California Analytical Methods Manual

For Dairy General Order Compliance – Nutrient Management Plan Constituents

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Preface

Methods for analysis of constituents required for compliance with the Monitoring and Reporting Program associated with the General Order for Existing Milk Cow Dairies was undertaken to have a process available to modify existing analytical methods in a reasonable timeframe. Appropriate analytical methods were identified in the document Approved Sampling Procedures for Nutrient and Groundwater Monitoring at Existing Milk Cow Dairies.

Process wastewater, irrigation water, and groundwater analysis shall be conducted by a laboratory certified for such analyses by the California Department of Health Services. These laboratory analyses shall be conducted in accordance with the Title 40 Code of Federal Regulations Part 136 (*Guidelines Establishing Test Procedures for the Analysis of Pollutants*) or other test methods approved by the Executive Officer.

Manure analyses shall be conducted by methods utilized by the Manure Analyses Proficiency (MAP) Testing Program or accepted by the University of California and laboratories participating in the MAP Testing Program or other programs whose tests are accepted by the University of California.

Plant tissue analyses shall be conducted by: methods utilized by the North American Proficiency Testing (NAPT) Program or accepted by the University of California; and laboratories participating in the NAPT Program or other programs whose tests are accepted by the University of California.

Analyses of soil shall be conducted by: methods utilized by the North American Proficiency Testing (NAPT) Program or accepted by the University of California; and laboratories participating in the NAPT Program or other programs whose tests are accepted by the University of California. This shall include analysis for nitrate-nitrogen and ammonium-nitrogen utilizing the 2 M potassium chloride extract of soil. Analyses of phosphorus in soil samples shall be performed using the method recommended by the University of California or the bicarbonate-P or Olsen-P test.

The MAP program has developed Recommended Methods of Manure Analysis for NH_4-N, Total Kjeldahl N, P, K, Ca, Mg and some trace elements. It does not have methodology for all analytes required in Table 2 of the Monitoring and Reporting Program. Additionally, it is not known if opportunities will exist in the future to modify the methods document to accommodate modifications in laboratory equipment, or additional analytes.

The NAPT program relies on accepted regional analytical methods. Again, uncertainty exists to fully understand options available should procedural modifications to methods be needed. This manual was developed in response to the uncertainty associated with potential modifications to the MAP and NAPT methods and the allowance in the Regional Board Sampling document to also allow methods accepted by the University of California.

As a result of the uncertainty associated with potential modifications to the MAP and NAPT methods and the allowance in the Regional Board Sampling document to also allow methods accepted by the University of California this manual of existing methods was undertaken.

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The organizations listed above were selected because of longstanding activity in the agriculture industry and this list is not intended to be an endorsement of any service these organizations may provide.

This manual was reviewed by individuals chosen for their diverse knowledge and technical expertise. The purpose of this independent review was to provide candid and critical comments to make the final manual as technically sound as possible and to ensure that it meets industry standards for laboratory protocols. We wish to thank the following individuals for their review of this report:

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Date	Summary of revision(s)	Location of revision(s)
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Introduction

This document is organized in the order presented in the Monitoring and Reporting Program of the General Order (Appendix 1). Laboratory analytical methods for each required chemical constituent are defined, with citations, to identify the basis for each method. For some constituents, no validated methods currently exist for use in manure analysis. In these cases, a place holder is included in this document to allow addition of validated methods at a future date.

The methods have been written in a fairly open format, leaving laboratories the ability to adapt the methods to existing laboratory equipment and procedures. However, the rationale and chemistry behind the methods should not be altered. Specifically, while sample amounts and extraction/digestion volumes may be altered, extraction ratios and type of extractant should not be modified. Before broad implementation, method modifications should be validated by successful testing of reference materials.

Each procedure identifies a sample holding time. This term includes the time the sample may be stored in a non-frozen state between collection and start of analysis. It is very important to identify if samples can be processed within the holding time or if the client should be notified that the sample has, or will, expire prior to analysis. Chain of custody documentation should always accompany samples. In addition, approved sample collection protocols must be used for data to be accepted for compliance with the General Order. Protocols approved by the Executive Officer are listed in Appendix 2, and may be downloaded at: www.cdqa.org/binder.asp (section 5)[1].

Methods specify quality control samples, but provide only general acceptance criteria in some cases. It is expected that laboratories will develop in-house acceptance criteria based on the charting of method performance over time (Appendix 4).

Laboratories participating in existing agricultural proficiency testing programs were invited to provide input into method development. Requests for modifications or revisions to these procedures, or addition of new procedures, may be made by contacting either Dr. Holstege or Dr. Meyer.

[1] List of approved sampling protocols is maintained by the Central Valley Regional Water Quality Control Board. http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/general_order_guidance/sampling_analysis/index.shtml .

Introduction – Manure

The Monitoring and Reporting Program in the General Order requires analysis of manure. This material is typically land applied through shank injection or trucks. Depending on application method, slurry manure may also be put into the process wastewater category.

Manure analyses shall be conducted using methods approved by the MAP Program or other programs whose tests are accepted by the University of California.

Required analyses	UC Method
[1]Percent moisture (if weight applied is reported) or Bulk density (if volume applied is reported)	M - 1.10 Total Solids and Moisture or M - 1.20 Bulk Density (method not available at this time)
[2]Total nitrogen	M - 2.10 Total Nitrogen by Combustion or M - 2.20 Total Nitrogen by Kjeldahl Method and M - 2.40 Ammonium Nitrogen
[2]Total phosphorus and total potassium [3]General minerals (calcium, magnesium, sodium, bicarbonate, carbonate, sulfate, and chloride)	M - 3.10 Total Phosphorus, Potassium, Calcium, Magnesium, Sodium, Sulfur; and M - 4.10 Total Sulfate by Turbidimetric Analysis, or M - 4.20 Total Sulfate by Anion Exchange Chromatography; and M - 5.10 Chloride; and M - 6.10 Carbonate and Bicarbonate (method not available at this time)

[1]Each offsite export
[2]Twice per year
[3]Once within 12 months of adoption of the General Order

The greatest challenge for laboratories is obtaining a representative aliquot from the sample. The variability of results for un-homogenized samples often requires that most samples be run in duplicate and precludes use of very small sample aliquots as sample size.

M – 1.10 Total Solids in Dairy Manure

1.0 Scope and Application

This method quantitatively determines the percent total solids, including organic and inorganic material, in dairy manure (slurry, semi-solid, or solid) based on gravimetric loss of volatiles on heating.

2.0 Method Summary

2.1 Description. Total solids (TS) percentage is determined gravimetrically based on the loss of volatiles, including free water associated with heating to 105 ^{o}C until mass remains constant (Peters et al., 2003). The method does not remove molecular bound water. The method is destructive with respect to the integrity of the material for additional testing. Total solids percent is used to convert analytical results from as-received to dry basis. Method is based on AOAC Method 990.15.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.006% - 0.03% TS.

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

*2.3.1*Method Reporting. Minimum reporting limit (MRL) for total solids is 0.1%.

*2.3.2*Total solids are reported as percent of as-received mass.

2.4 Interferences. Not applicable.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 10 g.

2.6 Laboratory Sample Storage. Store laboratory sample either under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be refrigerated up to 7 days as long as sample integrity is maintained.

2.8 Advantages. (1) Simple method requiring inexpensive equipment.

2.9 Disadvantages. (1) Test portion sample size may affect precision; (2) test portion sample size impacts drying time; and (3) potential loss of volatile compounds.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted material may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure materials containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for materials containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Semi-solid or solid laboratory samples may be processed by drying and grinding. Divide the sample in half, dry at 55°C ± 5°C, remove foreign material and grind. Record weight before and after drying for partial moisture content determination.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 7 days as long as sample integrity is maintained.

4.5.2 Frozen material may be stored for up to one year.

4.5.3 Dried and processed material (as described in section 4.2) may be held up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time.

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe; or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.4 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Weighing dishes. Aluminum or other low sided containers capable of withstanding 105 ºC.

5.7 Forced -air drying oven. Vented, capable of maintaining constant temperature of 105 ºC ± 5 ºC.

5.8 Desiccator chamber. Sealable chamber containing a desiccating agent.

6.0 Reagents

6.1 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh empty weighing dish. Record the mass (W_p) to the nearest 0.01 g.

7.2 Tare the balance with the empty weighing dish.

7.3 Transfer approximately 10 g of processed unknown into weighing dish and record wet mass (W_s) to the nearest 0.01 g.

7.4 Method blank. Include at least one empty weighing dish with each batch of unknowns.

7.5 Place unknowns and controls in forced-air drying oven pre-heated to 105 ºC ± 5 ^{o}C until weight remains constant (less 0.1% TS change from previous weight).

7.6 Remove all weighing dishes to a desiccator to cool.

7.7 Weigh unknowns and weighing dishes. Record dry weight (W_d) to the nearest 0.01 g.

8.0 Calculations and Reporting

8.1 Calculate the total solids content as percent (%) according to the following equation:

For primary results given in g:

$$TS = [(W_d - W_p) / W_s] \times 100\%$$

where

TS is the total solids content of the unknown or control (%)
W_d is the total weight of the unknown or control including the weighing dish, after drying (g)
W_p is the tare weight of the weighing dish (g)
W_s is the weight of the unknown or control, before drying (g)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. If this occurs, take corrective action and repeat.

9.2 Analyte carryover. Not applicable.

9.3 Reference Standard. Not applicable.

9.4 Drift Standard. Not applicable.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or by use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also

advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of these materials in accordance with local, state and federal regulations.

12.0 References

American Society of Agricultural Engineers. 2004. Uniform terminology for waste management, 292.5 pp 656-659 FEB04.

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC, 18th Edition. Moisture in Animal Feed (990.15). Arlington, VA.

International Organization for Standardization. 1994. Accuracy (trueness and precision) of measurement methods and results. Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method. Report 5725-2:1994 Geneva, Switzerland.

Peters, J. editor. 2003. Total Nitrogen. Recommended methods of manure analysis. ed J. Peters, pp14-17. University of Wisconsin Extension Publication. A3769.

Shreve et al. 2006. NFTA Method 2.1.4 – Dry Matter by Oven Drying for 3 hr at 105 ºC. National Forage Testing Association.

U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

M – 2.10 Total Nitrogen by Combustion in Dairy Manure

1.0 Scope and Application

This method quantitatively determines the total nitrogen content of dairy manure (liquid, slurry, semi-solid, or solid) by discrete combustion in an oxygen environment and quantification by thermal conductivity detector.

2.0 Method Summary

2.1 Description. The material is heated to a temperature of 950-1350°C in the presence of oxygen based on the method of Dumas (1831). Mineral and organic nitrogen compounds are oxidized and the combustion products passed through a catalyst reduction furnace where conversion of oxides of nitrogen (NO_x) to molecular nitrogen (N_2) occurs. Water vapor and CO_2 are removed and total nitrogen is quantified using a thermal conductivity detector (TCD). Method is comparable to the Total Kjeldahl Nitrogen (TKN, Method M-2.30) for nitrogen. The method is adapted from AOAC Method *990.3* and Peters *et al*. (2003).

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.001% - 0.005% N (dry basis, instrument dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Typical minimum reporting limit (MRL) for total nitrogen is 0.10% N.

2.3.2 Nitrogen concentration is reported as percent (%) on a dry basis.

2.4 Interferences. Volatilization losses of NH_3 may occur during processing. To reduce volatilization loss, minimize exposure to air and maintain unknown at ≤6°C.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is from 0.20 to 5.0 g (instrument specific).

2.6 Laboratory Sample Storage. Store as-received laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

2.8 Advantages. (1) Avoids use of concentrated acids; (2) fast automated analysis; and (3) complete recovery of NH_4-N, oxidized forms of N, and heterocyclic N compounds.

2.9 Disadvantages. (1) High cost of instrumentation; (2) limited test portion size may affect precision; (3) detection limit may be insufficient for liquid material; and (4) high instrument maintenance requirement.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

3.4 *Total Nitrogen* (TN). The sum total of all forms of nitrogen, inorganic plus organic, within the matrix.

3.5 *Total Kjeldahl Nitrogen* (TKN). The sum of organic nitrogen plus ammonia nitrogen (NH_4^{1+}). Does not include oxidized forms of nitrogen (NO_2^{1-} and NO_3^{1-}).

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure materials containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for materials containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Not applicable.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 7 days as long as sample integrity is maintained.

4.5.2 Frozen material may be stored for up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.4 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Micro-pipetter. 100 uL ± 5 uL volume.

5.8 Total Nitrogen Analyzer. Instrument utilizing a resistance furnace, catalyst, and scrubber system with thermal conductivity detector. Manufacturers include: Leco®; Elementar; Gerhardt Co.; Thermo; Costech; Exeter Analytical; or instrument of similar design capable of performing Dumas nitrogen determination.

6.0 Reagents

6.1 Acetic acid. Glacial acetic acid (CH_3COOH, 99.6%) ACS reagent.

6.2 Helium. Minimum grade: 4.5 (99.995%).

6.3 Oxygen. Minimum grade: 4.5 (99.995%).

6.4 Compressed air or nitrogen. 99.6%, free of oil and water, 6.2 ± 0.2 bar (90 ± 5 psi).

6.5 Consumables. Instrument specific, check manufacturer's recommendation.

6.6 Method blank solution. Empty, instrument-specific container.

6.7 Nitrogen calibration standard. Known composition and certified content. Recommended compounds: glycine p-toluene sulfonate ($C_9H_{13}O_6SN$, 5.67 %N); EDTA ($C_{10}H_{16}N_2O_8$, 9.59 %N); or acetanilide (C_8H_9NO, 10.36 %N). Available from commercial chemical vendors. Store in desiccator.

6.8 Nitrogen reference standard, certified content. Available commercially (should come from a different source than calibration standard).

6.9 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh and transfer 200 mg to 5000 mg test portion of the processed unknown into instrument-specific container. Record weight to nearest 0.1 mg if weighing <1000 mg, or to nearest 1.0 mg if weighing ≥1000 mg. Analyze each unknown in duplicate if test portion is <1000 mg. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific methodology.

7.2 Optional step for ammonium stabilization. Add glacial acetic acid (20 uL per 100 mg of unknown) to the surface of the test portion in the instrument container to stabilize the ammonium nitrogen in the test portion.

7.3 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing nitrogen calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicates. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the test portion total nitrogen content as percent (%) on a dry basis according to the following equation:

$$N = C/(TS/100\%)$$

where

N is the N content in the unknown (%, dry basis)
C is the N concentration result from the instrument (%)
TS is total solids of the test portion (%)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable.

Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is analyte carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s1 is the first unknown
s2 is the second unknown
h_{co} is analyte carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Instrument operates at very high temperatures.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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M – 2.20 Total Kjeldahl Nitrogen in Dairy Manure

1.0 Scope and Application

This method quantitatively determines the nitrogen content of dairy manure (liquid, slurry, semi-solid, or solid) through acid digestion and subsequent quantification of ammonium nitrogen using spectrophotometric, diffusion-conductivity, or distillation and titration analysis.

2.0 Method Summary

2.1 Description. This method is based on that of Kjeldahl (1883) and includes wet oxidation of dairy manure during sulfuric acid digestion with a catalyst. After digestion, ammonium (NH_4^{1+}) is determined either spectrophotometrically (660 nm) by flow injection analysis (FIA), diffusion-conductivity analysis (Carlson *et al.*, 1990); or by distillation of ammonia (NH_3) and subsequent titration. This method is adapted from Watson *et al*. (2003).

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.01% - 0.05% N (dry basis).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for TKN is 0.05% N.

2.3.2 Nitrogen concentration is reported as percent on a dry basis.

2.4 Interferences.

2.4.1 Volatilization losses of NH_3 may occur during processing. To reduce volatilization loss, minimize exposure to air and maintain material at 4 ^{o}C $\pm$ 2 °C.

2.4.2 The method does not quantitatively recover nitrogen from heterocyclic compounds and oxidized forms such as nitrate and nitrite (NO_3^{1-} and NO_2^{1-}).

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.1 g to 0.5 g g.

2.6 Laboratory Sample Storage. Store as-received laboratory sample either under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be refrigerated up to 7 days as long as sample integrity is maintained.

2.8 Advantages. (1) Moderate cost of instrumentation; (2) accommodates large test portion size; and (3) can handle wide range of moisture content.

2.9 Disadvantages. (1) Requires use of concentrated acid; (2) requires digestion catalyst; (3) incomplete recovery of nitrogen from heterocyclic carbon compounds; (4) long digestion time; and (5) labor intensive.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

3.4 *Total Nitrogen* (TN). The sum total of all forms of nitrogen, inorganic plus organic, within the unknown matrix.

3.5 *Total Kjeldahl Nitrogen.* The sum of organic nitrogen plus ammonia nitrogen (NH_4^{1+}). Does not include oxidized forms of nitrogen (NO_2^{1-} and NO_3^{1-}).

4.0 Sample Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure laboratory samples containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for samples containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Not applicable.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 7 days as long as sample integrity is maintained.

4.5.2 Frozen laboratory sample may be stored for up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time.

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe; or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.4 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Digestion block. Capable of 380 °C ± 10 °C and accommodating digestion tube (Section 5.8).

5.8 Digestion tubes and caps . Pyrex glass digestion tube with volumetric graduation mark (50 mL, 75 mL or 100 mL).

5.9 Acid resistant fume hood. Capable of minimum flow rate of 100 cubic feet per minute (CFM).

5.10 Vortex mixer. Standard laboratory mixer with tube adapter.

5.11 Ammonium nitrogen analysis instrument (select one).

5.11.1 Flow injection analyzer. Segmented flow or flow injection and analysis (FIA) type. Automated ammonium nitrogen analyzer (manufacturers include: Techincon Autoanlyzer II, Lachat Instruments, FIALab, Skalar) or other instrument of similar design capable of performing ammonium nitrogen analysis based on phenate or salicylate chemistry.

5.11.2 Diffusion-conductivity analyzer based on method described by Carlson (1990) (e.g. Timberline Instruments Method 4500-N D).

5.11.3 Micro-Kjeldahl steam distillation apparatus.

6.0 Reagents

6.1 Deionized (DI) water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Concentrated sulfuric acid (H_2SO_4). ACS Reagent.

