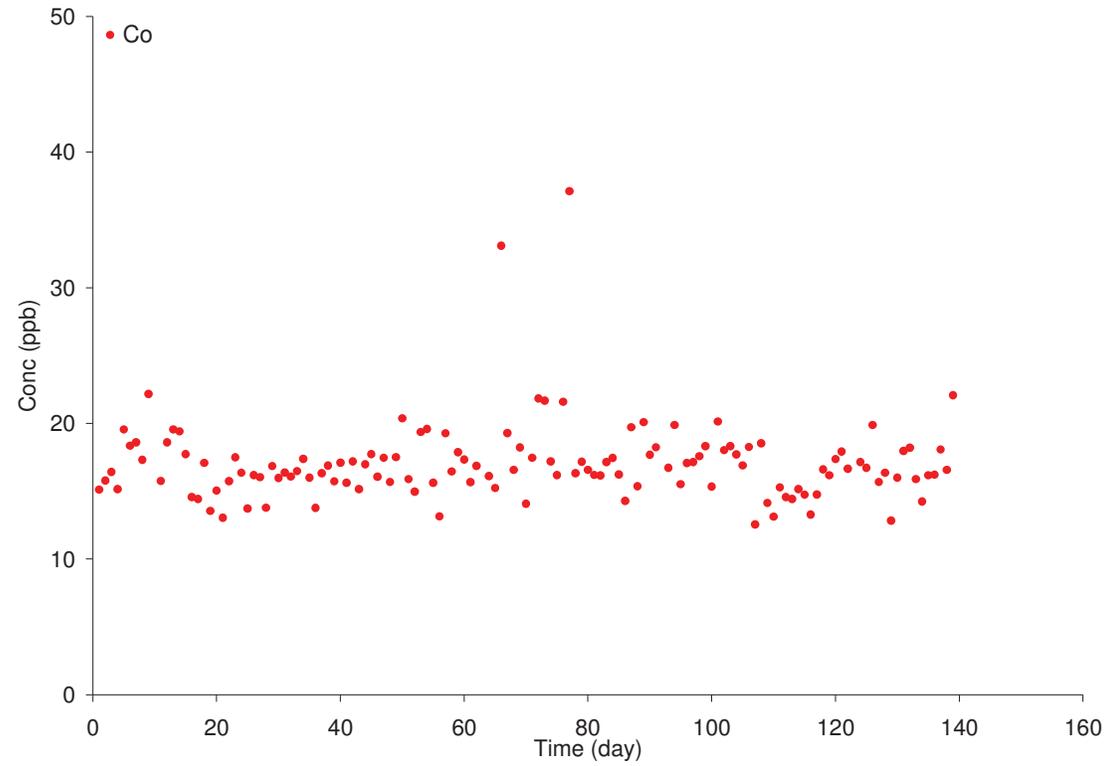
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



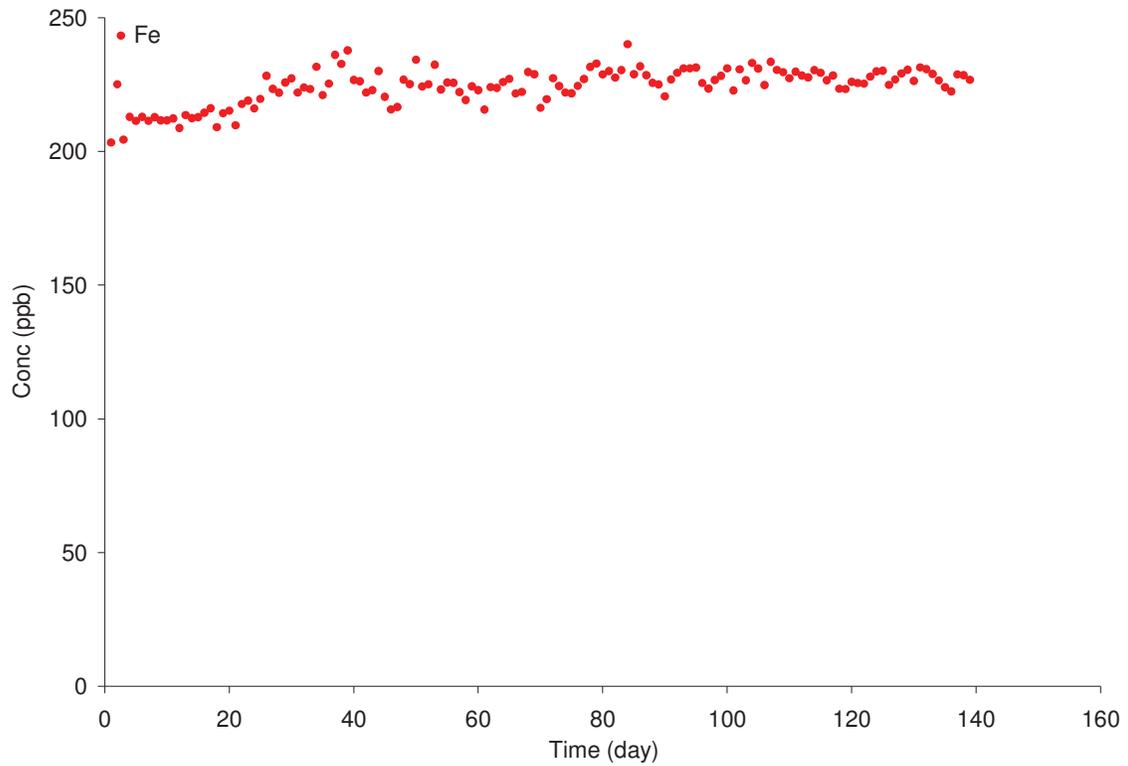
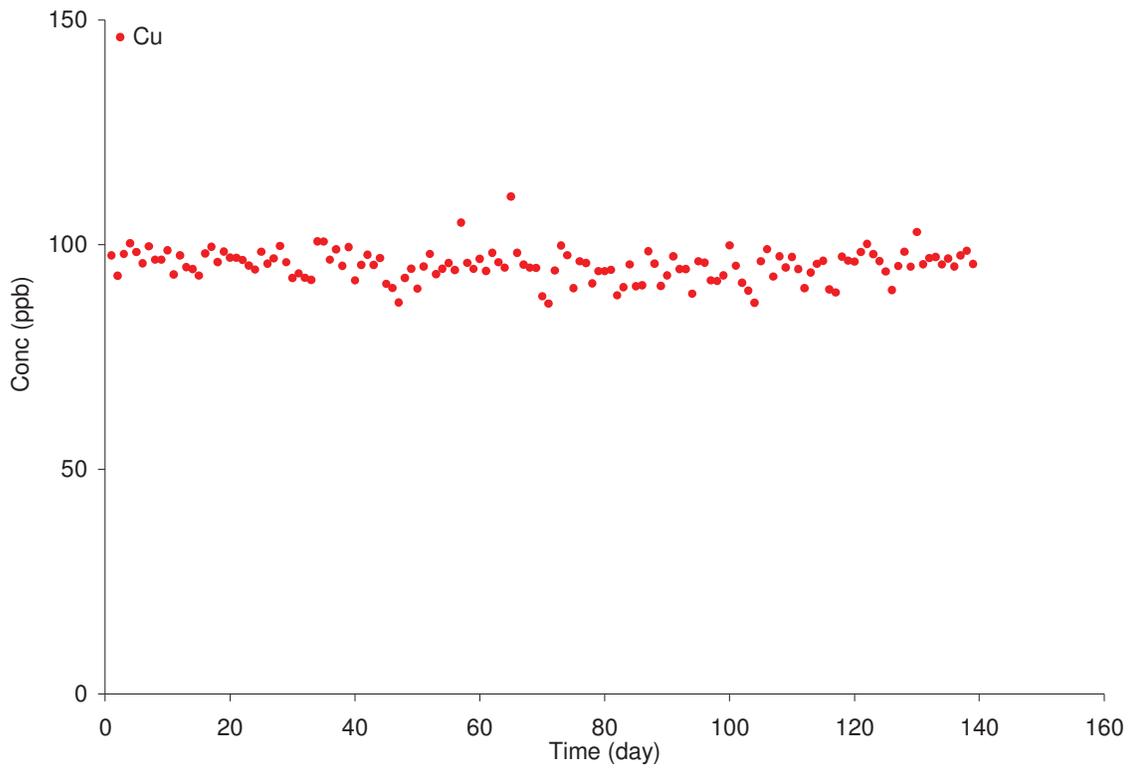
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



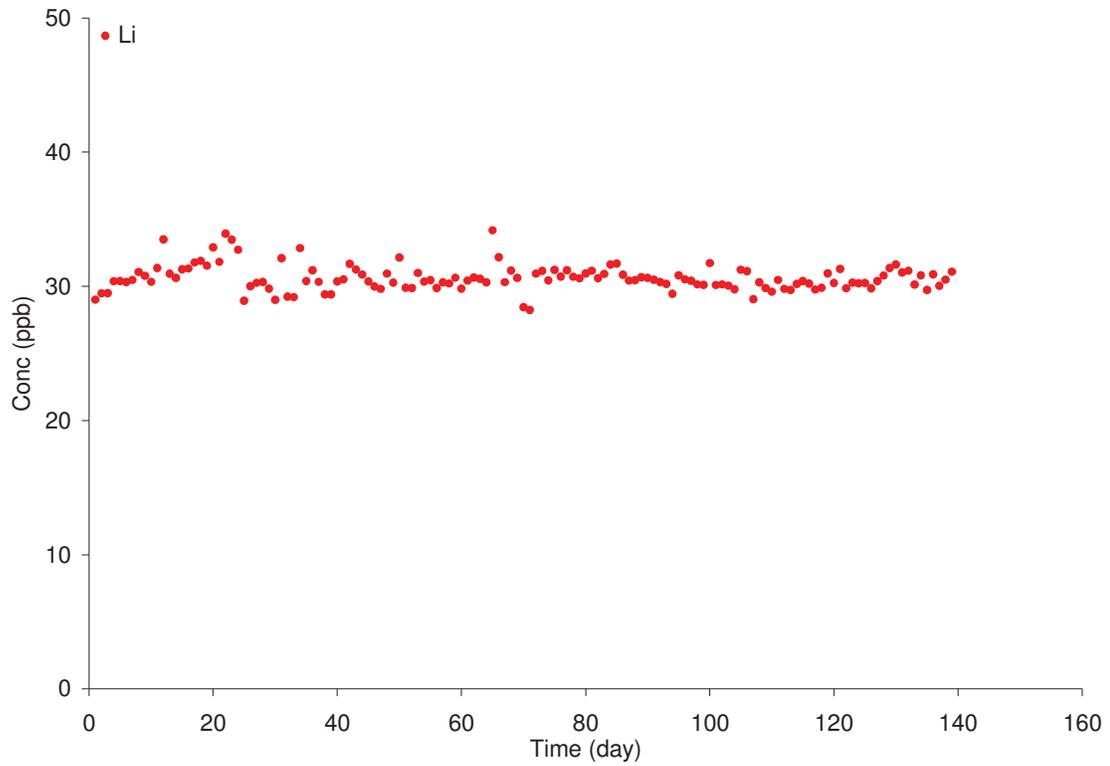
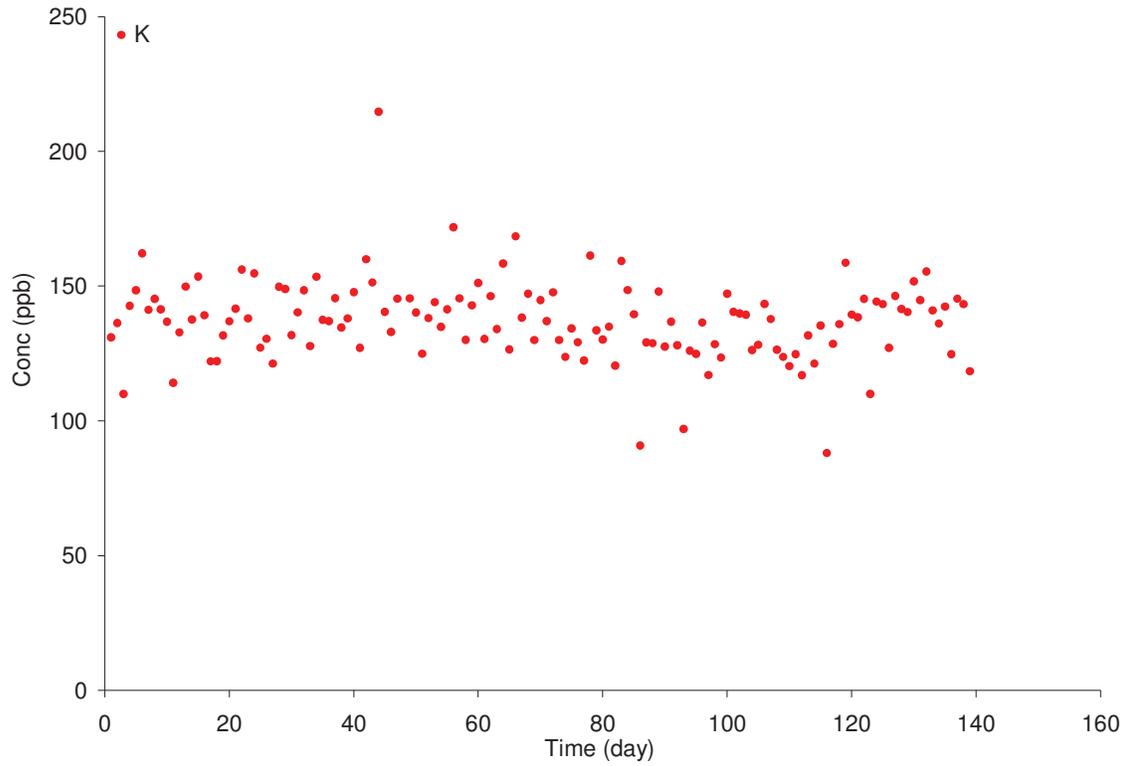
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



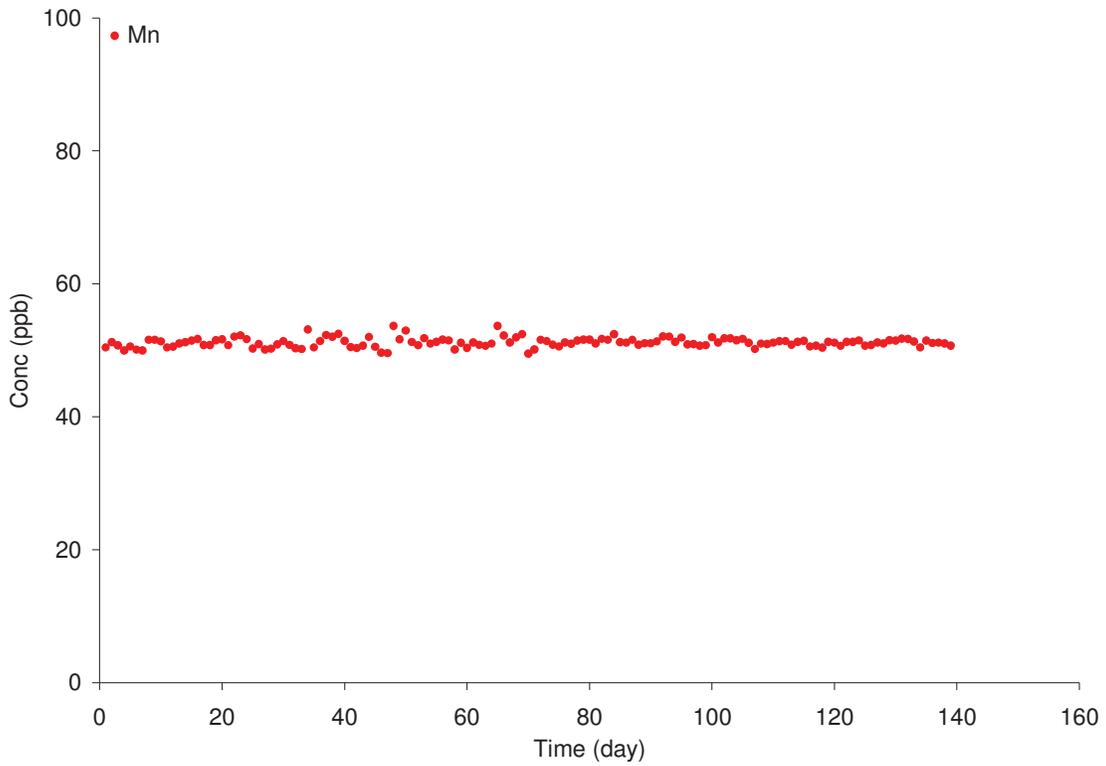
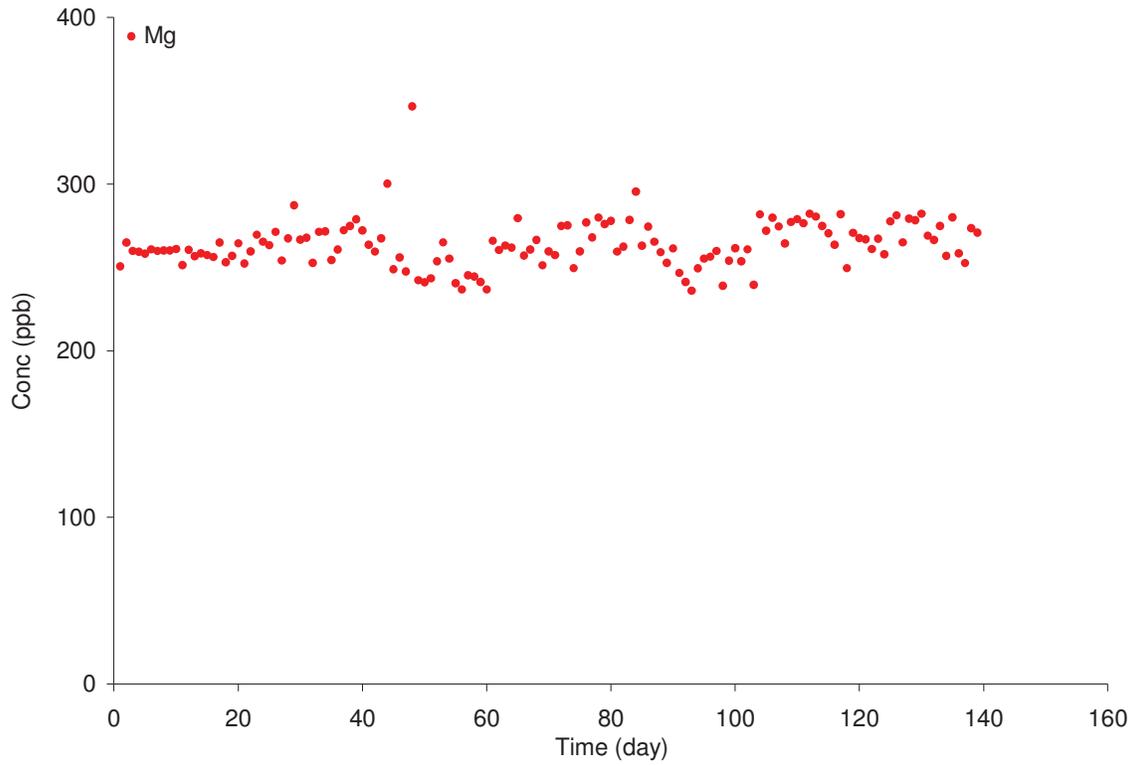
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



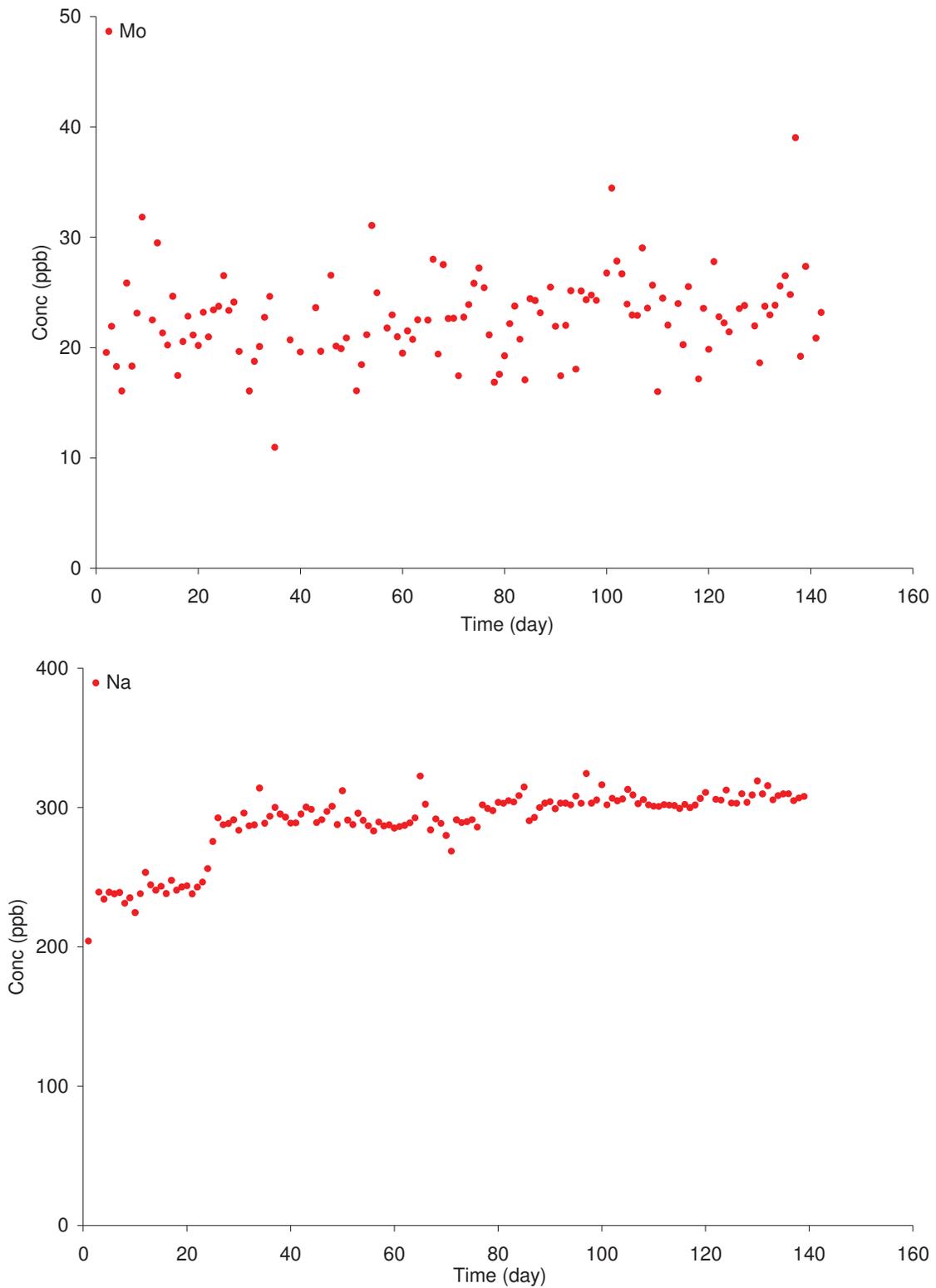
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



