Florida Department of Agriculture and Consumer Services
Division of Agricultural Environmental Services

Fertilizer Laboratory Methods Manual

Bureau of Agricultural Environmental Laboratories (BAEL)

Rev 01/31/25

Rule 5E-1.014, F.A.C.

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PREP 450
Riffling and Splitting of Fertilizer Sample

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for creating a homogenous portion of the dry fertilizer sample. This instruction allows for a portion that is representative of the entire sample but still easy to handle.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples prepared in the Bureau of Agricultural Environmental Laboratories (BAEL). This procedure applies to the converting of solid fertilizer into uniformly consistent analytical samples while preserving the integrity of the original material. The laboratory prepares three portions that represent the original sample as a whole.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves, and laboratory coat is required.

4. OUTLINE of PROCEDURE

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5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Bottle, plastic
6.2. Butcher paper
6.3. Camera
6.4. Cloth
6.5. Divider (small approximately 180-cubic inch, large approximately 450-cubic inch)
6.6. Downdraft Bench (with hiflow arm and compressed air hose)
6.7. Feeder
6.8. Spatula
6.9. Splitter

7. REAGENTS and MEDIA

7.1. N/A

8. ASSOCIATED DOCUMENTS

8.1. Form F00023, Storage Custody

9. SPECIFIC PROCEDURES

9.1. Sample Type Determination

9.1.1. The laboratory encounters different types of Fertilizer samples. The composition of the individual sample will control the riffling or splitting process to be used. Determine the sample type before beginning the riffling or splitting process.

9.1.2. A “normal” sample is defined as a dry, granular, or pelletized sample that is not dolomite, gypsum, custom blend, or fertilizer spike.

9.1.3. A pelletized dolomite or gypsum sample is treated as a “normal” sample.

9.2. Riffling “Normal” Sample

9.2.1. The bag containing the submitted sample and three plastic bottles (two 250mL and one 500mL) should be located next to the Downdraft Bench.

9.2.2. Place two trays beneath the smaller divider to catch the split sample. Ensure that the gate is closed.

9.2.3. Only one sample (bags 1-4) should be on the splitting bench during this process.

9.2.4. Match the sample number on the bag with all three bottles.

9.2.5. Take a picture of the bag and all three bottles. Ensure that the numbers are in focus.

9.2.6. Open the bag and pour the entire sample into the center of the feeder to create an elongated cone-shaped pile.

9.2.6.1. If the entire sample is contained in two bags, then both bags must be combined into the feeder at the same time.

9.2.6.2. If the entire sample does not fit into the top feeder, use the larger divider. Different sized dividers are located in the laboratory.

9.2.6.3. Alternatively, sample can be run through the divider to make room for more sample; but all the sample must be run through in the same riffling.

9.2.6.4. If there are particles too large to pass through the divider, break them up manually. If the particles are concrete or rock and not fertilizer, they can be discarded.

9.2.7. After the sample has passed through the divider, dislodge any trapped particles.

9.2.8. Once the sample is collected into the two trays, return both halves of the sample into the top feeder to be riffled again.

9.2.9. Riffle the sample a minimum of three times depending on sample size.

9.2.10. If the large divider is used, discard the right tray and riffle the sample in the left tray through the small divider three more times.

9.3. Splitting “Normal” Sample

9.3.1. Flip the power switch (located in the back of the feeder) to on.

9.3.2. Set the speed level to 30.

9.3.3. If the sample has trouble flowing through the tray, an adjustment of the vibration can be done within a 25 to 35 range for the speed level. The dial on the front of the machine adjusts the speed.

9.3.4. Flip the power switch (located at the back of the splitter) to on.

9.3.5. Press the power button on the front of the machine, and then press the start button. When the start button lights up, it indicates that the machine is on.

9.3.6. Screw the three labeled plastic bottles to the openings of the splitter.

9.3.7. Pour the entire contents of one tray into the top loader of the feeder. Keep the other tray (containing the other half of the sample) on the bench. Push start on the feeder.

9.3.7.1. If the sample has a pink sticker, place an alternate container under the splitter to collect the excess; and transfer to the designated hazardous waste container upon collection.

9.3.8. If all three bottles are not receiving sample, this indicates a problem. Suggestions on how to deal with the problem are addressed below.

9.3.9. After the entire tray of sample passes through the splitter, unscrew the bottles and cap them.

9.3.10. Place each bottle in the designated location.

Bottle	Location
250mL bottle with white label	grinding area
500mL bottle with white label	storage area for retained sample
250mL bottle with the red label	storage area for normal sample

9.3.11. Discard the sample in the other tray into the designated disposal bucket.

9.3.12. A yellow sticker indicates that calcium nitrate ($CaNO_3$) is present in the sample. If the bottles are identified with a yellow sticker, the 250mL bottle with the white label must be placed into the freezer until it is ready for grinding.

9.4. Riffling and Splitting Sample with Dolomite or Gypsum

9.4.1. Dolomite or gypsum samples are identified as such on the sticker.

9.4.2. Place butcher paper on the table.

9.4.3. Pour the entire sample in the center of the butcher paper creating a cone shape.

9.4.4. Mix the sample thoroughly, with a spatula, at least four times.

9.4.5. Divide the sample into four approximately equal portions. Dispose of two opposing portions and combine the remaining two portions.

9.4.6. Repeat the splitting process above until just enough remains to fill the three bottles. Of the remaining two portions, place one portion into the 500mL bottle, and divide the other portion between the two 250mL bottles.

9.4.7. Place bottle in the designated location.

Bottle	Location
250mL bottle labeled for moisture / sieve	weighing area *sample will be used in moisture test*
500mL bottle with white label	storage area for retained sample
250mL bottle labeled for oven	oven *sample will be ground after drying*

9.5. Splitting of Custom Blend Sample

9.5.1. Custom Blend samples are indicated as such by a sticker and accompanied by five plastic bottles (four 250mL and one 500mL).

9.5.2. Riffle the sample as per the riffling routine of a normal sample.

9.5.2.1. Process the first tray into the three bottles as per the splitting routine of a normal sample but

9.5.2.2. Do not discard the second tray and

9.5.2.3. Detach only two of the 250mL bottles and cap them.

9.5.3. Attach the remaining two 250mL bottles and process the second tray through the splitter.

9.5.3.1. Transfer the second set of bottles to the Pesticide Formulation Storage location. Use form F00023, Storage Custody, to indicate transfer.

9.6. Comminuting Fertilizer Spike Sample

9.6.1. Place the Fertilizer Spike sample in a clean bag.

9.6.1.1. Seal the bag by twisting.

9.6.1.2. Crush the sample to make it as homogeneous as possible. A hammer or maul may be used.

9.6.2. Transfer the sample to the grinding area

9.7. Problems during Splitting

9.7.1. If the bottle does not receive the sample, check that the rotating arm is spinning inside the splitter.

9.7.2. If a problem occurs during the sample's run through the machine, all bottles should be emptied, and the machine returned to its starting position.

9.7.3. When the second tray of sample is used, a clean designated disposal container should be placed under the splitter to catch all discarded sample. This will ensure sample purity in case the sample needs to be split a third time.

9.8. Cleaning of Riffling and Splitting Equipment

9.8.1. The feeder, splitter and divider shall be cleaned between each sample. Use the compressed air hose to blow out any trapped particles in the feeder, splitter or divider.

9.8.2. After all the samples are finished, wipe any accessible metal surfaces with a water-dampened cloth.

9.8.3. The following items are rinsed with water in a sink after all samples are finished: the divider, divider trays, bottom half of the splitter, tray of the vibratory feeder, and the funnel of the vibratory feeder.

10. WASTE MANAGEMENT

10.1. Fertilizer containing pesticide (indicated on the bottle by a pink sticker) should be retained separately and discarded as hazardous waste.

10.2. All other solid fertilizer is collected and may be used by employees or groundskeeping.

11. REFERENCES

11.1. SOP for Preparing Fertilizer Samples Appendix A, 6/26/2002

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	07.29.2015	Original Version	Sarah Smith	
2.0	11.30.2018	Changed to new format Added 9.1.2, 9.1.3, 9.2.3, 9.3.7.1, 9.8.3, Section 10, and 11.1 Minor grammatical fixes		Taleya Williams
3.0	11.25.2020	Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Quentin Cooper

13. SIGNATURE HISTORY

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1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the grinding of Fertilizer samples to be used for further analysis.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). This procedure applies to the pulverizing of a sample and converts dry solid fertilizer into homogeneous analytical samples while preserving compositional integrity of the original material.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Use of downdraft for dust control is required.