6.3 Digestion catalyst. Kjeltabs (Fisher Kjeltab ST-AUTO Kjeldahl tablets, 1.5 g K_2SO_4 plus 0.015 g Se), copper sulfate ($CuSO_4$) or equivalent.

6.4 Boiling Chips. Acid resistant.

6.5 Flow Injection Analysis Reagents (refer to instrument manufacturer's specific procedure for phenate or salicylate chemistry).

6.6 Diffusion-Conductivity Analysis Reagents (refer to instrument manufacturer's specific procedure).

6.7 Ammonium Distillation Analysis Reagents (refer to instrument manufacturer's specific procedure).

6.8 Method blank solution. Deoinized water.

6.9 Nitrogen calibration standard stock solution. Known composition and certified content. Recommended compounds: glycine p-toluene sulfonate ($C_9H_{13}O_6SN$, 5.67 %N); EDTA ($C_{10}H_{16}N_2O_8$, 9.59 %N); or acetanilide (C_8H_9NO, 10.36 %N). Available from commercial chemical vendors. Store in desiccator.

6.10 Nitrogen reference standard, certified content, available commercially (should be from a different source than calibration standard stock solution).

6.11 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Pre-heat digestion block to 160 °C in acid resistant fume hood.

7.2 Weigh and transfer approximately 100 mg to 500 mg test portion of processed unknown to a Kjeldahl digestion tube. Record weight to nearest 1 mg. Analyze each unknown in duplicate. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific processing methodology.

7.3 Prepare spikes, reference material, and blanks along with the unknowns for digestion.

7.4 In the fume hood, add 3 mL concentrated H_2SO_4, followed by one Kjeltab and a few boiling chips to each digestion tube.

7.5 Place digestion tube in 160 ^{o}C digestion block for 30 minutes.

7.6 Ramp temperature 5 ^{o}C min^{-1} to 380 ^{o}C, and hold for 60 minutes or until digestion is complete.

Remove from block and cool in acid resistant fume hood for 30 minutes.

7.7 Dilute to graduation mark with deionized water. Cap and mix using vortex mixer.

7.8 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the test portion TKN content as percent (%) on dry basis according to the following equation:

$$TKN = 100\% \times [(C - B) \times (V/m) \times D_f / 10000] / [TS / 100]$$

where,

TKN is the nitrogen content (percent, dry basis)
C is the digest NH_4^{1+}-N concentration after dilutions (mg L^{-1})
B is the method blank (mg L^{-1})
m is the test portion mass (g)
TS is the Total Solids of the test portion (%, method M-1.10)
V is the digest or distillate final volume (mL)
D_f is any additional dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Perform spike recovery with each batch of unknowns when the matrix differs significantly from the reference material. Spike the unknown with analyte reference standard at a concentration 4 to10 times the analyte concentration of the unknown or MDL, whichever is greater.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the method reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85% to 115%. Failure shall result in evaluation of reagents and equipment, correction of

problems, and re-analysis of spike recovery and all unknowns in the batch.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method requires use of high temperatures and concentrated acids. Neutralize acid spills with a solution of saturated sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$).

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

American Society of Agricultural Engineers. 2004. Uniform terminology for waste management, 292.5 pp 656-659 FEB04.

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M – 3.10 Phosphorus, Potassium, Calcium, Magnesium, Sodium and Sulfur in Dairy Manure

1.0 Scope and Application

This method quantitatively determines the phosphorus (P), potassium (K) calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S) content in dairy manure (liquid, slurry, semi-solid or solid) by acid wet digestion of the test portion with subsequent quantification by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.0 Method Summary

2.1 Description. The individual elemental content (P, K, Ca, Mg, Na, and S) of dairy manure is determined after digestion with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Elemental constituents of the digest are determined by ICP-OES. The method is adapted from Wolf et al. (2003).

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDLs are: 0.005% P, 0.005% K, 0.005% Ca, 0.005% Mg, 0.010% Na, and 0.0025% S (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limits (MRL) are: 0.01% P, K, Mg, and S; 0.05% Ca, and Na, (dry basis, instrument manufacturer dependent).

2.3.2 Elemental constituents are reported as percent (%) on a dry basis.

2.4 Method Interferences. None known.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.2 g to 1.0 g.

2.6 Laboratory Sample Storage. Store laboratory sample either under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

2.8 Advantages. (1) Fast automated analysis; (2) quantitation of all forms of P (inorganic and organic); and (3) and low detection limit.

2.9 Disadvantages. (1) High cost of instrumentation; (2) use of concentrated acids and reactive chemicals; and (3) high instrument maintenance cost.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure materials containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for materials containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Semi-solid or solid laboratory samples may be processed by drying and grinding. Divide the sample in half, dry at 55°C ± 5°C, remove foreign material and grind. Record weight before and after drying for partial moisture content determination.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

4.5.2 Frozen material may be stored for up to one year.

4.5.3 Dried and processed material (as described in section 4.2) may be held up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time.

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe; or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 ºC.

5.4 Freezer. Capable of maintaining constant temperature -20 °C ± 5 ºC.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Repipette dispenser. Dispenser calibrated to 100.0 mL ± 0.5 mL.

5.8 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 KPa ± 10 KPa).

5.8.1 Glass fiber filter

5.8.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.9 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.9.1 Centrifuge tubes. Compatible with centrifuge used.

5.10 Digestion tubes and caps. 100 mL volume, glass or Teflon, digestion block specific, capable of withstanding 200 °C.

5.11 Digestion block. Capable of a range of 80 °C to 140 °C ± 5.0 °C.

5.12 Acid resistant fume hood. Capable of minimum flow rate of 100 cubic feet per minute (CFM).

5.13 Micro pipette. Calibrated to dispense 1.0 mL ± 0.1 mL.

5.14 Vortex mixer. Standard laboratory mixer with tube adapter.

5.15 ICP-OES Instrument. Analytical wavelengths required: phosphorus - 178.3 or 213.6 nm; potassium - 404.7 nm; calcium - 396.5, 422.7 or 317.9 nm; magnesium - 279.6 or 285.2 nm; sodium - 589.0 nm; sulfur - 182.0 nm; or other instrument-specific wavelengths. Manufacturers include: Thermo-Fisher, Perkin-Elmer, Varian, Leeman and Spectro.

6.0 Reagents

6.1 Deionized (DI) water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Nitric Acid (HNO_3), concentrated, trace metal grade.

6.3 Hydrogen Peroxide (H_2O_2), 30% ACS Reagent.

6.4 Argon gas 99.5% purity.

6.5 Lithium chloride solution (1000 mg L^{-1} lithium chloride, LiCl, ACS reagent). Dissolve 2.03 g LiCl in 1.5 L of deionized water. Add 20 mL of concentrated nitric acid (HNO_3, trace metal grade) and bring to 2 L with deionized water.

6.6 Method blank solution. Digestion solution (6 mL concentrated HNO_3, 2 mL 30% H_2O_2, 92 mL deionized water).

6.7 Calibration standards. Prepare 3 or more multi-element calibration standard solutions in 2% HNO_3 solution. Available from commercial chemical vendors.

6.8 Multi-element reference standard, certified content. Available commercially (should come from a different source than calibration standard).

6.9 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Pre-heat digestion block to 80 ^{o}C ± 5 ^{o}C.

7.2 Transfer approximately 200 mg to 1000 mg size test portion of processed or dried unknown into a digestion tube. Record weight. Analyze all unknowns in duplicate. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific methodology.

7.3 Prepare method blank, reference material, drift control standards, and spike (if used) for digestion along with unknowns and duplicates.

7.4 Add 6.0 mL concentrated nitric acid using a repipette dispenser and swirl to thoroughly wet the material. Predigest at room temperature for a minimum of 20 minutes.

7.5 Place digestion tubes in digestion block for 10 minutes. Remove and cool for 2 minutes.

7.6 Increase digestion block temperature to 140 ^{o}C ± 5 ^{o}C.

7.7 Add 2 x 1.0 mL aliquots of 30% H_2O_2 solution. If foaming occurs, carefully swirl to clean off walls of digestion tube.

7.8 Return tubes to digestion block and digest for 60 minutes or until total digest volume is reduced to 2 mL to 3 mL.

7.9 Remove tubes from block and cool for 30 minutes.

7.10 Dilute each digest to 100 mL with deionized water. Cap and mix using vortex mixer. Digests exhibiting turbidity may require overnight settling, centrifugation or filtration to remove undissolved particulate matter prior to analysis.

7.11 Note: it may be necessary to add lithium chloride solution to all digests and standards in order to obtain a stable potassium reading.

7.12 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing multi-element calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the analyte content as percent (%) on dry basis, according to the following equation:

$$M = 100\% \times [(C - B) \times (V/m) \times D_f / 10000] / (TS / 100)$$

where

M is the element (P, K, Ca, Mg, or Na) concentration (%, dry basis)
C is the digest analyte concentration after dilutions (mg L^{-1})
B is the method blank concentration (mg L^{-1})
V is the digest final volume (mL)
m is the test portion mass (g)
D_f is any additional dilution factor
TS is the Total Solids of the test portion (%, method M-1.10)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

$$\text{MDL} = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$\text{RPD} = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the unknown duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all

personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes high temperatures and concentrated acids. Hydrogen peroxide may react violently with hot organic acid solution.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

American Society of Agricultural Engineers. 2004. Uniform terminology for waste management, 292.5 pp 656-659 FEB04.

Haeckel, R. 1991. Proposals for the description effects in clinical chemistry and measurement of carryover. Pure & Appl. Chem. 63: 301-306.

Haung, C.L. and E.E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Comm. Soil Sci. Plant Anal. 16: 945-958.

International Organization for Standardization. 1994. Accuracy (trueness and precision) of measurement methods and results. Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method. Report 5725-2 Geneva, Switzerland.

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U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

Wolf, A., M. Watson and N. Wolf. 2003. Digestion and dissolution methods for P, K, Ca, Mg and trace elements. J. Peters (ed) Recommended methods of manure analysis. University of Wisconsin Extension Publication. A3769.

Yang, W. M, R. Boles, and T. P. Mawhinney. 2002. Determination of phosphorus in fertilizers by inductively coupled plasma atomic emission spectrometry. J. AOAC 85,6: 1241-1246.

M – 4.10 Chloride in Dairy Manure

1.0 Scope and Application

This method quantitatively determines the chloride (Cl^-) content of dairy manure (liquid, slurry, semi-solid or solid) by chloridometer.

2.0 Method Summary

2.1 Description. The Cl^- content of dairy manure is determined by extraction with acetic acid solution and analysis by coulometric titration with a silver electrode. The concentration of Cl^- in solution is correlated with time of current flow. The method is adapted from Soil, Plant and Water Reference Methods for the Western Region B-3.10 and ISO 1575-2.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.002% - 0.01% Cl (dry basis).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit for chloride is 0.05% Cl.

2.3.2 Chloride concentration is reported as % on a dry basis.

2.4 Interferences. Iodide and bromide will combine with silver resulting in positive interference.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 1.0 g for as-received laboratory sample or 0.4 g processed material.

2.6 Laboratory Sample Storage. Store as-received laboratory sample either under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

2.8 Advantages. (1) Avoids use of concentrated acids; and (2) avoids hazardous reagents.

2.9 Disadvantages. (1) Moderate precision; and (2) slow analysis.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure materials containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for materials containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Semi-solid or solid laboratory samples may be processed by drying and grinding. Divide the sample in half, dry at 55°C ± 5°C, remove foreign material and grind. Record weight before and after drying for partial moisture content determination.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

4.5.2 Frozen material may be stored for up to one year.

4.5.3 Dried and processed material (as described in section 4.2) may be held up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time.

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of

homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe; or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.4 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.7 Repipette dispenser. Dispenser calibrated to 100.0 mL ± 0.5 mL.

5.8 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 KPa ± 10 KPa).

5.8.1 Glass fiber filter

5.8.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.9 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.9.1 Centrifuge tubes. 50 mL capacity, compatible with centrifuge used.

5.10 Chloridometer. Capable of performing chloride analysis based on coulometric titration chemistry. Manufacturers include: Labconco, FKGO; Ciba Corning; Haake Buchler or other instrument with equivalent capability.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Extraction solution (2.0% acetic acid). Dilute 20 mL glacial acetic acid (CH_3COOH, >99%) in 500 mL deionized water. Bring to 1 L final volume with deionized water.

6.3 Chloride acid solution (20% acetic acid, 2.4% nitric acid). Dilute 200 mL glacial acetic acid (>99%) with 200 mL deionized water. Add 12 mL concentrated nitric acid (HNO_3) and bring to 500 ml final volume with deionized water. For use in the chloridometer.

6.4 Method blank solution. Extraction solution.

6.5 Chloride calibration standards. Prepare 3 or more calibration standard solutions in extraction solution. Available from commercial chemical vendors.

6.6 Chloride reference standard, certified content. Available commercially (should come from a different source than calibration standard).

6.7 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh 1.0 g to 4.0 g unknown, or 0.4 g dried and ground unknown, into a 50 mL centrifuge tube. Record weight.

7.2 Add 40 mL of 2% acetic acid extraction reagent using a repipette dispenser.

7.3 Cap the tubes and shake on reciprocating shaker for 30 minutes at >100 opm.

7.4 Make dilutions according to instrument manufacturer's specification. Record dilution factor. This extract may also be used for the sulfate method (M – 8.10).

7.5 Clarify extract using filtration or centrifugation, if necessary.

7.6 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte results must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the test portion Cl^- content as % on a dry basis according to the following equation:

$$Cl = [(C \times (V/m) \times D_f \times 0.10]/(TS/100\%)$$

where

Cl is the Cl^- content (%, dry basis)
C is the extract Cl^- concentration (after dilutions, mg L^{-1})
V is the extract volume (mL)
m is the test portion mass (g)
TS is the total solids of the test portion (%, method M-1.10)
D_f is the dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank

typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s1 is the first unknown
s2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Perform spike recovery with each batch of unknowns when the matrix differs significantly from the reference material. Spike the unknown with analyte reference standard at a concentration 4 to 10 times the analyte concentration of the unknown or MRL, whichever is greater.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the method reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of	Degrees of freedom	t (cn-1,0.99
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85% to115%. Failure shall result in evaluation of reagents and equipment, correction of problems, and re-

analysis of spike recovery and all unknowns in the batch.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. High temperatures.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

American Society of Agricultural Engineers 2004. Uniform terminology for waste management. S292.5 FEB04 pp 656-659.

Gavlack, R. E. D. A. Horneck and R. O. Miller. 2005. Soil and Water Reference Methods for the Western Region, 3rd Edition. Western Regional Extension Publication WREP-125, Oregon State University.

Haeckel, R. 1991. Proposals for the description effects in clinical chemistry and measurement of carryover. Pure & Appl. Chem. 63: 301-306.

International Organization for Standardization. 1994. Accuracy (trueness and precision) of measurement methods and results. Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method. Report 5725-2:1994 Geneva, Switzerland.

International Organization for Standardization. 2000. Water quality - Determination of chloride by flow analysis (CFA and FIA) and photometric or potentiometric detection. Report 15682:2000. Geneva, Switzerland.

U.S. Environmental Protection Agency. 1986 Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

U.S. Environmental Protection Agency. 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses. Chapter 2. Document # EPA-823-B-01-002.

M – 5.10 Sulfate-sulfur in Dairy Manure by ICP-OES with Anion Exchange Cleanup

1.0 Scope and Application

This method quantitatively determines the sulfate-sulfur (SO_4-S) content of dairy manure (slurry, semi-solid, or solid) by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.0 Method Summary

2.1 Description. Sulfate-sulfur in dairy manure is extracted with acetic acid solution. Sulfate-sulfur is analyzed by ICP-OES following anion exchange cleanup. The extract is applied to the anion exchange column to remove non-ionic forms of sulfur. The column eluate is analyzed for sulfur by ICP-OES. The method is adapted from the method of Littlefield *et al.* (1990).

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.002% - 0.01% SO_4-S (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for SO_4-S is 0.05% SO_4-S.

2.3.2 Sulfate content is reported as percent (%) on a dry basis.

2.4 Interferences. Non-sulfate forms of sulfur can be a positive interference.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 1.0 – 4.0 g as-received, or 0.4 g dried and ground material.

2.6 Laboratory Sample Storage. Store as-received laboratory sample either under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

2.8 Advantages. (1) Avoids use of concentrated acids.

2.9 Disadvantages. (1) Moderate precision.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Prior to analysis dairy manure materials containing less than an estimated 14% total solids shall be homogenized for 60 seconds utilizing a rotor-stator homogenizer for liquid, slurry, and semi-solid materials (see Table 1). Use a blade homogenizer for 60 seconds for materials containing greater than estimated 14% solids.

4.1.2 For drier material (> 30% total solids) chop, divide, or mix to reduce heterogeneity. Results from testing precision (Section 10.2) should be used to determine if duplicates should be run on all unknowns.

4.2 Alternate Processing. Semi-solid or solid laboratory samples may be processed by drying and grinding. Divide the sample in half, dry at 55°C ± 5°C, remove foreign material and grind. Record weight before and after drying for partial moisture content determination.

Table 1. Dairy Manure Classification.

ASAE Designation	Estimated Total Solids Content (%) as Received
Liquid	<3 %
Slurry	3 - 8 %
Semi-Solid	8 - 14 %
Solid	> 14 %

(Adapted from ASAE S292.5 FEB04. 2004)

4.3 Total Solids Content. Determine total solids content on the test portion material according to Method M-1.10 and record.

4.4 Storage. Store laboratory sample either under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.5 Holding Time.

4.5.1 Laboratory sample may be refrigerated up to 28 days as long as sample integrity is maintained.

4.5.2 Frozen material may be stored for up to one year.

4.5.3 Dried and processed material (as described in section 4.2) may be held up to one year.

4.6 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process and complete analysis within defined holding time.

5.0 Apparatus

5.1 Rotor-Stator homogenizer. Laboratory unit capable of a minimum of 250 mL material. Manufacturers include: Pro Scientific, Max-Homogenizing; Omni, General Laboratory Homogenizer (GLH)***;*** Polytron®, Microtron, Silverson Laboratory Mixer, or equipment capable of homogenizing liquid, slurry, and semi-solid manure materials.

5.2 Blade homogenizer. Laboratory unit capable of mixing a minimum of 250 mL material. Manufacturers include: Biohomogenizer™; Jiffy® Mixer; Waring, Micronizer; Robot Coupe; or equipment capable of homogenizing solid manure materials.