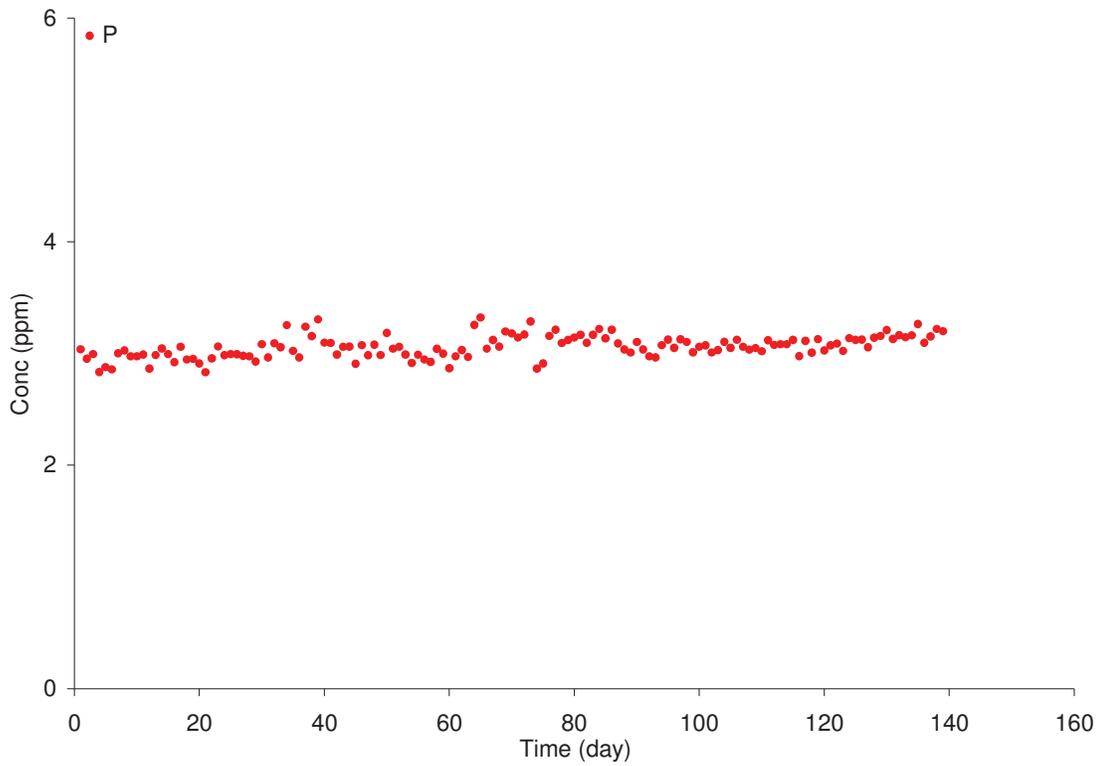
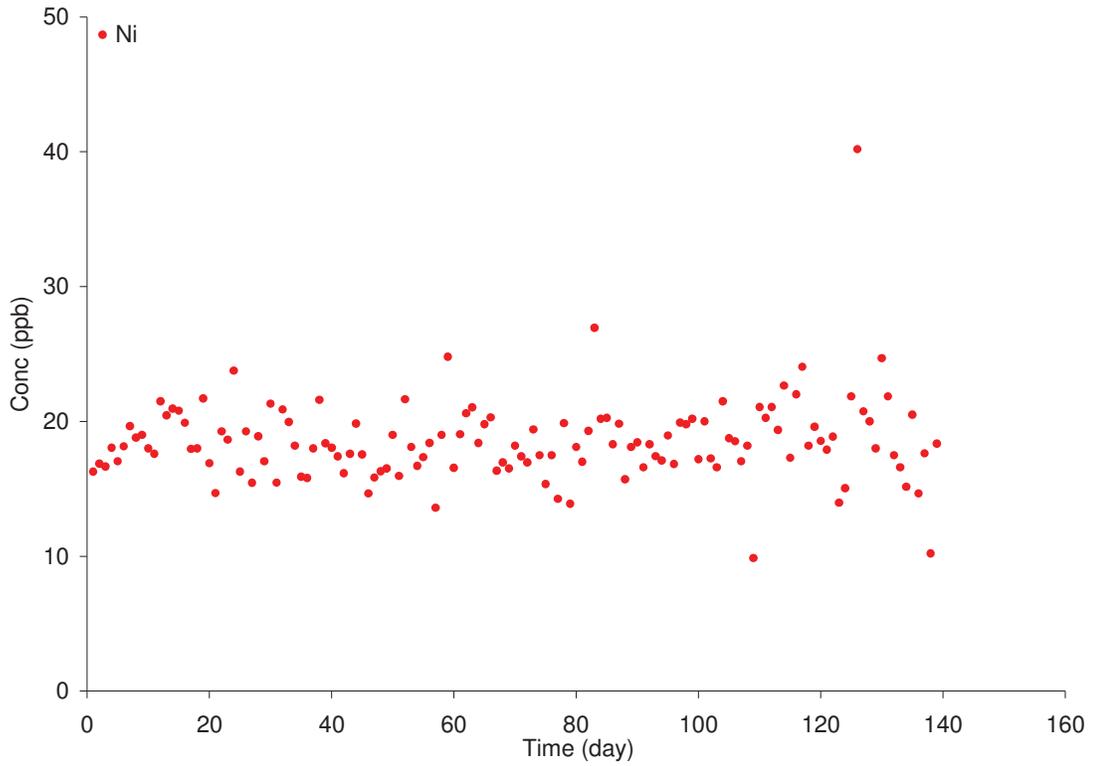
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



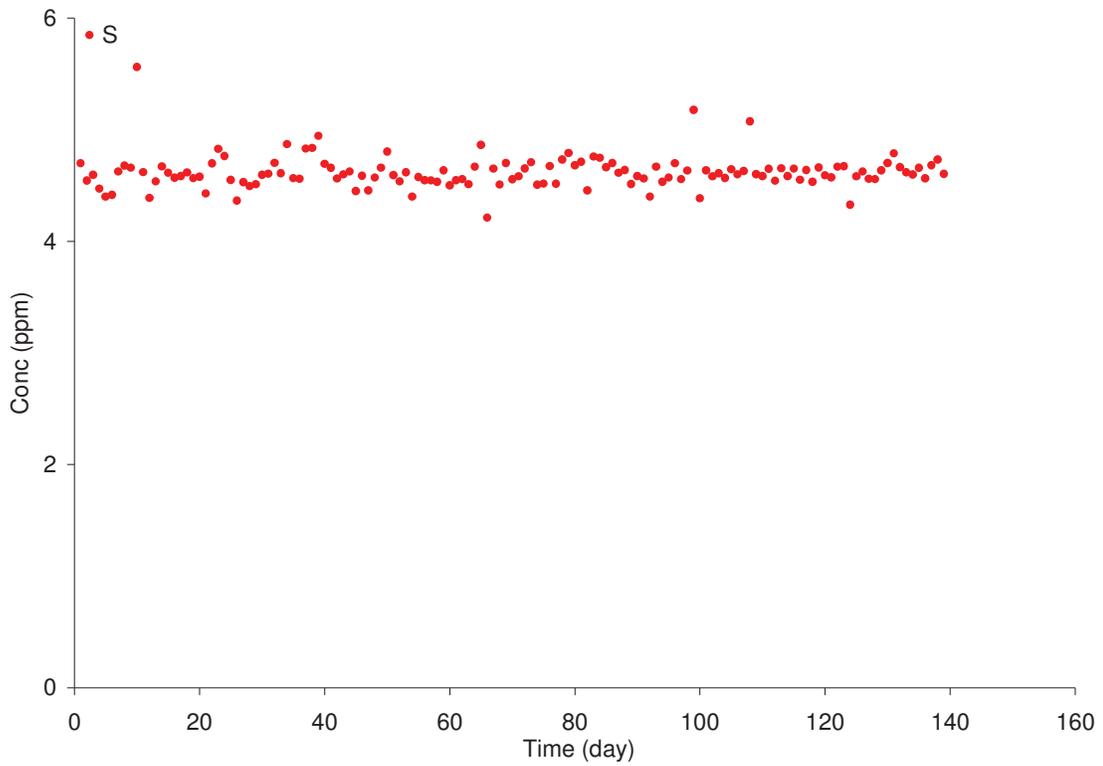
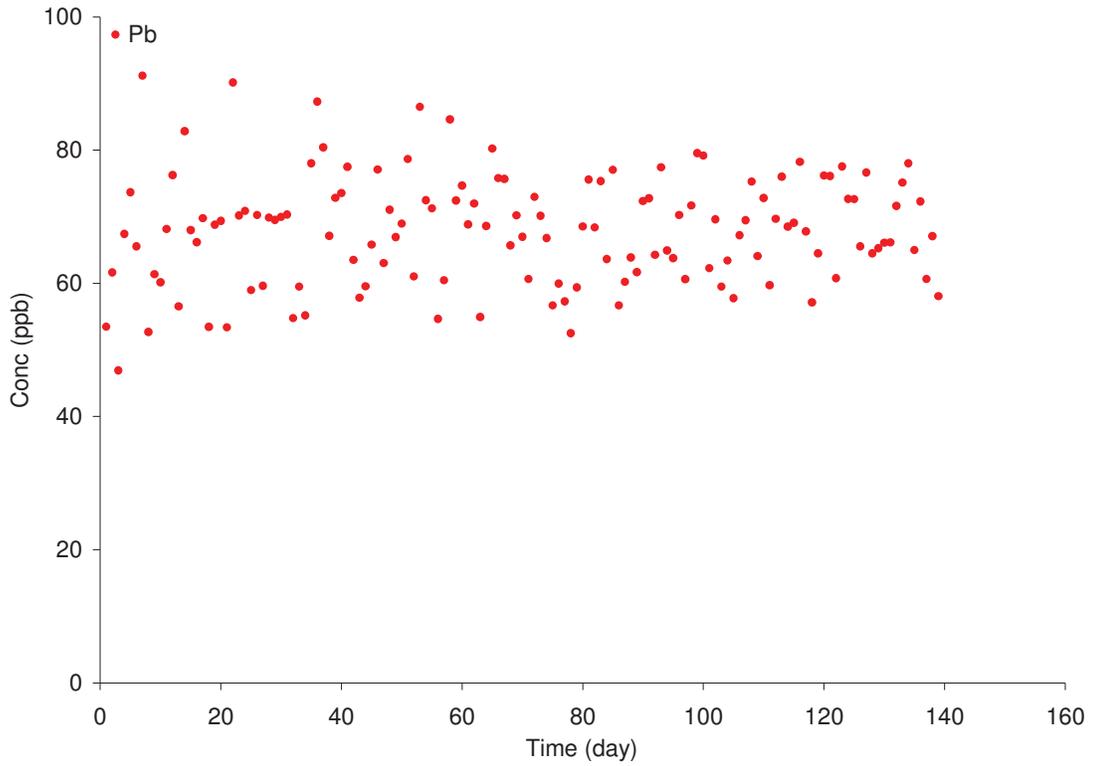
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



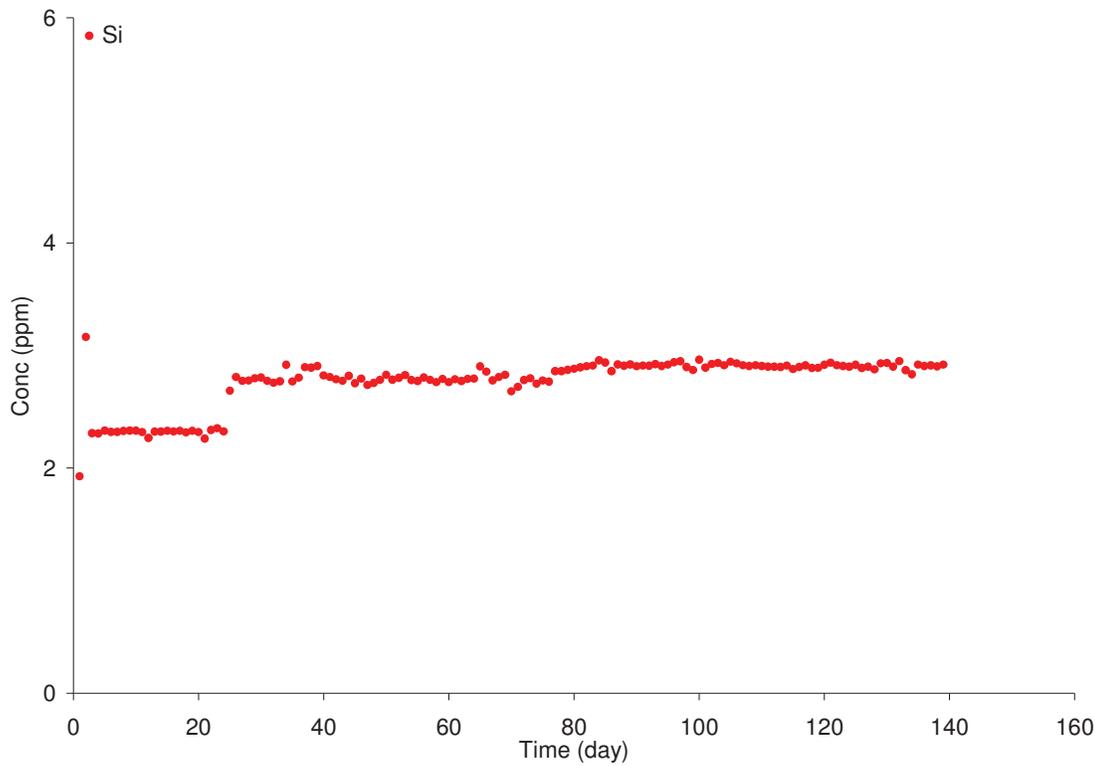
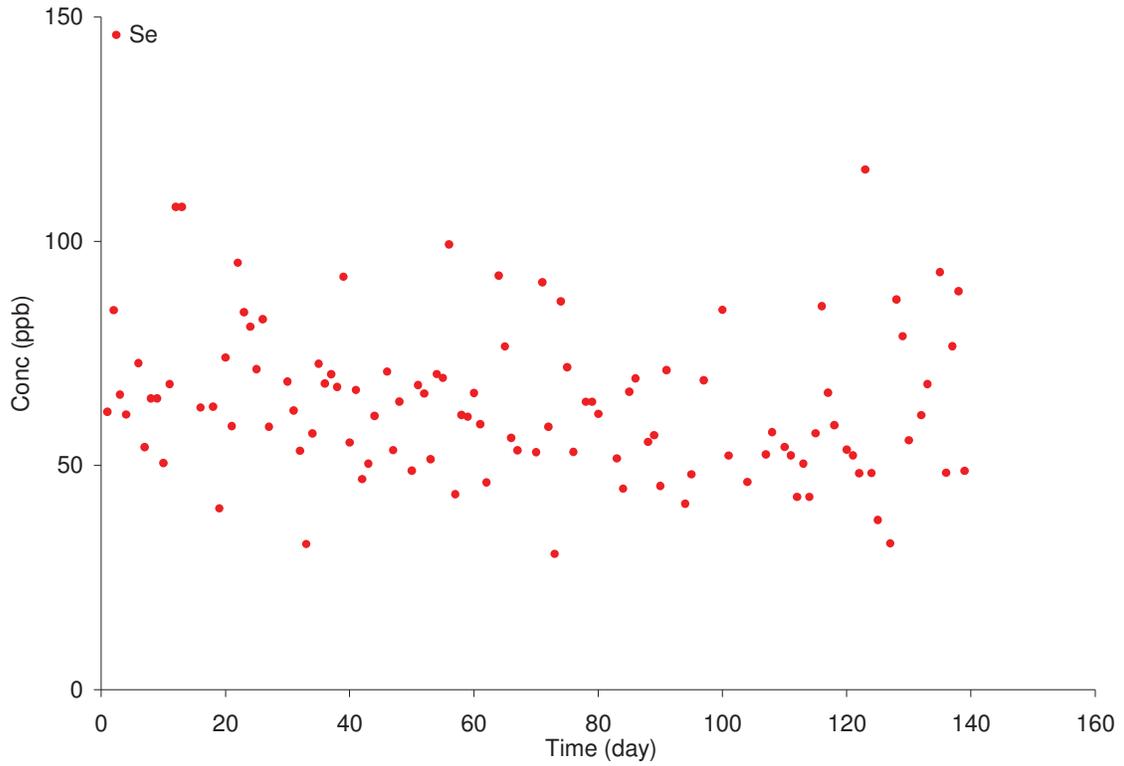
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



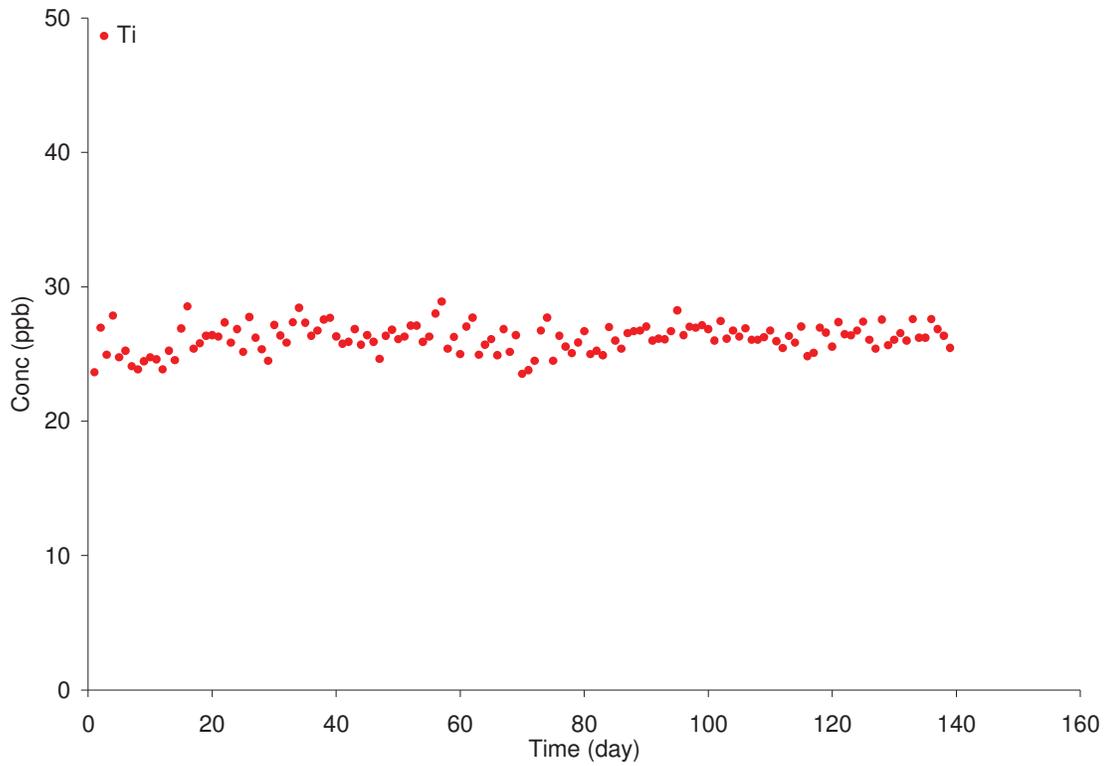
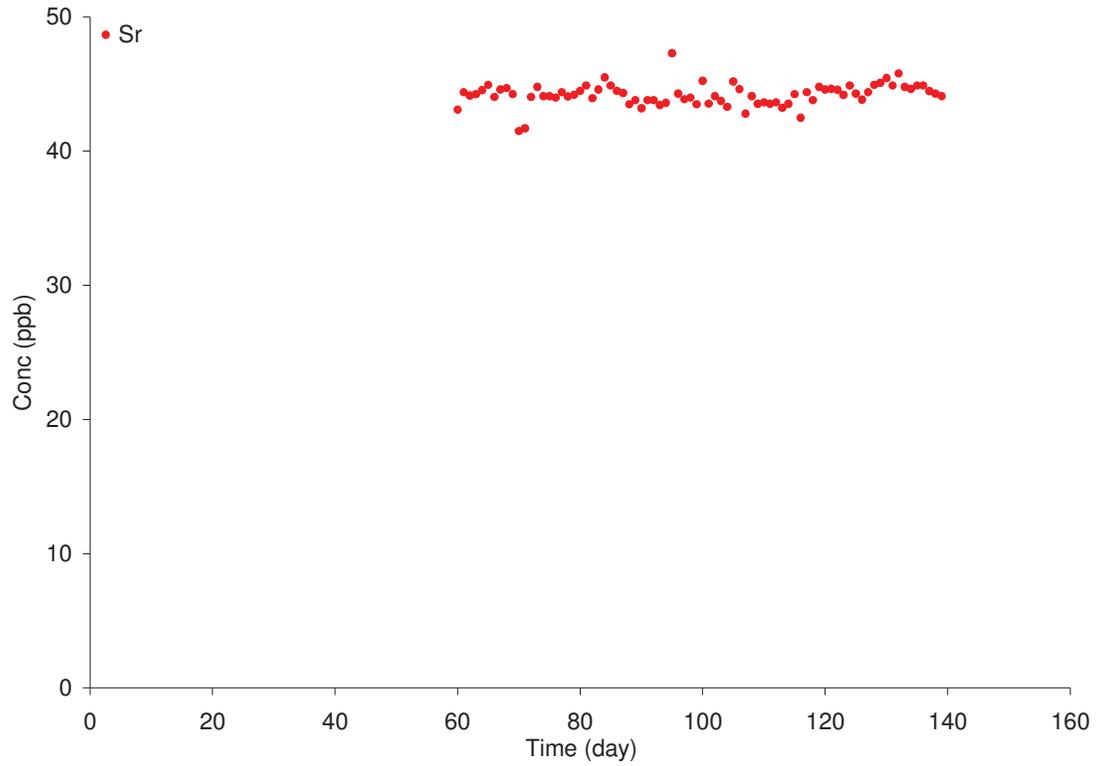
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



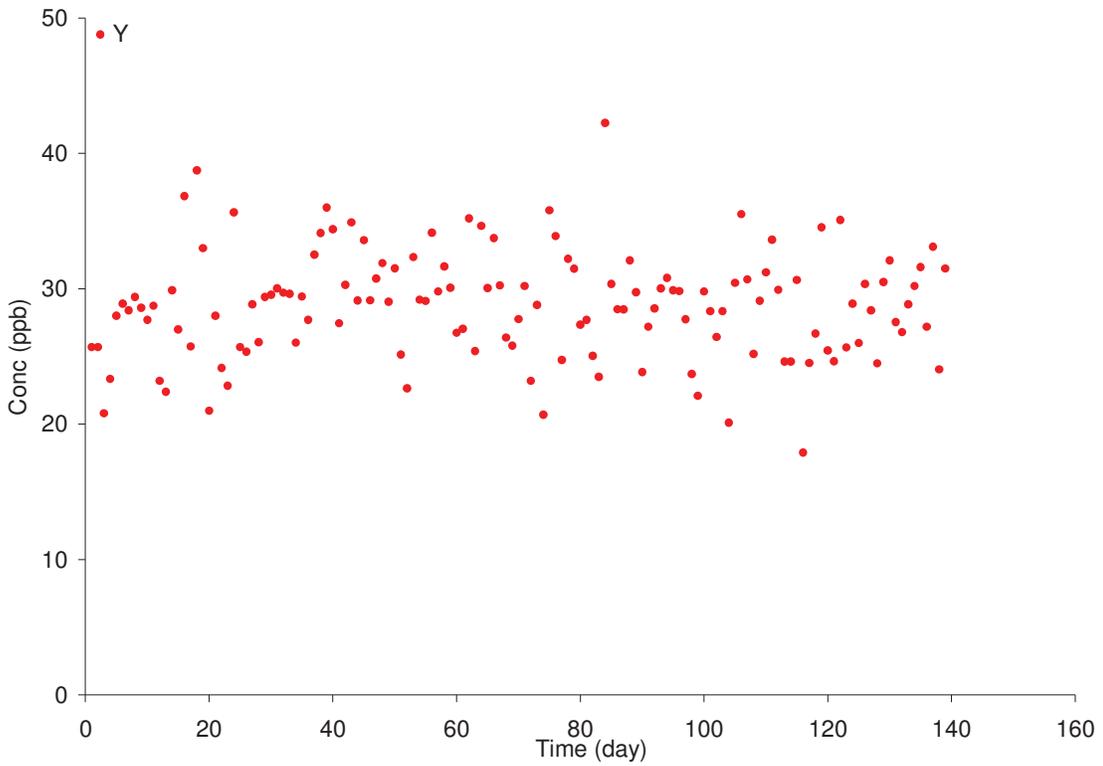
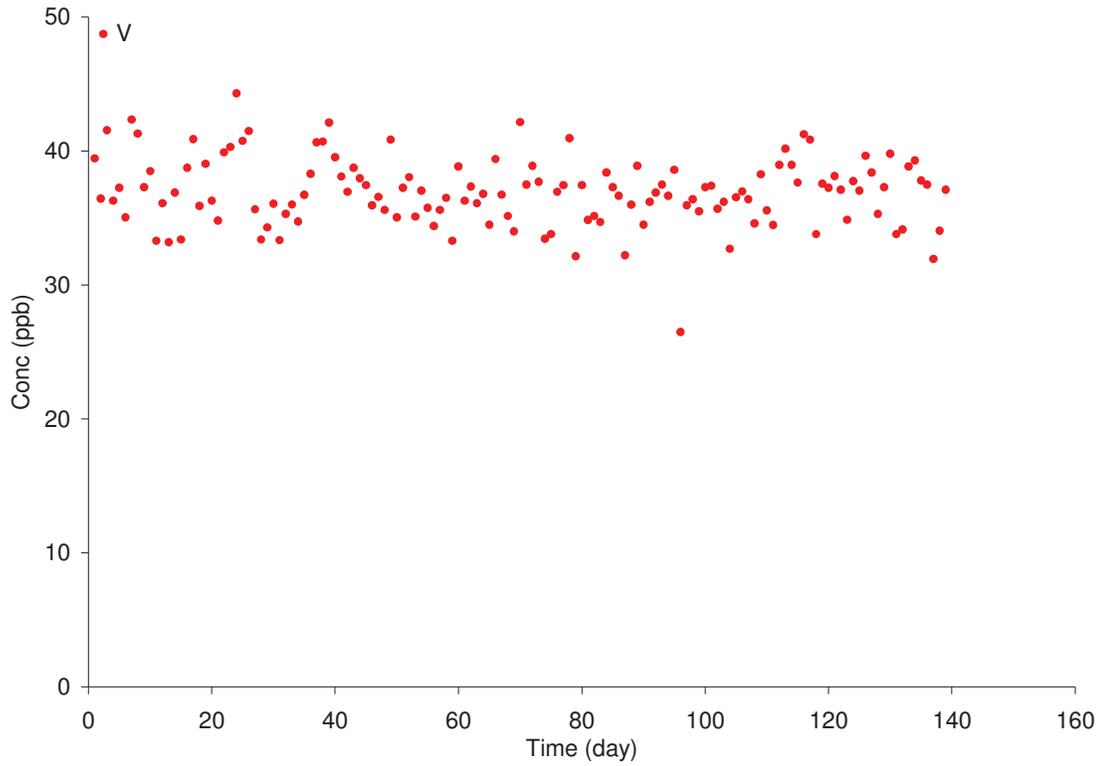
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