4. OUTLINE of PROCEDURE

4.1. 9.1 Setting Up the Grinding Mill
4.2. 9.2 Assembling the Grinding Tools
4.3. 9.3 Grinding a Sample
4.4. 9.4 Cleaning and Reassembly
4.5. 9.5 Laboratory Information Management System (LIMS) Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS

6.1. Bottle, wide mouth
6.2. Bottlebrush, large
6.3. Brush, wire brass wooden handle
6.4. Dehumidifier
6.5. Downdraft Unit
6.6. Funnel, medium plastic
6.7. Glazed butcher's paper, 24 inches by 36 inches
6.8. Humidity Monitor
6.9. Mortar and pestle
6.10. Retsch ZM 200 equipped with ring sieves (1.0mm, 0.75mm, 0.50mm, 0.25mm) (notched) or equivalent
6.11. Retsch ZM 200 Ultra Centrifugal Mill with anti-rebound fitting or equivalent
6.12. Shop vacuum with attachments
6.13. Sieve, U.S. Standard No. 20
6.14. Sieve, U.S. Standard No. 40
6.15. Spatula, large

7. REAGENTS and MEDIA

7.1. N/A

8. ASSOCIATED DOCUMENTS

8.1. ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer
8.2. Form F00041, Sieving Pulverized Samples

9. SPECIFIC PROCEDURES

9.1. Setting Up the Grinding Mill

9.1.1. At the beginning of each work period, activate the mill by turning the power on using the toggle switch at the lower, left rear of the unit.
NOTE: The mill cover will not open without the power on. To unlock the cover, use the pictogram that displays - "open unit."

9.1.2. Use the menu and setting button, to adjust the speed. 14,000 – 16,000 revolutions per minute (rpm) are most frequently used, with available range being 6,000 – 18,000.

9.1.3. The humidity is captured by the SensoScientific system, however the technician should visually check the humidity reading before starting grinding. The humidity in the Sample Room and the integrity of the sample will ultimately dictate the most effective rpm to use. Sample grinding should not take place when Sample Room humidity is above 40%.

9.2. Assembling the Grinding Tools

9.2.1. The labyrinth is placed with the torsion notch in the "6 o'clock" position.

9.2.2. The rotary blades are placed over the drive shaft.

9.2.3. Insert the collecting vessel (cassette) with the notch over the torsion lock of the labyrinth.

9.2.4. The ring sieve fits over the rotary blades, notch side down.

9.2.5. The ring sieve is placed with its notch over the torsion lock of the labyrinth (0.25mm for a First grind, Dolomite or Gypsum; 0.50mm for a Second grind; 0.75mm for a Calcium Nitrate and other designated samples).

9.2.6. The cassette cover is then installed.

9.2.7. Close the mill cover, using moderate pressure.

9.2.8. If the grinding tools have been properly assembled, a "ratcheting" sound can be heard.

9.2.9. The unit automatically locks itself.

9.2.10. If the unit will not close, use the pictogram to open the cover, inspect and/or reassemble the components until the unit's cover automatically closes.
NOTE: Unit will not close/lock if grinding tools are not correctly assembled.

9.3. Grinding a Sample

9.3.1. Samples requiring a First grind will be determined by checking the FERT_PREP section of the Fertilizer Prep backlog list. These samples will be ground using a 0.25mm ring sieve.

9.3.2. Samples requiring a Second grind will be determined by checking the FERT_GRIND_2 section of the Fertilizer Prep backlog list. These samples will be ground using a 0.50mm ring sieve.

9.3.3. Calcium Nitrate samples will be indicated on the sample backlog as "CA_NO3_FREEZE_PREP". These samples will be retrieved from the freezer and ground immediately using a 0.75mm ring sieve.

9.3.4. Once the mill is closed/locked, turn the downdraft bench on.

9.3.5. Select the "start" function on the mill display.

9.3.6. The performance screen is displayed and should be near zero percent.

9.3.7. Obtain the 8-ounce sample bottle.

9.3.8. Using the knob, adjust the rpm to the appropriate speed on the feeder and slowly feed the sample (while turning the bottle) into the filling hopper on the lid housing until all sample has been introduced to the mill.

9.3.9. Listen to the grinder and watch the display for any potential issues.

9.3.10. During grinding, the 100% range on the display should be avoided because the device is overloaded in this performance range and reproducibility of grind is no longer possible.

9.3.11. When the display indicates that the sample isn't grinding properly, evaluate and adjust the speed and screen size accordingly.

9.3.12. Compressed air should be blown into the filling hopper before you select the stop function, select the "stop" function.

9.3.13. The unit will unlock and begin to open. The lid can then be opened the rest of the way.

9.3.14. Remove the cassette lid. Using the bottle brush, carefully brush the pulverized sample from the lid onto the butcher's paper. Place lid aside.

9.3.15. Next, remove the ring sieve from the rotary blades and carefully brush the entire sample onto the butcher's paper. Place blades aside.

9.3.16. Remove the cassette, filled with pulverized sample from the assembly and empty the contents onto the butcher's paper. Brush the cassette to remove the entire sample. Place cassette aside.

9.3.17. Using a large spatula, thoroughly mix the pulverized sample by using a mix and roll technique. Ensure that all sample remains on the butcher's paper.

9.3.18. Fold the butcher's paper in half; set sample aside.

9.3.19. Quality Control (QC) of the grinding process is done each day that samples are to be ground. This is done for the first sample of the day on a First grind and for the first sample of the day on a Second grind.

9.3.19.1. After grinding the first sample of the day for a First grind, obtain the No. 40 sieve.

9.3.19.2. Place the sieve on top of a second piece of butcher's paper.

9.3.19.3. Retrieve the sample and pour the sample into the sieve; all pulverized sample must pass through the screen.

9.3.19.4. Sample trapped by the mesh must be re-pulverized by (a) grinding using a smaller ring sieve or (b) mortar and pestle.

9.3.19.5. After grinding the first sample of the day needing a Second grind, obtain the No. 20 sieve.

9.3.19.6. Place on top of a second piece of butcher's paper.

9.3.19.7. Retrieve the sample and pour the sample into the sieve; all pulverized sample must pass through the screen.

9.3.19.8. Sample trapped by the mesh must be re-pulverized by (a) grinding using a smaller ring sieve or (b) mortar and pestle.

9.3.19.9. Record the QC results on form F00041, Sieving Pulverized Sample.

9.3.20. Using a medium funnel pour pulverized sample back into 8-ounce bottle.

9.3.21. Place completed samples on a lab cart with a sign "Ready To Be Weighed".

9.4. Cleaning and Reassembly

9.4.1. After each sample, clean the cassette cover, ring sieve, rotor blades, cassette and butcher's paper using appropriate brush and compressed air.

9.4.2. Reassemble the grinding tools.

9.5. Laboratory Information Management System (LIMS) Entry

9.5.1. Log on to LIMS. Refer to ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer.

9.5.2. Select template FERT_LOGIN for First grind samples, or FERT_GRIND_2 for Second grind samples.

9.5.3. Scan or manually enter the sample ID's for the completed samples.

9.5.4. Enter "C" or appropriate sieve size in the GRIND-1 or GRIND-2 box for each completed sample.

9.5.5. In the GRIND-1 template, check to make sure codes such as CaNO3 and initial dilution codes are completed. If the grind code is grayed out, indicating test codes haven't been entered for the sample, the administrative Environmental Specialist 1 (ES1) will be notified.

9.5.6. The administrative ES1 should be notified to add missing tag codes. If the tag codes are missing, set the samples aside until the codes have been added.

10. WASTE MANAGEMENT

10.1. N/A

11. REFERENCES

11.1. Preparing "First" and "Miscellaneous" Samples Using Retsch ZM 200 Ultra Centrifugal Mill. FSFL-SOP 303, (01/09/2006).
11.2. Preparing Commercial and Deficient Samples ("Seconds") Using Retsch ZM 200 Ultra Centrifugal Mill. FSFL-SOP 304, (01/09/2006).
11.3. Standard Operating Procedures For Preparing Fertilizer Samples, (06/26/2002).
11.4. Operating Instructions Ultra Centrifugal Mill Type ZM 200 Retsch GmbH, (08/02/12).

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	2015.12.22	Original Version	George Taylor	
2.0	11.30.2018	Updated outline format Grammatical and verbiage fixes		Taleya Williams
3.0	12.09.2020	Added or equivalent to items in Section 6 Apparatus. Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Taleya Williams

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1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for dilution of liquid fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). This procedure designates how to properly dilute liquid fertilizer samples.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE OF PROCEDURE

4.1. 9.1 Check Balance
4.2. 9.2 Sample and Container Preparation
4.3. 9.3 Sample Dilution and LIMS data Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS and EQUIPMENT

6.1. Balance, top loader
6.2. Camera
6.3. Container, 1000 ml square Nalgene or other appropriate container
6.4. Labels
6.5. Laboratory Information Management System (LIMS)

7. REAGENTS and MEDIA

7.1. Deionized (DI) Water, Type 2

8. ASSOCIATED DOCUMENTS

8.1. ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer
8.2. Form F00010, Balance Verification
8.3. LABOP 122, Maintenance, Operation, and Program of Balances

9. SPECIFIC PROCEDURES

9.1. Check Balance

9.1.1. Balance should be clean and level.

9.1.2. Check balance calibration daily prior to first use, and record on form F00010, Balance Verification. Refer to LABOP 122, Maintenance, Operation, and Program of Balances.