5.3 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.4 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.8 Repipette dispenser. Dispenser calibrated to 40.0 mL ± 0.5 mL.

5.9 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 KPa ± 10 KPa).

5.9.1 Glass fiber filter

5.9.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.10 Serum separator tubes (optional in place of filtration apparatus), 16x150 mm (PN-02-657-3, Fisher Scientific Co.)

5.11 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.11.1 Centrifuge tubes with caps. Compatible with centrifuge used.

5.12 Magnetic stir plate and stir bar.

5.13 Anion Exchange Cleanup materials.

5.13.1 Resin Bio-Rad, AG 4x4, 100-200 mesh, free base form.

5.13.2 Bio-Rad Poly-Prep Chromatography Columns 0.8x4 cm or similar.

5.13.3 Boro-silicate test tubes, 16x150 mm with caps.

5.14 ICP-OES Instrument. Analytical wavelength required: 182.0 nm or other instrument-specific

wavelength. Manufacturers include: Thermo-Fisher, Perkin-Elmer, Varian, Leeman and Spectro.

6.0 Reagents

6.1 Deionized (DI) water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Extraction solution (2.0% acetic acid). Dilute 20 mL ACS reagent glacial acetic acid (CH_3COOH, 99%) in 500 mL deionized water. Bring to 1 L final volume with deionized water.

6.3 0.1 N HCl (column rinse solution). Add about 500 mL water to a 1 L volumetric flask, carefully add 8.34 mL concentrated HCl, swirl to mix, add water to the 1 L mark and mix thoroughly.

6.4 1.0 N HCl (column elution solution). Add about 500 mL water to a 1 L volumetric flask, carefully add 83.4 mL concentrated HCl, swirl to mix, add water to the 1 L mark and mix thoroughly.

6.5 Prepare resin slurry by adding 25 g of Resin Bio-Rad, AG 4x4G, 10 mL of concentrated HCl, and 167 mL water to a 250 mL Erlenmeyer flask. Place on stir plate with magnetic stir bar to mix.

6.6 Method blank solution. Extraction solution carried through the anion exchange procedure.

6.7 Sulfate-sulfur calibration standards. Prepare 3 or more calibration standard solutions in extraction solution. Available from commercial chemical vendors. Calibration standards should be applied to and eluted from the column to correct for slight losses. Preparation of standards in duplicate is recommended.

6.8 Sulfate-sulfur reference standard, certified content. Available commercially (should come from a different source than calibration standard).

6.9 Manure Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Transfer 1.0 g - 4.0 g test portion of processed unknown, or 0.4 g dried and ground unknown, into a 50 mL centrifuge tube. Record weight.

7.2 Add 40 mL of 2% acetic acid extraction reagent using a repipette dispenser.

7.3 Cap the tubes and shake on the reciprocating shaker for 30 minutes at >100 opm.

7.4 Make dilutions according to instrument specification. Record dilution factor. This extract may also be used for the chloride method (M – 5.10).

7.5 Clarify extract using filtration, centrifugation or serum separator tube, if necessary. Overnight settling is also typically required to remove fine particles that can plug the anion exchange column.

7.6 Anion Exchange Cleanup

7.6.1 Note: the anion exchange cleanup may be omitted when sample concentrations are under the reporting limit. Additionally, the laboratory may demonstrate (by testing 20 or more unknowns from different sources) that the omission of the cleanup results in less

than 25% error in the reported result.

7.6.2 During all column steps, ensure that solution can freely drip from columns. Wait for each solution to load onto resin before adding the next solution. It is not critical to have solution running through the columns at all times, but the columns should not be allowed to sit without flow through them for more than 30 minutes.

7.6.3 Column preparation. While resin slurry is mixing on a stir plate, pipette 4 mL slurry onto chromatography columns. Allow liquid to drip through column and collect in 16x150 mm disposable tube. Wash column with 2 mL 0.1 N HCl followed by 10 mL extraction solution.

7.6.4 Load 7.5 mL unknown extract onto column. Wash column with 10 mL 0.1 N HCl. Move column to clean 16x150 mm tube.

7.6.5 Elute with 6 mL 1 N HCl. Vortex eluate and pour into autosampler tubes for ICP analysis.

7.7 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the SO_4-S content as percent on a dry basis according to the following equation

$$SO_4\text{-}S = [C \times (V/m)] \times D_f \times 0.1/(TS/100)$$

where

SO_4-S is the SO_4-S content (%, dry basis)
C is the sulfate concentration from the analysis (after dilutions, mg L^{-1})
V is the extract volume (mL)
m is the test portion mass (g)
TS is the total solids of the test portion (%, method M-1.10)
D_f is the dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s1 is the first unknown
s2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown

9.6 Spike Recovery. Perform spike recovery with each batch of unknowns when the matrix differs significantly from the manure reference material. Spike unknown with analyte reference standard at a concentration 4-10 times the analyte concentration of the unknown or MDL, whichever is greater.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Method Detection Limit (MDL). The MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85% to115%. Failure shall result in evaluation of reagents and equipment, correction of problems, and re-analysis of spike recovery and all unknowns in the batch.

11.0 Safety and Disposal

11.1 Health and Safety**.** Dairy manure may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

American Public Health Association. 2005. Standard methods for the examination of water and wastewater, 18th edition. Washington, DC.

American Society of Agricultural Engineers. 2004. Uniform terminology for waste management, 292.5 pp 656-659 FEB04.

Gavlack, R. E. D. A. Horneck and R. O. Miller. 2005. Soil and Water Reference Methods for the Western Region, 3rd Edition. Western Regional Extension Publication. WREP-125. Oregon State University.

Haeckel, R. 1991. Proposals for the description effects in clinical chemistry and measurement of carryover. Pure & Appl. Chem. 63: 301-306.

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Littlefield, E. S., Miller, R. O, and Carlson, R. M. 1990. Determination of sulfate-sulfur in plant tissue by inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 21:1577-1586.

U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

U.S. Environmental Protection Agency. 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses. Chapter 2. Document # EPA-823-B-01-002.

Introduction – Process Wastewater

The Monitoring and Reporting Program in the General Order requires analysis of process wastewater. This material is typically land applied through pipelines, hoses, or via tanker system. Based on the definitions provided by the American Society of Biological and Agricultural Engineers, process wastewater has less than 3% total solids. Depending on application method, slurry manure may also fit into the category of process wastewater.

Process wastewater analyses shall be conducted in accordance with the Title 40 Code of Federal Regulations Part 136 (*Guidelines Establishing Test Procedures for the Analysis of Pollutants*) or manure analysis proficiency (MAP) program approved methods or other test methods approved by the Executive Officer. The required analyses and method numbers from the Guidelines document are summarized below.

The greatest challenge for laboratories is obtaining a representative aliquot from the sample. The variability of results for un-homogenized samples often requires that most samples be run in duplicate and precludes use of very small sample aliquots.

Required analyses:

- Quarterly during an application event:
    - Nitrate-nitrogen (only when retention pond is aerated)
    - Ammonium-nitrogen
    - Total Kjeldahl nitrogen
    - Total phosphorus
    - Potassium

- Annually for 2 years after groundwater monitoring wells are installed:
    - General minerals (calcium, magnesium, sodium, bicarbonate, carbonate, sulfate, and chloride)

Reference (method number or page)								
Parameter	**Methodology**	**EPA**[1]	**Standard methods (18th, 19th)**	**Standard methods (20th)**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Ammonium nitrogen	Manual, distillation (at pH 9.5) followed by	350.1, Rev. 2.0(1993)	4500–NH B3	4500–NH3 B	4500–NH3 B–97		973.49[2]	4.1 (pp. 25-26)
	Nesslerization		4500–NH3 C (18th)			D1426–98, 03 (A)	973.49[2] I–3520–85[3]	
	Titration		4500–NH3 C(19th) 4500–NH3 E (18th)	4500–NH3 C	4500–NH3 C–97			
	Electrode		4500–NH3 D or E(19th) 4500–NH3 F or G(18th)	4500–NH3 D or E	4500–NH3 D or E– 97	D1426–98, 03 (B)		4.2 (pp.26-28)
	Automated phenate	350.1, Rev. 2.0(1993)	4500–NH3 G (19th) 4500–NH3 H (18th)	4500–NH3 G	4500–NH3 G–97		I–4523–85[3]	
	Automated electrode Ion Chromatography						See footnote 4	
	Colorimetry using Auto-Analyzer							4.3 (pp. 28-29)

Reference (method number or page)									
Parameter	**Methodology**	**EPA**[1]	**Standard methods (18^{th}, 19^{th})**	**Standard methods (20^{th})**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Total Kjeldahl nitrogen	Digestion and distillation followed by:		4500–N_{org}-B or C, and 4500–NH_3 B	4500–N_{org} B or C and 4500– NH_3 B	4500–N_{org} B or C–97 and 4500– NH_3 B–97	D3590–89, 02 (A).		
	Titration, or		4500–NH_3 C (19^{th}) and 4500–NH_3 E (18^{th})	4500– NH_3 C	4500– NH_3 C–97	D3590–89, 02 (A)	973.48[2]	3.2 (pp 18-21)
	Nesslerization, or		4500– NH_3 C (18^{th})			D3590–89, 02 (A)		
	Electrode, or		4500– NH_3 F or G (18^{th}) and 4500–NH3 D or E (19^{th})	4500–NH3 D or E	4500– NH_3 D or E– 97	D3590–89, 02 (A)		2.3 (p. 19)
	Automated phenate colorimetric, or	351.1 (Rev.1978)					I–4551–78[5]	2.3 (p. 19)
	Semi-automated block digester colorimetric, or	351.2, Rev. 2.0 (1993)				D3590–89, 02 (B)	I–4515–91[6]	6 (p. 21)
	Manual or block digester, potentiometry, or					D3590–89, 02 (A)		2.3 (p. 19)
	Block digester, followed by auto distillation and						See footnote 7	
	Nesslerization, or						See footnote 8	
	Flow injection gas diffusion						See footnote 9	

Reference (method number or page)								
Parameter	**Methodology**	**EPA[1]**	**Standard methods (18th, 19th)**	**Standard methods (20th)**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Total Phosphorus	Persulfate digestion followed by:		4500–P B.5	4500–P B.5			973.55[2]	
	Manual ascorbic acid reduction, or	365.3 (1978)	4500–P E	4500–P E		D515–88(A)		
	Automated ascorbic acid reduction, or	365.1 Rev. 2.0 (1993)	4500–P F	4500–P F			973.56[2], I–4600– 85[3]	
	Semi–automated block digester.	365.4 (1974)				D515–88(B)	I–4610–91[10]	
	HCl digestion, or							5.2 (pp. 30-32)
	Microwave assisted acid digestion, or							5.3 (pp. 32-35)
	Nitric and HCl digestion with peroxide, or							5.4 (pp. 35-36)
	Nitric Acid digestion with peroxide using block digester							5.5 (pp. 36-38)
	Atomic absorption spectrophotometry (AAS), or							6.2 (pp. 39-41)
	inductively coupled plasma emission spectroscopy (ICP-AES), or							6.3 (pp. 41-43)
	Vanado-molybdophosphoric acid method (colorimetric)							3 (pp. 44-45)
	Ascorbic acid method (colorimetric)							4 (pp. 45-47)

Reference (method number or page)								
Parameter	**Methodology**	**EPA[1]**	**Standard methods (18th, 19th)**	**Standard methods (20th)**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Potassium	Digestion followed by: AA direct aspiration		3111 B		3111 B–99		973.53[2], I–3630– 85[3]	
	ICP-AES	200.7, Rev. 4.4(1994)	3120 B	3120 B	3120 B–99			Same as Total P
	Flame photometric, or		3500–K D	3500–K B	3500–K B–97			
	Colorimetric						317 B[11]	
	Ion Chromatography					D6919–03		
Calcium	Digestion followed by: AA direct aspiration		3111 B		3111 B–99	D511–93, 03(B)	I–3152–85[3]	
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B	3120 B	3120 B–99		I–4471–97[12]	Same as Total P
	DCP, or						See footnote 13	
	Titrimetric (EDTA)		3500–Ca D	3500–Ca B	3500–Ca B–97	D511–93, 03(A)		
	Ion Chromatography					D6919–03		
Magnesium	Digestion followed by: AA direct aspiration		3111 B		3111 B–99	D858–95, 02 (A or B)	974.27[2], I–3454–85[3]	
	ICP/AES	200.7, Rev. 4.4(1994)	3120 B	3120 B	3120 B-99		I–4471–97[12]	Same as Total P
	DCP or						See footnote[13]	
	Gravimetric			3500–Mg D				
	Ion Chromatography					D6919–03		

Reference (method number or page)								
Parameter	**Methodology**	**EPA**[1]	**Standard methods (18th , 19th)**	**Standard methods (20th)**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Sodium	Digestion followed by: AA direct aspiration		3111 B		3111 B–99		973.54[2], I–3735–85[3]	
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B	3120 B	3120 B–99		I–4471–97[12]	
	DCP, or						See footnote[13]	
	Flame photometry		3500–Na D	3500–Na B	3500–Na B–97			
	Ion Chromatography					D 6919–03		
Bicarbonate								
Carbonate								
Sulfate	Automated colorimetric	375.2, Rev. 2.0 (1993)						
	Gravimetric		4500–SO4 2–C or D	4500–SO4 2–C or D			925.54[2]	
	Turbidimetric					D516–90, 02	426C[2] 0	
	Ion Chromatography	300.0 Rev 2.1 (1993) and 300.1 Rev 1.0 (1997)	4110 B	4110 B	4110 B-00	D4327–97, 03	993.30[2]	
	CIE/UV						D6508, Rev. 2[14]	

Reference (method number or page)								
Parameter	**Methodology**	**EPA**[1]	**Standard methods (18th, 19th)**	**Standard methods (20th)**	**Standard methods (online)**	**ASTM**	**USGA/ AOAC/ other**	**MAP methods (Peters, 2003)**
Chloride	Titrimetric (silver nitrate) or		4500-Cl-B	4500-Cl-B	4500-Cl-B-97	D512–89(99) (B)	I–1183–85[3]	
	Titrimetric (Mercuric nitrate)		4500-Cl-C	4500-Cl-C	4500-Cl-C-97	D512–89(99) (A)	973.51[2], I–1184–85[3]	
	Colorimetric: manual or Ion Selective Electrode						I–1187–85[3]	
	Automated (Ferricyanide)		4500-Cl-E	4500-Cl-E	4500-Cl-E-97		I–2187–85[3]	
	Potentiometric Titration		4500-Cl-D	4500-Cl-D	4500-Cl-D-97			
	Ion Selective Electrode					D512–89(99)(C)		
	Ion Chromatography	300.0, Rev 2.1 (1993) and300.1, Rev 1.0 (1997)	4110 B	4110 B	4110 B-00	D4327–97, 03	993.30[2]	
	CIE/UV						D6508, Rev. 2[14]	
Nitrate	Ion Chromatography	2.1(1993) and 300.1, Rev	4110 B	4110 B	4110 B-00	D4327–97, 03	993.30 N[2]	
	CIE/UV						D6508, Rev. 2[14]	
	Ion Selective Electrode		4500-NO_3 D	4500-NO_3 D	4500-NO_3 D-00			
	Colorimetric (Brucine sulfate), or	352.1					973.50[2] 419D[15,4], p. 28[16]	
	Nitrate-nitrite N minus Nitrite N	See description in 40 CFR						

Table Notes:

[1] All EPA methods, excluding EPA Method 300.1, are published in *Methods for the Determination of Metals in Environmental Samples, Supplement I*, National Exposure Risk Laboratory-Cincinnati (NERL–CI), EPA/600/R–94/111, May 1994; and *Methods for the Determination of Inorganic Substances in Environmental Samples*, NERL–CI, EPA/600/R–93/100, August, 1993.

[2] Official Methods of Analysis of the Association of Official Analytical Chemists, Methods Manual, Sixteenth Edition, 4th Revision, 1998.

[3] Fishman, M. J., *et al.* "Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments," U.S. Department of the Interior, Techniques of Water-Resource Investigations of the U.S. Geological Survey,Denver, CO, Revised 1989, unless otherwise stated.

[4] Ammonia, Automated Electrode Method, Industrial Method Number 379–75 WE, dated February 19, 1976, Bran & Luebbe (Technicon) Auto Analyzer II, Bran & Luebbe Analyzing Technologies, Inc., Elmsford, NY 10523.

[5] The approved method is that cited in *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments*, USGS TWRI, Book 5, Chapter A1 (1979).

[6] Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Ammonia Plus Organic Nitrogen by a Kjeldahl Digestion Method, Open File Report 00–170.

[7] Nitrogen, Total Kjeldahl, Method PAI-DK01 (Block Digestion, Steam Distillation, Titrimetric Detection), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[8] Nitrogen, Total Kjeldahl, Method PAI–DK02 (Block Digestion, Steam Distillation, Colorimetric Detection), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[9] Nitrogen, Total Kjeldahl, Method PAI–DK03 (Block Digestion, Automated FIA Gas Diffusion), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[10] Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Total Phosphorus by Kjeldahl Digestion Method and an Automated Colorimetric Finish That Includes Dialysis, Open File Report 92–146.

[11] The approved method is that cited in *Standard Methods for the Examination of Water and Wastewater,* 14th Edition, 1976.

[12] Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Elements in Whole-water Digests Using Inductively Coupled Plasma-Optical Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry, Open File Report 98-165.

[13] Direct Current Plasma Optical Emission Spectrometric Method for Trace Elemental Analysis of Water and Wastes, Method AES0029, 1986, Revised 1991. Thermo Jarrell Ash Corporation, 27 Forge Parkway, Franklin, MA 02038

[14] Method D6508, Rev. 2, Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte, Waters Corp, 34 Maple St., Milford, MA, 01757.

[15] Methods for Chemical Analysis of Water and Wastes, Environmental Protection Agency, Environmental Monitoring Systems Laboratory–Cincinnati (EMSL–CI), EPA–600/4–79–020 (NTIS PB 84–128677), Revised March 1983 and 1979 where applicable.

[16] American National Standard on Photographic Processing Effluents, April 2, 1975. Available from ANSI, 25 West 43rd st., New York, NY 10036.