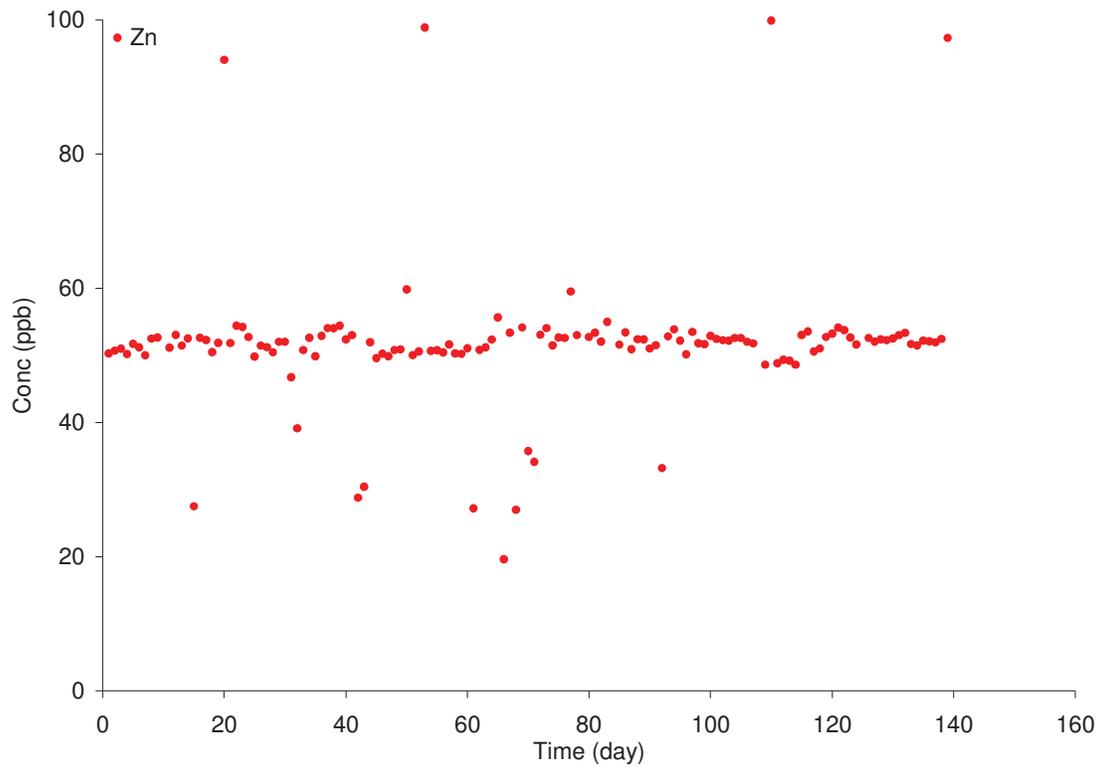
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



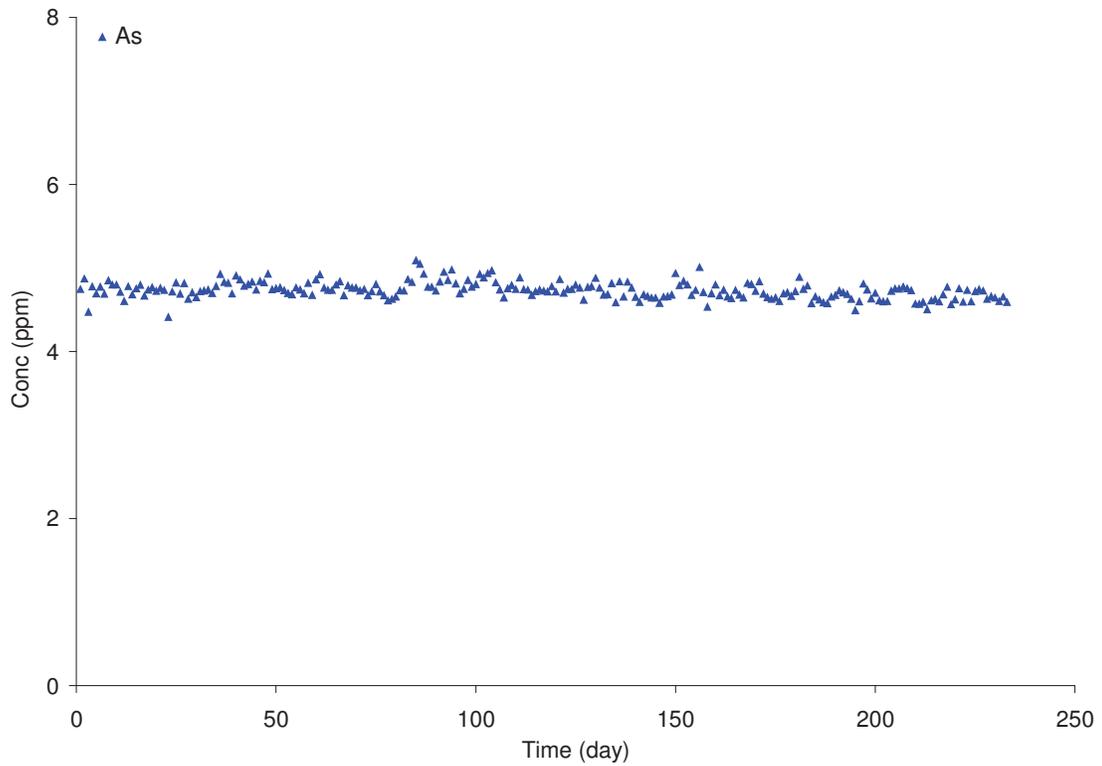
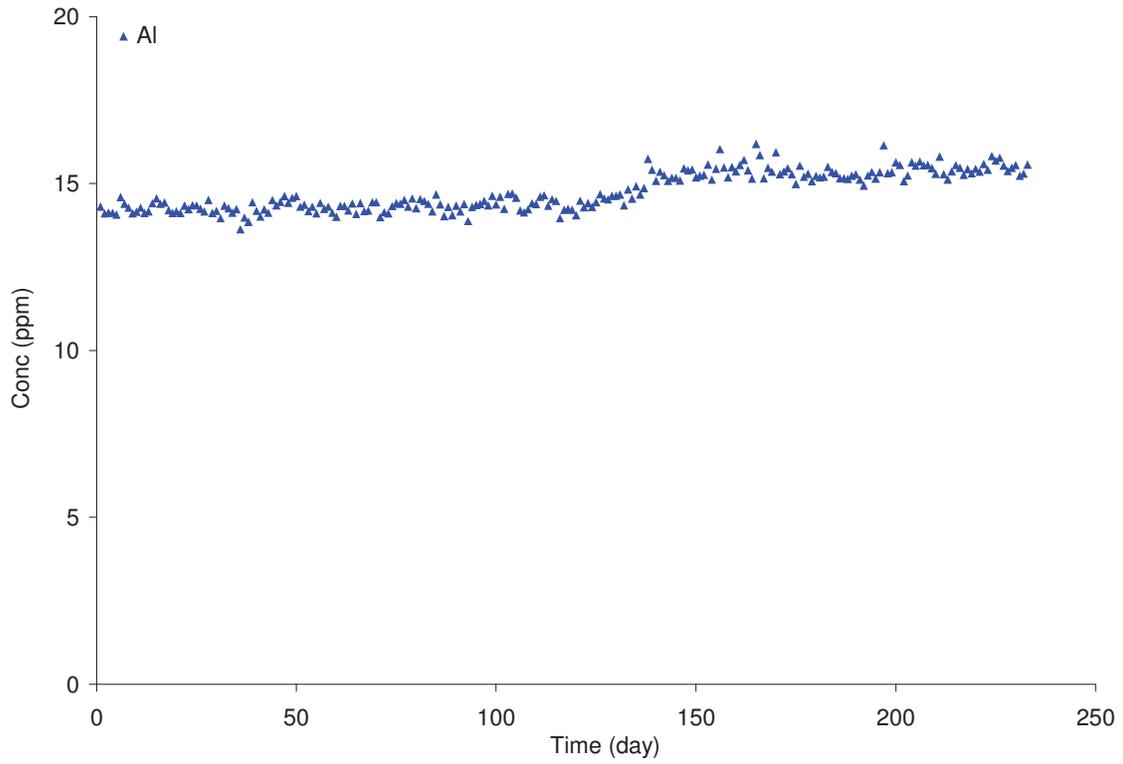
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-1: Analysis of elements at ppb level by TJA Iris ICP-OES (cont'd)



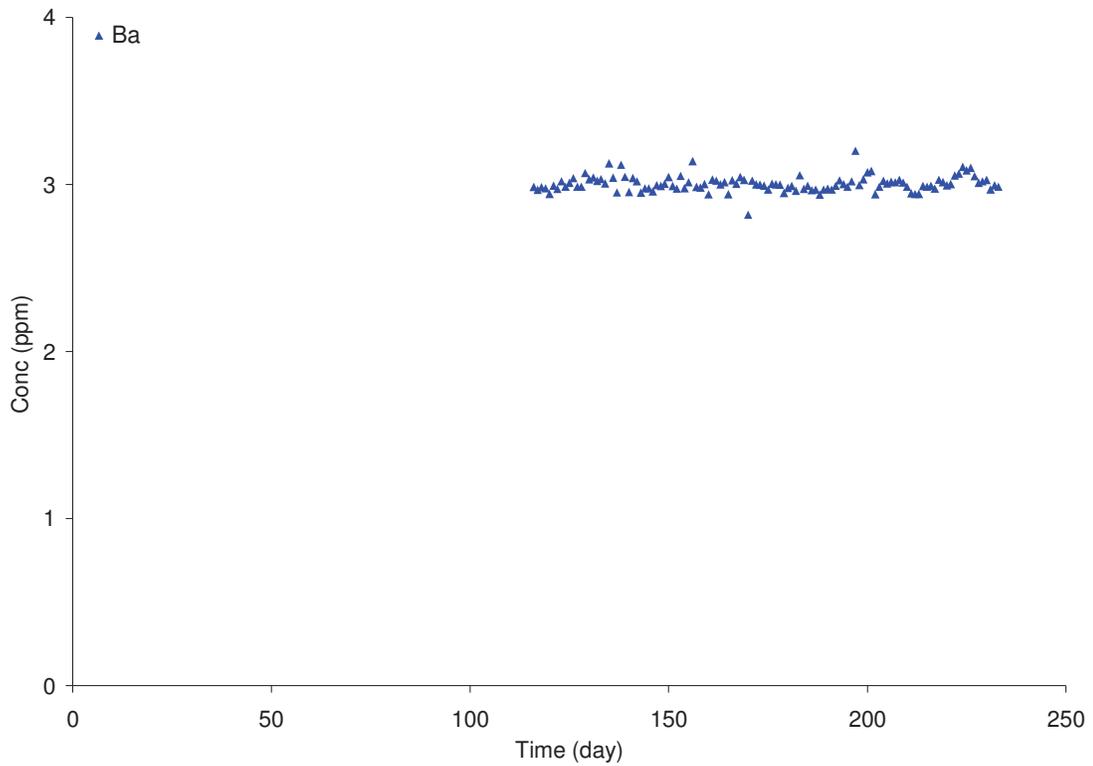
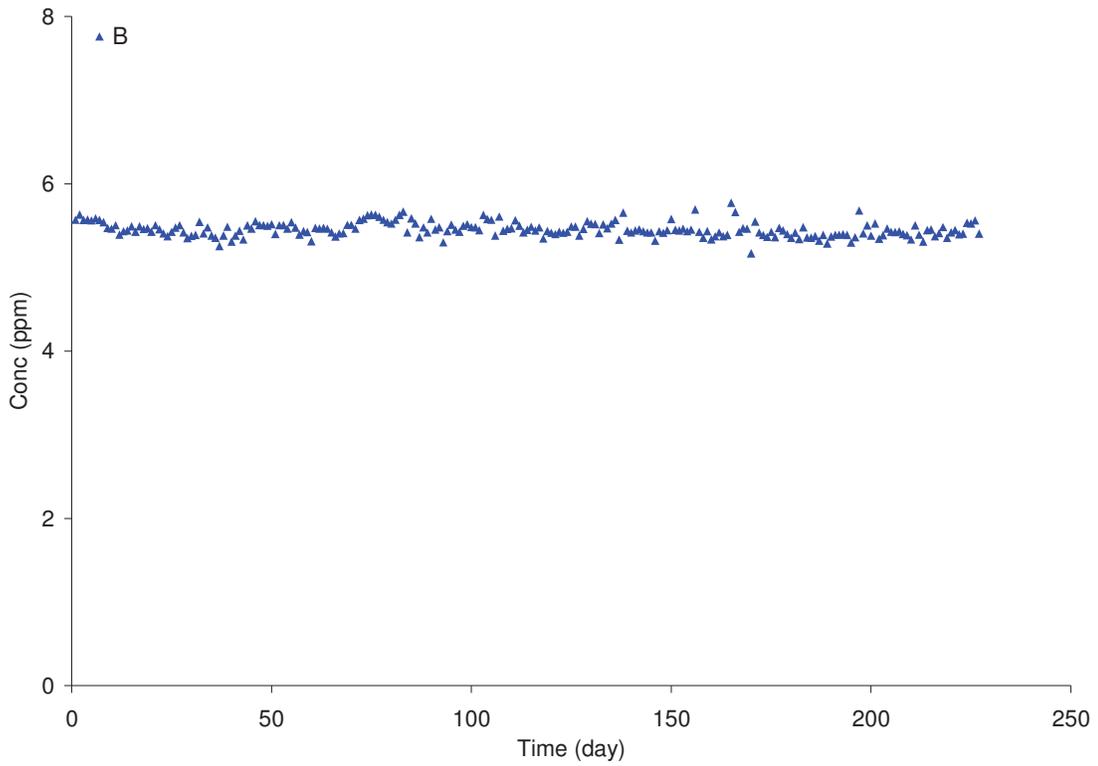
First day: 07/15/04. Day 25: Bottle switch. Day 94: Standard switch. Last day: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES



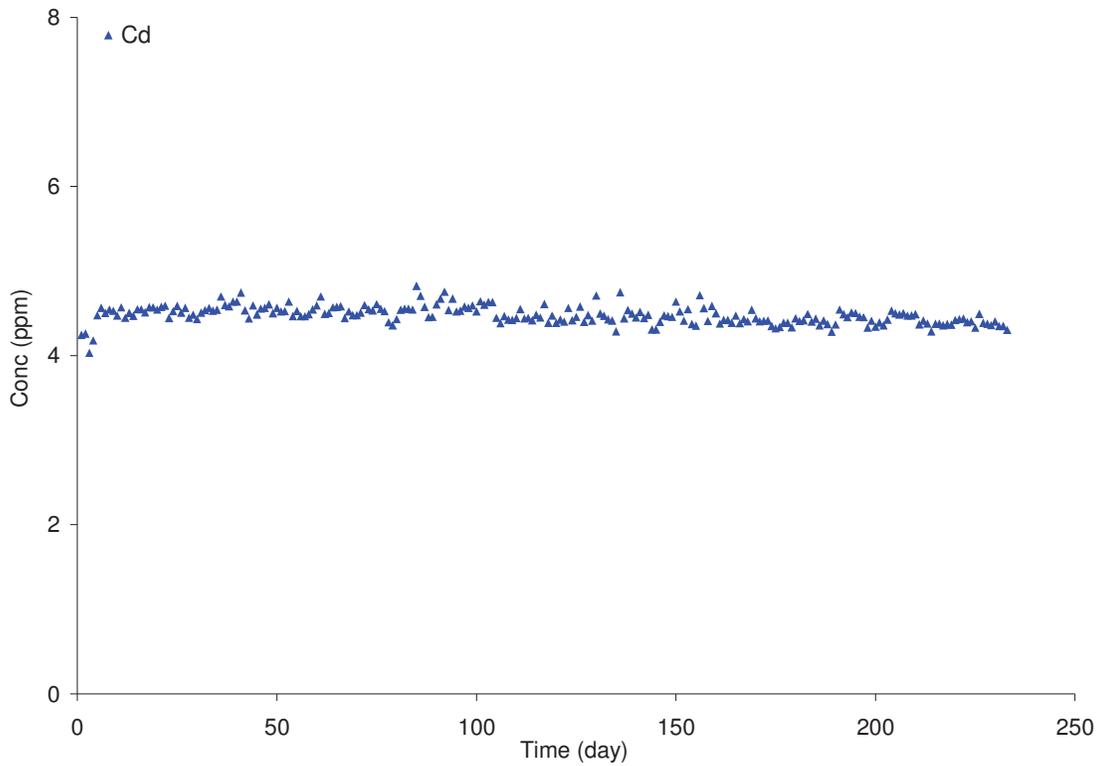
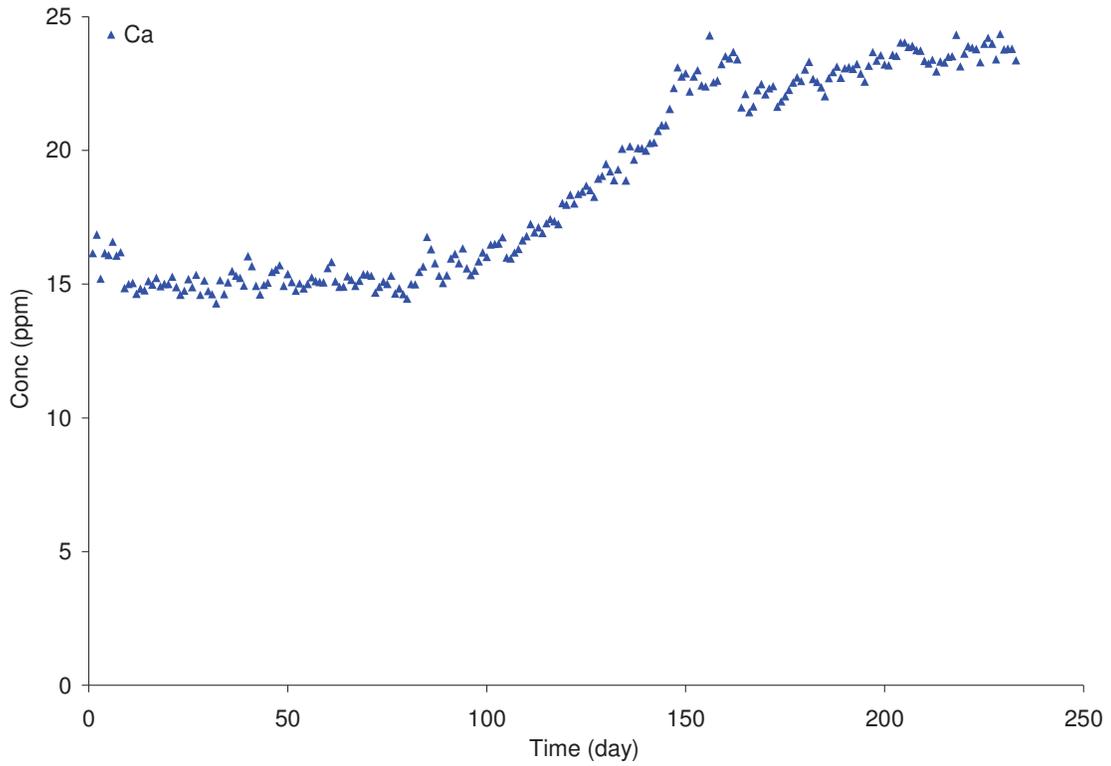
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



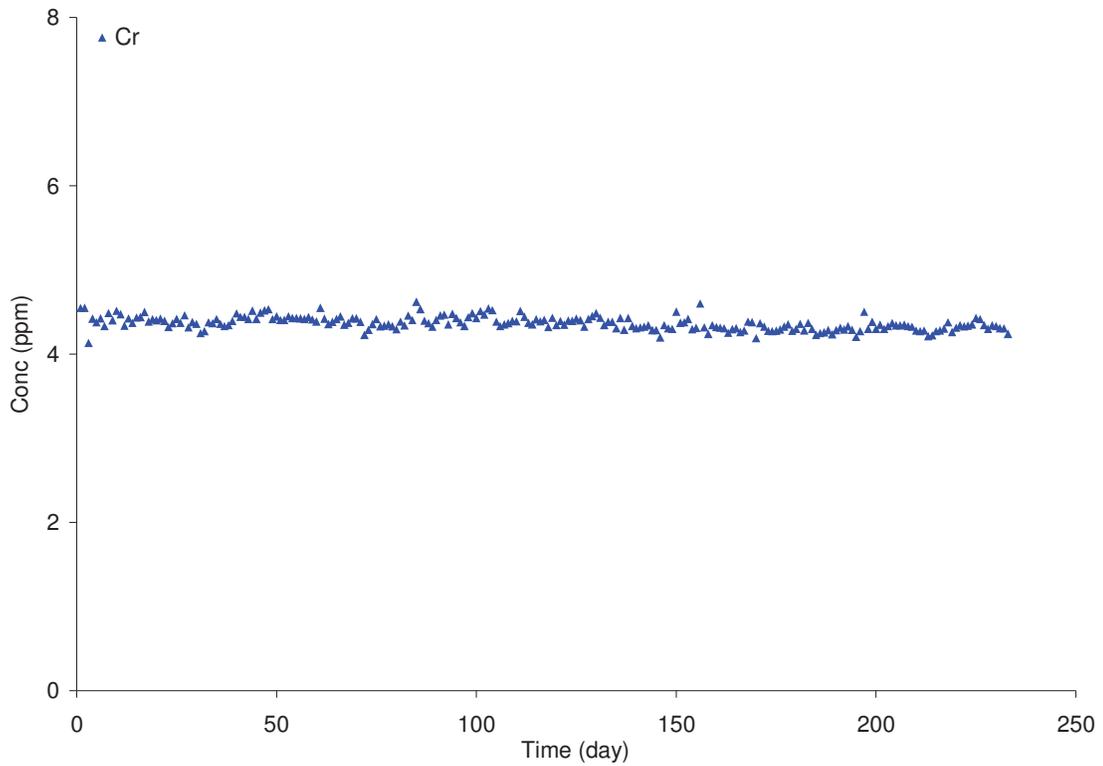
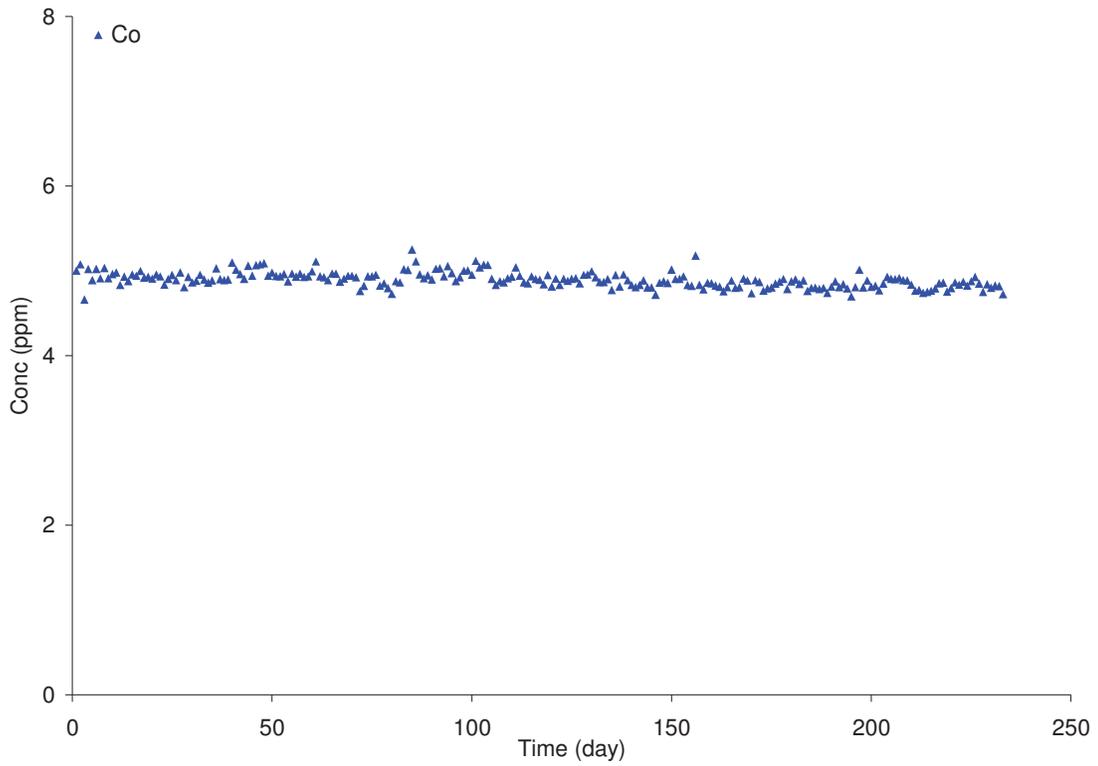
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