9.2. Sample and Container Preparation

9.2.1. Obtain a clean plastic 1000mL square container.

9.2.2. Inspect for discolorations, imperfections and integrity before use.

9.2.3. Examine original sample containment prior to opening for signs of leakage.

9.2.4. Should sample appear to be compromised, identify the problem and notify management via form F00021, Nonconformance and Deviation.

9.2.5. Remove sample container from shipping package.

9.2.6. Write sample number on original container if omitted in field.

9.2.7. Take photo of original containment and all new labeled containers for field identification verification purposes.

9.3. Sample Dilution and LIMS data entry

9.3.1. Remove cap and weigh original sample.

9.3.2. Record as “Total Weight” in LIMS

9.3.3. **Note: Remember to weigh and record all samples before proceeding to the next step.**

9.3.4. Tare new container and transfer the entire sample to the new container.

9.3.5. Rinse original container and lid with DI water enough times to sufficiently dissolve any existing crystals. The rinsate is added to the new container.

9.3.6. Add sufficient DI water to almost fill the new container.

9.3.7. Weigh new container and record as “New Weight” in LIMS.

9.3.8. Allow original sample container to dry, then weigh and record as “Bottle Weight” in LIMS.

9.3.9. If crystals are still present after dilution contact a manager for further instructions.

9.3.10. Place completed samples on a lab cart with a sign “Ready To Be Weighed”.

9.3.11. Record the “Dilution Factor” on sample container.

9.3.12. Refer to ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer.

9.3.13. Select template FERT_LOGIN.

9.3.14. Enter “C” in the GRIND-1 field for each completed sample.

9.3.14.1. If a liquid sample requires further analysis after the initial analysis, all subsequent analyses are performed on the original dilution. “Seconds” cannot be prepared on liquids. GRIND-2 has to be “completed” to trigger additional analyses.

10. WASTE MANAGEMENT

10.1. Original sample containers can be discarded in the trash after process is complete.

11. REFERENCES

11.1. N/A

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	03.09.2016	Original Version	Linda Giardelli	
2.0	11.30.2018	Updated outline format Added 9.3.8, 9.3.11, 9.3.13.1 Minor grammatical fixes		Taleya Williams
3.0	11/25/2020	Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Quentin Cooper
4.0	08.02.2024	Removed Signature History		Eddie Bello

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the weighing of fertilizer for sample preparation.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). See the Containers and Codes table in section 9 below for included preparation methods.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 9.1 Preparation for Weighing
4.2. 9.2 Login Laboratory Standards
4.3. 9.3 Weighing
4.4. Attachment A, Fertilizer Laboratory Standard and Batch Size

5. INTERFERENCE

5.1. Water and humidity can cause issues with this process; historical data has shown that performing these procedures with a humidity less than 40% is required.

6. APPARATUS and MATERIAL

6.1. Balance, analytical
6.2. Barcode Reader
6.3. Beakers, disposable, 50mL
6.4. Centrifuge tubes, 50mL
6.5. Computer
6.6. Erlenmeyer flask, 500mL
6.7. Laboratory Information Management System (LIMS)
6.8. Pipette, disposable
6.9. Spatula
6.10. Sticker, orange
6.11. Volumetric flask, 200mL
6.12. Volumetric flask, 250mL

7. REAGENTS and MEDIA

7.1. SRM 200b
7.2. SRM 695
7.3. SRM 88b

8. ASSOCIATED DOCUMENTS

8.1. ADMIN 023 Attachment C, LIMS Sample Login for Multi Sample
8.2. ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer
8.3. LABOP 122, Maintenance, Operation and Program of Balances

9. SPECIFIC PROCEDURES

9.1. Preparation for Weighing

9.1.1. Check that the balance is clean, level, and calibrated. Refer to LABOP 122, Maintenance, Operation, and Program of Balances.

9.1.2. Generate or print out either Fert_Prep_Weight or Fert_Prep_Weight2 sections from backlog report.

9.1.3. Obtain samples from grinding room.

9.1.4. No more than 10 samples should be scanned for weighing. Samples denoted as "Priority" should be weighed first. Priority samples may be included in smaller batches in order to expedite their preparation.

9.1.5. Program requirements for proficiency samples mandate two weights/preps on different days.

9.1.6. Using backlog, generate labels and line up appropriate weighing vessel behind each sample.

9.1.7. The table below shows the weighing vessel for each preparation method.

Method	Core Weight Code	Core Suggested Weight Code	Container
430 NAU	430_NAU_WT	430_XXX_WT_SUG	250mL volumetric flask
430 KJL	430_KJL_WT	430_XXX_WT_SUG	250mL volumetric flask
461	461_WT	461_APA_WT_SUG	500mL Erlenmeyer flask
505 APA	505_WT	505_WT_SUG	500mL Erlenmeyer flask
521 TP	521_WT	521_WT_SUG	500mL Erlenmeyer flask
541 CIP	541_WT	541_WT_SUG	500mL Erlenmeyer flask
604 SMGK	604_WT	604_WT_SUG	50mL disposable beakers
803 SB	803_WT	803_WT_SUG	50mL centrifuge tube
822 TOTAL	822_WT	822_WT_SUG	50mL centrifuge tube
825 CO/AL	825_WT	825_WT_SUG	50mL centrifuge tube
830 SMN	830_WT	830_WT_SUG	200mL volumetric flask
831 SCU	831_WT	831_WT_SUG	200mL volumetric flask
832 SFE	832_WT	832_WT_SUG	200mL volumetric flask
833 SZN	833_WT	833_WT_SUG	200mL volumetric flask
840 CFE	840_WT_F	840_WT_SUG_F	200mL volumetric flask
841 CFE	841_WT_F	841_WT_SUG_F	200mL volumetric flask
841 CMN	841_WT_M	841_WT_SUG_M	200mL volumetric flask
841 CCU	841_WT_C	841_WT_SUG_C	200mL volumetric flask
841 CZN	841_WT_Z	841_WT_SUG_Z	200mL volumetric flask
860 TCA	860_TCA_WT	860_WT_SUG_TCA	50 mL centrifuge tube
875 DOL LIME	875_WT	875_WT_SUG	50mL centrifuge tube
902 HM	902_WT	902_WT_SUG	50mL centrifuge tube

9.1.8. Log on to Laboratory Information Management System.

9.2. Login Laboratory Standards

9.2.1. To log in laboratory (lab) standards, refer to ADMIN 023 Attachment C, LIMS Sample Login for Multi Sample.

9.2.2. Print or record the lab standard sample numbers generated.

9.2.3. Suggested weights for lab standards will be listed at the top of that sample's column in the result entry template.

9.2.4. Refer to Attachment A, Fertilizer Laboratory Standard and Batch Size.

9.3. Weighing

9.3.1. In LIMS, open Result Entry using the appropriate entry template:

9.3.1.1. For zero weights, use template "FERT_PREP_WEIGHT_0"

9.3.1.2. For first, second, and referee weights, select "FERT_PREP_WEIGHT2"

9.3.2. Scan sample barcodes into appropriate template.

9.3.3. Place a labeled container on the balance and tare.

9.3.4. Confirm that the label on the sample bottle matches the sample number in the LIMS template.

9.3.5. For solid samples:

9.3.5.1. The sample must be mixed thoroughly by shaking and rotating the bottle back and forth to break up clumps before weighing the sample. A spatula may be used to break up the sample before the mixing.

9.3.5.2. Using a spatula, weigh the amount listed in LIMS under the section for suggested weight code. Wipe spatula between samples.

9.3.5.3. Confirm that the Initial_Liquid_Dilution in LIMS for a solid sample, including lab standard, is 1. If it is not, change it to 1.

9.3.5.4. If there is another value in the test code and this is not the first time the sample is being weighed (1st/2nd preps) staff should notify the section manager or administrative ESI because there may already be sample results calculated using the incorrect value and changing it here will not update the results in LIMS.

9.3.6. For liquid samples:

9.3.6.1. Shake the sample bottle vigorously, then inspect sample bottle for residual crystallization. If any crystals are found, notify supervisor and postpone weighing until further instructions are given.

9.3.6.2. Using a disposable pipette, weigh double the amount listed in LIMS under the suggested weight code for diluted samples. If the sample is not diluted, do not double the suggested weight.

9.3.6.3. Use a clean pipette for each sample.

9.3.6.4. Confirm that the liquid dilution factor on the bottle matches the liquid dilution code in LIMS.

9.3.6.5. If there is another value in the test code and this is not the first time the sample is being weighed (1st/2nd preps) staff should notify the section manager or administrative ESI because there may already be sample results calculated using the incorrect value and changing it here will not update the results in LIMS.