40 CFR Part 122, 136, 141, 143, 430, 455, and 465. Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; National Primary Drinking Water Regulations; and National Secondary Drinking Water Regulations; Analysis and Sampling Procedures; Final Rule pp. 11216-11226 (Federal Register / Vol. 72, No. 47 / Monday, March 12, 2007 / Rules and Regulations)

Introduction – Soil

The Monitoring and Reporting Program in the General Order requires analysis of soil. Analysis of total phosphorus every 5 years (beginning by May, 2008) is required. Additional analyses (more frequent or different constituents) may be done.

Soil analyses shall be conducted using methods utilized by the North American Proficiency Testing (NAPT) Program or accepted by the University of California; and laboratories participating in the NAPT Program or other programs whose tests are accepted by the University of California.

Required analyses	**UC Method**
Total phosphorus	S - 2.10 Total Phosphorus

The following soil tests are recommended but not required:	***UC Method***
Spring pre-plant for each crop:	
Laboratory analyses for:	
0 to 1 foot depth: Nitrate-nitrogen and organic matter	S - 1.10 Nitrate S - 4.10 Soil Organic Matter
1 to 2 foot depth: Nitrate-nitrogen	S - 1.10 Nitrate
Fall pre-plant for each crop:	
Laboratory analyses for:	
0 to 1 foot: Electrical conductivity, nitrate-nitrogen, soluble phosphorus, potassium and organic matter	S – 5.10 Electrical conductivity, S – 1.10 Nitrate, S – 2.20 Olsen-P, S – 3.10 Potassium, S – 4.10 Soil organic matter
1 to 2 foot: Nitrate-nitrogen	S – 1.10 Nitrate
2 to 3 foot: Nitrate-nitrogen	S – 1.10 Nitrate

S – 1.10 Nitrate Nitrogen in Soil

1.0 Scope and Application

This method quantitatively determines the nitrate nitrogen (NO_3-N) in soil by spectrophotometry after extraction with potassium chloride.

2.0 Method Summary

2.1 Description. Nitrate is determined by reduction to nitrite (NO_2^--N) via a cadmium reactor or utilizing another reductant. Nitrite is diazotized with sulfanilamide and coupled to N-(1-Napthyl)-ethylenediamine dihydrochloride to form an azochromophore, which can be measured spectrophotometrically at 520 nm. The Method is adapted from Soil, Plant and Water Reference Methods for the Western Region S-3.10, and ISO 13395:1996.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.01 mg kg^{-1} to 0.02 mg kg^{-1} NO_3-N (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for NO_3-N is 0.05 mg kg^{-1}.

2.3.2 Nitrate content is calculated as mg kg^{-1} NO_3-N and reported on a dry basis.

2.4 Interferences.

2.4.1 Nitrite (NO_2^--N) is a known interference with determination by cadmium reduction-spectrophotmetric method and results in positive bias.

2.4.2 Buildup of suspended matter in the reduction column will restrict flow resulting in low recoveries. Test solutions may therefore require additional filtration.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 4.0 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1) Avoids use of concentrated acids; and (2) fast automated analysis.

2.9 Disadvantages. (1) Hazardous reagent used (cadmium); and (2) requires high level of instrument maintenance.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit.* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 °C ± 5 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.2.3 The soil digest extract (test solution) may be refrigerated (4 °C ± 2 °C).

4.3 Sample Holding Time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be held for up to one year.

4.3.3 Processed, dry material may be held for one year.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.3 Force draft drying oven. Vented, capable of maintaining constant temperature of 40 °C ± 5 °C, flow capacity 100 ± 10 cfm.

5.4 Flail mill or plate grinder. Bico-Braun, or similar.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0%

error.

5.6 Extraction vessel. Polypropylene or glass test tube with cap, minimum volume 30 mL.

5.7 Repipette dispenser. Dispenser calibrated to 20.0 mL ± 0.2 mL.

5.8 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.9 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 ± 10 KPa).

5.9.1 Glass fiber filter

5.9.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.10 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.10.1 Centrifuge tubes. 50 mL capacity, compatible with centrifuge used.

5.11 Spectrophotometer. Automated ammonium nitrogen analyzer (Manufacturers include: Techincon Autoanlyzer II with nitrate analytical cartridge, Technicon Corp; QuikChem Method 10-107-06-1-A; Lachat Instruments; FIALab; Alpkem; Skalar) or instrument of similar design capable of performing nitrate nitrogen analysis based on reduction of NO_3-N to NO_2-N with determination by spectrophotometric analysis.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 $^{\circ}$C/µS • cm^{-1}.

6.2 Method blank solution. 2.0 N KCl solution filtered in the same manner as test portions.

6.3 Potassium chloride extraction solution (2.0 N KCl): Dissolve 149 g of KCl (FW 74.6 g $mole^{-1}$, >99%) in deionized water to 1 L final volume.

6.4 Nitrate calibration standard stock solution (1000 mg L^{-1} NO_3-N, certified content, commercially available). Alternatively, dissolve 7.218 g anhydrous potassium nitrate (KNO_3, FW 101.1 g $mole^{-1}$, >99.5% purity) in deionized water to 1 L final volume. Prepare calibration standard solutions by dilution of the stock solution in KCl extraction solution; suggested calibration range 0.05 to 20.0 mg L^{-1} NO_3-N.

6.5 Nitrate reference standard, certified content, available commercially (should be from a different source than calibration standard stock solution).

6.6 Soil Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh approximately 4.0 g ± 0.05 g of a representative test portion into a 30 mL test tube.

7.2 Add 20.0 mL ± 0.2 mL of 2.0 N KCl extraction solution using repipette dispenser.

7.3 Prepare reference material, blanks, and duplicates along with the unknowns for digestion.

7.4 Cap extraction vessel(s) and shake on reciprocating shaker for 1 hr at >100 opm.

7.5 Extracts exhibiting turbidity may require overnight settling, centrifugation or filtration to remove undissolved particulate matter prior to analysis. Extract may be refrigerated (4 $^{\circ}$C $\pm$ 2 °C) for up to 30 days from the time of extraction or frozen (-20 °C $\pm$ 5 °C) for up to 1 year.

7.6 Spectrophotometric Analysis. Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, and reference material. Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and unknown duplicates. Analyte concentrations must fall within the range of calibration standards. Analytes with concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analtyes with concentrations exceeding the highest calibration point require dilution and re-analysis.

8.0 Calculations and Reporting

8.1 Calculate the NO_3-N mg kg^{-1} concentration according to the following equation:

$$NO_3\text{-}N = [C \times (V/m)] \times D_f$$

where,

NO_3-N is the concentration of soil NO_3–N on a dry basis (mg kg^{-1});
C is the extract NO_3-N concentration after dilution (mg L^{-1})
V is the extract volume (mL)
m is the test portion mass (g)
D_f is any additional dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Analyte carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a NO_3-N calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$\text{MDL= Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Not applicable.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all

personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Cadmium is a hazardous material and must be disposed of properly.

11.4 Reagent Disposal. Dispose of reagents in accordance with local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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S - 2.10 Acid Recoverable Phosphorus in Soil

1.0 Scope and Application

This method determines the acid recoverable phosphorus (P) in soil by acid digestion and subsequent quantification by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.0 Method Summary

2.1 Description. Soil is digested with nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) at 140 - 160 °C with subsequent determination of elemental phosphorus by ICP-OES. The method is adapted from US-EPA method 3050 and Soil, Plant and Water Reference Methods for the Western Region S-15.10.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 2 mg kg^{-1} to 10 mg kg^{-1} P (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 10 mg kg^{-1} P.

2.3.2 Phosphorus content is calculated as mg kg^{-1} P and reported on a dry basis.

2.4 Interferences.

2.4.1 The method may provide incomplete recovery of acid insoluble geologic minerals.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.2 g to 1.0 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1) Fast automated analysis; and (2) low detection limit.

2.9 Disadvantages. (1) High cost of instrumentation; and (2) uses concentrated acids and reactive chemicals.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit.* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 ºC ± 5 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.2.3 The soil digest extract (test solution) may be refrigerated (4 °C ± 2 °C).

4.3 Holding Time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be stored for up to one year.

4.3.3 Processed, dry material may be held up to one year

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.3 Force draft drying oven. Vented, capable of maintaining constant temperature of 40 ºC ± 5 ºC, flow capacity 100 ± 10 cfm.

5.4 Flail mill or plate grinder. Bico-Braun, or similar.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Repipette dispenser. Dispenser calibrated to 6.0 mL ± 0.1 mL.

5.8 Digestion Tubes. Instrument specific glass, polypropylene, or teflon digestion tubes.

5.9 Digestion Block. Capable of 140 °C ± 5.0 °C.

5.10 Micropipettor. Calibrated to dispense 1000 uL ± 1.0 uL.

5.11 Vortex mixer. Standard laboratory vortex mixer.

5.12 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 KPa ± 10 KPa).

5.12.1 Glass fiber filter

5.12.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.13 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.13.1 Centrifuge tubes. Plastic Conical Centrifuge Tube. Graduated, capacity 50.0 mL ± 0.2 mL, compatible with centrifuge used.

5.14 ICP-OES spectrometer. Instrument capable of generating and detecting analytical wavelengths for phosphorus (178.3 or 213.6 nm). Manufacturers include: Thermo-Fisher, Perkin-Elmer, Varian, Leeman and Spectro; or instrument of similar design capable of performing phosphorus analysis.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 /µS • cm^{-1} Conductivity at 25 °C.

6.2 Nitric acid (HNO_3) concentrated, trace metal grade.

6.3 Hydrogen peroxide (H_2O_2), 30% solution.

6.4 Argon gas 99.5% purity.

6.5 Method blank solution.

6.6 Calibration standard stock solution (1,000 mg L^{-1} phosphorus, certified content, commercially available). Alternatively, dissolve 4.939 g of anhydrous monopotassium phosphate (KH_2PO_4, ACS reagent) in deionized water to 1 L final volume. Prepare working standards by dilution in 6% nitric acid.

6.7 Phosphorus Certified Reference Standard, certified content available commercially (should be from a different source than calibration standard stock solution).

6.8 Soil Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Pre-heat digestion block to 80 °C ± 5 °C.

7.2 Transfer approximately 0.2 g - 1.0 g of a representative test portion into a digestion tube and record mass to nearest 0.01 g. Weigh and analyze all unknowns in duplicate if weighing <1.0 g. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for unknowns smaller than 1.0 g is <15% using laboratory specific methodology.

7.3 Add 6.0 mL concentrated nitric acid using a repipette dispenser and swirl to thoroughly wet the test portion. Predigest at room temperature for a minimum of 20 minutes.

7.4 Place digestion tubes in 80 °C digestion block for 10 minutes. Remove and cool for 2 minutes.

7.5 Increase digestion block temperature to 140 °C ± 5 °C.

7.6 Add 2 x 1000 uL aliquots of 30% H_2O_2 solution to digestion tubes. If foaming occurs, carefully swirl to clean off walls of digestion tube.

7.7 Place digestion tubes in 140 °C digestion block and digest for 60 minutes or until total digest volume is reduced to 2 mL to 3 mL.

7.8 Remove tubes from block and cool for 30 minutes.

7.9 Dilute to 100 mL with deionized water. Cap and mix using vortex mixer. Digests exhibiting turbidity may require over night settling, centrifugation or filtration to remove undissolved particulate matter prior to analysis.

7.10 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and unknown duplicates. Analyte concentrations must fall within the range of calibration standards. Analytes with concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analytes with concentrations exceeding the highest calibration point require dilution and re-analysis.

8.0 Calculations and Reporting

8.1 Calculate the acid recoverable P concentration according to the following equation:

$$P = [C \times (V/m)] \times D_f$$

where,

P is the concentration of soil P on a dry basis (mg kg^{-1});
C is the digest P result in mg L^{-1}
V is the total digest volume in mL
m is the dry test portion mass in mg
D_f is the total dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Analyte carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Soil Standard Reference Material. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknown, or on each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Perform spike recovery with each batch of unknown when matrix differs significantly from soil reference material. Spike unknown with analyte reference standard at a concentration 4 to10 times the analyte concentration of the unknowns. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85% to115%. Failure shall result in evaluation of reagents and equipment, correction of problems, and re-analysis of spike recovery and all unknowns in the batch. Record results.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$MDL = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where,

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85%-115%. Failure shall result in evaluation of reagents and equipment, correction of problems, and re-analysis of spike recovery and all unknowns in the batch.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes high temperatures and concentrated acids. Hydrogen peroxide may react violently with hot organic acid solution.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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S-2.20 Bicarbonate Extractable Phosphorus in Soil

1.0 Scope and Application

This method determines extractable ortho-phosphate (PO_4-P) using the method of Olsen and Sommers (1982).

2.0 Method Summary

2.1 Description. Ortho-phosphate (PO_4-P) is extracted with 0.5 N $NaHCO_3$ based on the method developed by Olsen *et al*.,1954. Following reaction of ammonium molybdate with ascorbic acid in the presence of antimony, PO_4-P content is determined spectrophotometrically at 882 nm. The method is adapted from Soil, Plant and Water Reference Methods for the Western Region S-15.10.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.2 mg kg^{-1} to 1.0 mg kg^{-1} PO_4-P (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 1.0 mg kg^{-1} PO_4-P.

2.3.2 Orthophosphate content is calculated as mg kg^{-1} PO_4-P and reported on a dry basis.

2.4 Interferences.

2.4.1 High levels of arsenate will result in a positive bias.

2.4.2 Soil extractable PO_4-P increases 0.43 mg kg^{-1} per ^oC for temperatures between 15 and 30 ^oC for soils ranging from 5 - 40 PO_4-P mg kg^{-1}.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 1.0 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1) Fast automated analysis; and (2) low detection limit.

2.9 Disadvantages. (1) Extraction solution is unstable over time; (2) extraction chemistry is sensitive to changes in temperature; and (3) spectrophotometric determination is sensitive to changes in pH.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit.* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 ºC ± 3 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.3 Holding Time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be stored for up to one year.

4.3.3 Processed, dry material may be held for one year.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.3 Force draft drying oven. Vented, capable of maintaining constant temperature of 40 ºC ± 5 ºC.

5.4 Flail mill or plate grinder. Bico-Braun, or similar.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Extraction vessel. Polypropylene or glass test tube with cap, minimum volume 30 mL.

5.7 Repipette dispenser. Dispenser calibrated to 20.0 mL ± 0.2 mL.

5.8 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.9 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 ± 10 KPa).

5.9.1 Whatman No. 2 or equivalent highly retentive filter paper.

5.10 Centrifuge. Centrifuge (optional in place of filtration apparatus), centrifuge capable of 8000 x *g*.

5.10.1 Conical centrifuge tube. 50.0 mL, plastic wide mouth, compatible with centrifuge used.

5.11 Spectrophotometric Analyzer. Automated ortho-phosphate analyzer capable of determining orthophosphate using ammonium molybdate and ascorbic acid reduction at 882 nm. Manufacturers include: Technicon Corp; Lachat Instruments; FIALab; Alpkem; Astoria Pacific; Skalar; or instrument of similar design capable of performing ortho-phosphate analysis.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 $^{o}C/\mu S \bullet cm^{-1}$.

6.2 Sodium bicarbonate extraction solution (0.5 N $NaHCO_3$, pH 8.50). Dissolve 42.0 g $NaHCO_3$, ACS reagent, in about 900 mL of deionized water. Adjust the pH to 8.50 ±0.05 with 2.0 N NaOH. Bring to 1 L final volume with deionized water. This solution is unstable with regard to pH and should be prepared as required.

6.3 Method blank solution. Sodium bicarbonate extraction solution.

6.4 Phosphorus calibration standard (1,000 mg L^{-1} PO_4-P, certified content, commercially available). Alternatively, dissolve 7.218 g anhydrous monopotassium phosphate (KH_2PO_4 >99.5%) in deionized water to 1 L final volume. Prepare working standard solutions by dilution in 0.5N $NaHCO_3$ matrix. Suggested generic calibration range 0.05 to 4.00 mg L^{-1} PO_4-P.

6.5 Phosphorus reference standard, certified content, available commercially (should be from a different source than calibration standard stock solution).

6.6 Spectrophotometric Reagents (instrument manufacturer dependent).

6.7 Soil Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh and transfer approximately 1.00 g ± 0.05 g of test portion into a 30 mL extraction vessel.

7.2 Add 20.0 mL ± 0.2 mL of sodium bicarbonate extraction solution.

7.3 Prepare reference material, blanks, and duplicates along with the unknowns for digestion.

7.4 Cap extraction vessel(s) and shake on reciprocating shaker for 30 minutes at >100 opm.

7.5 Immediately filter extract using Whatman No. 2 filter paper or equivalent highly retentive paper. Re-filter if filtrate is cloudy.

7.6 Spectrophotometric analysis. Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, and reference material. Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and unknown duplicates. Analyte concentrations must fall within the range of calibration standards. Analytes with concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analytes with concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the sodium bicarbonate extractable PO_4-P concentration according to the following equation:

$$PO_4\text{-}P = [C \times (V/m)] \times D_f$$

where,

PO_4-P is the concentration of PO_4-P on a dry basis (mg kg^{-1});
C is the concentration after dilution (mg L^{-1})
V is the extract volume (mL)
m is the test portion mass (g)
D_f is the dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carry-over. Analyte carry-over analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carry-over as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carry-over (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carry-over effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carry-over values exceeding 5%.

Percent carry-over should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Soil Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 The Method Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$\text{MDL} = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$\text{RPD} = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where,

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Not applicable.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all

personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

Cowling, J.C., T.W. Speir and H.J. Percival. 1987. Potential problems with the determination of Olsen and microbial P of soils due to the instability of 0.5 M sodium bicarbonate. Comm in Soil Sci and Plant Anal. 18(6):637-652.

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US Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the analysis of pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

S - 3.10 Extractable Potassium in Soil

1.0 Scope and Application

This method determines extractable potassium (K) in soil using ammonium acetate with subsequent quantification by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.0 Method Summary

2.1 Description. Soil is extracted with 1.0 N ammonium acetate extraction solution followed by K analysis with ICP-OES. The method is quantitative for K associated with soil colloid exchange sites, but not for K associated with mineral structures. The method is adapted from Soil, Plant and Water Reference Methods for the Western Region S-8.10.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.2 mg kg^{-1} to 0.5 mg kg^{-1} K (dry basis).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 1.0 mg kg^{-1} K.