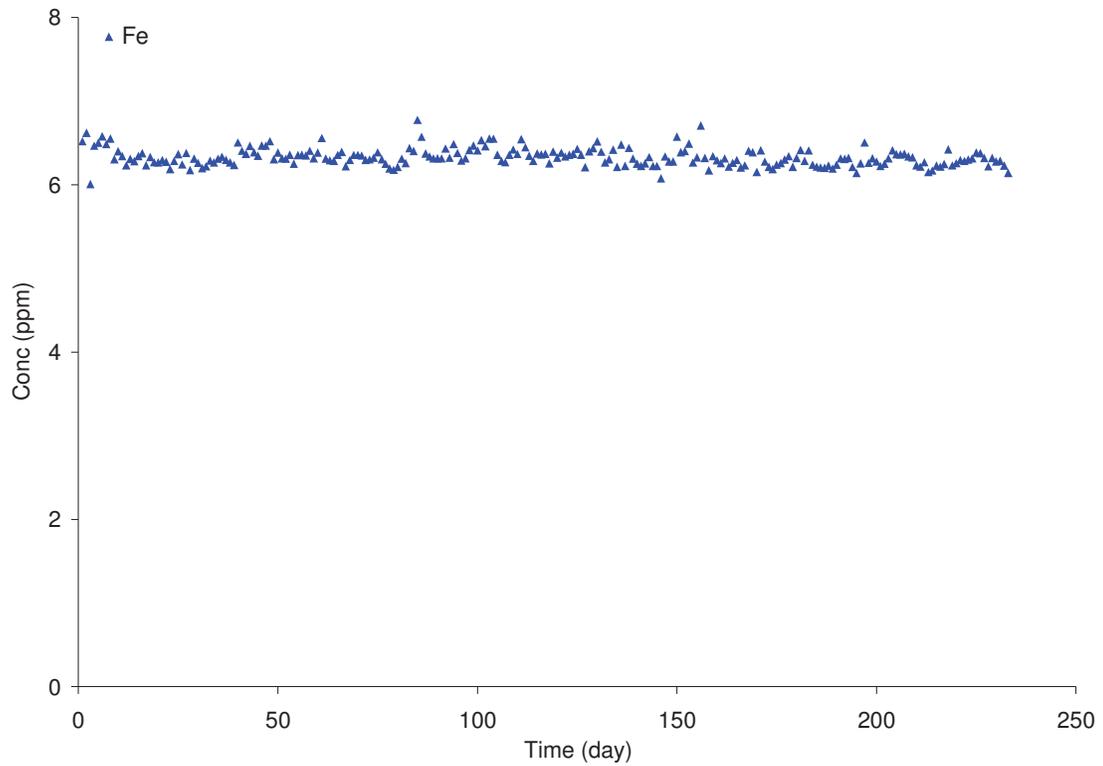
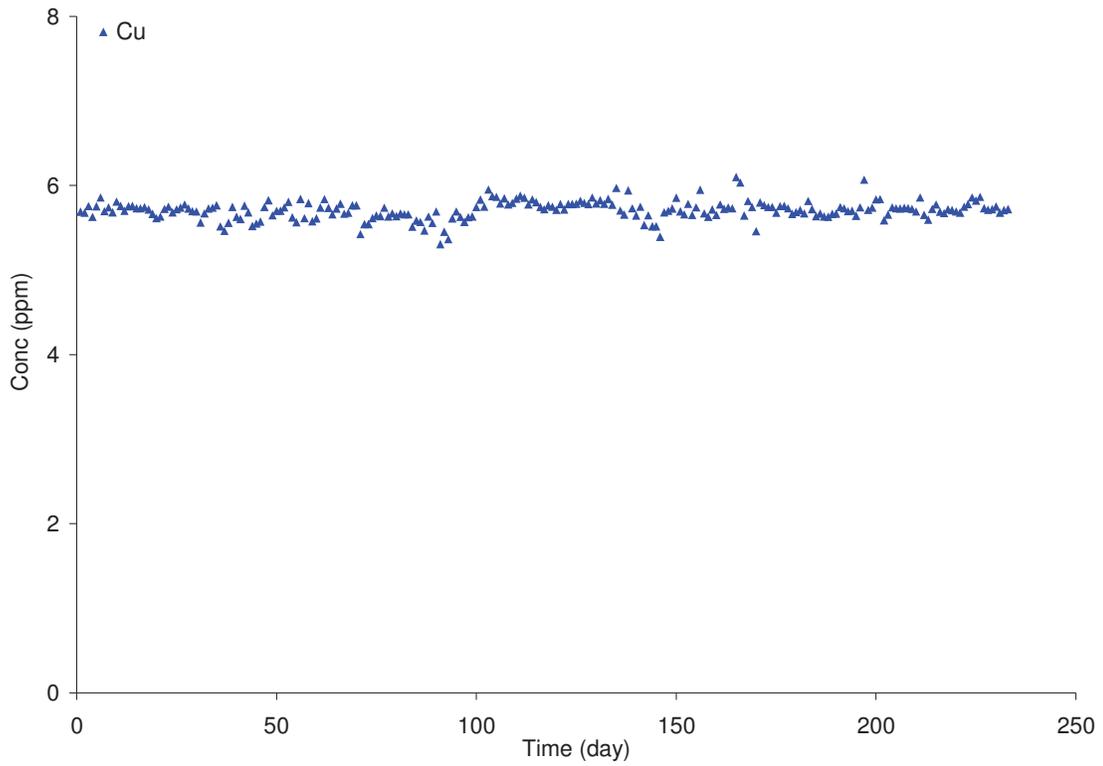
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



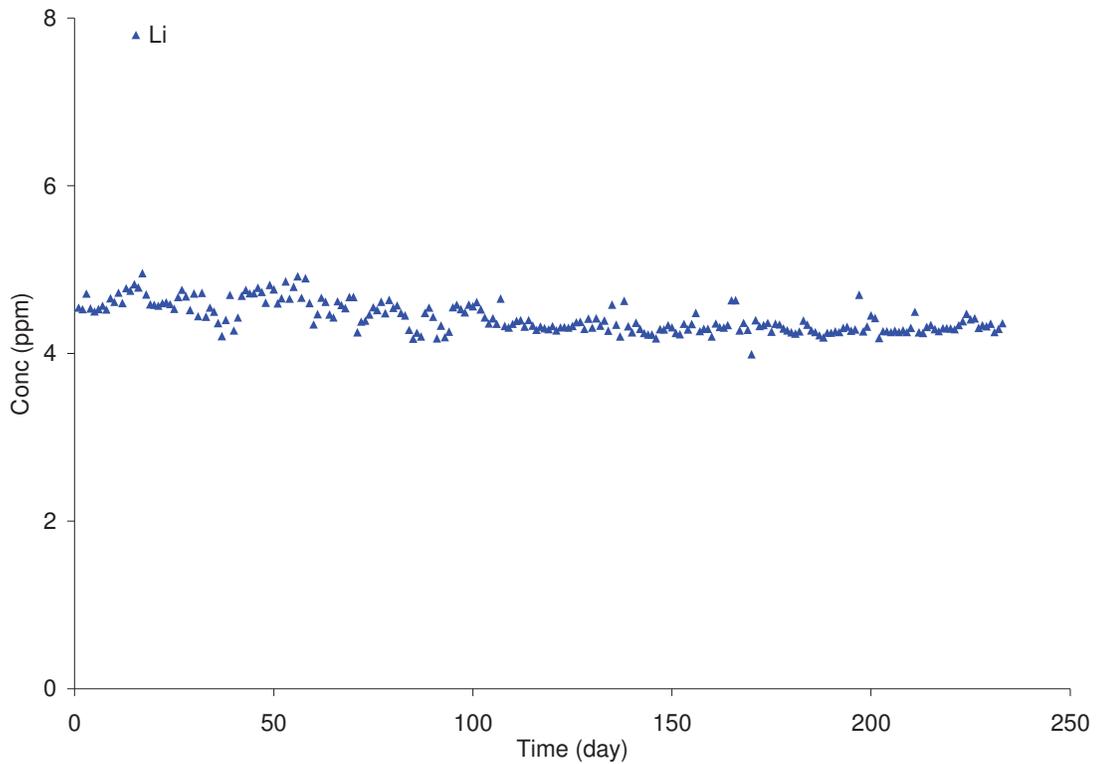
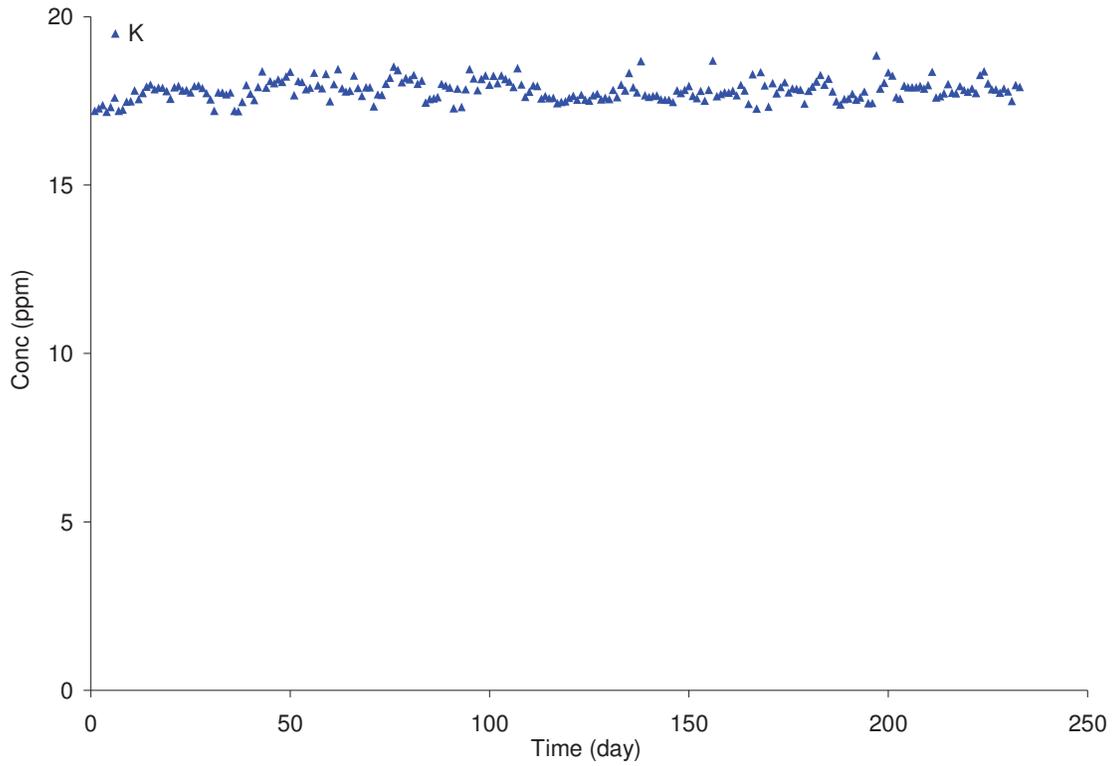
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



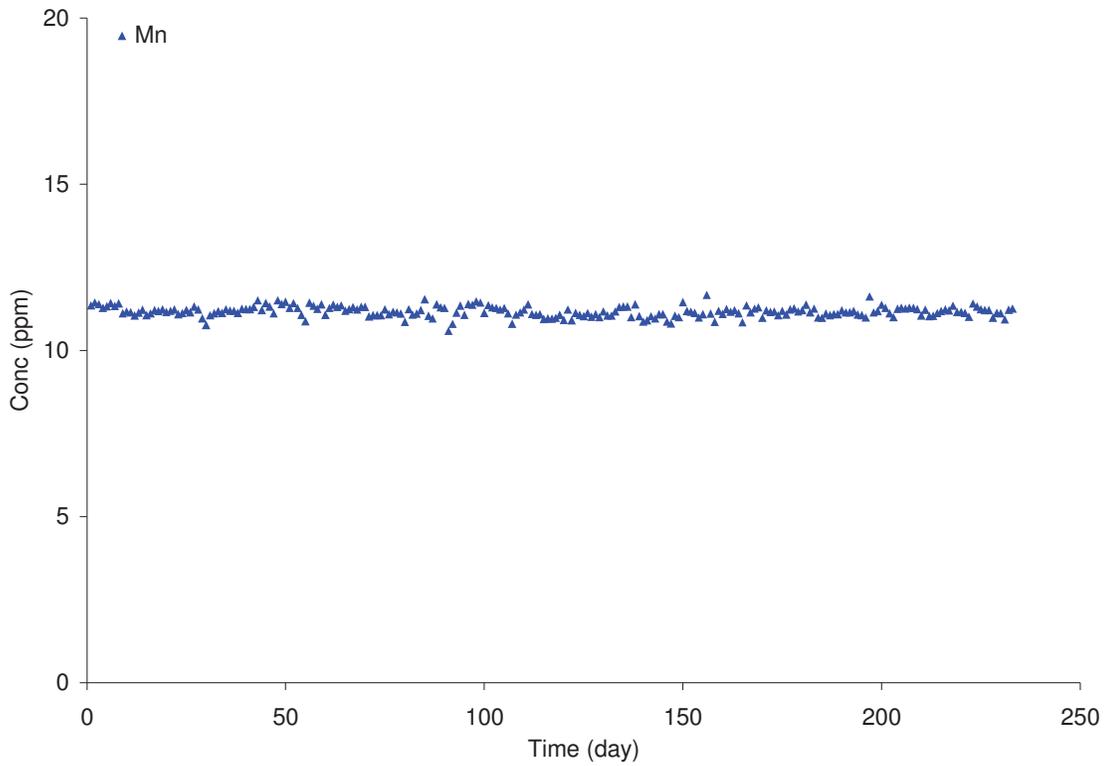
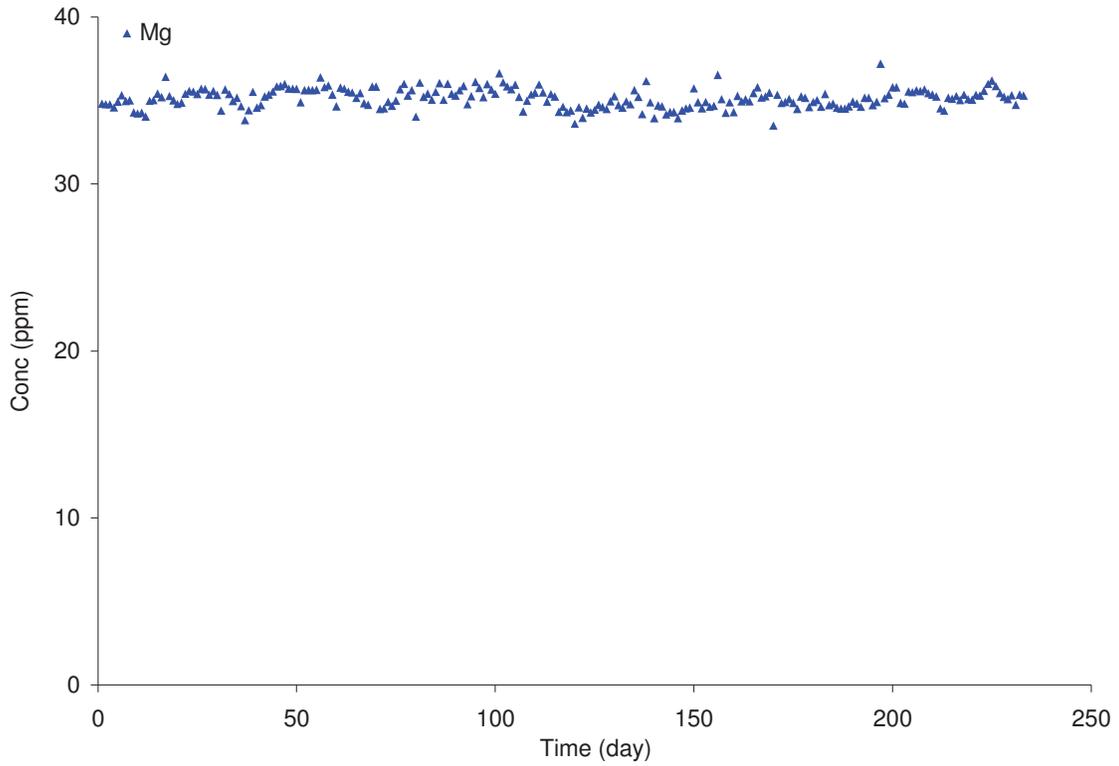
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



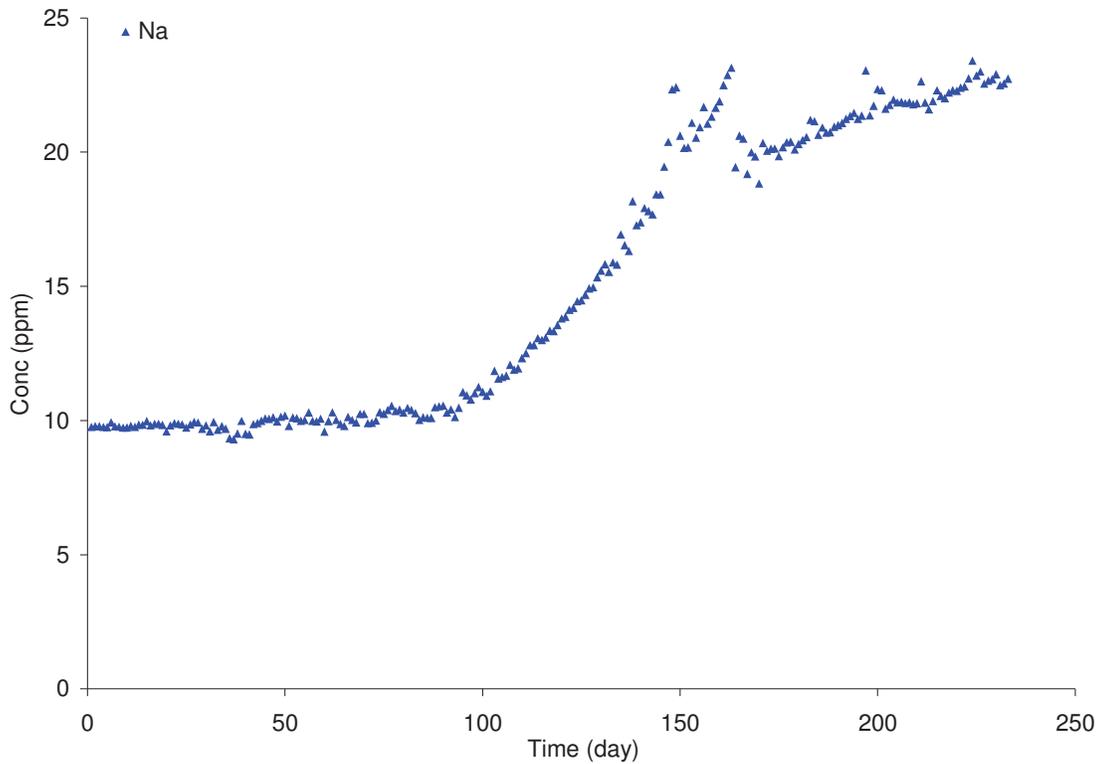
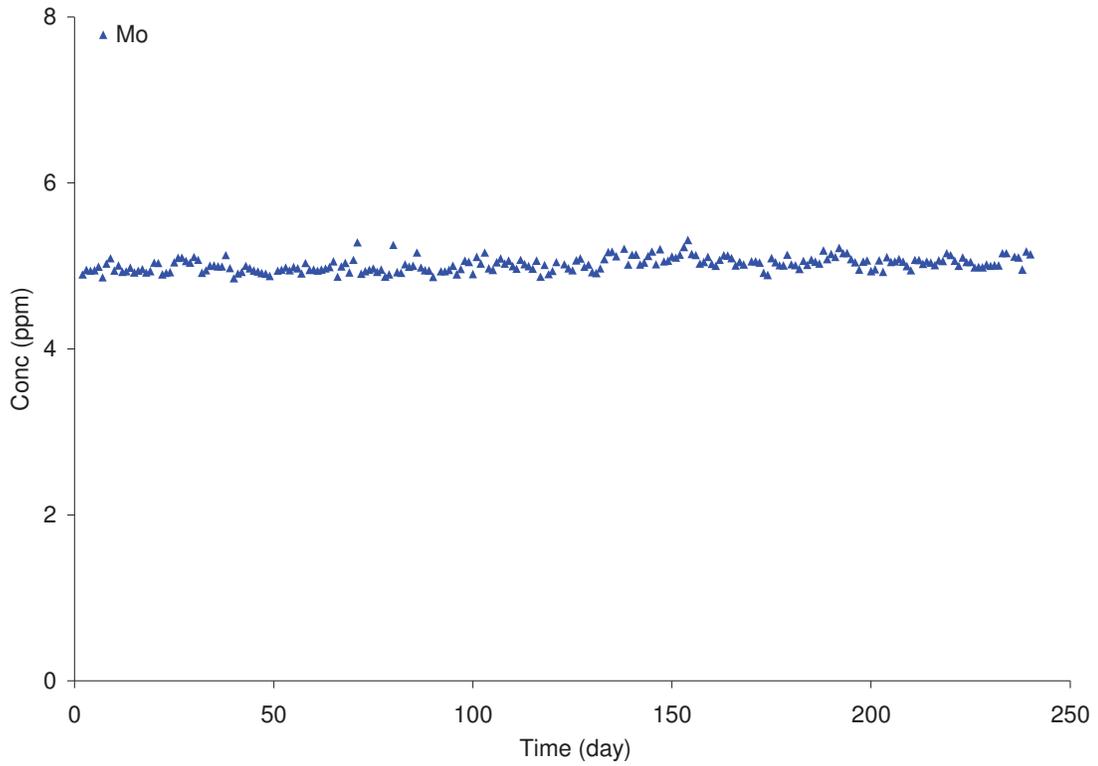
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



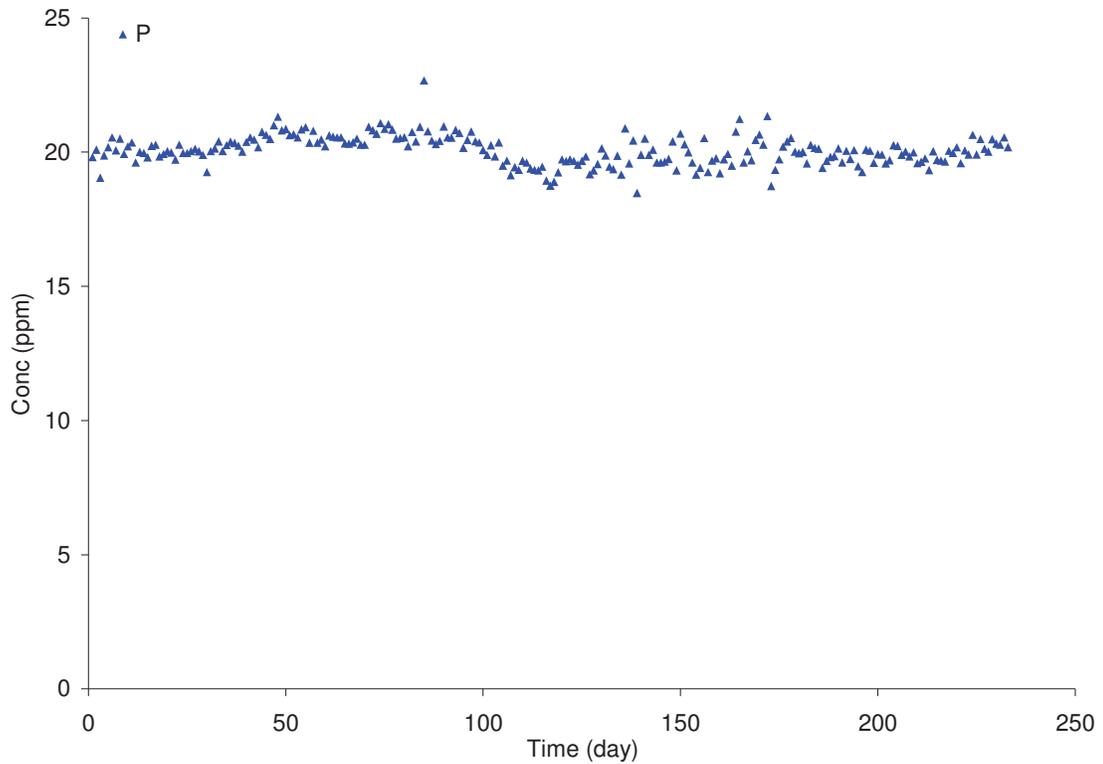
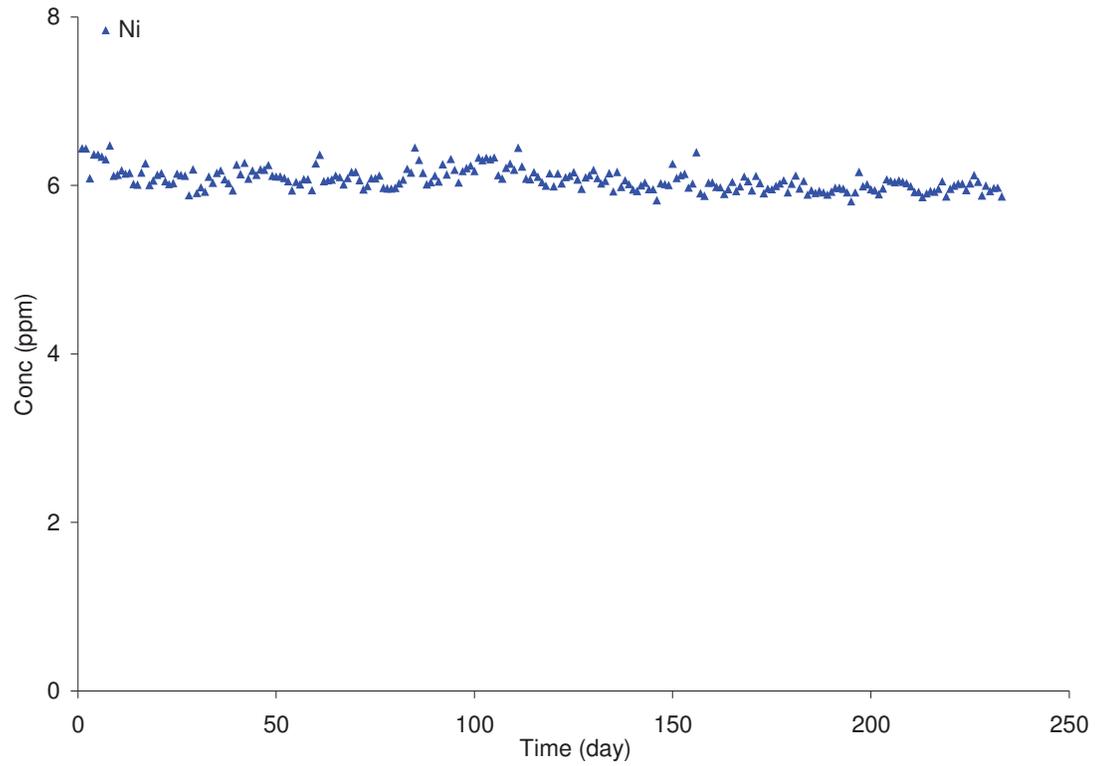
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