9.3.7. Wait for the weight reading on the balance to stabilize.

9.3.8. Press transfer button to load sample weight reading from balance directly into LIMS. If this function is unavailable, manually enter the weight as displayed by the balance.

9.3.9. Weigh out all requested analyses based on suggested weight for one sample at a time.

9.3.9.1. Core test codes may be followed by _1, _2, R_1, R_2

9.3.9.2. If %DIV/0 is in the suggested weight code, contact supervisor via email for correction of the problem.

9.3.9.3. If more than one suggested weight is shown, weigh the higher suggested weight.

9.3.10. Place each weighed sample vessel in a bin.

9.3.11. The laboratory standard must be weighed during the process of weighing for each batch and included in the bin for the corresponding batch.

9.3.12. Completed batches in bins should be placed on their appropriate shelves outside the balance room.

9.3.13. Be sure, at the end of each day, to return original sample bottles to the grinding room and place them in their correct numerical order on shelves. Certain samples, such as commercial and proficiency samples, may have their own specific location.

10. WASTE MANAGEMENT

10.1. N/A

11. REFERENCES

11.1. N/A

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	01.13.2016	Original Version	George Taylor	
2.0	12.05.2018	Update outline format Added 5.1, 9.3.5.4, 9.3.6.5 Minor grammatical fixes		Taleya Williams
3.0	11.25.2020	Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Quentin Cooper
4.0	08.02.2024	Removed Signature History. 9.1.4 Changed entire first sentence to a new one limiting the number of samples to “no more than 10”. 9.1.7. Corrected codes for method 461 9.3.8. Changed sentence to now refer to “transfer button”. 9.3.10. Simplified sentence to just end with “in a bin”.		Eddie Bello

PREP 453 Attachment A

Fertilizer Laboratory Standard and Batch Size

BAEL SOP	FM Method	Batch Size*	Location Code	Standard Material
METHOD 502	405 TKN	24	QA_FERT_LS_TKN_405	SRM 695
METHOD 505 METHOD 530	415 KJL	24	QA_FERT_LS_DIR_AMM_415	Ammonium Sulfate Std.
METHOD 504 METHOD 530	420 KJL	24	QA_FERT_LS_ DIR_AMM_420	Ammonium Sulfate Std.
METHOD 500 METHOD 531	425 KJL	24	QA_FERT_LS_ DIR_AMM_425	Ammonium Sulfate Std.
PREP 466	430 KJL	47	QA_FERT_LS_NAUL	SRM 695
PREP 466	430 NAU	47	QA_FERT_LS_NAUS	SRM 695
METHOD 506 METHOD 530	435 KJL	24	QA_FERT_LS_ DIR_AMM_435	SRM 695
METHOD 500 METHOD 531	440 KJL	24	QA_FERT_LS_ DIR_AMM_440	SRM 695
METHOD 501 METHOD 531	445 KJL	24	QA_FERT_LS_WIN_445	SRM 695
METHOD 461 P&K	N/A	21	QA_FERT_LS_PANDK	SRM 695
PREP 454	604 SMGK	18	QA_FERT_LS_SMGK	SRM 695
METHOD 510	611 CLO	20	QA_FERT_LS_CHLORINE	SRM 695
PREP 455	803 SB	47	QA_FERT_LS_SB	SRM 695
METHOD 520	805 CS 860 CS	21	QA_FERT_LS_SULFUR_COMB	Ammonium Sulfate Std.
METHOD 520	805 CS 860 CS	21	QA_FERT_LS_SULFUR_COMB	Calcium Thiosulfate Std.
METHOD 521	806 FS	8	QA_FERT_LS_SULFUR_FREE	Free Sulfur Std.
PREP 456	822 TOTAL 825 Co/AL 860 TCA 875 DOL LIME 902 HM	47	QA_FERT_LS_TOTAL	SRM 695
PREP 462	830 SMN	24	QA_FERT_LS_SMN	SRM 695
PREP 462	831 SCU	24	QA_FERT_LS_SCU	SRM 695
PREP 462	832 SFE	24	QA_FERT_LS_SFE	SRM 695
PREP 462	833 SZN	24	QA_FERT_LS_SZN	SRM 695
PREP 464	840 CFE	25	N/A	
PREP 464	841	25	N/A	

*Batch Size includes batch spike.

PREP 454
Sample Preparation for Water Soluble Potash and Magnesium Analysis
[FM 604]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of water soluble magnesium (Mg) and water soluble potash (K_2O) nutrients in fertilizer samples for analysis.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples in the Bureau of Agriculture Environmental Laboratories (BAEL). This preparation is for the analysis of water soluble potash and magnesium by inductively coupled plasma optical emission spectrometry.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Collection of Weighed Samples
4.2. 10.2 Digestion
4.3. 10.3 Final Volume
4.4. 10.4 Data Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS

6.1. Centrifuge tube, disposable (15mL)
6.2. Glass Bead
6.3. Hot plate
6.4. Parafilm
6.5. Rack
6.6. Volumetric flask (500mL)

7. REAGENTS and MEDIA

7.1. Water, Deionized (DI) Type II

8. REAGENT PREPARATION GUIDELINES

8.1. N/A

9. ASSOCIATED DOCUMENTS

9.1. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Weigh 0.5g of sample into a 50mL disposable beaker.

10.1.2. If samples are pre-weighed, Collect weighed samples.

10.1.3. Batch size and Laboratory Control Sample (LCS) material are defined in PREP 453 attachment A.

10.2. Digestion

10.2.1. Pre-heat hot plate by turning it on.

10.2.2. Record on each volumetric flask the appropriate sample number as listed on weigh vessel label.

10.2.3. Transfer sample from weigh vessel to labeled 500mL volumetric flask with thorough rinsing with DI water.

10.2.4. Add approximately 300mL of DI water and approximately 3-5 glass beads to each volumetric flask.

10.2.5. Place the volumetric flasks onto the hot plate.

10.2.6. Bring samples to a boil and continue boiling for at least one hour. The volumetric flasks should be consistently monitored to ensure all samples are vigorously boiling. Flasks can be rotated periodically to ensure proper heat distribution. Do not allow samples to dry out. More DI water can be added if necessary.

10.2.7. Remove flasks from the hotplate and allow cooling to room temperature.

10.3. Final Volume

10.3.1. Bring each sample to 500mL final volume with DI water and stopper the flask.

10.3.2. Mix the contents by thoroughly inverting the flask multiple times and allow to settle

10.3.3. Pour extracts into labeled the 15mL centrifuge tubes.

10.4. Data Entry

10.4.1. When preparation is complete, make sure all applicable preparation information is recorded in LIMS correctly in the appropriate template.

10.4.2. Refer to ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer, for result entry guidance.

10.4.3. Deliver samples and batch folder to the analysis section, for analysis by METHOD 513.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Digested Sample	Poured down the sink.
Glass Beads	Capture and dispose of in trash. Make sure they do not go down the sink.
Used Screening Columns	Dispose of in trash

12. REFERENCES

12.1. AOAC Official Method 937.02 *Magnesium (Water Soluble) in Fertilizers* 16th Edition Volume 1
12.2. AOAC Official Method 983.02 *Potassium in Fertilizers* Flame Photometric Method (Manual or Automated) 16th Edition Volume 1

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	08.12.2015	Original Version	Joshua Hartstein	
2.0	04.05.2019	Updated Outline Format Added section 7.1, 9.1, 10.1.2 Clarified 10.1.1, 10.2.6 Removed dilution section Renamed 10.4 and combined with 10.5 Added Waste Management Section, Section 11		Laketa Levy
3.0	10.16.2023	Section 10.1.1 and section 10.1.2. added weighing instructions. Section 10.2.6. expanded upon instructions to ensure vigorous boiling. Section 10.3.2. added instructions for the mixing of the sample. Section 10.3.3 added instructions for pouring aliquots into 15 mL tubes.		Taleya Williams

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of water soluble boron (B) micronutrients in Fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). This preparation is for the analysis of water soluble boron by inductively coupled plasma optical emission spectrometry.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. Collection of Weighed Samples
4.2. Digestion
4.3. Final Volume
4.4. Data Entry

5. INTERFERENCE

5.1. Glass apparatus can interfere with boron analysis.

6. APPARATUS and MATERIAL

6.1. Centrifuge tube, non-glass disposable (15mL)
6.2. Parafilm
6.3. DigiTUBE, 50mL designed for Digester
6.4. SCP DigiPREP hot block or equivalent brand
6.5. Watch glasses, plastic, disposable

7. REAGENTS and MEDIA

7.1. Water, Deionized (DI) Type II

8. REAGENT PREPARATION GUIDELINES

8.1. N/A

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer
9.2. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Weigh samples per the table below:

Guarantee	Weight
0.074%	1g
0.075%	0.8g

10.1.2. If samples are pre-weighed, collect weighed samples.

10.1.3. Batch size and Laboratory Control Sample (LCS) material are defined in PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.2. Digestion

10.2.1. Pre-heat hot block with temperature setting of 106 ± 1°C.

10.2.2. Make sure the tube with the temperature probe is filled at least to the 40mL mark with water or approximately 50% nitric acid. Approximately 50% nitric acid must be used if other samples that are prepped in acid will be digesting at the same time.