2.3.2 Potassium content is calculated as mg kg^{-1} K and reported on a dry basis.

2.4 Interferences.

2.4.1 Lithium chloride reagent may be added as an ionization buffer to improve the determination of potassium by ICP-OES.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 2 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1) Fast automated analysis; and (2) low detection limit.

2.9 Disadvantages. (1) Extraction solution is unstable with time.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit.* The defensible level above wh*ich re*sults are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 °C ± 5 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.2.3 The soil digest extract (test solution) may be refrigerated (4 °C ± 2 °C).

4.3 Sample Holding Time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be stored for up to one year.

4.3.3 Processed, dry material may be held up to one year

4.3.4 The soil saturated paste extract may held at 4 °C ± 2 °C up to 28 days from the time of extraction.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Flail mill or plate grinder. Bico-Braun, or similar.

5.4 Force draft drying oven. Vented, capable of maintaining constant temperature of 40 ºC ± 5 ºC, flow capacity 100 ± 10 cfm.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Extraction vessel. Polypropylene or glass test tube with cap, minimum volume 30 mL.

5.7 Repipette dispenser. Dispenser calibrated to 20.0 mL ± 0.2 mL.

5.8 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.9 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 ± 10 KPa).

5.9.1 Filter paper. Whatman No. 1, or equivalent highly retentive filter paper. Verify that filter paper is potassium free prior to use.

5.10 Centrifuge. Centrifuge (optional, if filtration apparatus is not used), centrifuge capable of 8000 x*g*.

5.10.1 Centrifuge tubes. 50 mL capacity, compatible with centrifuge used.

5.11 ICP-OES spectrometer. Analytical wavelength for potassium detection is 766.4 nm. Manufacturers include: Thermo-Fisher, Perkin-Elmer, Varian, Leeman and Spectro or instrument of similar design capable of performing potassium analysis.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 $^{o}C/\mu S \bullet cm^{-1}$.

6.2 Ammonium acetate extraction solution (1.0 N ammonium acetate, NH_4CH_3COOH, pH 7.0). Dissolve 57 mL of glacial acetic acid (CH_3COOH, 99%) in 500 mL of deionized water. Add 68 mL of concentrated ammonium hydroxide. Adjust pH to 7.0 $\pm$ 0.04 with 3.0 N glacial acetic acid or 3.0 N ammonium hydroxide and bring to 1 L final volume with deionized water. Confirm pH = 7.0 ± 0.04 prior to each use.

6.3 Ionization buffer solution (1000 mg L^{-1} lithium chloride (LiCl), ACS reagent). Dissolve 2.03 g LiCl in 1.5 L of deionized water. Add 20 mL of nitric acid (HNO_3, trace metal grade) and bring to 2 L with deionized water.

6.4 Method blank solution. Ammonium acetate extraction solution.

6.5 Calibration standard stock solution (1000 mg L^{-1} K, certified content, commercially available). Alternatively, dissolve 1.91 g of anhydrous KCl in deionized water and bring to 1 L final volume. Prepare working calibration solutions by dilution in 0.5 N ammonium acetate, 0.05% lithium chloride, 1.0% (v/v) nitric acid.

6.6 Potassium reference standard, certified content, available commercially (should be from a different source than calibration standard stock solution).

6.7 Soil reference material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh 2.0 g ± 0.02 g soil into an extraction vessel.

7.2 Prepare reference material, blanks, and duplicates along with the unknowns for digestion.

7.3 Add 20.0 ± 0.2 mL of extraction solution.

7.4 Cap extraction tube(s) and place on reciprocating mechanical shaker for 30 minutes at >100 opm.

7.5 Extracts exhibiting turbidity may require overnight settling, centrifugation or filtration to remove undissolved particulate matter prior to analysis. Extract may be refrigerated (4 °C ± 2 °C) for up to 30 days from the time of extraction.

7.6 Dilute extract 1:1 with ionization buffer.

7.7 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, and reference material. Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and unknown duplicates. Analyte concentrations must fall within the range of calibration standards. Analytes with concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analytes with concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the ammonium acetate extractable K concentration according to the following equation:

$$K = [C \times (V/m)] \times 2 \times D_f$$

where,

K is the concentration of soil K (mg kg^{-1});
C is the extract K concentration after dilution (mg L^{-1});
V is the digest volume (mL)
m is the test portion mass (g)
D_f is any additional dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carry-over. Analyte carry-over analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration

standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carry-over as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carry-over (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carry-over effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carry-over values exceeding 5%.

Percent carry-over should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carry-over (%)

9.3 Soil Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknown, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 The Method Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$\text{MDL} = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$\text{RPD} = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where,

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also

advised.

11.3 Caution. Method utilizes concentrated acids.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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U.S. Environmental Protection Agency. 1986 Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

S – 4.10 Soil Organic Matter Loss-on-Ignition

1.0 Scope and Application

This method determines soil organic matter (SOM) based on high temperature oxidation and subsequent analysis by gravimetric weight loss.

2.0 Method Summary

2.1 Description. Loss-on-Ignition (LOI) method determines the gravimetric weight change associated with high temperature oxidation of organic matter using a muffle furnace. The method is based adapted from Storer (1984) and is an estimate. LOI is poorly correlated with the Walkley-Black wet oxidation method for soils containing less than 3% organic matter. The Method is adapted from Soil, Plant and Water Reference Methods for the Western Region S-3.10 and TMECC 05.07-A Loss on Ignition Method.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL is in the range 0.02% to 0.10% SOM (dry basis).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 0.10% SOM.

2.3.2 Organic matter is calculated as % SOM and reported on a dry basis

2.4 Interferences.

2.4.1 Hygroscopic moisture associated with soil clay may result in high bias.

2.4.2 Combustion temperatures in excess of 360 °C may result in high bias for soils containing more than 1% carbonates.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 10 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1); avoids use of concentrated acids; and (2) inexpensive instrumentations.

2.9 Disadvantages. (1) slow; and (2) reduced accuracy on soils high in clay content.

3.0 Definitions

3.1 *Soil Organic Matter* (SOM) - the sum of solids in soil that contain organic carbon. The total

organic components in soil include un-decayed plant and animal tissue, and their partial decomposition products.

3.2 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.3 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.4 *Minimum Reporting Limit.* The defensible level above wh*ich re*sults are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 °C ± 5 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.3 Sample Holding time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be stored for up to one year.

4.3.3 Processed, dry material may be held up to one year.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Forced-air drying oven. Vented, capable of maintaining constant temperature of 40 ºC ± 5 ºC, flow capacity 100 ± 10 cfm.

5.4 Flail mill or plate grinder. Bico-Braun, or similar.

5.5 Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Crucibles. Carbon free, ceramic, quartz or alundum. Size 20 - 50 cm^3, capable of withstanding temperature in of 500 °C.

5.7 Desiccator. Sealable chamber containing desiccating agent (e.g. calcium chloride). Analytical balances.

5.8 Drying Oven. Capable of 105 °C ± 5 °C.

5.9 Muffle Furnace. Capable of 360 °C ± 10 °C. Verify muffle furnace temperature distribution across oven cavity and that it is within uncertainty limits.

6.0 Reagents

6.1 Method blank. Empty crucible.

6.2 Soil Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh crucible and record mass (W_C) to nearest 0.001 g.

7.2 Transfer approximately 10.0 g of representative test portion (as-received or dried) into the crucible.

7.3 Place in forced-air drying oven at 105 °C until weight is constant. Remove to desiccator to cool.

7.4 Weigh crucible and test portion to nearest 0.001 g and record mass (W_D).

7.5 Initialize and operate the muffle furnace according to manufacturer instructions. Place dried crucible with test portion in muffle furnace and heat to 360 °C. Hold temperature for two hours. Cool to handling temperature.

7.6 Place crucible in a desiccator until cool.

7.7 Weigh crucible to nearest 0.001 g and record mass (W_F).

8.0 Calculations and Reporting

8.1 Calculate the SOM% on a dry soil basis according to the following equation:

$$SOM\% = [(W_D - W_F)/(W_D - W_C)] \times 100\%$$

where,

W_D is the initial weight of the test portion plus crucible after drying oven (g)
W_C is the crucible weight (g)
W_F is the final weight of the test portion plus crucible after muffle furnace (g)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carry-over. Analyte carry-over analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carry-over as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carry-over (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carry-over effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carry-over values exceeding 5%.

Percent carry-over should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carry-over (%)

9.3 Soil Reference Material. Include a minimum of one soil reference material with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Not applicable.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknown, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 The Method Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$\text{MDL} = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$\text{RPD} = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where,
D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when

handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes high temperatures.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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S – 5.10 Soil Saturated Paste Extract Electrical Conductivity

1.0 Scope and Application

This method determines the electrical conductivity (EC_e) of a soil from a saturated paste extract.

2.0 Method Summary

2.1 Description. A paste is prepared from the soil sample by saturatation with water (U.S. Salinity Laboratory Staff, 1954 and Robbins, 1990), removal of excess water under vacuum, and electrical conductivity (EC_e) determined using a conductance probe. The Method is adapted from Soil, Plant and Water Reference Methods for the Western Region S-1.10.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL is 0.01 dS m^{-1}.

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 0.05 ds m^{-1}.

2.3.2 EC_e is reported as dS m^{-1}.

2.4 Interferences.

2.4.1 Platinum electrodes can degrade and cause erratic results.

2.4.2 Measured values may be influenced by contamination of the electrode.

2.4.3 Air bubbles on the electrode may perturb the measurement.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 150 g.

2.6 Laboratory Sample Storage. Store laboratory sample under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1).

2.7 Laboratory Sample Holding Time. Unprocessed laboratory sample may be held up to 7 days. Frozen material may be stored for up to one year.

2.8 Advantages. (1) Simple procedure; and (2) inexpensive instrumentation.

2.9 Disadvantages. (1) Moderate precision; and (2) uncertainty increases with concentrations less than 0.20 dS m^{-1}.

3.0 Definitions

3.1 *Soil Saturated Paste,* (SP%). A mixture of soil and water, whereby all soil pore space is occupied by water and no free water collects on the surface. At saturation the soil paste glistens as it reflects light, flows slightly when held at an angle, and slides freely from a spatula.

3.2 *Electrical Conductivity*, (EC_e). The measure of the ability of a material to conduct an electric current between two platinum electrodes. The International Standard (SI) unit is siemens per meter (S m^{-1}). Conductivity is dependent on concentration of total dissolved solids and is affected by temperature.

3.3 *Laboratory Sample Holding Time*. Maximum time the submitted sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.4 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.5 *Minimum Reporting Limit.* The defensible level above wh*ich r*esults are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing. Dry laboratory sample at 40 °C ± 5 °C, in a forced-air drying oven to a constant weight. Pulverize material using flail mill or plate grinder to pass through a 10 mesh sieve (< 2.0 mm).

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying, material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.2.3 The soil saturated paste extract may be refrigerated (4 °C ± 2 °C).

4.3 Sample Holding Time.

4.3.1 Unprocessed laboratory sample may be held up to 7 days.

4.3.2 Frozen material may be stored for up to one year.

4.3.3 Processed, dry material may be held up to one year

4.3.4 The soil saturated paste extract may held up to 7 days from the time of extraction.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 ºC ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 ºC ± 5 °C.

5.3 Force draft drying oven. Vented, capable of maintaining constant temperature of 40 ºC ± 5 ºC, flow capacity 100 ± 10 cfm.

5.4 Flail mill or plate grinder. Bico-Braun, or similar.

5.5 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.6 Containers with caps. 500 mL polypropylene container or 16 oz waxed paper cup.

5.7 Spatula. Blade 17.5 mm x 100 mm length.

5.8 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.9 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 ± 10 KPa).

5.9.1 Whatman No. 42 or equivalent highly retentive filter paper.

5.10 Test tube. Glass, 25 x 150 mm, with stopper; or equivalent.

5.11 Conductivity Meter with conductivity cell. Instrument equipped with an adjustable measuring range setting and (automatic) temperature correction with dynamic range from 0.01 to 30 dS m^{-1} and having an accuracy of 0.01 dS m^{-1} at 25 ^{o}C.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Method blank solution. Deionized water.

6.3 Potassium chloride standard calibration solution (0.01 N KCl, certified content, available commercially). Alternatively, dissolve 7.456 g KCl in deionized water to a final volume of 1.0 L. This solution will have an EC_e of 1413 dS m^{-1} @ 25 ^{o}C. Prepare working standards by dilution in deionized water.

6.4 Electrical conductivity reference standard, certified content, available commercially (should be from a different source than calibration standard stock solution).

6.5 Soil Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Transfer approximately 150 g representative test portion into a 500 mL container.

7.2 Gradually add deionized water and mix with spatula until material is of uniform consistency (free of partially wetted clumps).

7.3 Allow paste to equilibrate for 4 hours. Verify saturation characteristics and add soil or water as needed. At saturation, the soil paste:

7.3.1 Does not have free standing water on the surface of the paste.

7.3.2 Soil paste slides freely and cleanly off spatula (does not apply to high clay soils, > 40% clay).

7.3.3 Soil paste will flow slightly when the container is tipped to a 45 degree angle from horizontal.

7.3.4 Soil surface glistens as it reflects light.

7.3.5 Consolidates easily by tapping after a trench is formed in the paste with the flat side of a spatula (may not apply to sandy soils >70% sand).

7.3.6 Fine textured soils (> 40% clay) may puddle easily. To minimize, water should be added with a minimum amount of stirring, at initial stages of wetting. Some fine textured soils swell considerably upon addition of water.

7.4 Repeat equilibration and verification until the paste has stabilized.

7.5 Transfer soil saturation paste to the filtration apparatus and spread evenly over the surface of the filter paper. Apply vacuum and collect filtrate in collection tube or flask until sufficient filtrate is obtained.

7.6 Re-filter on a clean filtration apparatus if filtrate is turbid. Transfer filtrate to clean test tube and close cap.

7.7 Initialize and operate the conductivity meter (probe) according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, and reference material. Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and unknown duplicates. Rinse probe with deionized water after each measurement.

8.0 Calculations and Reporting

8.1 Report EC_e of soil saturated paste extract as dS m^{-1}.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carry-over. Not applicable.

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 The Method Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved .

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Not applicable.

11.0 Safety and Disposal

11.1 Health and Safety**.** Soil may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Solution Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

Gavlack, R. E. D. A. Horneck and R. O. Miller. 2005 3rd Ed. Soil and Water Reference Methods for the Western Region. Western Regional Extension Publication. WREP-125. Oregon State University.

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US Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

US-Environmental Protection Agency. 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the analysis of pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

Introduction – Plant Tissue

The Monitoring and Reporting Program in the General Order requires analysis of plant tissue removed from each field. Analysis of total nitrogen, phosphorus, and potassium (expressed on a dry weight basis), plus percent wet weight (if weight of harvested material is reported) or density (if volume of harvested material is reported) shall be performed for each harvest from each field where manure or process wastewater are applied.

Plant tissue analyses shall be conducted using methods utilized by the North American Proficiency Testing (NAPT) Program or accepted by the University of California; and laboratories participating in the NAPT Program or other programs whose tests are accepted by the University of California.

Required analyses	**UC Method**
Percent wet weight	PT – 1.10 Total Dry Matter
Total nitrogen	PT – 2.10 Total nitrogen or PT – 2.20 Total Kjeldahl nitrogen
Phosphorus Potassium	PT – 3.10 Total phosphorus and potassium
Fixed Solids (Ash)	PT – 4.10 Fixed Solids

PT – 1.10 Total Dry Matter in Plant Tissue

1.0 Scope and Application

This method quantitatively determines the percent dry matter, including organic and inorganic material, in plant tissue based on gravimetric loss of volatiles on heating.

2.0 Method Summary

2.1 Description. Dry Matter (DM) percentage is determined gravimetrically based on the loss of volatiles, including free water, associated with heating to 55 °C (Step 1—partial dry matter) and 105 °C (Step 2—laboratory dry matter) until mass remains constant (Peters et al., 2003). The method does not remove molecular bound water. Step 2 is destructive with respect to the integrity of the material for additional testing (fiber, lignin, acid detergent insoluble nitrogen, or nitrogen). Total solids percent is used to convert analytical results from as-received to dry basis. Method is based on AOAC Method 990.15 and National Forage Testing Association Method 2.2.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.006% - 0.03% DM.

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for dry matter is 0.1%.

2.3.2 Dry matter is reported as percent of as-received mass.

2.4 Interferences. Not applicable.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 25.0 g.

2.6 Laboratory Sample Storage. Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C ± 5 °C prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

2.8 Advantages. (1) Simple method requiring inexpensive equipment.

2.9 Disadvantages. (1) Test portion sample size may affect precision; (2) test portion sample size impacts drying time; and (3) potential loss of volatile compounds.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted material may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing.

4.1.1 Determine partial dry matter (PDM) of laboratory sample.

4.1.1.1 Weigh a weighing dish and record weight to the nearest 0.1 g (W_1). Tare the balance.

4.1.1.2 Transfer and weigh up to one-half the laboratory sample in the weighing dish (minimum 25 g). Record weight to the nearest 0.1 g (W_2). Include duplicates and method blanks for each batch of unknowns as described in section 9.0.

4.1.1.3 Dry at 55 °C ± 5 °C, in a forced-air drying oven to a constant weight.

4.1.1.4 Weigh the dry unknown and dish and record weight to the nearest 0.1 g (W_3). Calculate PDM as described in section 8.0.

4.1.1.5 Grind dry material using a blade grinder or mill.

4.2 Storage.

4.2.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.2.2 After drying (Step 4.1.1.3 above), material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.3 Sample Holding Time.

4.3.1 Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

4.3.2 Dried and processed material may be held up to one year.

4.4 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0%

error.

5.4 Weighing dishes. Aluminum or other low sided containers capable of withstanding 105 ºC.

Note: If the same dishes are to be used for both Total Dry Matter (current method) and Fixed Solids (Method #PT 4-10), then use glass or ceramic containers capable of withstanding 550 ºC.

5.5 Force draft drying oven. Vented, capable of maintaining constant temperature of 55 °C ± 5 °C and 105 °C ± 5 °C, flow capacity 100 ± 10 cfm.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Blade grinder or mill. Wiley #4 or UDE Mill equipment capable of grinding and homogenizing dry botanical samples to < 0.5 mm particle size.