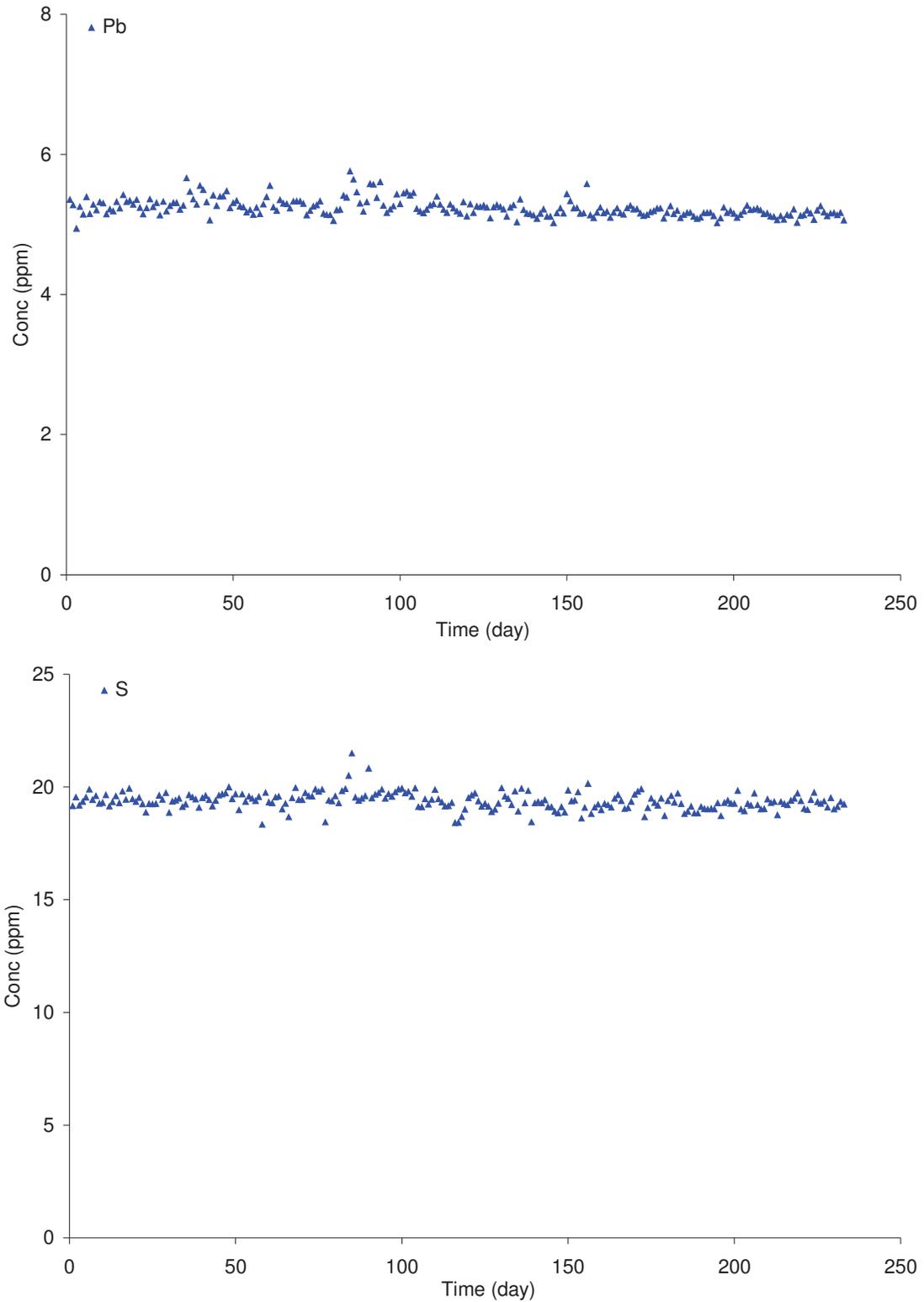
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

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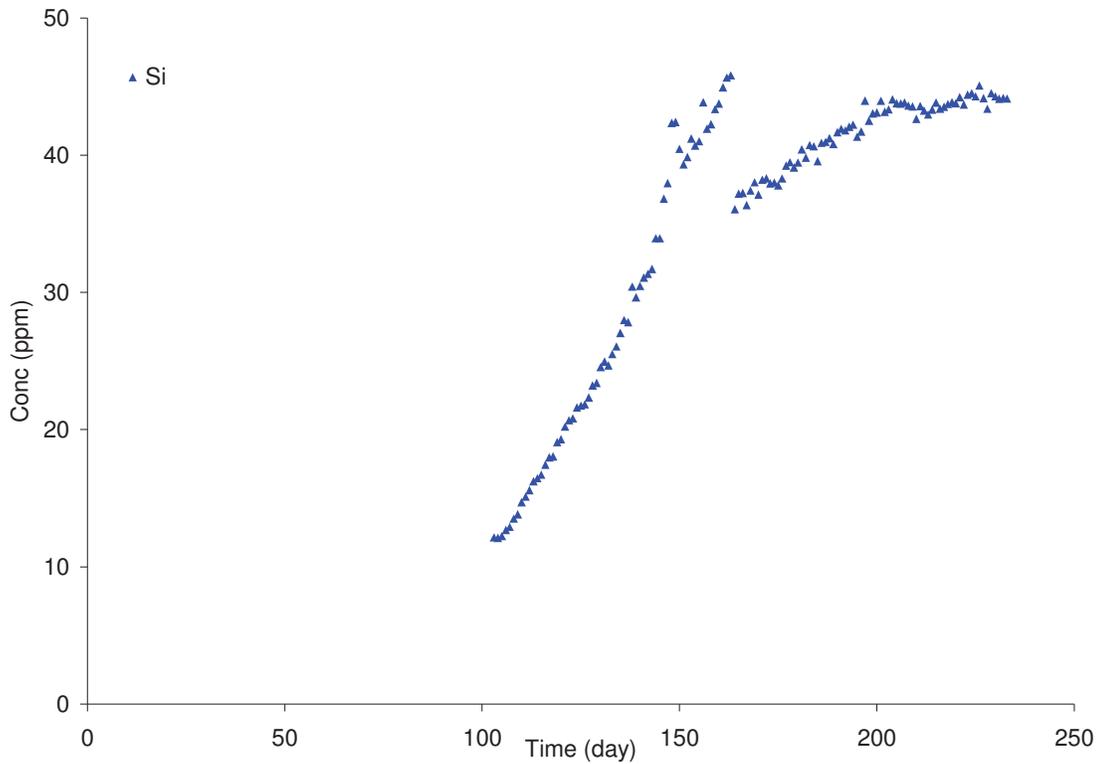
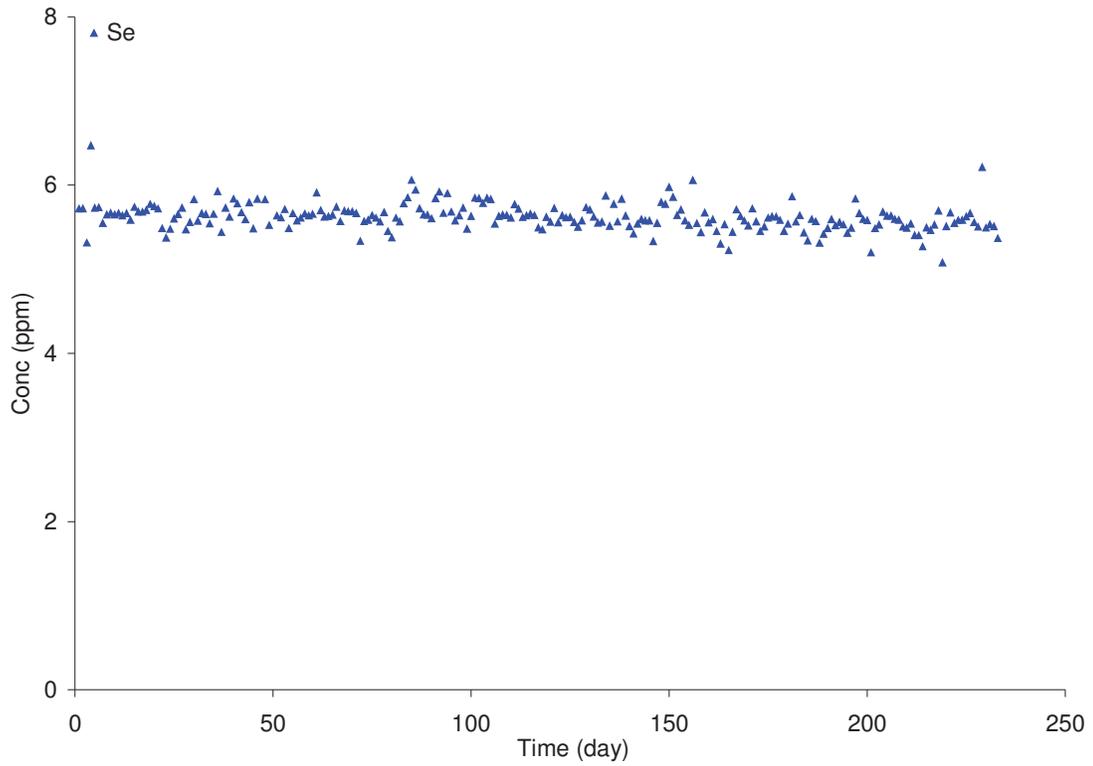
Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



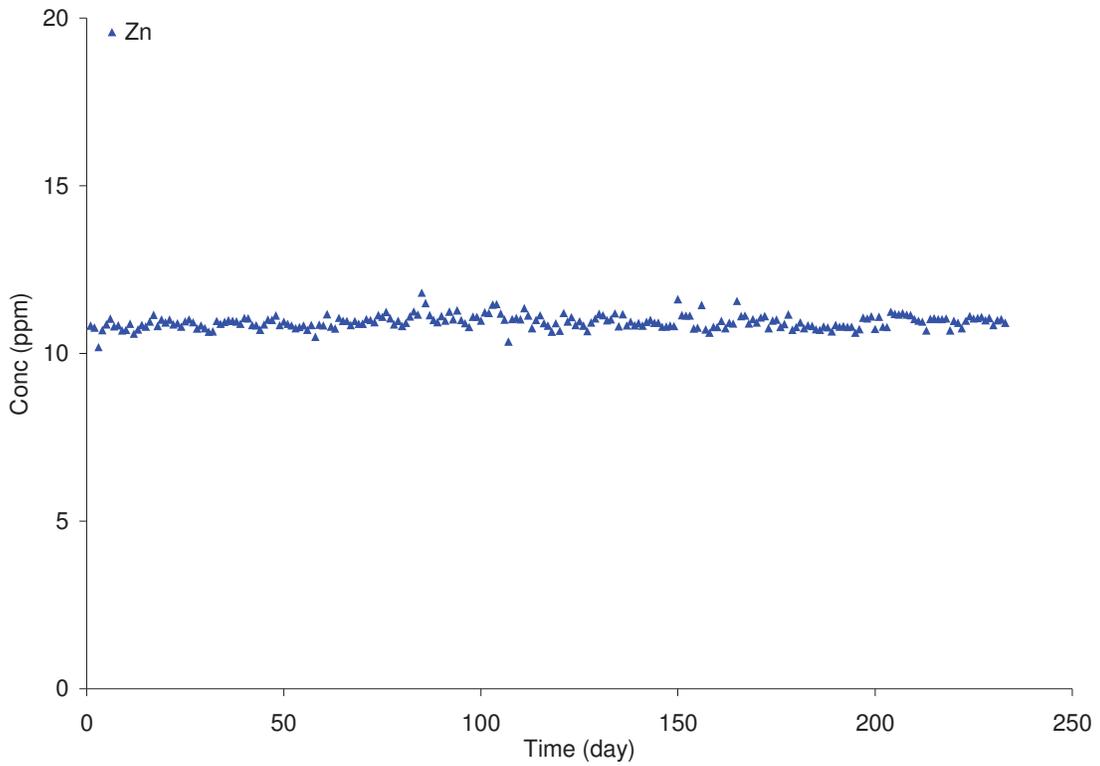
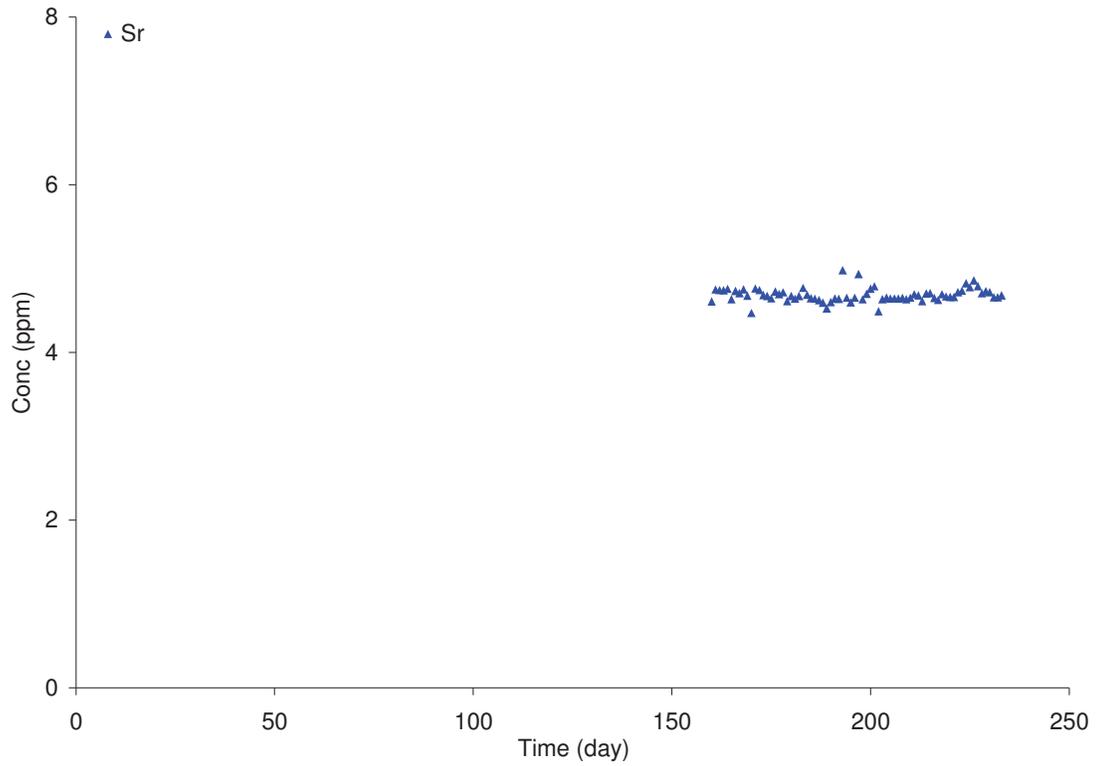
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Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Figure A3-2: Analysis of elements at ppm level by TJA Iris ICP-OES (con'd)



Day 1: 06/17/03. Day 6: new standard. Day 71: Lab moved. Day 102: new standard. Day 116: new bottle. Day 164: new bottle. Day 192: new standard. Day 233: 12/01/05.

Chloride (Soil, Tissue, and Water)

1. Application

This automated method is applicable to soil, tissue, waters (drinking, surface and saline waters), and domestic and industrial wastes.

2. Summary of Methods

Chloride is determined by an automatic chloride titrator (Digital chloridometer) by coulometric-amperometric titration with silver ions. In the chloride titrator, a constant direct current is passed between a pair of silver electrodes, causing release of silver ions into the titration solution at a constant rate. The silver ions react with chloride to precipitate ($\text{Ag}^+ + \text{Cl}^- \implies \text{AgCl}$). The end-point is after the increasing concentration of free silver ions cause a rising current to flow through a pair of silver indicator electrodes connected to a Meter-Relay. At a preset increment of indicator current the relay is actuated, stopping a timer, which runs concurrently with generation of silver ion. The amount of chloride precipitated is proportional to the elapsed time.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

- 4.1 Chloride from skin may contaminate samples, rubber gloves are necessary during sample handling.
- 4.2 Washed filters must be used when filtering samples for chlorides. Unwashed filters contain varying amounts of chloride and will affect results.
- 4.3 Iodine, bromide, ferricyanide and ferric iron cause high results and must be removed. Chromate and dichromate should be reduced to chromic state or removed where contamination is minor. Some contaminants can be destroyed by adding nitric acid.

5. Sample Collection, Preservation and Handling

Soil and tissue samples are oven-dried at 55°C and ground to pass a 2-mm screen and stored in paper boxes (soil sample) or plastic bottles (for plant tissues). Water samples are stored at 4°C until analysis. Water samples should be completed within 28 days of the date of sampling.

6. Apparatus and Materials

- 6.1 Digital Chloridometer (LabConCo model # 442-5000)
- 6.2 Erlenmeyer flasks (125 ml)
- 6.3 Funnel tubes (15 ml)
- 6.4 Acid washed filter paper (9 cm Whatman No. 2 or equivalent).
- 6.5 Time-controlled oscillating shaker (Eberbach) set at 160 excursions per minute.
- 6.6 Glass vials (5 ml)
- 6.7 Disposable examination gloves
- 6.8 Balance – capable of 0.01 g

7. Reagents

- 7.1 Deionized water
- 7.2 Concentrated standard solution 0.1 N HNO₃ 10% CH₃COOH solution
- 7.3 1:1 glacial acetic acid / deionized water
- 7.4 Nitric-acid reagent (for tissue only) (0.1 N HNO₃ and 10% glacial acetic acid): To 900 ml of water, add 6.4 ml of concentrated nitric acid and 100 ml of glacial acetic acid. Volumes are approximate. (Acids should be reagent grade). Mix thoroughly.
- 7.5 Gelatin reagent (to 100ml of hot deionized water add 0.62 g of gelatin mixture (LabConCo gelatin reagent #442-5064), mix until dissolved. Reagent is good for six months if kept refrigerated)
- 7.6 Standard Chloride solution (0.0141 N NaCl). Dissolve 0.8241 g pre-dried (140°C) NaCl in deionized water, dilute to 1 liter (1 ml = 0.5 mg Cl)
- 7.7 Reference solution (Environmental Resource Associates) Lot No. 99101

8. Methods

- 8.1 Weigh out 0.25-0.50 g (plant tissue), 0.4-0.5 g (for sandy soil) or 0.2-0.3 g (for silt, clay or muck soil) into a 125 Erlenmeyer flask. (For analysis of water, skip to step 8.4)
- 8.2 Add 10 ml of deionized water, plus 1 drop of 1:1 glacial acetic acid mixture to facilitate filtering.
- 8.3 Shake the suspension on an oscillating shaker, at 160 excursions per minute for 20 minutes, then filter. (Water samples high in suspended solids need filtering prior to analysis).
- 8.4 Transfer 3 ml of samples to glass vials, add 1 ml of (7.2) concentrated standard solution and 4 drops of (7.5) gelatin reagent.

8.5 Place glass vial on chloridometer, press start, when timer stops, record meg/L reading.

9. Calculations

Typical conditions for measuring low concentration of chloride over more than a hundredfold range are as follows:

ANTICIPATED CONCENTRATIONS* mEq/L

		0.05-0.9	0.9-5	5-30**
Conditions	Blank	1.8-3.2	33-177	177-1065ppm
mL sample	0	3.0	0.5	0.1
mL .4 N HNO ₃				
40% CH ₃ COOH	1.0	1.0	1.0	1.0
Drops gelatin	4	4	4	4
mL distilled H ₂ O	3	0	2.5	3.0
Total volume	4.0	4.0	4.0	4.1
Display reading	-	15-270	45-250	50-300
Correction factor	--	1/300	1/50	1/10

* Assumes using **LOW** range in all cases.

** Concentrations in this range can use the serial titration procedure and the standard acid solution.

CORRECTION FORMULA FOR CONVERTING DISPLAY UNITS

For solid samples: mEqCl/L to %NaCl:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{\text{original sample weight, grams}}$$

This assumes a known weight of a solid sample has been mixed in a known final volume of solution.

For liquid samples:

$$\%NaCl = \frac{(\text{reading})(5.85)(\text{final sample volumes, liters})}{(\text{sample volume, liters})(\text{density g/L})}$$

mEq Cl/L to mg NaCl/L: mg NaCl/L = (reading x 58.5)

mEq Cl/L to ppm Cl: ppm Cl = (reading) x (35.5)

10. Quality Control

10.1 Standard reference material from Lab Chem Inc. (LC13010-1) is analyzed with each run as well as reagent blanks.

11. Reporting

11.1 Samples are reported in ppm Cl \pm 0.5

12. Reference

Adapted from Standard methods for the Examination of Water and Wastewater, 15th Edition, 1980, (pgs 273-275). Method 407C

Ash

1. Application

This method covers the determination of ash from soil, tissue and waste samples.

2. Summary of Methods

Ash is determined by use of a muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

3. Safety

All chemical compounds should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

None

5. Sample Collection, Preservation and Handling

Samples are dried at 55°C

6. Apparatus and Materials

- 6.1 Muffle furnace
- 6.2 High temperature crucibles
- 6.3 Balance capable of reading to 0.001 g

7. Reagents

None

8. Methods

- 8.1 Record weight of high temperature crucible to 0.001 g
- 8.2 Weigh out 1 – 5 grams of soil, tissue, or waste into crucible and record weight of sample and crucible.
- 8.3 Place in muffle furnace set at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$. Sample must remain at $550^{\circ}\text{C} \pm 50^{\circ}\text{C}$ for 3 hours.

8.4 Remove samples from muffle furnace, cool and re-weigh to 0.001 g.

9. Calculations

Before ashing record:

$$\text{crucible weight} + (\text{crucible weight} + \text{sample weight}) = \text{sample weight}$$

After ashing record:

$$\text{crucible weight} + \text{sample weight}$$

$$\% \text{ ash} = \frac{\text{sample weight ash} - \text{crucible weight}}{\text{sample weight dry} - \text{crucible weight}} \times 100$$

10. Quality Control

10.1 Standard laboratory soil # 4

11. Reporting

11.1 Samples are reported in $\% \pm 0.1$

12. Reference

Standard Methods (for the examination of water and wastewater). 15th Edition, 1980 Pg. 97 (209G). Volatile and Fixed Matter in Nonfilterable Residue and in Solid and Semisolid Samples.

ABOUT THE PREPLANT SOIL NITRATE TEST



The preplant soil nitrate test (PPNT) has been available to Wisconsin corn growers since 1989. The PPNT is recommended on medium or finer textured soils when corn follows corn in the crop rotation and previous climatic and management conditions (see previous page) suggest that nitrate carry-over is likely.

Soil samples for the PPNT should be collected in the early spring after frost has left the soil and prior to planting or any preplant applications of N. Soil samples need to be collected in one foot increments to a depth of two feet. Previously, the suggested sampling depth was three feet. Sampling depth studies have shown a relationship between the nitrate content in the first two feet and the third that allows the nitrate content of the third foot to be estimated; thus, eliminating the need for the third foot sample. The 0–1 and 1–2 foot samples need to be separated. The best way to do this is to collect the soil samples in two buckets—each labeled

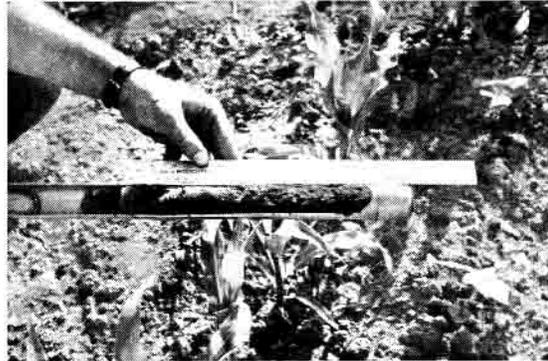
for the appropriate depth. A minimum of 15 soil cores taken randomly from 20 acres is the recommended sampling intensity. Separate samples need to be taken from field areas that differ in soil or past management practices. Mix the samples from each depth and obtain a composite 1-cup subsample. The subsample should be taken to a soil testing lab within a day. If this is not possible and the samples must be stored prior to lab delivery, they *must* be frozen or air-dried to prevent changes in nitrate content during storage. If moist soil samples are stored at warm temperatures, the nitrate content of these samples will increase and N recommendations based on soil nitrate test results will be too low.

The PPNT does not measure the N released from alfalfa when corn follows hay in the rotation. Likewise, the N supplied by fall, winter, or spring applications of manure will not be fully measured by the PPNT. This is due to the sampling period for the PPNT. With early spring sampling, soil temperatures have not warmed sufficiently to allow either of these organic forms of N to convert to plant-available forms of N, such as nitrate. Nitrogen credits for manure and legumes in these cases should be assessed using standard N crediting techniques (see Tables 3 and 4). Another option for assessing these credits could be the use of the pre-sidedress nitrate test (PSNT).

When the PPNT is used, N fertilizer recommendations (Table 1) are adjusted to reflect the residual soil nitrate present. Nitrogen fertilizer rates will decrease as the amount of soil nitrate increases. University of Wisconsin N fertilizer recommendations are adjusted for residual soil nitrate as follows:

- FOR SOILS TESTING 0 TO 200 LB N/ACRE:**
N Recommendation = Base N Rec. - (Soil test N - 50 lb N)
(Note: A minimum N application of 50 lb N/acre is recommended)
- FOR SOILS TESTING OVER 200 LB N/ACRE:**
N Recommendation = 0

ABOUT THE PRE-SIDEDRESS SOIL NITRATE TEST



The pre-sidedress nitrate test (PSNT) is another soil test available to corn growers for improving the efficiency of N applications. Soil samples for PSNT are taken after planting at a time when the mineralization of organic N sources to plant-available forms of N has occurred. Consequently, the PSNT can predict the amount of N released from previous legumes, fall, winter, or spring manure applications, and soil organic matter as well as residual nitrate in the top foot of soil. The PSNT can be a valuable tool for a grower wanting to confirm the amount of N credited from manure or previous legume crops.

PSNT samples should be taken when corn plants are 6 to 12 inches tall, usually four to six weeks after planting. Unlike preplant nitrate test (PPNT) samples, PSNT soil samples are collected only to a depth of one foot. The sampling intensity is similar to PPNT as are the sample storage and handling techniques. As with the PPNT, the PSNT is not recommended on sandy soils (sands and loamy sands).

Soil Sample Preparation

1. Application

Soil samples are dried, ground and sieved prior to analysis. The grinding and sieving operations should ensure a homogeneous mixture for analysis.