10.2.3. Add approximately 30-40mL of DI water to each sample tube.

10.2.4. Place the sample tube onto hot block and place a plastic disposable watch glass on each tube.

10.2.5. Visually verify that the heating block is at 106 ± 1°C.

10.2.6. Digest samples for a minimum of 60 minutes at 106 ± 1°C. The time can be reset if needed to ensure 60 minutes digestion.

10.3. Final Volume

10.3.1. After digestion is complete, remove the samples from the hot block and allow the samples to cool to room temperature.

10.3.2. Remove the watch glasses and bring each sample up to final volume of 50 mL with DI water.

10.3.3. Cap and invert each sample. Allow samples to settle.

10.4. Data Entry

10.4.1. When preparation is complete, make sure all applicable preparation information is recorded in LIMS correctly in the appropriate template.

10.4.2. Refer to ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer, for result entry guidance.

10.4.3. Deliver samples and batch folder to analysis section for analysis by applicable method.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Sample not used	Dispose as non-hazardous waste down the drain
Watch Glasses	Dispose of in trash
Used Screening Columns	Dispose of in trash

12. REFERENCES

12.1. E00142, Boron Analysis, FM 803, (02/06/2003)
12.2. E00143, Hot Block Preparation of Boron Samples for ICP-OES Analysis. FSFL-SOP 513 (12/02/2005)
12.3. E00163, Boron (Acid and Water Soluble) in Fertilizers. *AOAC 16th Edition* Method 982.01, (1995)

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	03.09.2016	Original Version	Joshua Hartstein	
2.0	04.05.2019	Updated Outline Format Added section 5.1, 9.2, 10.1.1, 10.1.2, 10.2.6, 10.3.1, 10.3.2, 10.3.3 Clarified 6.1, 10.2.2 Renamed 10.3 Renamed 10.4 and combined with 10.5 Added Waste Management Section, Section 11		Taleya Williams
3.0	01.16.2024	Removed 10.3.4-5 Added weight table Removed screening column from materials Removed signature page Added external document references		Taleya Williams

PREP 456

Sample Preparation for Total Micronutrients and Heavy Metal Analysis [FM 822, FM 825, FM 860, FM 875, FM 902]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of acid soluble micronutrients and heavy metals [Aluminum (Al), Arsenic (As), Cadmium (Cd), Calcium (Ca), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Selenium (Se), and Nickel (Ni) and Zinc (Zn)] in fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to Fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). Acid soluble micronutrient metals and heavy metals are extracted from fertilizer sample by digesting the sample with nitric and hydrochloric acids using a hot block. The sample extract is then analyzed by inductively coupled plasma optical emission spectrometry.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. The acids may react with samples to produce noxious nitrogen oxides. This is accelerated with heat. Work in the fume hood to avoid breathing these vapors.

4. OUTLINE of PROCEDURE

4.1. 9.1 Preparation and Set up
4.2. 9.2 Sample Digestion
4.3. 9.3 Data Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Autodiluter
6.2. Centrifuge tube, disposable glass (15mL)
6.3. Heating block, DigiPREP MS or equivalent
6.4. DigiTUBE, plastic 50mL designed for Digester or equivalent
6.5. Parafilm
6.6. Tube rack
6.7. Watch glass (50mL), disposable

7. REAGENTS and MEDIA

7.1. Hydrochloric acid (HCl), trace metal grade or equivalent
7.2. Nitric acid (HNO_3), trace metal grade or equivalent
7.3. Water, Deionized (DI) Type I

8. ASSOCIATED DOCUMENTS

8.1. ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer
8.2. Form 00026, Dispenser/Diluter Verification
8.3. LABOP 120 Attachment C, Maintenance and Operation of Hot Block
8.4. PREP 453, Weighing of Fertilizer Sample
8.5. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

9. SPECIFIC PROCEDURES

9.1. Preparation and Set up

9.1.1. Make sure hood is on and the ribbon indicates flow.

9.1.2. Turn on heating block and set the temperature to 106 ± 1°C and set the heating time for 90 minutes. Please refer to LABOP 120 Attachment C, Maintenance and Operation of Hot Block for instructions of use of hot block.

9.1.3. Check the DigiPROBE storage well container to ensure there are no cracks, holes, or that the container is not melted. If there are any cracks or holes, replace with new 50 mL DigiTUBE. If the tube shows signs of melting, the hot block should not be used.

9.1.4. Make sure the probe storage tube is filled with approximately 30 mL HNO_3 and 10 mL HCl to at least the 40 mL mark.

9.1.5. Collect weighed samples. Samples are weighed according to PREP 453, Weighing Fertilizer.

9.1.6. Batch size and Laboratory Control Sample (LCS) material are defined in PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size.

9.2. Sample Digestion

9.2.1. Weigh 0.5g of fertilizer into a 50mL digestion tube.

9.2.2. **Under a fume hood:** Add 4.5mL of trace metal grade HNO_3 allowing samples to react before adding 1.5mL of trace metal grade HCl to each sample.

9.2.3. Place the samples in the hot block and cover each sample tube with a disposable watch glass.

9.2.4. Visually verify that the heating block is at 106 ± 1°C.

9.2.5. Digest samples for a minimum of 60 minutes (without boiling) at 106 ± 1°C. The time can be reset if needed to ensure 60 minutes digestion.

9.2.6. After digestion is complete, remove the samples from the hot block. Leave the watch glasses in place and put the samples in a tube rack. Leave the tube rack in the hood and allow the samples to cool to room temperature.

9.2.7. Remove and rinse the watch glass with DI water into the sample and bring the sample up to 50mL with DI water.

9.2.8. Cap and mix each sample. Allow samples to sit for particulates to settle.

9.3. Data Entry

9.3.1. When preparation is complete, make sure all applicable preparation information is recorded in LIMS correctly in the appropriate template.

9.3.2. Refer to ADMIN 023 Attachment Q, Manual Result Entry for Fertilizer, for result entry guidance.

9.3.3. Deliver samples and batch folder to analysis section for analysis by applicable METHOD 509.

10. WASTE MANAGEMENT

10.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Screening Column	Dispose of in trash
Acid Waste	Neutralized and disposed down sink

11. REFERENCES

11.1. Secondary/Micronutrient Analysis. FM 822, (05/20/2002) External 00144

11.2. Preparation and Analysis of Acid Soluble (Total) Micronutrients by ICP-OES FSFL-SOP 504, (04/15/2009) External 00145

11.3. *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, **Method 937.02**, ww.eoma.aoac.org External 00135

11.4. *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, **Method 2017.02**, ww.eoma.aoac.org External 00135

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	06.01.2016	Original Version	George Taylor	
2.0	04.19.2019	Updated Outline Format Combined with PREP 457 and added applicable information Reformatted section 9.1, 9.4, to make consistent with other PREP SOPs Added Waste Management, section 10 Removed different preparations for 0s,1s, and 2s Grammatical fixes and clarification throughout		Taleya Williams / Madeline Funaro
3.0	2020.09.18	Updated Scope Updated Health and Safety Updated references to include AOAC Updated to include procedures for the digestion of fertilizer samples with Nitric and Hydrochloric acid		William Meeks Jr.
4.0	2020.10.26	Modified temperature settings for heat blocks through out to reflect standard method.		William Meeks, Jr.
5.0	2023.12.05	Removed signature page Removed steps related to final filatration of samples Added external document numbers		Taleya Williams

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of available phosphorous (APA) nutrients in fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). This is a double extraction for the determination of available phosphoric acid in fertilizer samples that can be hydrolyzed to orthophosphate and have a range of 0 to 20% P_2O_5. The preparation is achieved by extracting the phosphate from the fertilizer sample with neutral ammonium citrate solution at 65°C. The range may be increased or decreased by changing the sample weight and/or the use of dilutions.