6.0 Reagents

6.1 Plant Tissue Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh empty weighing dish. Record the weight (W_d) to the nearest 0.01 g

7.2 Tare the balance with the empty weighing dish.

7.3 Weigh 1.0 to 2.0 g of partially dry unknown into the weighed dish, recording weight to the nearest 0.01 g (W_4). Include a method blank (empty pan), duplicates, and standard reference material with each batch of unknowns.

7.4 Dry in forced-air drying oven at 105 °C ± 5 °C until weight remains constant (less 0.1% DM change from previous weight, typically >3 hours).

7.5 Remove all unknowns and controls to a desiccator to cool.

7.6 Weigh unknowns and controls, recording weights (W_5) to the nearest 0.01 g.

8.0 Calculations and Reporting

8.1 Partial dry matter content. Calculate PDM as percent (%) according to the following equation:

$$PDM = [(W_3 - W_1) / W_2] \times 100\%$$

where

PDM is the partial dry matter content of the laboratory sample or control (%)
W_1 is the weight of the dish (g)
W_2 is the unknown or control weight, before drying (g)
W_3 is the unknown or control and dish total weight, after drying (g)

8.2 Laboratory dry matter (LDM) content. Calculate the LDM as percent (%) according to the following equation:

$$LDM = [(W_5 - W_d) / (W_4)] \times 100\%$$

where

LDM is the Laboratory dry matter content of the partially dried sample or control (%)
W_d is the weight of the dish (g)
W_4 is the initial weight of the test portion or control (g)
W_5 is the dry weight of the test portion or control, and dish (g)

Calculate the total dry matter of the laboratory sample as percent (%) according to the following equation:

$$DM = PDM \times LDM / 100$$

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.3 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Not applicable.

9.3 Reference Standard. Not applicable.

9.4 Drift Standard. Not applicable.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown. Relative percent difference (RPD) is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$MDL = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using standard reference material. Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Plant tissue may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Portion Disposal. Dispose of these materials in accordance with local, state and federal regulations.

12.0 References

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC, 18th Edition. Moisture in Animal Feed (990.15). Arlington, VA.

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Undersander, D., D.R. Mertens, and N. Thiex. 1993. Forage Analyses Procedures. Method 2.2 Two Step Total Dry Matter Determination of Wet Samples. National Forage Testing Association. Available http://www.foragetesting.org/files/LaboratoryProcedures.pdf .

U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

PT – 2.10 Total Nitrogen by Combustion in Plant Tissue

1.0 Scope and Application

This method quantitatively determines the total nitrogen content in plant tissue by discrete combustion in an oxygen environment and quantification by thermal conductivity detector.

2.0 Method Summary

2.1 Description. The material is heated to a temperature of 950-1350°C in the presence of oxygen based on the method of Dumas (1831). Mineral and organic nitrogen compounds are oxidized and the combustion products passed through a catalyst reduction furnace where conversion of oxides of nitrogen (NO_x) to molecular nitrogen (N_2) occurs. Water vapor and CO_2 are removed and total nitrogen is quantified using a thermal conductivity detector (TCD). The method is comparable to the Total Kjeldahl Nitrogen (TKN, Method PT-2.30) for nitrogen. The method is adapted from AOAC Method *990.3*.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.001% - 0.005% N (dry basis, instrument dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for total nitrogen is 0.05% N.

2.3.2 Nitrogen concentration is reported as percent (%) on a dry basis.

2.4 Method Interferences. Volatilization losses of NH_3 may occur during sample processing. Material drying temperature should not exceed 60 °C.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.1 g to 5.0 g (instrument specific).

2.6 Laboratory Sample Storage. Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C $\pm$ 5 °C prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

2.8 Method Advantages. (1) Avoids use of concentrated acids; (2) fast automated analysis; and (3) complete recovery of NH_4-N, oxidized forms of N, and heterocyclic N compounds.

2.9 Method Disadvantages. (1) High cost of instrumentation; (2) limited test portion size may affect precision; and (3) high instrument maintenance requirement.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted laboratory sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

3.4 *Total Nitrogen* (TN). The sum total of all forms of nitrogen, inorganic plus organic, within the matrix.

3.5 *Total Kjeldahl Nitrogen* (TKN). The sum of organic nitrogen plus ammonia nitrogen (NH_4^{1+}). Does not include oxidized forms of nitrogen (NO_2^{1-} and NO_3^{1-})

4.0 Processing and Storage

4.1 Processing.

4.1.1 Determine partial dry matter (PDM) of laboratory sample.

4.1.1.1 Weigh a weighing dish and record weight to the nearest 0.1 g (W_1). Tare the balance.

4.1.1.2 Transfer and weigh up to one-half the laboratory sample in the weighing dish (minimum 100 g). Record weight to the nearest 0.1 g (W_2). Include duplicates and method blanks for each batch of unknowns as described in section 9.0.

4.1.1.3 Dry at 55 °C ± 5 °C, in a forced-air drying oven to a constant weight.

4.1.1.4 Weigh the dry unknown and dish and record weight to the nearest 0.1 g (W_3). Calculate PDM as described in section 8.0.

4.1.1.5 Grind dry material using a blade grinder or mill.

4.2 Dry Matter Content. Determine total dry matter content on the test portion material according to Method P-1.10 and record.

4.3 Storage.

4.3.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.3.2 After drying (Step 4.1.1.3 above), material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.4 Sample Holding Time.

4.4.1 Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

4.4.2 Dried and processed material may be held up to one year.

4.5 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.4 Weighing dishes. Aluminum or other low sided containers capable of withstanding 105 ºC.

5.5 Force draft drying oven. Vented, capable of maintaining constant temperature of 55 °C ± 5 °C, flow capacity 100 cfm ± 10 cfm.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Blade grinder or mill. Wiley #4 or UDE Mill equipment capable of grinding and homogenizing dry botanical samples to < 0.5 mm particle size.

5.8 Total Nitrogen Analyzer. Dumas based instrument utilizing a resistance furnace, catalyst, and scrubber system with thermal conductivity detector. Minimum sample size 200 mg. Manufacturers include: Leco®; Elementar; Gerhardt Co.; Thermo; Costech; Exeter Analytical; or instrument of similar design capable of performing Dumas nitrogen determination.

6.0 Reagents

6.1 Helium. Minimum grade: 4.5 (99.995%).

6.2 Oxygen. Minimum grade: 4.5 (99.995%).

6.3 Compressed air or nitrogen. 99.6%, free of oil and water, 6.2 ± 0.2 bar (90 ± 5 psi).

6.4 Consumables. Instrument specific, check manufacturer's recommendation.

6.5 Method blank. Empty, instrument-specific container.

6.6 Nitrogen calibration standard. Known composition and certified content. Recommended compounds: glycine p-toluene sulfonate ($C_9H_{13}O_6SN$, 5.67 %N); EDTA ($C_{10}H_{16}N_2O_8$, 9.59 %N); or acetanilide (C_8H_9NO, 10.36 %N). Available from commercial chemical vendors. Store in desiccator.

6.7 Nitrogen reference standard, certified content, available commercially (different source than calibration standard stock solution).

6.8 Plant Tissue Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Weigh and transfer 100 mg to 5000 mg test portion of the processed unknown into instrument-specific container. Record weight to nearest 0.1 mg if weighing <1000 mg, or to nearest 1.0 mg if weighing ≥1000 mg. Analyze each unknown in duplicate if test portion is <1000 mg. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific methodology.

7.2 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing nitrogen calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicates. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis. Record dilution factor.

8.0 Calculations and Reporting

8.1 Calculate the total nitrogen concentration as percent (%), on a dry basis, according to the following equation:

$$N = C/(DM/100\%)$$

where,

N is the plant tissue test portion nitrogen content (% N, dry matter basis);
C is the N result from the instrument (% N);
DM is the test portion dry matter (%)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknown samples. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or each unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Peformance

10.1 Minimum Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

11.0 Safety and Disposal

11.1 Health and Safety**.** Plant tissue may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes high temperatures.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Portion Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

Association of Official Analytical Chemists. 2005. Official Methods of Aalysis of AOAC, 18th Edition. Protein (Crude) in Animal Feed: Combustion Method (990.03). Arlington, VA.

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International Organization for Standardization. 1998. Soil Quality – Determination of total nitrogen content by dry combustion ("elemental analysis"). Report 13878:1998(E). Geneva, Switzerland.

Reeuwijk, L.P. van; V.J.G Houba. 1998. Quality of analytical Procedures. Guidelines for quality management in soil and plant laboratories. pp 85-104. Reeuwijk, L.P. van (ed.) Food and Agriculture Organization of the United Nations Soils Bulletin 74 (1998) 121-128. Rome, Italy.

U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

U.S. Environmental Protection Agency. 1993. Methods for Chemical Analysis of Waters and Wastes Method 353.2, Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. EPA-600/R-93-100 (NTIS PB 84-120821).

PT – 2.20 Total Nitrogen by Kjeldahl Digestion in Plant Tissue

1.0 Scope and Application

This method quantitatively determines the nitrogen content of plant tissue through acid digestion and subsequent quantification of ammonium nitrogen using spectrophotometric, diffusion-conductivity, or distillation and titration analysis.

2.0 Method Summary

2.1 Description. Plant tissue is digested with sulfuric acid and hydrogen peroxide in the presence of a catalyst according to the method of Kjeldahl (1883). Salicylic acid and sodium thiosulfate are also included to convert nitrate nitrogen (NO_3-N). Resulting ammonium nitrogen (NH_4^+) may be determined spectrophotometrically, by diffusion-conductivity analysis, or by distillation and titration. The method is adapted from Soil, Plant and Water Reference Methods for the Western Region B-15.10.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.01% to 0.05% N (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 0.05% N.

2.3.2 Nitrogen content is calculated as % N and reported on a dry basis.

2.4 Interferences. The method does not quantitatively recover nitrogen from heterocyclic compounds (bound in carbon rings), nitro compounds, hydrazones, oximes, semicarbazones and oxidized forms such as nitrate (NO_3^{1-}).

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.1 g to 0.5 g.

2.6 Sample Stability. Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C ± 5 °C prior to processing.

2.7 Sample Holding Time. Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

2.8 Advantages. (1) Moderate cost of instrumentation; (2) accommodates large sample size; and (3) can handle wide range of moisture content.

2.9 Disadvantages. (1) Requires use of concentrated acid; (2) requires digestion catalyst; (3) incomplete recovery of nitrogen from heterocyclic carbon compounds; (4) long digestion time; and (5) labor intensive.

3.0 Definitions

3.1 *Sample Holding Time*. Maximum time a sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum reporting limit. The def*ensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing.

4.1.1 Determine partial dry matter (PDM) of laboratory sample.

4.1.1.1 Weigh a weighing dish and record weight to the nearest 0.1 g (W_1). Tare the balance.

4.1.1.2 Transfer and weigh up to one-half the laboratory sample in the weighing dish (minimum 100 g). Record weight to the nearest 0.1 g (W_2). Include duplicates and method blanks for each batch of unknowns as described in section 9.0.

4.1.1.3 Dry at 55 °C ± 5 °C, in a forced-air drying oven to a constant weight.

4.1.1.4 Weigh the dry unknown and dish and record weight to the nearest 0.1 g (W_3). Calculate PDM as described in section 8.0.

4.1.1.5 Grind dry material using a blade grinder or mill.

4.2 Dry Matter Content. Determine total dry matter content on the test portion material according to Method P-1.10 and record.

4.3 Storage.

4.3.1 Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C ± 5 °C prior to processing.

4.3.2 After drying (Step 4.1.1.3 above), material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.4 Sample Holding Time.

4.4.1 Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

4.4.2 Dried and processed material may be held up to one year.

4.5 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Balances used for weighing reagents must have less than 1.0% error.

5.4 Weighing dishes. Aluminum or other low sided containers capable of withstanding 105 ºC.

5.5 Forced draft drying oven. Vented, capable of maintaining constant temperature of 55 ºC ± 5 ºC, flow capacity 100 cfm ± 10 cfm.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Blade grinder or mill. Wiley #4 or UDE Mill equipment capable of grinding and homogenizing dry plant samples to < 0.5 mm particle size.

5.8 Digestion block. Capable of 380 °C ± 10 °C and accommodating digestion tube (Section 5.8).

5.9 Digestion tubes with caps. Pyrex glass digestion tube with volumetric graduation mark, 50 mL, 75 mL or 100 mL.

5.10 Acid resistant fume hood. Capable of minimum flow rate of 100 cubic feet per minute (cfm).

5.11 Repipette dispenser. Dispenser calibrated to 3.0 mL ± 0.1 mL.

5.12 Vortex mixer. Standard laboratory mixer with tube adapter.

5.13 Ammonium nitrogen analysis instrument (select one).

5.13.1 Flow injection analyzer. Segmented flow or flow injection and analysis (FIA) type. Automated ammonium nitrogen analyzer (e.g. Techincon Autoanlyzer II, Lachat Instruments, FIALab, Skalar, or other instrument of similar design capable of performing ammonium nitrogen analysis based on phenate or salicylate chemistry).

5.13.2 Diffusion-conductivity analyzer based on method described by Carlson (1990) (e.g. Timberline Instruments Method 4500-N D).

5.13.3 Micro-Kjeldahl steam distillation apparatus.

6.0 Reagents

6.1 Deionized (DI) water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Concentrated sulfuric acid (H_2SO_4). ACS Reagent.

6.3 Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \bullet 5H_2O$). Powder crystals of sodium thiosulfate, pentahydrate, 60 mesh.

6.4 Digestion catalyst. Kjeltabs (Fisher Kjeltab ST-AUTO Kjeldahl tablets, 1.5 g K_2SO_4 plus 0.015 g Se), copper sulfate ($CuSO_4$) or equivalent.

6.5 Boiling Chips. Acid resistant.

6.6 Ammonium nitrogen analysis instrument specific reagents (refer to instrument manufacturer's specific procedure for phenate or salicylate chemistry).

6.7 Method blank solution. Deoinized water.

6.8 Nitrogen calibration standard. Known composition and certified content. Recommended compounds: glycine p-toluene sulfonate ($C_9H_{13}O_6SN$, 5.67 %N); EDTA ($C_{10}H_{16}N_2O_8$, 9.59 %N); or acetanilide (C_8H_9NO, 10.36 %N). Available from commercial chemical vendors. Store in desiccator.

6.9 Nitrogen reference standard, certified content, available commercially (different source than calibration standard stock solution).

6.10 Plant Tissue Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Transfer approximately 100 to 500 mg of representative test portion into a Kjeldahl digestion tube. Record weight to nearest 0.1 mg. Record weight to nearest 0.1 mg. Analyze each unknown in duplicate. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific methodology.

7.2 Prepare spikes, reference material, and blanks along with the unknowns for digestion.

7.3 In the fume hood, add 3 mL concentrated H_2SO_4 to each digestion tube.

7.4 Nitrate reduction step. Note: this step may be omitted if nitrate is being quantitatively determined separately (Method B-3.10 from Gavlack et al., 2005 or similar). Add nitrate concentration to TKN for total nitrogen determination.

7.4.1 Pre-heat digestion block to 120 °C in acid resistant fume hood.

7.4.2 In the fume hood, add 1.0 g sodium thiosulfate crystals via long-stem funnel. Digest in digestion block at 120 °C until frothing ceases.

7.4.3 Cool to safe handling temperature.

7.5 Add one Kjeltab and a few boiling chips to each digestion tube.

7.6 Place digestion tube in 160 °C digestion block for 30 minutes.

7.7 Ramp temperature 5 °C min^{-1} to 380 °C, and hold for 60 minutes or until sample digestion is complete. Remove from block and cool in hood for 30 minutes.

7.8 Dilute to graduation mark with deionized water. Cap and mix using vortex mixer.

7.9 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte concentrations must fall within the range of calibration standards. Analyate concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis.

8.0 Calculations and Reporting

8.1 Calculate the TKN content as % on dry basis according to the following equation:

$$TKN = 100\% \times [(C - B) \times (V/m) \times D_f / 10000] / [DM / 100]$$

where,

TKN is the plant tissue nitrogen content (%, dry basis)
C is the digest NH_4^{1+}-N concentration after dilutions (mg L^{-1})
B is the method blank (mg L^{-1})
m is the test portion mass (g)
DM is the test portion dry matter (%)
V is the digest or distillate final volume (mL)
D_f is any additional dilution factor

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where,

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where,

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknowns. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Perform spike recovery with each batch of unknowns when the matrix differs significantly from the reference material. Spike the unknown with analyte reference standard at a concentration 4 to 10 times the analyte concentration of the unknown or method reporting limit, whichever is greater.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Method Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to 5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

$$\text{MDL} = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$\text{RPD} = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first sample replicate
D_2 is the concentration of analyte in the duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using either standard reference material or use of spike recovery and analysis.

10.3.1 Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

10.3.2 Spike recovery. Calculate percent spike recovery as follows:

$$R = [(C_s - C_u)/S_p] \times 100\%$$

where

R is the spike recovery (%)
C_s is the result of the spiked unknown
C_u is the result of the unknown
S_p is the concentration of the spike

Spike recovery should fall within a laboratory-determined acceptable range or 85%-115%. Failure shall result in evaluation of reagents and equipment, correction of problems, and re-analysis of spike recovery and all unknowns in the batch.

11.0 Safety and Disposal

11.1 Health and Safety**.** Plant tissue may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes concentrated acid solutions and high temperature.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Portion Disposal. Dispose of materials in accordance with local, state and federal regulations.

12.0 References

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PT – 3.10 Total Phosphorus and Potassium in Plant Tissue

1.0 Scope and Application

This method quantitatively determines the phosphorus or potassium content in plant tissue by acid wet digestion followed by quantification by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.0 Method Summary

2.1 Description. The individual elemental content (P and K) of plant tissue is determined after digestion with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Elemental constituents of the digest are determined by ICP-OES. The method is adapted from Wolf et al. (2003).

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.002% to 0.01% P and K (dry basis, instrument manufacturer dependent).

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) is 0.01% for P and K.

2.3.2 Elemental constituents are reported as percent (%) P or K on a dry basis.

2.4 Interferences. None known.

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 0.2 g to 1.0 g.

2.6 Laboratory Sample Storage. Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C $\pm$ 2 °C) or frozen (-20 °C $\pm$ 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C $\pm$ 5 °C prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be held until material integrity has become compromised.