2. Summary of Methods

Soil samples are dried at 50 °C in cardboard boxes. The dried soil is ground in a mechanical mortar and pestle and passed through a 12-mesh (approximately 2 mm) screen. Routine testing for pH, lime requirement, phosphorous, potassium and organic matter is designed to handle the analyses in series of groups of ten. The soil samples, at the time they are received, are recorded and placed in trays holding five rows of ten boxes each (boxes are 2.5" x 3" x 3" deep), making a total of 50 samples. Each tray is lettered or numbered and sample identification follows each set of numbered racks through the entire analysis. Boxes in sample trays, shaker flasks, funnel-top filter tubes, colorimeter tubes and racks for pH and pipette batteries are all spaced at 2.5" center-to-center. Soil for analysis is measured by volume rather than by weight.

3. Safety

A dust collection system should be connected to the soil grinder. Dust masks and ear protection plugs should be used. Basic precautions regarding mechanical equipment and electric motors, and involving common sense, must be followed.

4. Interferences

Drying about 50 °C can result in release of nonexchangeable K from illitic minerals and entrapment (fixation) of K by vermiculite. If micronutrient analyses are to be performed, all surfaces contacting the material should be made of stainless steel, plastic or wood. Rubber, paint and galvanized metal must be avoided if Zn is to be analyzed. Air or oven-drying samples can lead to significant changes in the ammonium or nitrate contents of soils. However, the changes in ammonium content of soils have been more pronounced than the changes in nitrate content. Drying and storage of soil samples after drying leads to a marked increase in their content of exchangeable ammonium.

5. Sample Preparation and Handling

Soil samples usually are received in a moist, aggregated state, unsuitable for analysis. The volume of the soil sample containers are 22 cubic inches (about 375 cc). Many samples are larger than this as received. Such samples must be subsampled to ensure as representative a sample as possible of appropriate volume.

Dried and ground soil is measured using a calibrated scoop. The scoop volume is based on the weight of a light-colored silt loam soil such that an acre of the soil to a depth of 7 inches weighs 2 million pounds. A heaping scoop of the required volume of soil is removed from the soil box, the scoop is tapped three times lightly on the handle with the spatula and the soil is leveled off with the spatula. The soil is then transferred to the appropriate container using a stainless steel or polypropylene funnel.

6. Apparatus and Materials

- 6.1 Soil sample trays accommodating five rows of ten sample boxes
- 6.2 Soil sample boxes, cardboard, 2.5" x 3" x 3"
- 6.3 Mechanical soil grinder with a 10- or 12-mesh stainless steel screen
- 6.4 Dust collection system (attached to soil grinder)
- 6.5 Forced air drying cabinet, thermostatically controlled at 50°C

7. Reagents

Not applicable.

8. Methods

- 8.1 Place soil samples in cardboard boxes, with location of sample in tray recorded on a lab data sheet.
- 8.2 Place tray of samples in drying cabinet.
- 8.3 Dry 24 to 48 hours at 50°C. (Wet clays might require a longer drying period.)
- 8.4 Grind entire sample to pass a 10-mesh screen.

9. Calculations

Not applicable.

10. Quality Control

- 10.1 The first sample in each tray should be a standard soil of known analyses. This sample is used to check each procedure. If the analysis is outside the known range, corrective action must be taken.

11. Reporting

Not applicable.

12. References

- 12.1 Evans, D.D., J.L. White, *et. al.* 1965. Methods of Soil Analysis, Part 2 (Chemical and Microbiological Properties), p1182.

- 12.2 Gelderman, R.H., and A.P. Mollarino. 1998. Soil sample preparation. In J.R. Brown (ed.), Recommended Chemical Soil Test Procedures for the North Central Region (Revised). Missouri Agr. Exp. Sta. SB1001. Columbia, MO.
- 12.3 Corey, R.B., and C.B. Tanner. 1961. Soil Sci. Soc. Am. Proc. 25:326-327.



Guidelines for Optimizing Accuracy and Consistency in the NIRSC Laboratory

INTRODUCTION

1. NIRSC Organizational Goals

The NIRSC Forage and Feed Testing Consortium (NIRSC) was formed in 1992 through the efforts of several universities and commercial entities because of their interest in promoting consistent and quality forage testing results for the agricultural public. The group took on official form by electing a Board of Directors, adopting by-laws, and incorporating in June of 1998 and by becoming a federal non profit in 2002 as a 501(c)(6). The mission of the NIRSC is to improve the accuracy and understanding of NIRS (near-infrared reflectance spectroscopy) testing of forages and feeds.

NIRSC carries out its mission by supporting the overall proficiency of our members. The organization does this through several activities: standardizing instruments, managing an instrument monitoring program, supporting member operator skills through workshops, monitoring and updating equations, developing new equations, participating in forage quality testing research, exchanging information between members, and promoting optimal practices. These activities improve business conditions for all of the forage and feed testing industry by promoting accuracy and reliability of the NIRS instrument through uniformity, standardization, and good practices. The last activity, promoting optimal practices, is the focus of this document and collection of resources.

2. Goals of this Collection/Document

The NIRSC includes laboratories that process a tremendous volume of feed and forage samples. As such the consortium possesses the potential to significantly

influence the quality and direction of NIRSC analysis throughout the country. This potential may be limited by the homogeneity and unity of member organizations in their processing of samples and application of NIRSC equations. The quality of internal operations at each member lab can likewise influence the consortium's ability to speak and act (or be perceived to do so) with a single, reliable, and authoritative voice.

The NIRSC has assembled this document to help guide members toward optimum performance. There are two parts to this document. Part I describes NIRSC's recommended internal monitoring methods and external monitoring methods. Internal monitoring includes those actions taken within an NIRSC lab at that lab's discretion to monitor equation and/or instrument performance. External monitoring are methods set up and carried out by NIRSC to monitor equation performance and to monitor and maintain NIRSC member instruments. Part II of this document outlines specific steps and procedures recommended when handling samples for subsequent NIR analysis.

There are several specific goals we plan to achieve through this document.

- 1) Reduce as much as possible the variation in results from member labs by optimizing our collective accuracy in sample analysis. This can be achieved by promoting proper sample handling and processing and by discouraging practices that may reduce the accuracy and applicability of NIRSC equations.
- 2) Improve each member's ability to monitor internal performance by providing resources to assist with quality assurance and quality control (QA/QC). A corollary to this goal is to encourage members to utilize such resources.
- 3) Provide clear definitions of NIRSC equations and proper report notation to properly represent NIRSC member participation to clients. Please see Appendix I—Integrity of Equation Use for the official NIRSC Statement of Use and Definitions.

3. Need for this Collection of Sample Handling Methods for Quality Assurance

The NIRSC has historically supported the overall proficiency of its members, but a comprehensive description of sample handling from sample collection through sample processing for the specific purpose of NIRSC analysis has been lacking. In order for there to be confidence in results generated from NIRSC equations, uniform sample handling methods must be used based on authoritative resources. The following document aims to help the NIRSC lab identify and describe sample handling steps and provide useful authoritative resources on protocol for each step.

PART I: DESCRIPTION OF NIRSC'S INTERNAL AND EXTERNAL MONITORING MEASURES

A. INTERNAL MONITORING MEASURES

Many laboratories have internal monitoring programs. These suggestions are not meant to replace but rather to augment existing programs. They are also directed toward new member labs to provide them with the tools necessary to maintain the high quality results that are expected of NIRSC member laboratories. Finally, members new to NIR analysis may be unfamiliar with the variety of methods available to monitor and insure quality results. The reputation of the NIRSC as a whole can be no better than the reputation of any single member.

NIRSC members are not required to implement internal monitoring programs. However, any laboratory with a commitment to providing accurate and reliable results should also commit to tracking internal performance of their instrument(s) and equations.

First and foremost, it should be stated that all sample introduction material and ground sample should be maintained at the same temperature as the instrument to limit variation in spectra caused by temperature fluctuation.

There are three measures the NIRSC advocates for maintaining optimum internal monitoring of NIR results beyond those recommended by the instrument manufacturer.

- 1) Use of NFTA samples for both instrument and equation monitoring.
- 2) Use of NIR spectra available to members through the NIRSC website for equation monitoring.
- 3) Use of NFTA bulk samples as internal standards or check cells for both instrument and equation monitoring.

Each of these measures is explained in detail below.

1. NFTA Samples for Quality Monitoring

The NFTA results from each participating NIRSC member are recorded, compiled, and analyzed. All labs' submissions are confidential. Summaries of results are coded so that lab identity and anonymity is preserved.

The goals of this project are:

- a. To determine how consistent NIRSC labs are in their predictions. We need to include a protocol here for labs to work with Paolo when having trouble with NFTA samples and to make a log or history of NFTA sample failures.

- b. To determine whether an equation could perform better on certain samples and/or DM, CP, ADF or NDF prediction.

2. Spectra for Member Internal Checks

It is a possibility that some type of corruption could occur, though rare, in an NIRSC equation. NIRSC has spectra available on its website so that members can test that NIRSC equations are functioning properly and are not corrupted and that instruments are functioning properly, and to highlight possible standardization problems. Spectra files of several products with acceptable ranges of prediction values are posted on NIRSC's website. Instructions on how members may download the files and test the equations are posted with the spectra. NIRSC offers as much support as possible to facilitate the process, but labs must perform checks on their own schedule. This creates the opportunity for users to have ownership of the process and responsibility to check that results reported to their customers are accurate.

3. Use of Bulk Samples as Internal Quality Control Standards

Rationale

Labs that participate in the National Forage Testing Association (NFTA) Proficiency Program have a supplemental quality control method available to them. The use of NFTA bulk samples as quality control standards can help monitor the performance of their NIR calibrations and/or instrument.

Recommended Procedures

When using NFTA samples as quality control standards, it is prudent to find a sample that is representative of samples typically received by your lab. The NFTA samples all have Reference Method Averages (RMAs) for Dry Matter, Crude Protein, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF), against which participating labs are statistically graded. The RMAs are determined from the results of all labs using an NFTA approved reference method. This is indicated by each yearly questionnaire.

When using NFTA samples as standards, a portion of the NFTA bulk sample is packed and sealed in a NIR Sample Disk. This sample should be mixed with, and repacked from an NFTA sample on a weekly basis, so as to eliminate sample deterioration possibilities. Each time the sample is then scanned, the results are measured against the corresponding RMAs. It is recommended to scan the sample at least weekly; however, a daily scan would be better for detecting drift. If you are in need of sample, NFTA will send duplicate samples bags of specifically requested certification samples for a fee of \$25.00 per bag. RMAs are also available for Phosphorus, Calcium, Potassium, Magnesium, Sulfur, ADF-CP, and Lignin for labs that measure these constituents by NIR.

NIRSC commercial laboratory membership requires participation in the NFTA Proficiency Program. If you are interested in finding out more information, this can be found on the NFTA website at <http://www.foragetesting.org>.

Resources

NFTA Website

<http://www.foragetesting.org>

B. EXTERNAL MONITORING MEASURES

Here we will describe NIRSC's instrument monitoring program. Although it is limited to FOSS instruments at this time, the theory of instrument monitoring may be described. We have some documents from Paolo from early 2000's on that. Here we will also describe instrument standardization. Again limited to FOSS instruments, we can describe the theory of checking instrument integrity over several instruments and over time and how this is important in achieving accurate predictions from NIRS equations.

PART II: SAMPLE HANDLING STEPS AND RECOMMENDED PROCEDURES

Sample Collection

Rationale

The usefulness and applicability of any analytical results are limited first and foremost by the quality of the sample submitted to the laboratory. The best practices in any lab cannot compensate for an improperly collected sample.

Though we ultimately have no control over the sampling procedures employed by our clientele, we must make every effort to emphasize the importance of proper sampling techniques to our customers.

The following resources may help educate customers about both the importance of proper sampling and the techniques that facilitate representative sample collection.

Recommended Procedures

The NFTA website (www.foragetesting.org) provides a 10-step protocol for proper hay sampling. There is also an online test to become a certified hay sampler that

requires the applicant to correctly answer a series of pertinent questions about the hay sampling process.

For silage and mixed rations, the University of Wisconsin Extension service offers a publication titled “Sampling hay, silage, and total mixed rations for analysis.” This document is available online (<http://learningstore.uwex.edu/pdf%5CA2309.pdf>) at no charge. Copies can also be ordered at a cost of \$1 each. Search for publication A2309 at <http://cecommerce.uwex.edu/OrderPubLookup.asp>.

Labs must be proactive in their approach to informing clients about proper sampling. The NFTA offers instructional posters that can be displayed at your place of business. When clients bring improperly collected samples to the lab (e.g. extremely small samples, grab samples, or cores from a single bale), take advantage of the opportunity to provide guidance and instruction. Direct the client to relevant posters, publications, and the NFTA website. Potential discrepancies between laboratories can be minimized by the use of these proactive measures rather than dealing with sampling issues in a reactive manner.

Resources

Sampling hay, silage, and total mixed rations for analysis
<http://www.uwex.edu/ces/crops/uwforage/Feeding.htm>

NFTA website
<http://www.foragetesting.org>

Subsampling Undried Forages in the Lab

Rationale

The National Forage Testing Association (NFTA) website in the Laboratory Procedures/Sample Preparation section, describes sample preparation as the following: “Laboratory sample preparation is the process of converting the sample received at the laboratory into a homogeneous material suitable for analysis. This process generally involves drying and/or grinding.” In a perfect laboratory world, all samples received would be of the perfect size to allow drying and grinding of the entire sample. However, we know that reality forces most labs to subsample large samples on a daily basis.

Recommended Procedures

The NFTA website states that most forage samples fall into one of three categories:

1. Those dry enough to grind and analyze immediately, >90% Dry Matter(DM).
2. Those dry enough to be coarsely ground but too wet to be finely ground, 85% - 90% DM.
3. Those samples <85% DM which need to be partially dried before the sample can be coarsely ground.

It is the premise of NIRSC that all of the samples that are received will be analyzed by NIRS. Following that premise, NIRSC will follow the recommended procedure for samples <85% dry matter. The samples will be partially dried enough to be finely ground and packed into a NIR disk for analysis. The procedure for this method is as follows:

- Remove sample from shipping container and discard any roots from plants and brush off dirt. Note and report removed material and any other sample manipulation.
- Chop samples of whole plants into about half-inch pieces using either hand clippers or a laboratory forage chopper. Cut open stalk or corn cob pieces to facilitate drying. Include any ears attached to the plants. When using the laboratory chopper, be sure to brush any sample adhering to the sides of the chopper into the receiving tray. Silages and haylages generally have particle lengths less than 1 inch and do not require chopping.
- Place the chopped sample into a clean dishpan or on a clean plastic sheet. Mix thoroughly.
- The NFTA recommendation for subsampling includes coarse grinding all material if the entire sample submitted is greater than 75 grams.
- If the entire sample cannot be dried, reduce the sample size by making a cone of sample and quartering. Save opposite quarters. Repeat mixing, coning and quartering until the volume is reduced to an appropriate size. Make certain that representative ratios of leaf and stem occur in each pile.
- Transfer reduced sample to a tared container for drying.
- Dry the reduced sample using either forced-air oven or microwave oven.
- Grind the partially dried sample to fineness desired for analysis in appropriate grinder.
- Thoroughly mix the ground sample. Transfer to an airtight container and label immediately.

A more detailed description of this procedure can also be found on the NFTA website in the Laboratory Procedures/Sample Preparation section. Also included is a list of needed equipment and safety precautions.

AAFCO also discusses mass reduction in *Guidelines for Preparing Laboratory Samples*, noting that sample mass may be reduced by splitting or subsampling. AAFCO recommends a selecting a minimum of 30 increments of a sample for

preparation. Heterogeneity errors are reduced with more increments, and fewer than 10 increments is never recommended. Grinding less than ¼ of the submitted sample prior to mass reduction is not acceptable.

Resources

National Forage Testing Association (NFTA) Website
<http://www.foragetesting.org>

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Drying

Background

Sample integrity must be maintained throughout all aspects of laboratory testing. Sample drying is important for all samples that come into the laboratory. No matter how dry samples come into the laboratory, they will need to be dried to meet the NIRSC sample prep method used in calibration development.

Recommended Procedure

For all forages, it is necessary to partially dry them prior to fine grinding. Drying can be done by either a 55-60 degree C forced-air oven or a microwave oven. Drying at higher temperatures (>60 degrees C) can cause chemical changes in the sample that will adversely affect the subsequent fiber, lignin or acid detergent insoluble nitrogen analysis. The drying method for a sample to be analyzed by NIRS should be consistent with the drying method used for samples in the NIRS calibration.

NIRSC Equation	Acceptable Drying Method(s)	
	Equation Variant I	Equation Variant II
Alfalfa Hay	oven/microwave	oven
Grass Hay	oven/microwave	oven
Mixed Hay	oven/microwave	oven
Haylage	oven/microwave	oven
Corn Silage	oven/microwave	oven
Alfalfa Breeders Equation	oven	

The Alfalfa Hay, Grass Hay, Mixed Hay, Haylage, and Corn Silage equations all have variants of the equation that contain a digestible fiber parameter. Please note that oven drying is always the acceptable method and it is the mandatory method for fiber digestibility.

NFTA Forage Analysis Procedures, July 1993 offers an excellent reference to this procedure.

Section 2.2.1.1 Partial Dry Matter Using Forced Air Ovens

Section 2.2.1.2 Partial Dry Matter Using Microwave Ovens

These procedures are summarized below.

Partial Dry Matter Using Forced Air Drying Ovens-Part 1

Step 1: Dry empty pans at 55-60 degrees C for 2 hours.

Step 2: Weigh empty pans on a top loading balance and record the weight (W1) to the nearest 0.01g.

Step 3: Place a representative amount of forage into the pan. Fill pan to a maximum sample depth of 1.5 inches. Record weight of pan and wet forage to the nearest 0.01g (W2).

Step 4: Dry in a forced air drying oven at 55-60 degrees C for 16-24 hours. Be sure to allow air flow between the pans.

Step 5: Remove pans from oven and allow to air equilibrate for about 15 minutes, then weigh pan and dry forage to the nearest 0.01g (W3).

Partial Dry Matter Using a Microwave Oven-Part 1

Step 1: Dry paper boats in microwave oven for 3 minutes on full power.

Step 2: Weigh empty boat to the nearest 0.01g (W1).

Step 3: Place a representative sample in the boat and record the weight of the boat and the sample to the nearest 0.01g (W2).

Step 4: Dry in the microwave carefully to avoid hot spots, charring and fire. For the average hay, initially dry for 2 ½ minutes at 30% power. Remove from microwave and stir to allow moisture to escape. When cool, weigh the boat and forage. Place back in microwave and dry for 2 ½ minutes at 20% power. Repeat until successive weighings are less than 0.7 grams.

Step 5: After drying is complete, equilibrate to room temperature a final time until cool. Record the final dry weight (W3) of the boat and dry forage to the nearest 0.01g.

Dry matter of partially dried forages should be between 85-95%.

Laboratory Dry Matter of Partially-Dried (Oven) Forages-Step 2

Step 1: Dry aluminum dish with cover at 104 degrees C for at least 1 hour.

Step 2: Remove dishes and covers from oven and place in a dessicator and allow to cool. Weigh dishes with cover (W4) to nearest 0.1mg.

Step 3: Add approximately 2 grams ground sample to each dish and record the weight of dish with cover and sample (W5) to the nearest 0.1mg. Gently distribute the sample uniformly in the dish to expose the maximum area for drying.

Step 4: Dry in a preheated oven to 104 +/- 1 degree C for 3 hours +/- 5 minutes. Place dishes in oven so that air can circulate freely.

Step 5: Remove dishes from oven and place cover back on each dish when transferred to the dessicator. Allow to cool.

Step 6: Weigh dish with cover and dried sample (W6) to the nearest 0.1mg.

Determination of Total Dry matter using the Two-Step Procedure

$$\text{Partial DM} = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

$$\text{Lab DM} = \frac{(W6 - W4)}{(W5 - W4)} \times 100$$

$$\% \text{ Total DM} = \text{Partial DM} \times \text{Lab DM}$$

Where:

- W1= empty weight of container in grams
- W2= wet weight of forage and container in grams
- W3= dry weight of forage and container in grams
- W4= empty weight of dish with cover in grams
- W5= initial weight of sample & dish with cover in grams
- W6= dry weight of sample & dish with cover in grams

Calculation: Percent Total Moisture

$$\% \text{ Total Moisture} = 100 - \% \text{ Total DM}$$

Resources

NFTA Forage Analysis Procedures, July 1993.

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Intrasoft International. 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

Grinding

Background

Good analytical data requires that samples be representative of the whole lot and that their integrity has been ensured during transport to the lab and during their preparation.

Recommended Procedure

Regardless of grinding method, all material for NIRS analysis must pass through a 1 mm screen of a cyclone mill (UDY, Cyclotec or equivalent). It is important to make sure the same grinding method is used in developing the calibration as well as for routine analysis. It maybe necessary to reduce sample bulk by grinding through a cutting mill first, then to regrind through a cyclone mill.

NFTA Forage Analysis Procedures, July 1993 offers an excellent reference to this procedure.

Section 1.1 Grinding with a Cutting Mill

Make sure the mill is clean, then insert appropriate screen into the mill. If a fine grind is desired, most samples are ground with a 1 mm screen. If large samples are to be ground, you may grind them using a 4 or 6 mm screen and then regrind using the 1 mm screen. Following your mill's operating instructions, place the entire sample into the grinder and allow it to completely pass through. A higher pitched "empty" sound, may be noted. Remove the sample container and hold it beneath the grinding chamber as you open the mill. Sweep any incompletely ground residue from the mill into the container. Remove the screen and transfer residue from it into the sample container. Mix thoroughly before analysis. Clean entire mill using air and/or a brush.

Section 1.2 Grinding with a Cyclone Mill

Make sure the mill is clean, then insert 1 mm screen to be used to properly grind all NIR samples. Insert a clean sample bottle beneath the clear plastic cyclone and turn the mill on. Following your mill's operating instructions, present the sample to the mill and allow it to grind the entire sample. Shut off the mill. Mix thoroughly before analysis. Clean entire mill using air and/or a brush.

Resources

NFTA Forage Analysis Procedures, July 1993.

Intrasoft International, 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

Mixing a Dried & Ground Sample

Rationale

Mixing a dried and ground sample is an important step before NIRS analysis or wet chemical analysis. Grinding stratifies a sample and mixing is necessary afterwards. If the dried and ground sample is not mixed properly, the resulting analyses will not be representative of the sample.

Recommended Procedures

The NIRSC recommends the following methodologies. Two different methods are listed.

Method One

US Dairy Forage Research Center: Dave Mertens, 2006:

Place a sheet of paper approximately 18" square on a flat surface and pour the entire ground sample near the center but offset slightly towards one corner. Mix the sample by pulling the corner nearest the sample pile towards the diagonally opposite corner, so that the sample mixes as it rolls. You will observe the sample color becoming more uniform as you mix. Usually 12 rolls are sufficient, but it is impossible to 'over-mix' the sample.

If the entire sample cannot be used, take at least 3 subsamples from different parts of the mixed pile using a putty knife. Ensure that the knife runs along the paper surface so that fines are included in the subsample. One advantage of this technique is that fines are rolled throughout the sample and tend not to be lost at the bottom.

Place the sample in a cup or bag. Cups are preferred because they allow the user to re-mix the sample before packing NIR cups. The body of the cup, and not just the lid, should be labeled so that accidental lid-switching cannot cause problems.

Method Two

University of Minnesota Forage Quality NIRS Laboratory, 2001

Sample becomes stratified due to fine grinding (cyclone) and needs remixing. A 15-gallon plastic drum is used as a sample tumbler. Place 70 - 100 samples (in 4 oz. plastic cups with sealed lids) into tumbler for 15 minutes. The drum rotates at 15 rpm and contains a rod that lifts and drops the bottles to provide a random tumbling.

In tests using NIR, subsamples from the same bottle were nearly identical. Standard errors for laboratory chemical procedures also decreased due to the more uniform subsampling from the tumbler.

Whirlpaks can also be tumbled by sealing open end with tape and providing an air space for sample movement.

If samples sit longer than 4 weeks before analysis, tumbling is repeated due to possible moisture stratification within the sample.

Resources

Packing and Scanning a Sample for NIRS

Rationale

Loading a dried and ground sample into a sample cell (ring cup) for NIRS scanning is an important last process in obtaining analysis scan results. If the dried and ground sample is not handled properly while packing a sample cell, the resulting

analysis will not be representative of the sample. For example, a non-homogeneous ground sample will result in a distorted sample prediction, while a dusty cell window or instrument window will produce a faulty artifact in the sample prediction.