2.2. This method is applicable to fertilizer samples containing any amount of calcium present in the sample. The calcium is extracted from the sample with water at 65°C and prevented from forming insoluble calcium/magnesium phosphate complexes by the addition of nitric acid to the flask prior to the citrate extraction. This procedure must also be used when triple super phosphate or super phosphate are present in the sample.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Collection of Weighed Samples
4.2. 10.2 Calcium Extraction
4.3. 10.3 Citrate Extraction
4.4. 10.4 Final Volume and Filtration
4.5. 10.5 Data Entry into Laboratory Information Management System
4.6. 10.6 Transfer of Extracts

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Centrifuge tube, disposable (15mL)
6.2. Filter disk, 7 cm Whatman No. 1 or equivalent
6.3. Flask, 500mL Kohlrausch (class “A”)
6.4. Flask, Erlenmeyer (250mL)
6.5. Funnel, Buchner 7cm
6.6. Label maker
6.7. Laboratory Information Management System (LIMS)
6.8. Printer
6.9. Rack, tube
6.10. Screening column
6.11. Shaker Bath, Constant Temperature Circulating
6.12. Stopper, Two Hole for 500mL Kohlraush Flask
6.13. Stopper, One Hole for 250mL Erlenmeyer flask
6.14. Thistle Tube, Polyethylene
6.15. Motorized Stirrer with ~1½ foot reach
6.16. Timer
6.17. pH meter

7. REAGENTS and MEDIA

7.1. Ammonium citrate dibasic, ACS grade or equivalent
7.2. Ammonium hydroxide, ACS grade or equivalent
7.3. Citric acid monohydrate, ACS grade or equivalent
7.4. Nitric acid, ACS grade or equivalent
7.5. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Nitric Acid 1:1

8.1.1. Pour 1.0L of DI water into a 2L pre-labeled container. Place on a large magnetic stir plate. Turn on stir plate. Slowly add 1.0L of nitric acid to the container. Allow to stir for 10 minutes.

8.2. Neutral Ammonium Citrate Solution

8.2.1. Add 400g of ammonium citrate dibasic to carboy, add approximately 20L of hot DI water. Add 114mL of ammonium hydroxide to citrate solution. Stir solution and allow to cool completely before testing. Adjust the pH to 7.00 by adding small increments of ammonium hydroxide or citric acid monohydrate.

8.3. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.4. The specific ingredient amounts can vary to accommodate the batch size.

8.5. Expiration Date: 6 months from date of preparation unless otherwise specified.

8.6. Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment P, Manual Result Entry
9.2. ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.3. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size
9.4. Paradigm 3, Document Control Software, Improvement Module

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Pick up pre-weighed sample batches from Completed Sample Weighing shelf.

10.1.2. The batch size should not exceed the limit specified in PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.2. Calcium Extraction

10.2.1. Preheat shaker bath to 65ºC.

10.2.2. Add 100mL of hot DI water to Erlenmeyer flask containing the sample, stopper with 1 hole stopper containing thistle tube. Place in 65ºC shaker bath and set timer for 1 hour.

10.2.3. Add 10mL of Nitric acid 1:1, to Kohlrausch flasks.

10.2.4. Set up Buchner funnel – Kohlrausch flask – vacuum apparatus.

10.2.5. After one hour, remove Erlenmeyer flask from shaker bath, rinse thistle tube and stopper with hot DI water into Erlenmeyer flask.

10.2.6. Place filter disk into the Buchner funnel and wet with DI water.

10.2.7. Transfer contents of Erlenmeyer flask to wetted filter disk in Buchner funnel – Kohlrausch flask – vacuum apparatus.

10.2.8. Thoroughly rinse Erlenmeyer flask with hot DI water through filter disk in bottom of Buchner Funnel.

10.2.9. Vacuum dry filter disk.

10.2.10. Perform one additional rinse of the Buchner funnel to ensure that any remaining sample is collected.

10.2.11. Carefully remove filter disk and place in Erlenmeyer flask.

10.3. Citrate Extraction

10.3.1. Add 100mL of hot Neutral Ammonium Citrate Solution, to Erlenmeyer flask.

10.3.2. Seal with 1 hole stopper containing a thistle tube. Place in 65ºC shaker bath and set timer for 1 hour.

10.3.3. After a minimum of 90 minutes, remove Erlenmeyer flask from shaker bath, rinse thistle tube and stopper with hot DI water into Erlenmeyer flask.

10.3.4. Place filter disk into the Buchner funnel and wet with DI water

10.3.5. Transfer contents of Erlenmeyer flask to wetted filter disk in Buchner funnel-Kohlrausch flask – vacuum apparatus.

10.3.6. Thoroughly rinse Erlenmeyer flask with hot DI water through filter disk in bottom of Buchner Funnel.

10.3.7. Vacuum dry filter disk.

10.3.8. Perform one additional rinse of the Buchner funnel to ensure that any remaining sample is collected.

10.3.9. Remove the filter disk and discard.

10.3.10. Remove top of funnel.

10.3.11. Rinse the bottom funnel into the Kohlrausch flask.

10.4. Final Volume and Filtration

10.4.1. Allow extracts to cool for approximately 30 minutes, then dilute to 500mL with DI water.

10.4.2. Shake well.

NOTE: 30-45 seconds of vigorous shaking may be required to mix sample completely.

10.4.3. Filter approximately 12-14mL of sample extract into a disposable tube using a screening column.

10.5. Data Entry into Laboratory Information Management System (LIMS)

10.5.1. Using the applicable LIMS result entry template, complete the APA flask volume test codes in the LIMS. Refer to ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates, for a list of Result Entry templates and Attachment P, Manual Result Entry, for result entry instructions.

10.5.2. Print a copy of the LIMS result entry screen to include as the paperwork for the prep batch.

10.6. Transfer of Extracts

10.6.1. Transfer all extracts and paperwork to designated area in building 5 using a laboratory approved sample carrier.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Remaining Sample Extract from 10.4	Can be discarded down drain with sufficient flushing
Screening Columns and Filter Disks	Discarded in regular waste bin

12. REFERENCES

12.1. Available Phosphorus (Dual Extraction) – Flow Injection. FM 505, (07/15/2009)
12.2. APA (P_2O_5) Extraction –Citrate Soluble for Flow Injection. FSFL-SOP 500, (02/03/2006).

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	10.12.2016	Original Version	Sylvia Mehr	
2.0	04.15.2019	Updated outline format Revised scope to remove FM504 Added Section 8 Added Section 11 Minor grammatical fixes and clarifications throughout		Taleya Williams

14. SIGNATURE HISTORY

_____
Patricia Lucas
Chief, Technical Director

Date_____

_____
Teresa Rygiel
Laboratory Director

Date_____

_____
Quentin Cooper
Quality Assurance Officer

Date_____

_____
Taleya Williams
Wet Chemistry and Preparation Manager / Editor

Date_____

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of total phosphorous (TP) nutrients in fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). This preparation is for the analysis of total phosphorous by flow injection analysis. Phosphorus that can be hydrolyzed to orthophosphate with a concentration range of 0 to 20% P_2O_5 may be determined by this method. The range may be increased by decreasing sample weight and/or the use of dilutions. Laboratory Information Management System (LIMS) suggested weights must be followed. This method is applicable to fertilizer samples containing any amount of calcium.

2.2. Available Phosphorous: This method is also performed on a sample that has failed the normal available extraction methods. The following APA source materials will almost always fail the citrate soluble APA extractions: bone meal, basic slag, ammonium polyphosphate, and phosphorous acid (not to be confused with phosphoric acid).

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. Acid digestions must be performed under a fume hood.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weigh Sample
4.2. 10.2 Handling Pre-Weighed Samples
4.3. 10.3 Acid Digestion
4.4. 10.4 Citrate Extraction
4.5. 10.5 Final Volume
4.6. 10.6 Data Entry into Laboratory Information Management System
4.7. 10.7 Transfer of Extracts

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Centrifuge tube, disposable (15mL)
6.2. Erlenmeyer flask, 250mL
6.3. Filter disk, 7cm Whatman No. 1 or equivalent
6.4. Flask, 500mL Kohlrausch (class “A”)
6.5. Funnel, Buchner 7cm
6.6. Hot plate
6.7. Laboratory Information Management System (LIMS)
6.8. pH meter

6.9. Rack, tube
6.10. Shaker bath, constant temperature circulating
6.11. Stopper, one hole for 250mL Erlenmeyer flask
6.12. Stopper, two hole for 500mL Kohlraush Flask
6.13. Thistle tube, polyethylene
6.14. Timer

7. REAGENTS and MEDIA

7.1. Ammonium citrate dibasic, ACS grade or equivalent
7.2. Ammonium hydroxide, ACS grade or equivalent
7.3. Citric acid monohydrate, ACS grade or equivalent
7.4. Water, deionized (DI) Type II
7.5. Concentrated Hydrochloric acid, (HCl) ACS grade or equivalent
7.6. Concentrated Nitric acid, (HNO_3) ACS grade or equivalent

8. REAGENT PREPARATION GUIDELINES

8.1. 1:1 Nitric Acid

8.1.1. Directions: Using a clean 1000mL graduated cylinder, measure 1000mL of DI Water and pour into the 2L storage container. Using a clean 1000mL graduated cylinder measure 1000mL of concentrated HNO_3. Slowly add the HNO_3 to the DI water, cap the container and mix thoroughly using stir bar for approximately 10 minutes. Diluting the HNO_3 can cause heat; be careful not to add the acid too fast.