2.8 Advantages. (1) Fast automated analysis; and (2) complete recovery of all forms of P (inorganic and organic); (3) and low detection limit.

2.9 Disadvantages. (1) High cost of instrumentation; (2) use of concentrated acids and reactive chemicals; and (3) high instrument maintenance.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time a sample may be held in a non-frozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing and Storage

4.1 Processing.

4.1.1 Determine partial dry matter (PDM) of laboratory sample.

4.1.1.1 Weigh a weighing dish and record weight to the nearest 0.1 g (W_1). Tare the balance.

4.1.1.2 Transfer and weigh up to one-half the laboratory sample in the weighing dish (minimum 100 g). Record weight to the nearest 0.1 g (W_2). Include duplicates and method blanks for each batch of unknowns as described in section 9.0.

4.1.1.3 Dry at 55 °C ± 5 °C, in a forced-air drying oven to a constant weight.

4.1.1.4 Weigh the dry unknown and dish and record weight to the nearest 0.1 g (W_3). Calculate PDM as described in section 8.0.

4.1.1.5 Grind dry material using a blade grinder or mill.

4.2 Dry Matter Content. Determine total dry matter content on the test portion material according to Method P-1.10 and record.

4.3 Storage.

4.3.1 Store laboratory sample under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing.

4.3.2 After drying (Step 4.1.1.3 above), material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.4 Sample Holding Time.

4.4.1 Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

4.4.2 Dried and processed material may be held up to one year.

4.5 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Balances used for weighing reagents must have less than 1.0% error.

5.4 Weighing dishes. Aluminum or other low sided containers capable of withstanding 105 ºC.

5.5 Force draft drying oven. Vented, capable of maintaining constant temperature of 55 °C ± 5 °C, flow capacity 100 cubic feet per minute (cfm) ± 10 cfm.

5.6 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.7 Blade grinder or mill. Wiley #4 or UDE Mill equipment capable of grinding and homogenizing dry plant samples to < 0.5 mm particle size.

5.8 Reciprocating horizontal mechanical shaker. Capable of 180 oscillations per minute (opm).

5.9 Acid resistant fume hood. Capable of minimum flow rate of 100 cfm.

5.10 Digestion block. Capable of a range of 80 to 140 °C ± 5.0 °C.

5.11 Repipette dispenser. Dispenser calibrated to 6.0 mL ± 0.1 mL.

5.12 Micropipettor. Calibrated to dispense 1 mL ± 0.01 mL.

5.13 Filtration apparatus. Funnel, vacuum flasks, and associated vacuum system. Filter assembly (preferably plastic) and vacuum system (capable of -80 KPa ± 10 KPa).

5.13.1 Glass fiber filter

5.13.2 Whatman No. 42 or equivalent highly retentive filter paper.

5.14 Centrifuge. Centrifuge (optional in place of filter apparatus), centrifuge capable of 8000 x*g*.

5.14.1 Centrifuge tubes with caps. 50 mL capacity, compatible with centrifuge used.

5.15 Vortex mixer. Standard laboratory mixer with tube adapter.

6.0 Reagents

6.1 Deionized water. ASTM Type I grade, < 0.056 Conductivity at 25 ^{o}C/µS • cm^{-1}.

6.2 Nitric Acid (HNO_3), concentrated, trace metal grade.

6.3 Hydrogen Peroxide (H_2O_2), 30% ACS Reagent.

6.4 Argon gas 99.5% purity.

6.5 Lithium chloride solution (1000 mg L^{-1} lithium chloride, LiCl, ACS reagent). Dissolve 2.03 g LiCl in

1.5 L of deionized water. Add 20 mL of concentrated nitric acid (HNO_3, trace metal grade) and bring to 2 L with deionized water.

6.6 Method blank solution. Digestion solution (6 mL concentrated HNO_3, 2 mL 30% H_2O_2, 92 mL deionized water).

6.7 Calibration standards. Prepare 3 or more multi-element calibration standard solutions in 2% HNO_3 solution. Available from commercial chemical vendors.

6.8 Reference standard, certified content, available commercially (different source than calibration standard stock solution).

6.9 Plant Tissue Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Pre-heat digestion block to 80 °C ± 5 °C.

7.2 Transfer a test portion of approximately 200 mg – 1000 mg of each unknown into a digestion tube. Record weight to nearest 0.1 mg if weighing <1000 mg, or to nearest 1.0 mg if weighing ≥1000 mg. Analyze each unknown in duplicate if test portion is <1000 mg. The requirement to duplicate each unknown may be waived if the laboratory has documented that typical RPD for replicates of test portions smaller than 1000 mg is <15% using laboratory specific methodology.

7.3 Prepare method blank, reference material, drift control standards, and spike (if used) for digestion along with unknowns and duplicate unknowns.

7.4 Add 6.0 mL concentrated nitric acid using a repipette dispenser and swirl to thoroughly wet the sample. Predigest at room temperature for a minimum of 20 minutes.

7.5 Place digestion tubes in digestion block at 80 °C ± 5 °C for 10 minutes. Remove and cool for 2 minutes.

7.6 Increase digestion block temperature to 140 °C ± 5 °C.

7.7 Add 2 x 1 mL aliquots of 30% H_2O_2 solution. If foaming occurs, carefully swirl to clean off walls of digestion tube.

7.8 Return tubes to digestion block and digest for 60 minutes or until total digest volume is reduced to 2 mL to 3 mL.

7.9 Remove tubes from block and cool for 30 minutes.

7.10 Dilute each digest to 100 mL with deionized water. Cap and mix using vortex mixer. Digests exhibiting turbidity may require overnight settling, centrifugation or filtration to remove undissolved particulate matter prior to analysis.

7.11 Note: it may be necessary to add lithium chloride solution to all digests and standards in order to obtain a stable potassium reading.

7.12 Initialize and operate the analysis instrument according to manufacturer's instructions. Calibrate instrument utilizing analyte calibration standards. Analyze method blank, reference standard, reference material, and spike (if used). Analyze drift control standard, batch of unknowns, followed by duplicate drift control standard and duplicate unknowns. Analyte concentrations must fall within the range of calibration standards. Analyte concentrations lower than the lowest calibration point may require re-analysis at a reduced dilution level; or, in the case where this is not applicable, report as <MRL. Analyte concentrations exceeding the highest calibration point require dilution and re-analysis.

8.0 Calculations and Reporting

8.1 Calculate the analyte content as percent (%) on dry basis according to the following equation:

$$M = 100\% \times [(C - B) \times (V/m) \times D_f / 10000] / (DM / 100)$$

where,

M is the plant tissue P or K concentration (%, dry basis)
C is the digest P or K concentration after dilutions (mg L^{-1})
B is the method blank concentration (mg L^{-1})
V is the digest final volume (mL)
m is the test portion mass (g)
D_f is any additional dilution factor
DM is the test portion dry matter (%)

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Carryover analysis should be determined annually. Analyze two consecutive high calibration standard solutions, followed by three consecutive low calibration standard solutions, repeated in triplicate. Calculate the means of the first and third low calibration standard solution results and calculate carryover as follows:

$$h_{co} = (b_1 - b_3)/b_3 \times 100$$

where

h_{co} is carryover (%)
b_1 is the mean result of the first low calibration standard solution
b_3 is the mean result of the third low calibration standard solution

Carryover effect is significant if h_{co} exceeds 5%. Lab instrumentation should be serviced or adjusted for carryover values exceeding 5%.

Percent carryover should be used to evaluate the validity of test results where a low concentration unknown follows a high concentration unknown. In instances where the difference between consecutively analyzed unknowns multiplied by h_{co} is greater than 10% of the second unknown result, the second unknown must be re-analyzed; i.e., re-analysis is required when:

$$(s_1 - s_2) \times h_{co} \geq 0.10 \times s_2$$

where

s_1 is the first unknown
s_2 is the second unknown
h_{co} is carryover (%)

9.3 Reference Standard. Include a minimum of one analyte reference standard with each batch. Results should be within reference specified uncertainty limits. Record results.

9.4 Drift Standard. Assess instrument drift utilizing a calibration standard analyzed at the beginning and again at the end of each batch. For larger batch sizes, it is recommended that additional drift standards be analyzed throughout. Concentration of the drift standard should be near the midpoint of the calibration curve. Drift standards should measure within 5% of the known value. Failure shall result in re-analysis of the batch of unknown samples. Record results.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the North American Proficiency Testing Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit (MDL). MDL shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 2), and calculated using the following equation:

MDL = Student's *t* (n-1,1-α=0.99) value × *s.d.*

A MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 2. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first sample replicate
D_2 is the concentration of analyte in the duplicate

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using standard reference material. Standard reference material (SRM). Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Plant tissue may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Method utilizes concentrated acids and high temperature.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Sample Disposal. Dispose of samples in accordance with local, state and federal regulations.

12.0 References

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Yang, W. M, R. Boles, and T. P. Mawhinney. 2002. Determination of phosphorus in fertilizers by inductively coupled plasma atomic emission spectrometry. J. AOAC 85:6, 1241-1246.

PT – 4.10 Fixed Solids in Plant Tissue

1.0 Scope and Application

This method quantitatively determines total fixed solids by combustion at 550 C in the presence of excess air.

2.0 Method Principle

2.1 Description. Fixed solids (FS) percentage is determined gravimetrically based on the loss of volatiles, including organic matter, associated with heating to 550 C. The ash residue is the inorganic (mineral) fraction of the plant tissue.

2.2 Performance.

2.2.1 Minimum Detection Limit (MDL). Typical MDL range is 0.01 – 0.05 %FS.

2.2.2 Precision. Duplicate analyses should yield a relative percent difference (RPD) less than or equal to 15%.

2.3 Reporting.

2.3.1 Method Reporting. Minimum reporting limit (MRL) for fixed solids is 0.1%.

2.3.2 Fixed solids is reported as a percent of dry matter (DM).

2.4 Interferences. Not applicable?

2.5 Test Portion Size Requirement. Minimum test portion size for each replicate is 1.0 g of dried and processed material.

2.6 Laboratory Sample Storage. Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C ± 5 °C prior to processing.

2.7 Laboratory Sample Holding Time. Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

2.8 Method Advantages. (1)Simple method requiring minimal equipment.

2.9 Method Disadvantages. (1) Test portion size may affect precision.

3.0 Definitions

3.1 *Laboratory Sample Holding Time*. Maximum time the submitted material may be held in a nonfrozen state. Holding time is calculated as the total time from collection to the initiation of extraction or analysis, minus time frozen.

3.2 *Batch.* The set of quality control and unknown test portions analyzed at the same time for a single constituent (see Section 9.0).

3.3 *Minimum Reporting Limit (MRL).* The defensible level above which results are reported by the laboratory. MRL must be greater than 2x MDL.

4.0 Processing, Preservation and Storage

4.1 Processing. Determine partial dry matter according to Method PT-1.10, section 4.1.

4.2 Dry Matter Content. Determine total dry matter content on the test portion material according to Method P-1.10 and record.

4.3 Storage.

4.3.1 Store laboratory samples with estimated moisture content >20% under refrigeration (4 °C ± 2 °C) or frozen (-20 °C ± 5 °C) prior to processing (see Section 4.1). Material with estimated moisture content <20% may be stored at 20 °C ± 5 °C prior to processing.

4.3.2 After drying (Step 4.1 of Method PT-1.10), material may be stored in a sealed container at 20 °C ± 5 °C (room temperature).

4.4 Sample Holding Time.

4.4.1 Laboratory sample may be held up to 7 days as long as sample integrity is maintained.

4.4.2 Dried and processed material may be held up to one year.

4.5 Defrost. Place frozen material at 4 °C ± 2 °C for 48 hours, process as described in section 4.1 and complete analysis within defined holding time.

5.0 Apparatus

5.1 Refrigerator. Capable of maintaining constant temperature 4 °C ± 2 °C.

5.2 Freezer. Capable of maintaining constant temperature -20 °C ± 5 °C.

5.3 Analytical balances. Analytical balances used for weighing standards or unknowns must have less than 0.1% error. Other balances used for weighing reagents must have less than 1.0% error.

5.4 Weighing dishes. Glass or ceramic containers capable of withstanding 550 ºC.

5.5 Force draft drying oven. Vented, capable of maintaining constant temperature of 55 °C ± 5 °C and 105 °C ± 5 °C, flow capacity 100 ± 10 cfm.

5.6 Forced air muffle furnace. Vented, capable of maintaining constant temperature of 550 °C ± 10 °C.

5.7 Desiccator chamber. Sealable chamber containing a desiccating agent.

5.8 Blade grinder or mill. Wiley #4 or UDE Mill equipment capable of grinding and homogenizing dry botanical samples to < 0.5 mm particle size.

6.0 Reagents

6.1 Plant Tissue Reference Material (RM). Standard reference material sources are listed in Appendix 3.

7.0 Procedure

7.1 Pre-heat the muffle furnace to 200 °C.

7.2 After determining total dry matter according to Method PT-1.10, place weighing dishes containing unknowns and controls in the pre-heated muffle furnace.

7.3 Slowly (over approximately 2-3 hours) ramp the temperature to 550 °C, then hold for 2 hours, followed by a ramp down to 200 °C.

7.4 Transfer weighing dishes containing unknowns and controls to a dessicating chamber to cool to room temperature.

7.5 Weigh and record gross weight of each dish and unknown or control (AshW) to the nearest 0.01 g.

8.0 Calculations and Reporting

8.1 Fixed solids (FS) content. Calculate the FS content as percent (%), on a dry basis according to the following equation:

FS = AshW/LDM x 100

where

LDM is the laboratory dry matter determined using Method PT-1.10.

Round final result to three significant digits. Do not report significant digits less than the MRL.

Results less than minimum reporting limit (MRL), report as <MRL.

8.2 Client Reports. Report matrix, method reference identification, MDL, duplicate relative percent difference (RPD), reference standard result, and method anomaly observation(s) as applicable. Data should be flagged when holding time is exceeded.

9.0 Quality Control

9.1 Method blank. A method blank shall be performed with every batch. The value of the blank typically should not exceed the MDL. In cases where the value of the blank exceeds the MDL, the value of the blank should be subtracted from the analytical result. The blank value should fall within the laboratory normal range. If it does not, take corrective action and repeat.

9.2 Analyte carryover. Not applicable.

9.3 Reference Standard. Not applicable.

9.4 Drift Standard. Not applicable.

9.5 Laboratory Duplicate Analysis. Duplicate analyses shall be performed on a minimum of 10% of unknowns, or every unknown if homogeneity exceeds 15% of the mean value of the replicates of the unknown. Relative percent difference (RPD) is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The suggested level of performance for RPD is under 15%.

9.6 Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory shall participate in the Manure Analysis Proficiency Program or other programs accepted by the University of California.

10.0 Method Performance

10.1 Minimum Detection Limit. Shall be determined initially and any time instrument is replaced or significant change in the method occurs, based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136, using a standard deviation (*s.d.*) of a minimum of 7 unknowns at 2 to5 times the estimated limit of detection, a Student's t 99% confidence interval (Table 1), and calculated using the following equation:

$$MDL = \text{Student's } t \text{ (n-1,1-}\alpha\text{=0.99) value} \times s.d.$$

An MDL less than or equal to the minimum reporting limit specified in Section 2.2 must be achieved.

Table 1. Table of Student's *t* Values at the 99 percent confidence level.

Number of replicates	Degrees of freedom (n-1)	t (cn-1,0.99)
7	6	3.145
8	7	2.998
9	8	2.896
10	9	2.821

www.statsoft.com/textbook/sttable.html

10.2 Precision. Relative percent difference (RPD) between duplicate analyses is calculated according to the following equation:

$$RPD = \{(D_1 - D_2)/[(D_1 + D_2)/2]\} \times 100\%$$

where

D_1 is the concentration of analyte in the first unknown replicate
D_2 is the concentration of analyte in the duplicate unknown

The laboratory should develop criteria for evaluation of duplicate performance based on performance of the method over time. The recommended level of performance for RPD is under 15%.

10.3 Accuracy. Accuracy may be assessed using standard reference material. Analytical result of the SRM should fall within the established range of the material used.

11.0 Safety and Disposal

11.1 Health and Safety**.** Plant tissue may contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing, latex gloves, and eye protection when handling this material.

11.2 Laboratory Safety. The laboratory is responsible for maintaining a current awareness file of local, state and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.3 Caution. Not applicable.

11.4 Reagent Disposal. Dispose of reagents in accordance with and local, state and federal regulations.

11.5 Laboratory Sample and Test Portion Disposal. Dispose of these materials in accordance with local, state and federal regulations.

12.0 References

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC, 18th Edition. Moisture in Animal Feed (990.15). Arlington, VA.

International Organization for Standardization. 1994. Accuracy (trueness and precision) of measurement methods and results. Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method. Report 5725-2:1994 Geneva, Switzerland.

Reeuwijk, L.P. van; V.J.G Houba. 1998. Quality of analytical Procedures. Guidelines for quality management in soil and plant laboratories. pp 85-104. Reeuwijk, L.P. van (ed.) Food and Agriculture Organization of the United Nations Soils Bulletin 74 (1998) 121-128. Rome, Italy.

Shreve et al. 2006. NFTA Method 2.1.4 – Dry Matter by Oven Drying for 3 hr at 105 ºC. National Forage Testing Association.

Undersander, D., D.R. Mertens, and N. Thiex. 1993. Forage Analyses Procedures. Method 2.2 Two Step Total Dry Matter Determination of Wet Samples. National Forage Testing Association. Available http://www.foragetesting.org/files/LaboratoryProcedures.pdf .

U.S. Composting Council. 2001. Test Methods for the Examination of Composting and Compost. Method 03.02 Ash. US Composting Council, Ronkonkoma, NY.

U.S. Environmental Protection Agency. 1986. Title 40 Code of Federal Regulations Part 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants. Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.

Appendix 1. Sampling Requirements from the General Order

(Extracted from MRP pages 2 through 4 of the General Order)

Nutrient Monitoring

Starting no later than 12 months after adoption of this Order, the Discharger shall begin monitoring process wastewater, manure, and plant tissue produced at the facility, soil in each land application area, and irrigation water used on each land application area for the constituents and at the frequency as specified in Table 2 below. This information is for use in conducting nutrient management on the individual land application areas and at the facility on the whole. It must be used to develop and implement the Nutrient Management Plan. The Discharger is encouraged to collect and use additional data, as necessary, to refine nutrient management.