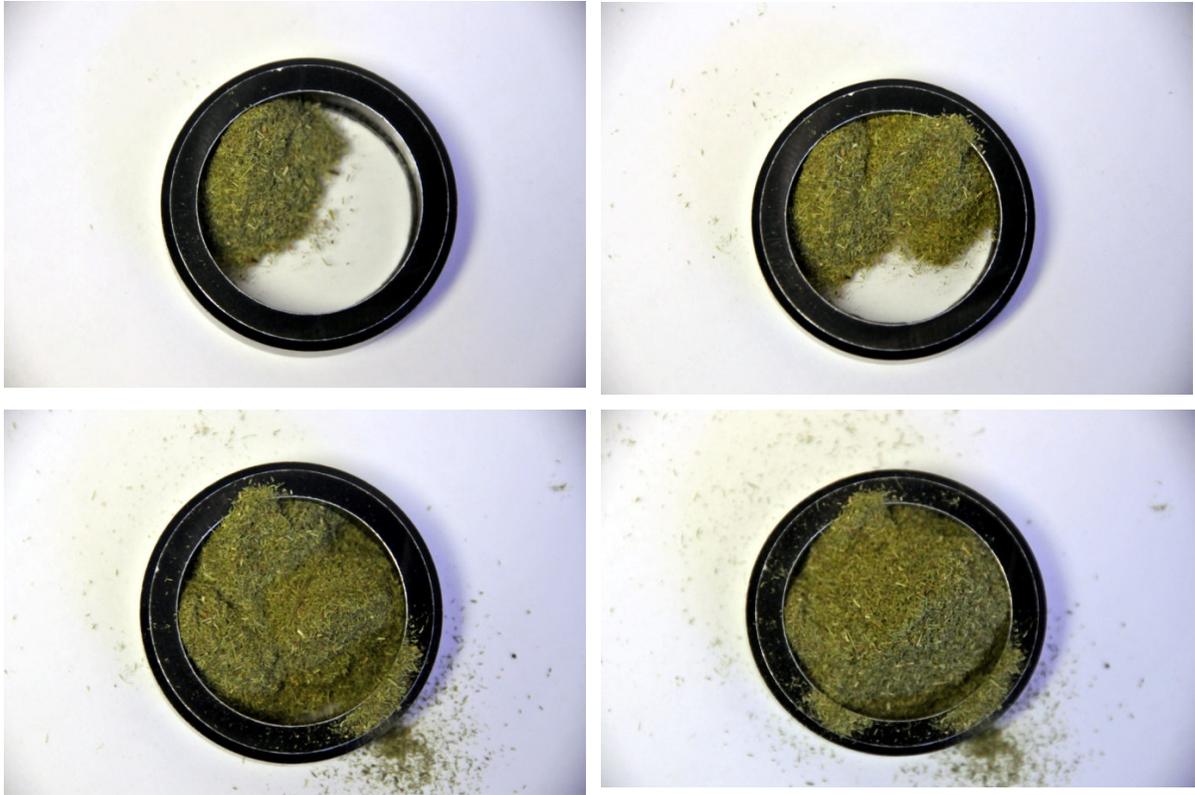
Consistency and thoroughness in packing a sample cell for scanning will help produce NIRS analyses representative of the original sample. In addition, consistency among NIRSC labs in preparing samples for NIRS scan will help reduce discrepancies between laboratories.

Recommended Procedures

Packing a dried and ground sample is described by NFTA under laboratory procedures information (www.foragetesting.org) beginning with section 2.2.2.4 “Dry Matter by Near Infrared Reflectance Spectroscopy.” Here general drying to 90-95% dry matter and grinding to pass a 1mm cyclone screen are covered, as well as subsampling the ground sample and filling a sample cup for NIRS scan. But see the sections on drying, grinding, and mixing in this document for recommendations on these methods specifically related to NIRSC equation use.

The NIRSC recommends the following methodology, which is the protocol used when scanning samples on the NIRSC Master Instrument.

1. **Mix Sample:** for samples in bags, stir with a spatula; for samples in cups, tumble specimen cups 20 half-turns. Mixing of dried and ground samples is important in order to alleviate any settling and stratification that might have occurred from grinding. Stratified samples produce varying size fractions in the ground sample and in turn produce different analyses results.
2. **Subsample:** Using a spatula, take three subsamples by scooping from separate areas of a sample bag or specimen cup. Scoop from three locations vertically as well as horizontally. If samples are in cups, rotate cups as subsampling occurs. Fill a ring cup in three quadrants with these subsamples. Overfill the remainder of the ring cup with additional subsamples.



3. Prepare the Ring Cup: Strike off excess sample from the ring cup with a straight spatula edge. Press the cardboard back onto the ring cup and tap the cup gently on its edge twice to dislodge dust. (Tapping more than twice may cause stratification.) Brush all cup surfaces with a soft brush to avoid dust transfer to the inside of the instrument. Brushing dust off the external cup surfaces is extremely important when using an autosampler (see recommendation #4). Load the cup into the instrument and scan.
4. Cleaning the Instrument scanning Window: Clean weekly. High T-values from check cell predictions are an indicator of dust on the window and that cleaning is overdue.
 - a. Autosampler: Remove the autosampler first, then rest autosampler on a soft surface, remove window, and wipe both sides of the window with a Kimwipe moistened with glass cleaner. **WARNING!! The white ceramic is usually positioned under the window. Don't touch the white ceramic!! Use a very soft brush and a vacuum cleaner to remove all the dust that may be deposited inside the autosampler.** Be sure to also apply vacuum/compressed air alternately two or three times through the apertures for entrance and exit of ring cups.

Dust may have spread around and gotten trapped in some dead corner.

- b. Spinning drawer: Remove the white ceramic (two screws) and if it is dusty, gently brush it off with a soft brush. Vacuum the inside of the drawer.
-
5. Duplicate Scans: This is a more advanced and time consuming procedure than single scans. It is used by NIRSC for critical spectra collection such as master scans for calibrations. Pack two cups of the same sample at the same time and scan consecutively. After scanning, label the duplicate spectra records with the same number or name. Move the duplicates into two spectra files (such as Rep_1 file and Rep_2 file) and use the Contrast Spectra function in WinISI to look for spectra that differ considerably. NIRSC recommends re-running samples in which the two replicates exhibit RMSC >3500.

Resources

NFTA website

<http://www.foragetesting.org>

Equation Use

Rationale

NIRS analysis of feed and forages is a rapid and effective method of determining sample composition. There are limits to its application, and these limits must be observed. Failure to observe these limits can lead to inaccurate results.

The NIRSC offers nine different equations to analyze a wide variety of feeds and forages. Each equation was developed to analyze a particular group of materials, such as alfalfa hay, grass hay, legume and grass haylages, or fermented corn silage. For NIRS results to be reliable, it is imperative to limit equation application to appropriate materials.

Recommended Procedures

NIRSC equations are supplied with appropriate files to report Global H (GH) and Neighborhood H (NH) values for all analytes. It is important to monitor these

values and deal appropriately with samples yielding GH and NH values beyond acceptable thresholds.

For Global H, the limit of reliable results is 3.00. Any sample with a GH higher than 3.00 should be acknowledged as having a poor NIRS match. Wet chemistry analysis may be necessary to confirm analyte values.

Different sources cite different values for the acceptable limit for Neighborhood H values, though they are typically between 0.6 and 1.2.

Samples yielding high values for GH and/or NH are excellent samples to add to the equation calibration. Spectra from such samples should be saved and sent to the NIRSC for possible inclusion in the calibration file update.

Reporting results for samples that yield high GH and/or NH values without discussing the situation with the client or checking the values with wet chemistry analysis is a disservice to the client. This practice may also lower the quality and reputation of the laboratory, and by extension, the NIRSC.

NIRSC equations are developed using sample sets that have been dried and ground. These sets include samples that have been dried and ground according to multiple methods (e.g. microwave and oven drying, single- and multi-step grinding). Thus NIRSC equations are robust and multiple methods of sample drying and grinding may be successfully employed for use with NIRSC equations. However, all samples should be dried and ground in a manner consistent with the methods used to prepare samples for the NIRSC equation development.

Note: Oven drying must be used for fiber digestibility and RUP analysis on corn silage, hay, and haylage samples.

Please refer to Appendix I for details regarding equation definitions, spectra ownership, and NIRSC logo usage.

Resources

Foss International, NIR Instrument Maintenance and Diagnostics Workshop Manual.

Infrasoft International. 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

NIRSC. 2002. NIRS Consortium Annual Meeting. NIRS—Getting Started.

Sample Storage

Rationale

Most labs retain feed and forage samples. It is important to prevent sample deterioration during this storage interval so that additional analyses may be performed or previous results confirmed.

Recommended Procedures

Effective storage of feed and forage samples is simple. Because samples prepared for NIRS analysis have been dried to below 10 percent moisture, sample degradation is of relatively small concern. Basic principles of effective storage for dried and ground feed and forage samples include storage in airtight containers out of direct light at room temperature. Many laboratories use cups and/or sealed plastic bags for archiving samples. Such containers are typically adequate to maintain the integrity of samples. Where possible, it is advisable to further restrict exposure to air and moisture by storing samples cups/bags in larger airtight containers. Even with such measures in place, it is possible for samples to absorb measurable amounts of moisture from the atmosphere. However, other analyte values remain largely consistent, and samples can often be stored effectively in this manner for several years.

Resources

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Nelson, C. J., and D. Smith. 1972. Changes in carbohydrate and nitrogen concentrations during storage of heat and freeze-dried alfalfa root tissue. *J. Agric. Food Chem.* 20:125.

Appendix I: Intellectual Property Standards Listed in NIRSC Bylaws

1. Definition of an “NIRSC Equation”

An NIRSC equation is defined as- an NIRS equation (calibration) derived from NIRSC aggregate spectra and chemical data submitted to the NIRSC. The equation (calibration) can not be biased or modified in any way.

2. Use of NIRSC Aggregate Materials

Use aggregate spectra (SPECTRA), chemistry data (CHEMISTRY), and/or forage and feed samples (SAMPLES) submitted to NIRSC:

1. Spectra shall be defined in this case as being the absorbance or reflectance data collected by a Near Infrared Reflectance instrument, used to describe chemical, biological, or physical properties of a sample. Chemistry data shall be defined in this case as being the data collected from chemical analysis to describe chemical, biological, or physical properties of a sample. Samples shall be defined in this case as being the physical samples from any physical material used in predicting spectra or determining chemical data.
2. NIRSC Aggregate spectra shall be defined as spectra pooled by NIRSC from Spectra submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
3. NIRSC Aggregate chemistry shall be defined as chemistry pooled by NIRSC from chemistry data submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
4. NIRSC Aggregate samples shall be defined as samples pooled by NIRSC from samples submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
5. NIRSC shall not sell, lease, or donate any aggregate spectra, chemistry, or samples to any non-member of the NIRSC without a vote of the membership. Approval of sale, lease or donation of aggregate spectra, chemistry, or samples will require at least a 75% approval of NIRSC voting members. NIRSC aggregate spectra, chemistry, and samples can be used for the formulation of NIRSC global calibrations, expandable calibrations, validation sets, etc.
6. NIRSC aggregate spectra, chemistry, or samples may be used by instrument manufacturers for the purpose of evaluation of their instruments, or spectral transfer to their particular format. This will require an approval from the NIRSC Board of Directors. Any research group or instrument manufacturer receiving approval for use of the NIRSC spectra, chemistry, or samples will be required to complete a Research Agreement.

3. NIRSC Logo Usage

An important goal for NIRSC membership is to maintain performance, and any logo use should reflect this. Two logo concepts have been implemented by NIRSC that would both represent support for the forage/feed and agriculture industry, but differentiate level of NIRSC participation.

1. The first logo represents that an entity is a participating NIRSC member. Under the logo is stated, "Working for better forage analysis."
2. The second logo represents that an entity uses NIRSC equations. Under the logo is stated, "Proudly using NIRSC equations." With this second logo option, the member's analysis or results sheet would need to footnote which equations are being used and sign an agreement to this effect. "Using" NIRSC equations means doing work for a 2nd party or doing some kind of reporting for a 2nd party. Method of footnoting is flexible as long as members report on which NIRSC equations are being used. The intent is to keep misrepresentation of NIRSC equations from happening while also benefiting the NIRSC.

Appendix II: Creation of a Permanent Check Cell

If creating a permanent check cell is something your lab wishes to do, it is very important to remember that it can be a very difficult process to perform correctly. Be aware of the following factors:

- The sample must be packed tightly enough to not allow sample particle shifting. This must be done without packing too tightly and breaking glass in the cell.
- The cell must be sealed well enough to prevent moisture migration, and must hold up to handling and temperature changes.

With these factors in mind, the committee feels it would be most prudent to contract InfraSoft International (ISI) to perform this task for you. It may save a large amount of time and glass.

Sample Preparation for Manure

1. Application

A subsample of all manure samples received at the lab are thoroughly mixed, sub-sampled, weighed, dried, and ground prior to analysis. The mixing and sub-sampling operations help to ensure a homogeneous mixture for analysis.

2. Summary of Methods

3. Safety

Basic precautions regarding mechanical equipment and electric motors must be followed. All electrical equipment is properly grounded and installed and maintained by qualified electricians. Dust masks, safety glasses and ear protection plugs should be used when grinding forages.

4. Interferences

5. Sample Collection, Preservation, and Handling

Solid manure samples (>15% DM) typically are received in a fresh, aggregated state, unsuitable for most analysis without homogenization. The amount of sample needed for analysis is generally around 50 grams for solid manure and around 100 grams for liquid manure samples (<15% DM). Many samples are larger than this as received. Such samples must be sub-sampled properly to ensure that a representative sample is obtained for further analysis.

6. Apparatus and Materials

- 6.1 Cabinet-type, forced-air drying oven at 55 C, \pm 3 C
- 6.2 Analytical electronic balance, accurate to 0.1 mg
- 6.3 Plastic hexagonal tray, approximately 10 cm diameter, 3 cm deep
- 6.4 Bucket or bin, if necessary, for mixing and sub-sampling
- 6.5 Wiley mill, 1 mm size
- 6.6 Sample tray rack accommodating five rows of ten sample cups
- 6.7 Sample cups with covers, plastic, 6 cm in diameter, 8 cm deep

7. Reagents

None.

8. Methods

- 8.1 Record tare weight of plastic tray.
- 8.2 Thoroughly mix the sample. Shake if liquid or use spatula or hands if solid.
- 8.3 Sub-sample around 50 grams of solid manure or around 100 grams of liquid manure. If liquid manure is very low in solids (approx. <2% DM) a slightly larger sample size may be required.
- 8.4 Record initial weight of sample plus tray.
- 8.5 Place the remaining sample in a refrigerator/freezer until all analyses are complete.
- 8.6 Place trays in drying oven for 24-48 hours.
- 8.7 Weigh trays back to record dry weight of sample plus tray.
- 8.8 Grind sample thru 1 mm Wiley mill and place in sample cup, cap and store in a sample tray.

9. Calculations

Percent Lab Dry Matter (% DM):

$\% \text{ Lab DM} = \{(\text{Dry Weight of Sample and Tray} - \text{Tare Weight of Tray}) / (\text{Initial Weight of Sample and Tray} - \text{Tare Weight of Tray})\} \times 100$

Percent Lab Moisture:

$\% \text{ Lab Moisture} = 100 - \% \text{ DM}$

10. Quality Control

11. Reporting

Results are reported as % Lab Dry Matter and % Lab Moisture on an as received basis.

12. References

DATE: 9/21/2011

Carbon (Total, Organic, and Inorganic)

1. Application

This method covers the determination of total carbon (TC), organic carbon (OC) and inorganic carbon (IC) concentrations in soil, plant tissues and manures by dry combustion using a LECO CNS-2000 analyzer.

The LECO CNS-2000 Carbon, Nitrogen and Sulfur Analyzer is a non-dispersive, infrared, microcomputer based instrument, designed to measure the total carbon, nitrogen, and sulfur content in a wide variety of materials (soil, plant tissue, fertilizers, meat products, dairy products, seeds, food, resins, and environmental wastes) in a nominal 200 mg sample weight.

2. Summary of Method

Total carbon and organic carbon contents of a sample are determined in two separate combustion conditions/profiles. The first combustion profile will maximize the recovery of TC while the second profile will minimize the decomposition of carbonate C and maximize the recovery of OC. The two main variables of these profiles are the furnace temperatures and the oxygen flow rate. The furnace temperatures are set at 1350°C and 900°C for the TC and OC profiles, respectively. IC is calculated as the difference between the TC and OC values.

Although SPAL uses 900°C for the determination of OC, temperatures between 375 and 1000°C are found in the literature. Based on this, SPAL could accommodate specific requests from clients to run OC samples at a specific temperature.

In some cases hydrochloric acid has been used for the decomposition of carbonates. However, this treatment generates Cl gases that can damage the infrared detector and requires the use of scrubbing substrates in the system to prevent damage. Therefore, if a client needs to determine organic and inorganic carbon (by difference) the first option given is the OC at 900°C, the second option is OC at a temperature defined by the client, and as a last option (and therefore more expensive) the use of hydrochloric acid (by the client or by SPAL) to remove carbonates previous to the OC determination.

3. Safety

3.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

3.2 Follow the manufacturer's recommendation for safe operation of the instrument.

3.3 Secure compressed gas cylinders and use the proper gas regulators.

3.4 Sample boats being unloaded from the furnace are extremely hot - do not handle them until they cool down.

4. Interferences

4.1 Fineness of the ground sample affects sample combustion and thus analysis results. All samples should be ground to pass an 18 mesh sieve (1-mm) or finer.

4.2 Sample boats will be contaminated with inorganic carbonates (IC) following the analysis for organic carbon. To remove the carbonates place the contaminated boats in a muffle furnace at 1000°C for one hour or run the boats as blanks at 1350°C in the Leco CNS-2000

5. Sample Collection, Preservation and Handling

5.1 Soil and plant samples are dried at 55°C and 65°C, respectively. The dried soil sample is then ground to pass a 12 mesh screen and the plant tissue is ground to pass a 2 mm screen.

5.2 Acid-digested or acid-treated samples should not be run on the Leco CNS-2000

5.3 The Leco COM-CAT combustion accelerator can be used to insure complete combustion when large samples are used or when total sulfur determination is required

6. Apparatus and Materials

6.1 Scale 0.0001 g

6.2 Leco CNS-2000 Carbon, Nitrogen and Sulfur Analyzer

6.3 Autoloader Assembly with 49-position sample rack

6.4 Printer

6.5 Sample spatula

7. Reagents

7.1 COM-CAT combustion catalyst (Tungsten Tri-oxide, Leco 501-426)

7.2 Anhydron (Anhydrous Mg perchlorate, Leco 501-171)

7.3 Lecosorb (Sodium hydroxide, Leco 502-174)

7.4 Sulfamethazine (Leco 502-304), EDTA (Leco 502-092) or soil standards (Leco 502-309, 502-308)

7.5 Glass wool

7.6 UHP helium gas

7.7 UHP oxygen gas

7.8 Compressed air (low water content)

8. Methods

8.1 Operate instrument according to manufacturer's instructions. The following are generalized instructions:

8.1.1 Turn furnace on (or take off standby).

8.1.2 Turn gas regulators to desired flow rate (40 psi).

8.1.3 Select the appropriate method for either TC or OC.

8.1.4 Wait until the furnace has stabilized at the set temperature.

8.1.5 Test for leaks in the helium lines, ballast tank and combustion system.

8.1.6 Define the standard by entering the appropriate carbon content of the pure primary standard.

8.1.7 Include ten blanks and three dried (or desiccated pure) primary standards at the beginning of each run to calculate the calibration factor for determining carbon (to correct for drift).

8.1.8 Weigh out 0.15 to 2.0g of dried soil or plant tissue, respectively, in a clean sample ceramic crucible (boat). 1.0g of COM-CAT accelerator can be added to the boats before the sample is weighed. Weights are automatically transferred to the microprocessor by pressing the print button on the scale pad.

8.1.9 Transfer the weighed samples to the 49-position sample rack and load the rack onto the autoloader.

8.1.10 Run the samples.

9. Calculations

The inorganic carbon content is calculated as the difference between total carbon and organic carbon as follows:

$$\% \text{ IC} = \% \text{ TC} - \% \text{ OC}$$

10. Quality Control

10.1 The method's analysis range (lower limit is based on 3x standard deviation of the blank) is 0.02 - 200 mg carbon. Analysis precision is RSD 0.4%.

10.2 At least 10 blanks must be analyzed daily before each run and the blank with the value closest to zero should be selected and used for blank correction. Three to five standards should be analyzed and the one with the value closest to the real value of the standard should be used to correct for drift in the calibration curve.

10.3 At least one standard of the same material as the samples should be run with every ten unknowns and at the end of each run to verify calibration.

11. Reporting

11.1 Data is reported as %C for soil and plant tissue on a dry weight basis.

11.2 The detection limit is 0.020 mg C.

12. References

12.1 Leco Corporation. 2002. CNS-2000 elemental analyzer. CNS-2000 instruction manual.

12.2 Organic Carbon Dry Combustion method, Leco CN-2000, FP 2000, or CNS-2000 (JD Schwab, Personal communications).

12.3 Islam, K.R. and R.R. Weil. 1998. A rapid microwave method for colorimetric measurement of soil organic carbon. *Commu- soil-sci-plant-anal.* 29 (15/16):2269-2284.

12.4 Matejovic, I. 1997. Determination of carbon and nitrogen in samples of various soils by dry combustion. *Commu-soil- sci-plant-anal.* 28(17/18):1499-1511.

12.5 Leco Corporation 2003. Organic application note. Carbon, nitrogen and sulfur testing in soil/plant tissue.

Carbon (Organic, and Inorganic)

1. Application

This method covers the determination of total organic carbon (TOC), and inorganic carbon (IC) concentrations in soil, plant tissues and manures by dry combustion using a Leco CNS-2000 analyzer. The Leco CNS-2000 Carbon, Nitrogen and Sulfur Analyzer, is a non-dispersive, infrared, microcomputer based instrument, designed to measure the total carbon (nitrogen and sulfur) content in a wide variety of organic materials (soil, plant tissue, fertilizers, meat products, dairy products, seeds, food, resins, and environmental waste) in a nominal 200 mg sample weight.

2. Summary of Method

Total carbon (TC) and total organic carbon (TOC) and inorganic carbon (IC) contents of a sample are determined in two separate runs. The first run total carbon will be determined as per the routine dry combustion method (refer to Total carbon analysis method on our website). In the second run, total organic carbon will be determined after the sample is acid-treated to remove the carbonate carbon. The inorganic carbon is calculated as the difference between the TC and TOC values. The two main differences between TC and the TOC methods are in the oxygen profile used in each and the use of a halogen trap materials used in the TOC method. The furnace temperature is set at 1350°C for both methods.

3. Safety

- 3.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.
- 3.2 Follow manufacturer's recommendation for safe operation of the instrument.

- 3.3 Secure compressed gas cylinders and use proper gas regulators.
- 3.4 Sample boats being unloaded from the furnace are extremely hot do don't handle them until they cooled down.

4. Interferences

- 4.1 Fineness of sample grind affects sample combustion and thus analysis results. All samples should be ground to pass an 18 mesh sieve (1-mm) or finer.

5. Sample Collection, Preservation and Handling

- 5.1 Soil and plant samples are dried at 55°C, 65°C, respectively. The dried sample is then finely ground using a shatter-box grinder.
- 5.2 Use extreme caution when handling acid or acid-treated samples and follow all appropriate safety precautions.

6. Apparatus and Materials

- 6.1 Scale 0.0001 g
- 6.2 Leco CNS-2000 Carbon, Nitrogen and Sulfur Analyzer
- 6.3 Autoloader Assembly with 49-position sample rack or manual loader
- 6.4 Combustion boats
- 6.5 Nickel liners
- 6.6 Hotplate
- 6.7 Printer
- 6.8 Sample spatula and eye dropper

7. Reagents

- 7.1 COM-CAT combustion catalyst (Tungsten Tri-oxide, Leco 501-426)
- 7.2 Anhydron (Anhydrous Mg perchlorate, Leco 501-171)
- 7.3 Lecosorb (Sodium hydroxide, Leco 502-174)
- 7.4 Sulfamethazine (Leco 502-304), EDTA (Leco 502-092) or soil standards (Leco 502-309, 502-308)
- 7.5 Glass wool
- 7.6 UHP helium gas
- 7.7 UHP oxygen gas
- 7.8 Compressed air (low water content)

- 7.9 Hydrochloric acid
- 7.10 De-ionized water
- 7.11 Halogen trap material (antimony metal and F-Cl absorbent)
- 7.12 Synthetic carbon standard

8. Methods

- 8.1 Prepare instrument according to manufacturer's operator instruction manual (follow the Lab user's guidelines as a quick reference during operation)
 - 8.1.1 Install halogen trap materials into secondary reagent tube following the attached diagram.
 - 8.1.2 Turn furnace on (or take off standby)
 - 8.1.3 Turn gas regulators to desired flow rate (40 psi)
 - 8.1.4 Select the appropriate method either for TC or OC
 - 8.1.5 Wait until furnace have stabilized at set temperature
 - 8.1.6 Run ambient monitor test
 - 8.1.7 Test for leaks in the helium lines, ballast tank and combustion system
 - 8.1.8 Define the standard by entering the appropriate carbon content of the pure primary standard.
 - 8.1.9 Analyze blanks until instrument is stable then analyze 3 to 5 boats containing 1.5 g COM-AID and 3 to 5 empty combustion boats.
 - 8.1.10 Weigh approximately 0.25 g of standard material and analyze 3 to 5 times then drift correct using these values.
 - 8.1.11 Weigh out 0.25 g of dried soil or plant tissue in a clean sample ceramic crucible lined with nickel liners. Also weigh 0.25 g of synthetic carbon. The synthetic carbon is used as a check for the acid treatment procedure. Weights can automatically be transferred to the microprocessor by pressing the print button on the scale pad
 - 8.1.12 Using an eye dropper, add 1:1 (HCl: water by volume) solution to samples and synthetic carbon standard until completely wetted. Try to use approximately the same amount of acid solution on each blank and sample.
 - 8.1.13 Place on hotplate on and heat on a low setting until dry. Then remove and cool the sample. Repeat the acid addition and the heating to dryness until no reaction occurs.
 - 8.1.14 Analyze samples and synthetic carbon standard to determine TOC contents.

8.1.15 Analyze a standard at the end of the run to verify calibration.

9. Calculations

The inorganic carbon content is calculated as the difference between total carbon and organic carbon as follows:

$$\% \text{ IC} = \% \text{ TC} - \% \text{ OC}$$

10. Quality Control

- 10.1 The method's analysis range (lower limit based on 3X standard deviation of blank) is 0.02 - 200 mg carbon. Analysis precision is RSD 0.4%
- 10.2 At least 10 blanks must be analyzed daily before each run and the blank with the value closest to zero should be selected and used for blank correction. Three to five standards should be analyzed and the one with the value closest to the real value of the standard should be used to drift correct the calibration curve
- 10.3 At least one standard of the same material as the samples should be run with each 10 unknowns and at the end of the run.