8.2. Neutral Ammonium Citrate Solution

Directions: Add 600g of ammonium citrate dibasic to a 30L vessel. Add approximately 24L of hot DI water to the vessel and turn on stir bar. Add 100mL of ammonium hydroxide to the citrate solution. Allow solution to stir for approximately 2 hours. Adjust the pH to 7.00 by adding small increments of ammonium hydroxide or citric acid monohydrate.

8.3. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.4. The specific ingredient amounts can vary to accommodate the batch size.

8.5. Expiration Date: 12 months from date of preparation

8.6. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment P, Manual Result Entry
9.2. ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.3. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Weigh Sample

10.1.1. Weigh samples as per the table below:

Guarantee	Weight
<5%	3g
5-10%	2g
10-20%	1g
20-30%	0.5g
>30%	0.25g

10.2. Handling Pre-Weighed Samples

10.2.1. If samples are pre-weighed, collection of Weighed Samples

10.2.2. Pick up pre-weighed sample batches from Completed Sample Weighing shelf.

10.2.3. The batch size should not exceed the limit specified in PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.3. Acid Digestion

10.3.1. Into 250mL Erlenmeyer flask, with weighed sample, add 20mL of Nitric Acid 1:1 and 5mL of concentrated HCl.

10.3.2. Place the sample with solution on hot plate, boil approximately 15 minutes or until solution is clear. Do not let the sample go dry.

10.3.3. Allow the sample to cool, then proceed to 10.4, Citrate Extraction.

10.4. Citrate Extraction

10.4.1. Preheat the shaker bath to 65ºC.

10.4.2. Add 100mL of hot (approximately 65ºC) Neutral Ammonium Citrate Solution to Erlenmeyer flask.

10.4.3. Seal with 1 hole stopper containing a thistle tube. Place in 65ºC shaker bath and set timer for 15 minutes.

10.4.4. After 15 minutes remove Erlenmeyer flask from shaker bath, rinse thistle tube and stopper with hot DI water into Erlenmeyer flask.

10.4.5. Place filter disk into the Buchner funnel and wet with DI water.

10.4.6. Transfer contents of Erlenmeyer flask onto wetted filter disk in Buchner funnel-Kohlrausch flask – vacuum apparatus.

10.4.7. Thoroughly rinse Erlenmeyer flask with hot DI water through filter disk.

10.4.8. Vacuum dry filter disk.

10.4.9. Perform one additional rinse of the Buchner funnel to ensure that any remaining sample is collected.

10.4.10. Remove the filter disk and discard.

10.4.11. Remove top of funnel.

10.4.12. Rinse the bottom of the funnel into the Kohlrausch flask.

10.5. Final Volume

10.5.1. Allow extracts to cool to room temperature and dilute to 500mL.

10.5.2. Mix the contents by thoroughly inverting the flask multiple times and allow to settle.

10.5.3. Pour approximately 12-14mL of sample extract into a disposable centrifuge tube.

10.6. Data Entry into Laboratory Information Management System

10.6.1. Using the applicable Laboratory Information Management System (LIMS) result entry template, complete the APA flask volume test codes in the LIMS. Refer to ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates, for the list of result entry templates and Attachment P, Manual Result Entry, for result entry instructions.

10.6.2. Print a copy of the LIMS result entry screen to include as the paperwork for the prep batch.

10.7. Transfer of Extracts

10.7.1. Transfer all extracts and paperwork to designated area in building 5 using a laboratory approved sample carrier.

10.7.2. Record initials and date on the paperwork.

11. WASTE MANAGEMENT

11.1. Remaining sample extract from 10.5 can be discarded down the drain with sufficient flushing using tap water.

12. REFERENCES

12.1. E00164, Total Phosphorus -Flow Injection. FM 521, (07/15/2009)
12.2. E00146, Total P_2O_5 Sample Preparation, FSFL-SOP 501, (02/03/2006)

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	10.12.2016	Original Version	Sylvia Mehr	
2.0	12.05.2018	Updated Outline Format Moved Reagents back into SOP (Section 8) Minor grammatical fixes and clarifications		Taleya Williams
3.0	1/13/2021	Clarified language in section 2.2 Removed section 10.3 Acid Digestion for Deficient Samples Containing Basic Slag, Iron or Aluminum Phosphate, or Liquid Samples and renumbered sections accordingly. Removed Reagent 01 and Reagent 02 through to align with other SOPs Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Taleya Williams
4.0	02.01.2024	Removed signature page Removed steps related to final filtration Added weight table Added external document numbers		Taleya Williams

PREP 461

Sample Preparation for Available Phosphorus and Soluble Potash in Fertilizer

1. PURPOSE

1.1 This standard operating procedure (SOP) provides instruction for the preparation of available phosphorous (APA) and soluble potash (K_2O) nutrients in fertilizer samples.

2. SCOPE

2.1 The procedure delineated below is applicable to fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL). APA (P_2O_5) and K_2O are extracted from fertilizer with a citrate-EDTA while shaking at 65°C. After the extract is brought to volume with deionized water, the extract is analyzed by ICP-OES.

2.2 This method is adapted from AOAC Method 2015.18. The method described in this SOP deviates from Method 2015.18 by bringing the samples to a final volume of 500 mL instead of 250 mL, as stated in Method 2015.18. Thus, the citrate-EDTA of the sample is diluted prior to analysis, eliminating the need to dilute the sample inline by increasing the tubing size of the buffer/internal standard peristaltic tube and reducing the tubing size of the sample peristaltic tube as described in Method 2015.18. Sample weight was increased from 0.5 g to 0.75 g to achieve required detection limit.

3. HEALTH and SAFETY

3.1 A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Extraction
4.2. 10.2 Data Entry into Laboratory Information Management System
4.3. 10.3 Transfer of Extracts

5. INTERFERENCE

5.1. Fertilizers containing phosphite will also be extracted by this method.

5.2. Due to the susceptibility of the citrate-EDTA solution to microbial degradation, the citrate-EDTA solution has a 2-week expiration when stored in the dark and a 16-hour expiration for sample extracts. An inhouse longevity study demonstrated microbial degradation could be mitigated by utilizing refrigeration. The expiration dates found in this SOP have been extended based on this study.

6. APPARATUS and MATERIAL

6.1. Centrifuge tube, disposable (15-ml)
6.2. Flask, 500 mL Kohlrausch (class “A”) or Volumetric Flask
6.3. Flask, Erlenmeyer (250 mL)
6.4. Label maker
6.5. Magnetic stir bar
6.6. pH meter
6.7. Rack, tube
6.8. Shaker Bath, Constant Temperature Circulating
6.9. Water Bath

6.10. Beaker
6.11. Balance Stir Plate
6.12. Stopper, rubber for 500 mL Kohlrausch Flask
6.13. Thistle Tube, Polyethylene
6.14. Time

7. REAGENTS and MEDIA

7.1. onium citrate dibasic, ACS grade or equivalent
7.2. DTA, disodium salt, dihydrate, >99%
7.3. Ammonium hydroxide, ACS grade or equivalent
7.4. Nitric acid, ACS grade or equivalent
7.5. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Citrate-EDTA Extraction Solution

8.1.1. Directions: Add 25 g disodium EDTA and 50 g dibasic ammonium citrate to a 2L volumetric flask containing ~1500 mL DI water. Adjust to near neutral by adding 30 mL of a solution of ammonium hydroxide-water (1+1, v/v), under a hood. (Volumes are scalable as needed.)

8.1.2. Using a pH electrode while stirring, adjust the final pH to 7.00 using a pH by adding the ammonium hydroxide-water (1+1, v/v) solution, or 1:1 Nitric Acid dropwise. After a stable pH of :7.00 ±0.02 has been attained, dilute to volume with DI water and mix.

8.1.3. Citrate-EDTA solution has a **2-months expiration** when stored refrigerated.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment P, Manual Result Entry
9.2. DMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.3. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Extraction

10.1.1. Fill shaker bath until water level covers the flask holders.
10.1.2. Turn on shaker bath and set temperature to 65º C.
Note: It takes roughly 90 min to heat to 65º C from room temperature.
10.1.3. Fill the water bath approximately two-thirds full.
Note:
10.1.4. Turn on water bath and set temperature 65º C.
10.1.5. Heat citrate-EDTA to reach 65 ±2ºC
Note: It takes at least 30 min for the citrate-EDTA to reach 65 ±2ºC.
10.1.6. Weigh 0.75 g of fertilizer into a separate 250-mL Erlenmeyer flask. See PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size for batch size and Laboratory Standard information.

10.1.7. Add 100 mL of the 65 ±2ºC preheated citrate-EDTA extraction solution to the 250-mL Erlenmeyer flask containing the sample, stopper with a rubber stopper with thistle tube and place in the 65 ±2ºC shaker bath. Shake at 200 reciprocations/min. for 1 hour.

10.1.8. After one hour, remove the 250-mL Erlenmeyer flask from the shaker bath, rinse rubber stopper with thistle tube with DI water into the 250-mL Erlenmeyer flask, and cool to room temperature.