Table 2. NUTRIENT MONITORING

Process Wastewater

Each application:
Record the volume (gallons or acre-inches) and date of process wastewater application to each land application area.

Quarterly during one application event:
Field measurement of electrical conductivity.

Laboratory analyses for nitrate-nitrogen (only when retention pond is aerated), ammonium-nitrogen, total Kjeldahl nitrogen, total phosphorus, and potassium.

Once within 12 months and annually for two years after groundwater monitoring wells are required:
Laboratory analyses for general minerals (calcium, magnesium, sodium, bicarbonate, carbonate, sulfate, and chloride).

Manure

Each application to each land application area:
Record the total volume (cubic yards) applied and density (pounds per cubic foot) or total weight (tons) applied and percent moisture.

Once within 12 months:
Laboratory analyses for general minerals (calcium, magnesium, sodium, bicarbonate, carbonate, sulfate, and chloride).

Twice per year:
Laboratory analyses for total nitrogen, total phosphorus, potassium, and density (if volume manure applied is reported) or percent moisture (if weight manure applied is reported).

Each offsite export of manure:
Record the total volume (cubic yards) exported and density (grams per liter) or total weight (tons) exported and percent moisture.

Laboratory analyses for density (if volume manure exported is reported) or percent moisture (if weight manure exported is reported).

Annually:
Record the total dry weight (tons) of manure applied annually to each land application area and the total dry weight (tons) of manure exported offsite.

Plant Tissue

At harvest:

Record the total weight (tons) and percent wet weight or volume (cubic yards) and density (grams per liter) of harvested material removed from each land application area.

Laboratory analyses for total nitrogen, phosphorus, and potassium (expressed on a dry weight basis), and percent wet weight (if weight of harvested material is reported) or density (if volume of harvested material is reported).

The following test is only required if the Discharger wants to add fertilizer in excess of 1.4 times the nitrogen expected to be removed by the harvested portion of the crop (see Attachment C for details): Mid-season, if necessary to assess the need for additional nitrogen fertilizer during the growing season.

Laboratory analyses for total nitrogen, expressed on a dry weight basis.

Soil

Beginning in the summer of 2008 and then once every 5 years from each land application area:

Laboratory analyses for:

Total phosphorus

The following soil tests are recommended but not required:

Spring pre-plant for each crop:

Laboratory analyses for:

0 to 1 foot depth: Nitrate-nitrogen and organic matter.

1 to 2 foot depth: Nitrate-nitrogen.

Fall pre-plant for each crop:

Laboratory analyses for:

0 to 1 foot: Electrical conductivity, nitrate-nitrogen, soluble phosphorus, potassium and organic matter.

1 to 2 foot: Nitrate-nitrogen.

2 to 3 foot: Nitrate-nitrogen.

Irrigation Water[1]

Each irrigation event for each land application area:

Record volume (gallons or acre-inches)[2] and source (well or canal) of irrigation water applied and dates applied.

One irrigation event during each irrigation season during actual irrigation events:

For each irrigation water source (well and canal):

Electrical conductivity and total nitrogen.[3]

Data collected to satisfy the groundwater monitoring requirements (below) will satisfy this requirement.

[1] The Discharger shall monitor irrigation water (from each water well source and canal) that is used on all land application areas.
[2] Initial volume measurements may be the total volume for all land application areas. Volume measurements for each irrigation source for each land application area shall be recorded no later than **1 July 2011**.
[3] In lieu of sampling the irrigation water, the Discharger may provide equivalent data from the local irrigation district.

Appendix 2. Sampling Guidelines

Collection of laboratory samples to comply with the Monitoring and Reporting requirements of the General Order must be done by methods and at frequencies defined in the facility's Sampling and Analysis Plan. This Plan defines the minimum sampling frequency, sampling method, source description of the material being sampled, and minimum analyses required both in the field and by the analytical laboratory. Sampling Methods must be approved by the Central Valley Regional Water Quality Control Board's Executive Officer. A list of approved methods is available at: http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/general_order_guidance/sampling_analysis/index.shtml. Procedures for obtaining method approval are described on the web page. The list below contains direct electronic links to each of the approved sampling methods.

Laboratory sample holding time is defined as the maximum time a sample can be held, from collection to the start of testing, and still yield valid results. Table 1 lists the University of California Method guidelines for delivery and holding times approved by the Executive Officer.

Table 1. Time allowed froms ampling to delivery to analytical laboratory and maximum holding time.

Material	Collection to laboratory delivery	Okay to freeze prior to delivery?	Holding time (total)
Manure	Same day	Yes	7 days
Process wastewater	24 hours	No	N/A
Plant tissue	72 hours	No	7 days
Soil	As soon as possible	No	7 days
Irrigation water	24 hours	No	N/A

Individuals collecting and transporting samples must complete a chain of custody form to present to the analytical laboratory. This form specifies the time the laboratory sample was collected and is used by the laboratory to determine whether valid results can be obtained before the holding time expires. The laboratory should notify the individual submitting the sample if a valid result cannot be obtained. If analyses are still requested, the holding time exceedance must be noted on the client report.

Upon receipt, laboratory staff will log the sample into the laboratory data system and record quality of sample received. If sample anomalies exist (warm or off color or odiferous, for example), this will be noted and included in the client report.

The following documents are available for download at www.cdqa.org/binder.asp, section 5 – "How-to" Instruction Documents.

Sampling Supply Wells and Subsurface (Tile) Drainage Systems

Compiled by Thomas Harter and Deanne Meyer, August 2007
University of California, Davis
CDQAP-WDR General Order Reference Binder TAB 5.1, Version 8-07

Solid Manure Sampling Protocol

Deanne Meyer and Denise Mullinax
University of California, Davis and the California Dairy Quality Assurance Program
CDQAP-WDR General Order Reference Binder TAB 5.2, Version 2-29-08

Process Wastewater (Liquid Manure) Sampling Protocol

Deanne Meyer
University of California, Davis
CDQAP-WDR General Order Reference Binder TAB 5.3, Version 2-29-08

Soil Sampling Protocol

Marsha Campbell Mathews & Carol Frate
UCCE Farm Advisors, Stanislaus and Tulare Counties
CDQAP-WDR General Order Reference Binder TAB 5.5, Version 2-29-08

Irrigation (Fresh) Water Sampling Protocol

Carol Frate and Marsha Campbell Mathews
UCCE Farm Advisors, Tulare and Stanislaus Counties
CDQAP-WDR General Order Reference Binder TAB 5.6, Version 2-29-08

Sampling Protocol for Plant Tissue Corn and Winter Forage Silage

Marsha Campbell Mathews & Carol Frate
UCCE Farm Advisors, Stanislaus and Tulare Counties
CDQAP-WDR General Order Reference Binder TAB 5.5, Version 2-29-08

Sampling Protocol for Irrigated Pastures

Josh Davy, Morgan Doran, Betsy Karle and Deanne Meyer
UCCE Livestock and Natural Resources Program Representative - Tehama, Glenn, Colusa
CUCCE Livestock and Natural Resources Program Advisor - Yolo, Solano, Sacramento
UCCE Dairy Program Representative - Tehama, Glenn
UCCE Livestock Waste Management Specialist
CDQAP-WDR General Order Reference Binder TAB 5.7, September 2008

Solid Manure Moisture Content Determination-Microwave Method for Exported Solid Manures

Deanne Meyer, Patricia Price and Betsy Karle
Department of Animal Science, UC Davis
UCCE Dairy Program Representive - Tehama, Glenn
CDQAP-WDR General Order Reference Binder TAB 5.8, September 2008

Sampling Protocol for Alfalfa Hay, Greenchop, and Silage (Haylage)

Shannon Mueller and Dan Putnam
UCCE Farm Advisor - Fresno
UCCE Specialist
CDQAP-General Order Reference Binder TAB 5.9, September 2008

Appendix 3. Sources of Reference Material (RM).

Manure

WEPAL

P.O. Box 8005
NL-6700 EC Wageningen
The Netherlands
Phone: +31 317 482337/482349
Info.Wepal@wur.nl
www.wepal.nl

Soil

Agricultural Laboratory Proficiency (ALP)

P.O. Box 650820
Sterling, VA 20165-0820
USA
Phone: 571-434-1925
alp@cts-interlab.com
www.collaborativetesting.com

NIST

100 Bureau Drive, Stop 2300
Gaithersburg, MD 20899-2300
USA
Phone: 301-975-2200
srminfo@nist.gov
www.nist.gov

Other

LECO Corporation

3000 Lakeview Avenue
St. Joseph, MI 49085
Phone: 800-292-6141 or 269-985-5496
info@leco.com
www.leco.com

Elemental Microanalysis

C/O KE Developments
The Mount, Toft
Cambridge CB23 2RL
UK
Phone: +44 (0)1837 54446
info@microanalysis.co.uk
www.microanalysis.co.uk

EuroVector

Via Tortona, 5
20144 Milan
Italy
Phone: 0039.02.839.4736
admin@eurovector.it
www.eurovector.it

Appendix 4. Quality Assurance

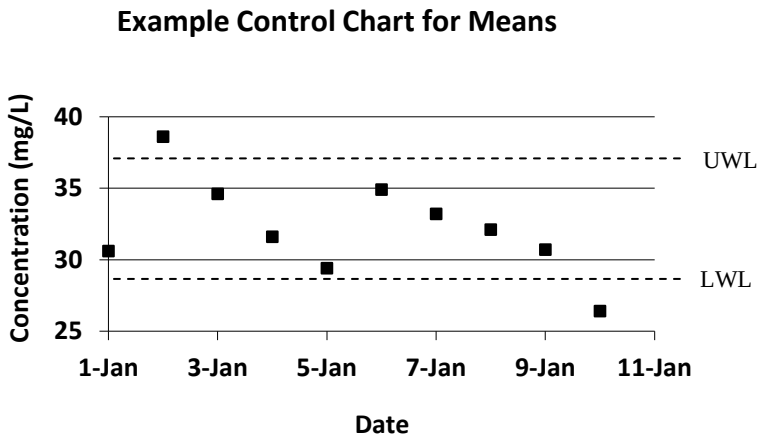
An analytical laboratory must have a written quality assurance plan and follow the guidelines of that plan in order to produce data of known and defensible quality. At a minimum, the quality assurance plan should include written standard operating procedures for each method performed in the laboratory, a written schedule for equipment maintenance and calibration, internal quality control activities, and data assessments for bias and precision.

Standard Operating Procedures (SOP). A SOP is a document that describes an analytical method in sufficient detail that an experience technician could perform the method without close supervision.

Equipment Maintenance and Calibration. The manufacturers of laboratory equipment generally make reasonable recommendations for routine maintenance, repair, and calibration of their instruments. Good laboratory practice dictates that manufacturers' recommendations be followed. This will also ensure maximum efficiency in the lifetime use of the equipment.

Internal Quality Control Activities. Not all quality control activities can be applied to each method. Therefore, section nine of each method includes several activities, with the expectation that the laboratory would exercise good judgment in selecting the elements to run on a routine basis to ensure the generation of good quality data.

> *Control Charts.* Control charts are a graphical representation of the routine measurements of standards, reference materials, spike samples, blanks, and replicate analyses. Control charts should be constructed with upper and lower warning limits marked prior to population with data. Warning limits may be set at 2-3 standard deviations from the stated value for standard reference materials or from replicate analyses of a calibration check standard. If concentration varies, convert results to percentages.



Data Assessments for Bias and Precision. Bias and precision are the terms used to evaluate the accuracy of a given data set. If data are described as low in bias and high in precision, then accuracy is ensured.

Bias. Bias is a measurement of the error in a method. Error may arise from the method itself (instrument limitation) or from the laboratory's use of the method. Method bias is measured best by comparison of results from samples of known concentration such as standardized reference materials or by analyzing samples fortified to a known concentration.

Precision. Precision measures how closely the results of multiple analyses of a single sample cluster with one another. Precision may be assessed by multiple analyses of a single sample or by repeated analyses of the same standard material.

References

American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th edition. Washington, DC.

Holstege, D.C. 2006. ANR Analytical Lab Quality Assurance Manual, 2nd edition. Davis, CA.

O'Bryan, E.F. 2001. Analytical Laboratory Quality Control Charting. Presentation, American Chemical Society Regional Meeting, June 11-13, 2001. Grand Rapids, MI.

U.N./E.C.E. Task Force on Laboratory Quality Management and Accreditation. 2002. Technical Report: Guidance to Operation of Water Quality Laboratories. Geneva, Switzerland.

U.S. Environmental Protection Agency. 2005. Manual for the Certification of Laboratories Analyzing Drinking Water, Criteria and Procedures, Quality Assurance, 5th edition. Cincinnati, OH.

QA/QC Plan Checklist for Method # _____

Documentation:

SOP ☐ Started Date _____
☐ In Process
☐ Completed Date _____

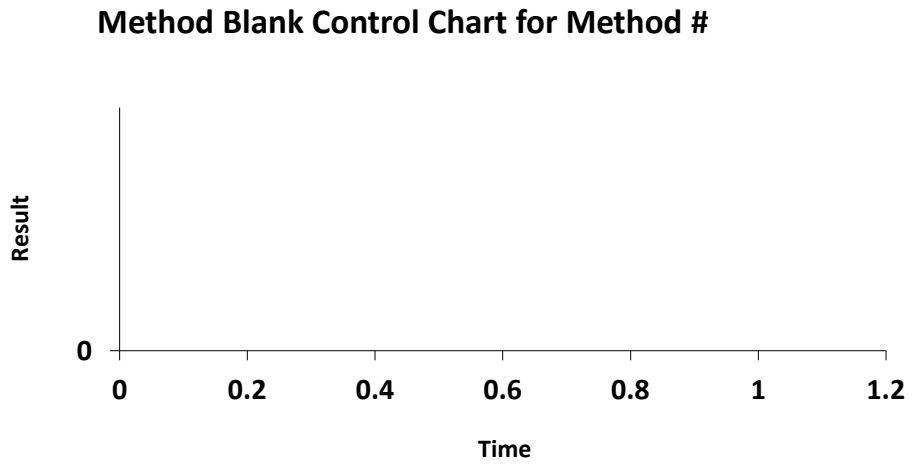
Equipment Maintenance & Calibration Program (list all equipment used for the method named above)

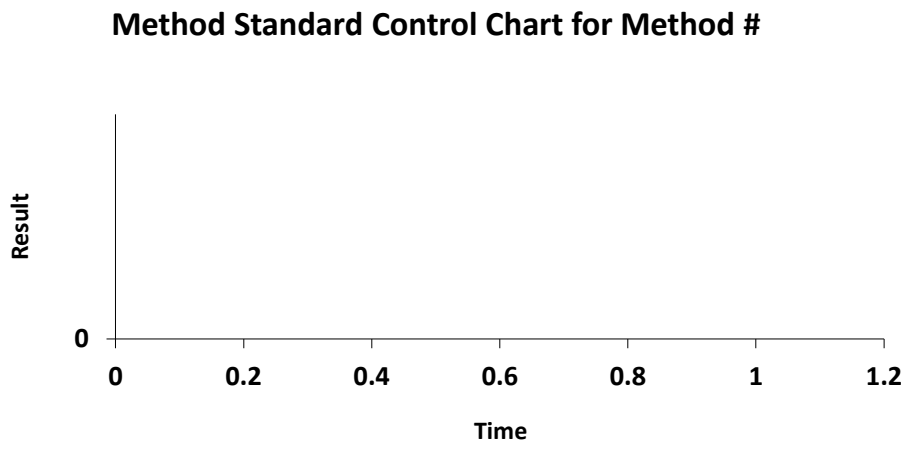
Equipment Identification	Maintenance Frequency (Circle one)	Calibration Frequency (Circle one)	Completed?
_____	Annual Monthly Weekly Daily Other:	Annual Monthly Weekly Daily Other:	☐ Yes ☐ No
_____	Annual Monthly Weekly Daily Other:	Annual Monthly Weekly Daily Other:	☐ Yes ☐ No
_____	Annual Monthly Weekly Daily Other:	Annual Monthly Weekly Daily Other:	☐ Yes ☐ No

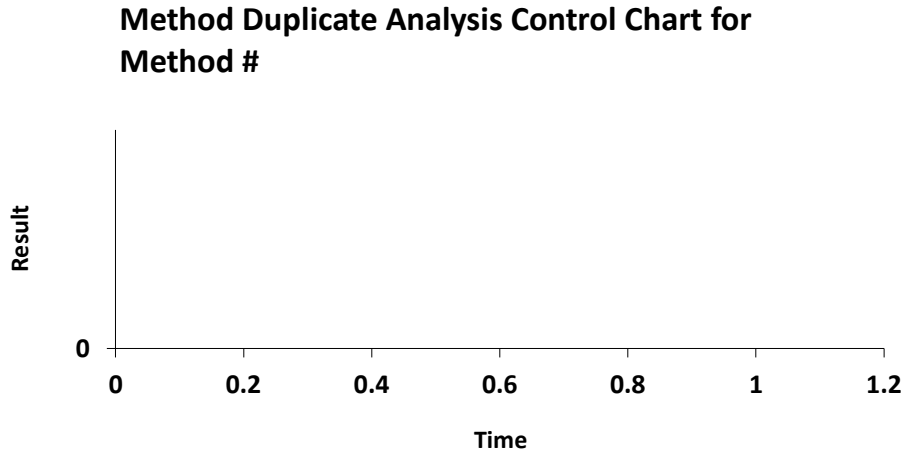
Internal Quality Control Activities (complete the table, as appropriate, for the method listed above)

Method Section	Quality Control Element	Frequency	Acceptance Criteria
9.1	Method Blank		Result below LOQ
9.2	Sample Carry-Over		h_{co} < 5%
9.3	Matrix Reference Standard		Result within uncertainty limits
9.4	Drift Standard		Result within 5% of known value
9.5	Laboratory Duplicate Analysis		RPD < 15% for samples > 5 x LOQ
9.6	Spike Recovery		R with 85%-115%

Control Charts. Determine upper and lower warning limits (UWL, LWL) and mark them for each chart. Plot all results from internal quality control activities listed above and evaluate for bias, accuracy and precision every 5-10 data points.







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