11. Reporting

- 11.1 Data is reported as % C for soil and plant tissues on a dry weight basis.
- 11.2 Detection limit is 0.020 mg C

12. References

- 12.1.1 Leco Corporation 2002. CNS-2000 elemental analyzer. CNS-2000 instruction manual.
- 12.1.2 Leco Corporation 2003. Organic application note; total/organic carbon and nitrogen in soils.
- 12.1.3 Organic Carbon Dry Combustion method, Leco CN-2000, FP 2000, or CNS-2000 (JD Schwab, Personal communications)

- 12.1.4 Islam, K.R. and R.R. Weil. 1998. A rapid microwave method for colorimetric measurement of soil organic carbon. *Commu-soil-sci-plant-anal.* 29 (15/16):2269-2284
- 12.1.5 Matejovic, I. 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. *Commu-soil-sci-plant-anal.* 28(17/18):1499-1511

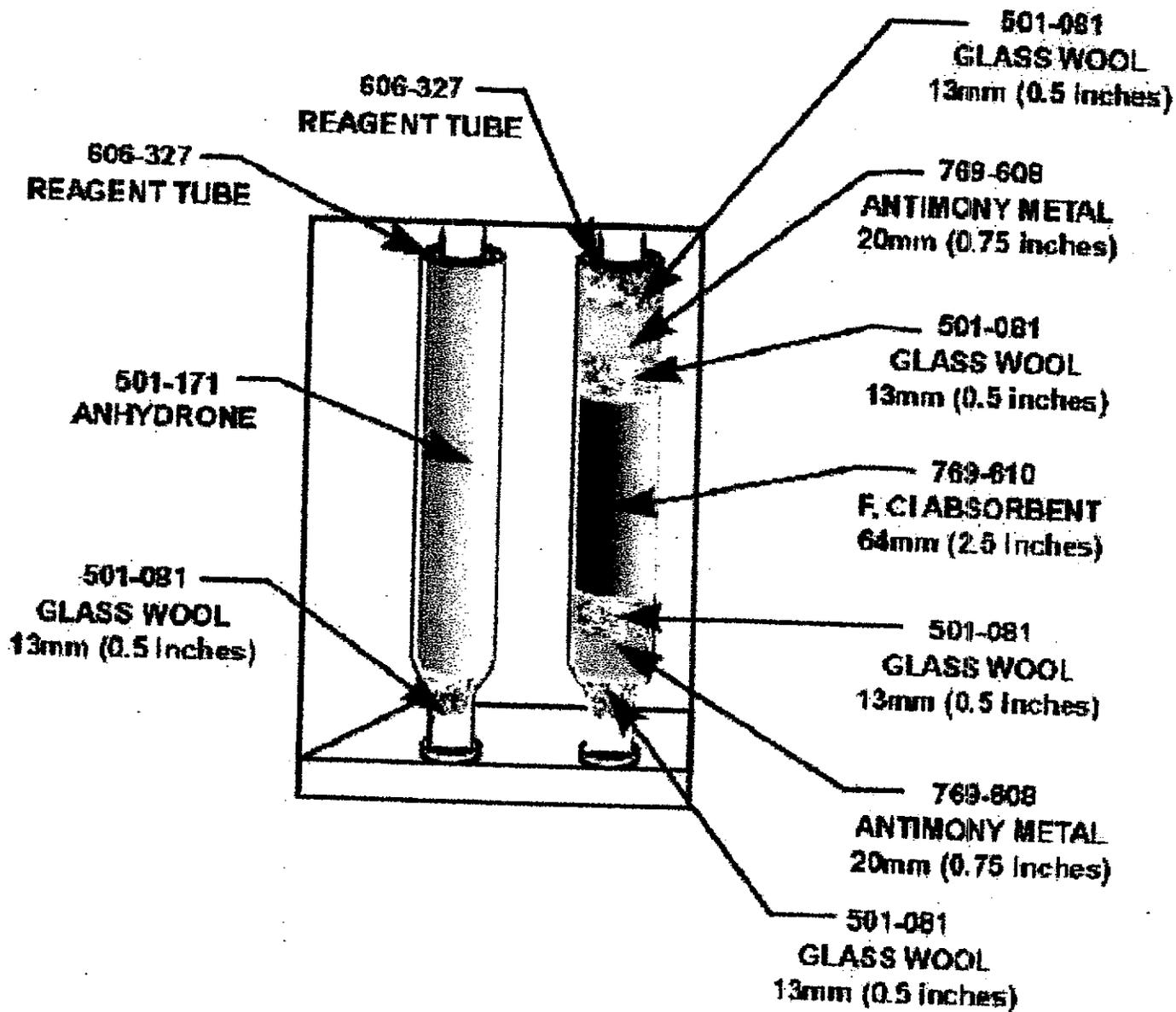


Fig. 1

Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Soil and Sediment Samples with ICP-OES and ICP-MS

Soil & Plant Analysis Laboratory
University of Wisconsin – Madison
<http://uwlab.soils.wisc.edu>

October 2005

1. Application

This method covers the digestion of soil and sediment samples for the analysis of *leachable* components (major, minor, and trace elements or total minerals, heavy metals, and micro-nutrients) by ICP-OES (TJA Iris Advanced ICP-OES) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus ICP-MS).

- 1.1 Soil and sediment samples contain major (Si, Al, Fe, Ti, Mn, Ca, Mg and Na), minor, and trace components. Alternatively, soil and sediment samples contain fraction one or structural components which are held within aluminum-silicate minerals and fraction two components which are held in soil and sediment by other mechanisms (precipitated, replaced, absorbed, complexed, exchanged, etc).
- 1.2 If a soil/sediment sample is totally dissolved, such as with a mixture of hydrofluoric acid (HF) and other acids, the measured components include both fraction one and fraction two components and the measured concentrations are “total

concentrations” of a sample. These concentrations are comparable to the concentrations obtained by other methods such as XRF methods and NAA methods.

- 1.3 The exclusive analysis of fraction two components has more applications than the analysis of total concentrations in agricultural or environmental areas, since fraction one components are “inert” while fraction two components are “active” and “available” in agricultural or environmental processes.
 - 1.3.1 Fraction two components are supposed to be “all-leached” out by treating samples with concentrated acids (except HF acid) at a high temperature and the measured concentrations are “total leachable concentrations.” These leachable concentrations are often referred as “total concentrations” or “total minerals,” although these “total concentrations” are conceptually not true total concentrations at all.

1.3.2 The total leachable concentrations are not directly comparable to XRF or NAA results, since the XRF or NAA concentrations are true total concentrations. However, this is highly element-dependent and may be sample-dependent. For example, the leachable concentration of silicon is far less than the total concentration of silicon, but the leachable concentration of mercury is usually close to (>95%) the total concentration of mercury in samples.

1.4 The total (leachable) components seem simple and well defined conceptually but the analysis of these leachable components is actually defined operationally. The measured results could be widely variable if a given sample is processed (leached) with different procedures or conditions. The results of leachable concentrations in soil or sediment samples should be interpreted carefully, keeping these considerations in mind.

1.5 There are unlimited versions of procedures available in literature for the process of soil and sediment samples, considering the numerous combinations of sample weight, acid type, acid amount, acid concentration, digestion time duration, digestion temperature, digestion pressure, and equipments. Since the measured results could be variable if a given sample is processed with different/alternative procedures or conditions, *a procedure without alternative steps* is preferred, developed, and used at this laboratory to achieve the greatest consistency in analyzing

different types of samples and/or samples at different times. In general, results obtained by a consistent method are comparable mutually.

2. Summary of method

2.1 A dried and ground sample (0.5 gram) and 5 mL of concentrated nitric acid are added into a 50-mL Folin digestion tube. The mixture is heated at 120-130 °C for 14-16 hours and then is treated with hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is further 1:1 diluted for the analysis of major and minor components by ICP-OES and further 1:9 diluted for the analysis of minor and trace components by ICP-MS.

2.2 After solid samples are converted into solutions samples, the procedures of “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS” are followed.

3. Safety

All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.

4. Interference

4.1 This method covers the analysis of over 30 elements by ICP-OES and ICP-MS. Even a general discussion of interferences is lengthy but not necessarily relevant to a specific element/isotope. The analysis of

metals and non-metals by ICP-OES and ICP-MS has been established and there is an enormous amount of literature available relevant to this subject. Reading the published articles is recommended.

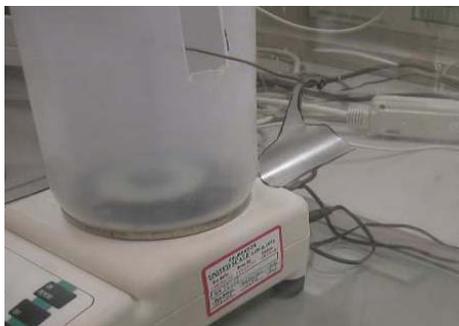
- 4.2 In this method, the solution for ICP-OES analysis contains < 500 ppm of dissolved solid and the solution for ICP-MS analysis contains <100 ppm of dissolved solid. The major components are Fe, Al, K, Ca, Mg and Mn. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences in general are not expected, although some specific element/isotope may be interfered.

5. Sample collection, preservation and handling

A representative sample of soil/sediment is dried and ground. A five-gram vial or equivalent is used to hold a sub sample for airtight storage.

6. Apparatus and device

- 6.1 Analytical balance (accurate to 1 milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.



- 6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.
- 6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate, Model: HP 53014C).



- 6.4 Ten-mL universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

- 6.5 ICP-OES: TJA Iris Advantage ICP-OES.
- 6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat #2110 by Perfector Scientific) for the ICP-OES autosampler are used “as is.”
- 6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).
- 6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes, Cat #14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water for several times. The tubes are air-dried before use.

7. Reagents

- 7.1 Concentrated nitric acid (> 68%) (e.g. TraceMetal grade. Fisher A509-212).
- 7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).
- 7.3 Single-element and multi-element primary standard solutions.

8. Pre-digestion

- 8.1 Dry samples at 60 °C for two days. Large stones/rocks or plant materials are removed. Grind the samples (Calcareous samples may be ground to very fine powders). Small-size samples are wrapped in

plastic film and broken or ground to avoid contamination of normal grounding. Extremely small size samples are used “as-is.” Store in a five-gram vial or other appropriate container for airtight storage. Note: Samples may be dried at 60 °C or at 110 °C. The water content could be different.

- 8.2 Weigh 0.50±0.01 g of the sample (unknown samples, in-house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried digestion tubes (Finely ground calcareous sample powders: 0.25 gram, sandy samples: 1.00 gram). Make one to three digestion blanks.
- 8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an IRS (internal reference standard) for the analysis by ICP-OES. Spike 0.2 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.
- 8.4 Carefully add drops of 20–30% (v/v) nitric acid to moisten the samples. This is especially important for calcareous samples to prevent them from foaming over.
- 8.5 After the samples have been moistened with the diluted nitric acid, add 5 mL of concentrated nitric acid. Soak at room temperature for 2-3 hours.

Note: A digestion with perchloric acid should be avoided for safety concerns. Samples digested with HClO₄ are not good for the analysis of V, Cr, As, ⁷⁷Se, Rb and

several other isotopes using quadrupole ICP-MS.

9. Hot plate digestion

9.1 Place all of the digestion tubes in a block heater. Cover the tubes with plastic film to retard water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

9.2 Set the block heater at 130°C (Block Heater Lindberg Blue: t = 115°C at mark 2.5, t = 130°C at mark 3.0, t = >170°C at mark 7). Turn the power on.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this could cause higher blank concentrations for several elements.

9.3 The temperature will ramp up to 120-130°C after 1.5 hours. Keep heating at 120-130°C for 14-16 hours.

9.4 Remove film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is very important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H₂O₂ are not good for Sn analysis if the hydrogen peroxide is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add hydrogen peroxide (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Leave overnight to let particles settle down. After this digestion (1st dilution), nominal dilution factor = (50 mL/0.5 gram) = 100. Y = 8 ppm. Rh = 40 ppb.

Note: A typical digestion time table at SPAL – start heating in the afternoon (3 pm), heat overnight with plastic film cover, take the cover off in the early morning (7 am) the next day, and add hydrogen peroxide afterwards.

Note: Samples may not be heated above 130-140°C. Localized overheating may cause a sample to boil over and be lost.

Note: Soil/sediment samples may contain MnO₂. Hydrogen peroxide reacts with MnO₂ quickly. Hydrogen peroxide also reacts with some other components quickly in a hot nitric acid medium. Therefore, add hydrogen peroxide only after the sample tubes have been cooled.

Note: After a soil sample is digested with concentrated acid (without HF) at a high temperature, the majority of the sample remains as a solid and 5-10% of the sample is leached into solution (this ratio

is much higher for calcareous soil samples). If a sample is digested at a dilution factor (DF) of 100 (e.g. 0.5 gram of soil sample is digested and diluted to 50 mL) the solution does not contain 1% of the total dissolved solid (TDS) but contains <0.1% of the TDS. This kind of solution can generally be directly introduced to ICP-OES or ICP-MS. However, most components may still be significantly higher than “optimum” concentration ranges. In SPAL, the solution is analyzed by ICP-OES with a further 1:1 dilution for major and most minor elements. With the SPAL’s specific model of the ICP-MS instrument (VG PlasmaQuad PQ2 Turbo Plus ICP-MS), this kind of solution is analyzed with a further 1:9 dilution for minor and trace elements. One may argue that why not to use less amount of soil at the start so that the second dilution or any further dilution is avoided. Firstly, as it is pointed out in section 1 (Application), any “alternative” steps should be avoided as much as possible in order to achieve a consistent analysis. The leaching efficiency would be different if the acid to soil ratio is changed. Secondly, larger-size sample is more “representative” than smaller-size sample for samples such as soil or sediment which is usually fairly “inhomogeneous.” Thirdly, the size of half a gram of sample is widely used in other procedures. The size of a sample of course can be changed if the consistency is not an issue in some special projects.

10. Measurement by ICP-OES

10.1. Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube. Mix. After this 2nd dilution (for ICP-OES), nominal dilution factor = $(6 \text{ mL}/3 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 200$. Y = 4 ppm.

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using up-side down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in “Elemental analysis of solution samples with ICP-OES”).

10.2. Measurement by ICP-OES

10.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-OES.”

10.2.2 Digestion blanks are also measured with other samples.

10.3. Reporting after ICP-OES

10.3.1 The details are given in “Elemental analysis of solution samples with ICP-OES.”

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1st dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2nd dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Add sample solutions (1 mL) to the 14-mL Falcon tubes containing 9 mL of 2% nitric acid. Mix well. After this dilution (2nd for ICP-MS), total dilution factor = $(10 \text{ mL}/1 \text{ mL}) \times (50 \text{ mL}/0.5 \text{ gram}) = 1,000$. Rh = 4 ppb.

11.1.2 Depending on sample matrix and analyte concentration, the sample may be diluted in other ratios.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in “Elemental analysis of solution samples with ICP-MS.”

11.2.2 Digest blanks are also measured with other samples.

11.2.3 In the menu, select “soil” and edit it if needed.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in “Elemental analysis of solution samples with ICP-MS.”

11.3.2 The overall DF is 1,000, after this procedure is followed exactly. Otherwise, adjust the DF accordingly.

Scenario 1: 10 mg/kg (10 ppm or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.2 mL of 10 ppm Rh is digested and diluted to 50 mL (1st DF = 100). This 1st solution (X = 100 ppb, and Rh = 40 ppb) is further diluted by 1:9 (2nd DF = 10) to contain 10 ppb of X and 4 ppb of Rh in a 2nd solution (overall DF = 1000). This 2nd solution is measured against a standard containing 1 ppb of X and 4 ppb of Rh and the measured result is 10 ppb. After applying the overall dilution factor of 1000, the concentration of X in the solid material is $10 \text{ ppb} \times \text{DF } 1000 = 10,000 \text{ ppb} = 10 \text{ ppm}$.

Scenario 2: Element X in the 2nd solution (X = 10 ppb and Rh = 4 ppb) is still much higher than the standard (X = 1 ppb and Rh = 4

ppb). This 2nd solution is diluted by 5 times (3rd DF = 5, total DF = $100 \times 10 \times 5 = 5000$) to contain 2 ppb of X and 0.8 ppb of Rh and this 3rd solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3rd solution (2 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (1 ppb X/4 ppb Rh) and the concentration in the 3rd solution is calculated to be 10 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is $10 \text{ ppb} \times 1000 = 10 \text{ ppm}$. Option 2: At step 13.2, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3rd solution), X in this 3rd solution will be calculated to be 2 ppb against a standard of 1 ppb X with 4 ppb Rh. Now the total DF is 5000 and X in the solid sample is $2 \text{ ppb} \times 5000 = 10 \text{ ppm}$.

12. Quality assurance (QA) and quality control (QC)

ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application, yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure. Some basics are given in “Elemental analysis of solution samples with ICP-OES” and “Elemental analysis of solution samples with ICP-MS.”

– End –

Lime

1. Application

This procedure covers the analysis of aglime and other liming material, and the calculation of the neutralizing index.

2. Summary of Methods

A sample of limestone is reacted with an excess of HCl. After the first reaction is complete, the H^+ remaining is titrated with NaOH. The $CaCO_3$ is calculated as the amount of acid neutralized by the sample compared with the amount neutralized by an equal weight of pure $CaCO_3$.

A dried sample of limestone is passed through a nest of 8, 20, 60, and 100-mesh sieves. The amount retained on each sieve is used to calculate the neutralizing index (NI) of the material. In Wisconsin, aglime is sold and applied on the basis of its neutralizing index. Fine lime reacts more quickly than coarse lime, so less is required to affect a given increase in pH in a three-year period. Fine lime is more costly to produce, however, and does not have as much residual activity as coarse lime.

3. Safety

Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

There are few interferences in this procedure. Errors may arise if insufficient time is allowed for reaction of the sample in HCl or if the sample is not shaken adequately in the sieve analysis.

5. Apparatus and Materials

- 5.1 Erlenmeyer flasks (500 ml)
- 5.2 Burette (50 ml)
- 5.3 Shaker (Ro-Tap® Model # CL 340)
- 5.4 Sieves (ASTM, 8, 20, 60, 100-mesh)
- 5.5 Fischer filter paper, P-8 coarse, funnel (for filtering opaque samples)

6. Reagents

- 6.1 Standard NaOH (1.00 N): Fisher #SS266-20
- 6.2 1% phenolphthalein indicator solution (dissolve 1 g of phenolphthalein in 100 ml of 95% ethanol)
- 6.3 Standard HCl (1.00 N) Fisher #SA48-20

7. Methods

CaCO₃ Equivalent:

- 7.1 Grind lime sample to pass a 60-mesh sieve.
- 7.2 Weigh 1.000 g of the ground sample, and transfer to a 500 ml Erlenmeyer flask.
- 7.3 Add, by means of pipette, 25 ml of standard HCl solution.
- 7.4 Place the 500 ml flask on a hot plate, and heat just below boiling for 30 minutes \pm 10 min. Do not boil. Remove from hot plate, cool to room temperature, dilute to approximately 150 ml with distilled water. Rinse condensate from sides of flask while diluting. Filter sample if opaque.
- 7.5 Add two drops of phenolphthalein indicator.
- 7.6 Titrate to a pink color with standard NaOH. Record volume to 0.1 ml. Be sure buret is at zero to begin.

Ca/Mg Analysis:

- 7.7 Pour titration into a 500 ml cylinder and dilute to 500 ml with deionized water.
- 7.8 Stir (vortex) diluted sample
- 7.9 Place 8 ml of diluted sample into a plastic test tube and analyze by inductively coupled plasma emission spectroscopy (ICP-OES).

Particle Size Distribution:

- 7.10 Weigh a separate 100 g sample of dried, but not ground aglime.
- 7.11 Transfer to the top of the nest of sieves (8, 20, 60, and 100-mesh)
- 7.12 Place the nest of sieves on the Ro-Tap shaker, and shake for 10 minutes.
- 7.13 Weigh the sample retained on each sieve and contained in the bottom pan of the 100, 60, and 20-mesh sieves.

8. Calculations

- 8.1 Calculate the CaCO₃ equivalent of the sample:

$$\text{CaCO}_3 = \frac{\text{mEq acid neutralized by 1.0 g of liming material} \times 100}{\text{mEq acid neutralized by 1.0 g pure CaCO}_3}$$

$$= \frac{(\text{ml HCl} \times \text{N HCl}) - (\text{ml NaOH} \times \text{N NaOH}) \times 100}{\text{sample weight g mEq}}$$

8.2 Calculate the neutralizing index (NI) of the liming material:

$$\text{NI} = [0.2(\% \text{ on 20-mesh sieve}) + 0.6(\% \text{ on 60-mesh sieve}) + 1.0(\% < 60\text{-mesh sieve})] \times \text{CaCO}_3 \text{ equivalent.}$$

9. Quality Control

- 9.1 Standard lime sample – A 1.00 g sample of pure CaCO₃ is analyzed with each batch of samples to check procedural accuracy. This standard should require 5.0 ± 0.1 ml of standard NaOH to titrate to the end point.
- 9.2 Calcium Carbonate (CaCO₃) Fisher # 664-500

10. Reporting

The percent of the sample retained on each sieve and the percent finer than 60-mesh is reported, along with the CaCO₃ equivalent of the liming material. The neutralizing index (NI) is calculated and reported.

11. References

- 11.1 *Calcium Carbonate Equivalent*: See a standard quantitative analysis text for preparation of standard NaOH and HCl; e.g. Blaedel, W. J., and V. M. Meloche. 1963. *Elementary Quantitative Analysis*. Harper and Row, New York. Pages 355-370.
- 11.2 *Particle Size Distribution*: Adapted from H. L. Motto and S. W. Melsted. The Efficiency of Various Particle-Size Fractions of Limestone, 1960, *Soil Sci. Soc. Am. Proc.* 24: 488-490.

Greenhouse Potting Mix

1. Application

Potting mixes used in commercial greenhouses contain high proportions of peat and artificial materials such as perlite and vermiculite. Nutrients are supplied as a nutrient solution when watering in most cases. Therefore, little attention is given to nutrient supplying ability of such mixes. Under greenhouse management, plants are using primarily the nutrients in the water of the soil mix. This testing procedure measures the *intensity* factor (nutrients in solution). The procedure has been termed the “saturated extract” method.

2. Summary of Methods

Potting mixes are wetted with 100ml of 0.005M DTPA solution per 400cc of soil. The mixture is then saturated with distilled water to the point where the water content is about twice the field capacity. The greenhouse solution is extracted from the saturated phase by suction. The pH is determined as a subsample without DTPA solution added. The extract is analyzed for soluble salts, NO₃-N, P, K, Ca, and Mg.

NO₃-N is determined by flow injection.

The elements P, K, Ca, and Mg are analyzed by inductively-coupled plasma (ICP) emission spectroscopy.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

Normal care must be taken to avoid contamination. For interferences with individual analyses, see the procedures for those analyses in soil. Spectral interferences are less severe in the ICP analysis compared to flame emission of atomic absorption spectroscopy.

5. Apparatus and Materials

- 5.1 Plastic beakers (600-ml)
- 5.2 Spatula
- 5.3 Suction manifold
- 5.4 Buchner funnels, 111 mm inside diameter.
- 5.5 Filter paper (Whatman No. 1 or 2 or equivalent)
- 5.6 Suction flasks, 500-ml Erlenmeyer flasks with tubulation
- 5.7 Vacuum pump
- 5.8 Vials, 100-ml, with caps

6. Reagents

DTPA (Diethylenetriaminepentaacetic acid), 40.95g of DTPA per 22 L of deionized water.

See individual procedures for pH, soluble salts (electrical conductivity), and NO_3N .

7. Methods

- 7.1 Place 200 to 400 cc of greenhouse “soil” in a 600-ml plastic beaker.
- 7.2 Add 100 ml DTPA
- 7.3 Add deionized water until the soil is just saturated, mixing with a large spatula. At saturation the soil paste glistens as it reflects the light. The soil mixture will flow slightly when the beaker is tipped. The paste slides freely and cleanly from a spatula. Free water should not collect on the surface.
- 7.4 Determine the pH of the soil using the procedure for Soil pH and Lime requirement.
- 7.5 Filter the paste, using suction and Buchner funnels fitted with 11-cm Whatman No.1, No. 2 or equivalent filter paper, 1 to 4 hours after water is first added to the greenhouse mix. Collect the filtrate in 500-ml suction flasks.
- 7.6 Transfer the filtrate from the suction flasks to 100-ml vials. If the filtered extract is not analyzed the same day, refrigerate the vials.
- 7.7 Measure the electrical conductivity of the saturation extract.
- 7.8 Determine NO_3^- -N in the filtered extract by flow injection.
- 7.9 Analysis of P, K, Ca, and Mg is done by (ICP) inductively-coupled plasma spectroscopy.
- 7.10 Analyze solutions for P, K, Ca, and Mg.

8. Calculations

No calculations are required unless dilutions are required: if so, multiply results by the dilution factor.

9. Quality Control

- 9.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 9.2 Standard soil – One or more standard soils of known analysis is analyzed with each batch of samples to check instrument calibration and procedural accuracy.

10. Reporting

Results for pH are given without units. The term “pH” means $-\log [H^+]$. Electrical Conductance is reported in $mhos \times 10^{-5}/cm$. Values for NO_3^- -N, P, K, Ca, and Mg are reported as ppm in solution.

11. References

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Mound Sand

ASTM C33, Section 5.2

Sieve Size or Number	ASTM C 33 Specifications Percent Passing
3/8 Inch	100
No. 4	95 - 100
No. 8	80 - 100
No. 16	50 - 85
No. 30	25 - 60
No. 50	5 - 30
No. 100	0 - 10
No. 200	

Total Nitrogen, Nitrate and Ammonium in Soil and Tissue

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