10.1.9. Transfer the extract into a 500-mL Kohlrausch flask or volumetric flask, rinsing the flask with DI water a minimum of 3 times to ensure a complete transfer. Dilute to volume with DI water; stopper and mix by inverting 10 to 12 times.

10.1.10. Transfer approximately 15 mL of the extract into a disposable 15-mL centrifuge tube. Filter or centrifuge any extract containing suspended debris.

10.1.11. Extracts must be stored refrigerated and are to be analyzed within **3 days**. Note: Storage location is in Building 5, Refrigerator 6

10.2. Data Entry into Laboratory Information Management System (LIMS)

10.2.1. Using the applicable LIMS result entry template, complete the P&K flask volume test codes in the LIMS. Refer to ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates, for a list of Result Entry templates and Attachment P, Manual Result Entry, for result entry instructions.

10.2.2. Print a copy of the LIMS result entry screen to include as the paperwork for the prep batch.

10.3. Transfer of Extracts

10.3.1. Transfer all samples and paperwork to the designated area in building 5 using a laboratory-approved sample carrier.

11. REFERENCES

11.1 *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, **Method 2015.18**, ww.eoma.aoac.org, External Document 00135.

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	2021.09.17	Original Version	William Meeks	
2.0	10.16.2023	Section 6 updated Apparatus and Material list. Section 8.1.2. added option to use Nitric Acid to adjust pH. Added section 10.1.4. and 10.1.5. and related note. Added instructions for the rubber stopper with thistle to section 10.1.8. Added note to section 10.1.11. Added external document number to reference in section 11.1 Removed signature page		Taleya Williams Jonnie Carpenter

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of soluble iron, manganese, copper, and zinc in fertilizers by Atomic Absorption analysis.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer that is analyzed in the Bureau of Agricultural Environmental Laboratories (BAEL).

2.2. Soluble metal samples are extracted at acidic pH. Depending on the analyzed metal, the sample is solubilized in a solution of a specific pH, at which metal oxides are slightly soluble and are not a source of significant positive bias (ferric oxides, 1-2% solubility; manganese oxides, 1-1.5% solubility; copper oxides, 4-5% solubility). The respective extraction solutions also ensure that all the metal sulfate sources are fully recoverable. In samples containing high phosphate (%P_2O_5 guarantee ≥ 10), from monoammonium, diammonium or polyammonium phosphate sources, the soluble metals can be precipitated as metal ammonium phosphate. The most probable reaction between the soluble micronutrient and the phosphate salt is a recombination reaction which forms a stable (reciprocal) salt pair. This reaction proceeds in an irreversible manner to near completion, in which the salt pair is of much lower solubility than the original compounds.

$$MeSO_4 + \underset{\text{DAP}}{(NH_4)_2HPO_4} \text{ ----> } MeNH_4PO_4 + NH_4HSO_4$$

$$MeSO_4 + \underset{\substack{\text{Ammonium Polyphosphate}\\(\text{pyrophosphate})}}{(NH_4)_4P_2O_7} \text{ ----> } Me(NH_4)_2P_2O_7\ H_2O + (NH_4)_2SO$$

$$(Me = Mn^{+2}, Cu^{+2}, Fe^{+2}, Zn^{+2})$$

This reaction has been documented in Lehr (1972), where he listed several such reactions that may take place during the manufacture or storage of fertilizers. Many reactions require only water which is attracted to the hygroscopic fertilizer ingredients. Thus, samples must be protected from moisture, before extraction, and must be analyzed as soon as possible following extraction. After appropriate dilution, samples are analyzed by atomic absorption (A.A.) spectroscopy.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Collection of Weighed Samples
4.2. 10.2 Extraction of Samples
4.3. 10.3 Transfer of Samples
4.4. 10.4 Data Entry into Laboratory Information Management System (LIMS)
4.5. 10.5 Delivery

5. INTERFERENCE

5.1. Polyphosphate that is incompletely solubilized and superphosphates interfere with SCu and SZn extraction.

6. APPARATUS and MATERIAL

6.1. Graduated Centrifuge Tube, 15mL
6.2. Syringe with Luer-Lok tips (without needle), 20mL
6.3. Analytical Balance (accuracy to 0.0001g)
6.4. Aqueous 0.45µm nonsterile Syringe Filters 25mm disc

6.5. Bin to accommodate 25 200mL volumetric flasks
6.6. Filter screen column, 200 to 300µm or equivalent
6.7. Glass pipets (Class A)
6.8. Green rubber stoppers, size 3
6.9. Magnetic Stir Bar (approximately 3 inches)
6.10. Nalgene Container, 20L
6.11. Parafilm
6.12. pH meter (accuracy to 0.01 pH units)
6.13. 100mL graduated cylinder
6.14. Volumetric flasks Class A 200mL
6.15. Centrifuge

7. REAGENTS and MEDIA

7.1. Sodium Hydroxide Solution, 1N, purchased
7.2. 2,2-Bis(hydroxymethyl)-2,2',2"–nitrilotriethanol, $C_8H_{19}NO_5$, 99%+ (BIS-TRIS)
7.3. Solution for pH meter calibration, pH 4, Certified
7.4. Solution for pH meter calibration, pH 7, Certified
7.5. Hydrochloric acid (HCl) Certified A.C.S. grade or equivalent
7.6. Potassium Hydrogen Phthalate (KHP) Certified A.C.S grade or equivalent
7.7. Sodium Hydroxide (NaOH), pellets
7.8. SRM695 (NIST)
7.9. Sulfuric Acid, Certified A.C.S grade or equivalent
7.10. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Soluble Iron Buffer Solution

8.1.1. Directions: 200mL of Sulfuric Acid, Certified A.C.S grade or equivalent in a 20L calibrated reservoir containing 10L of DI Water. Fill up to the 20L mark and mix well.

8.2. 1N Sodium Hydroxide Solution

8.2.1. Directions: Add 8g of NaOH pellets to approximately 100mL of DI Water in a 200mL volumetric flask. Cool to room temperature. Bring to volume with DI Water and mix well. A commercially made 1N NaOH solution can be used.

8.3. Soluble Manganese Buffer Solution (0.05M KHP)

8.3.1. Preparation of 1L: Dissolve 10g of KHP in 800mL of DI Water. Adjust pH (4.95 to 5.0) by adding 20mL of 1.0N Sodium Hydroxide and bring to volume with DI Water; mix well.

8.3.2. Preparation of 20L: Dissolve 100g KHP in approximately 3L of DI Water in a 4L Beaker with handle. Transfer to the 20-L reservoir containing 10L DI Water. Repeat with 100g of KHP again, and transfer to the 20-L container. Fill to the 19L mark. Add 390mL of 1.0N Sodium Hydroxide, mix well and ensure that pH is adjusted (4.95-5.0). Fill with DI Water to the 20L mark; mix well.

8.4. Soluble Zn/Cu Buffer Solution (0.24M BIS-TRIS)

8.4.1. Preparation of 1L: Dissolve 50g of 2,2-Bis(hydroxymethyl)-2,2',2"-nitrilotriethanol in 700mL of DI water. Add 4mL of concentrated HCl and mix well. Verify the final pH of the buffer solution (5.95-6). Adjust final pH by adding 1:1 HCl, or 1:1 NaOH, if needed. Bring solution to 1L with DI Water and mix well.

8.4.2. Preparation of 20L: Dissolve 500g of BIS-TRIS in approximately 3L of DI Water. Transfer to the 20L reservoir containing 10L of DI Water. Repeat with 500g of TRI-BIS. Mixing well, add 250 mL of 1:1 HCL. Adjust final pH to 6.0 by adding concentrated HCL or 1:1 NaOH, if needed, and bring solution to 20L with DI water. Mix well .

8.5. 1:1 Hydrochloric Acid\Water (v\v) Solution

8.5.1. Directions: Add 250mL hydrochloric acid to approximately 200mL of DI Water in a 500mL volumetric flask. Bring to volume with DI Water, and mix well

Caution: Always add the acid to the water. Adding water to the acid causes the acid to spatter!

8.6. 1:1 Sodium Hydroxide\water (w\v) Solution

8.6.1. Directions: Add 250g of NaOH to approximately 200mL DI Water in a 500mL volumetric flask, bring to volume DI Water, and mix well.

8.7. Label the container appropriately, Label L003 indicates required information. Add relevant information to the Reagent Preparation, Form F00015.

8.8. The specific ingredient amounts can vary to accommodate the batch size.

8.9. Expiration Date: 12 months from date of preparation, unless otherwise specified.

8.10. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates.
9.2. F00015, Reagent Preparation
9.3. L003, Reagent
9.4. PREP 453, Weighing of Fertilizer Sample
9.5. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Samples are weighed according to PREP 453, Weighing of Fertilizer Sample.

10.1.2. The recommended sample weights are found in the following tables:

SCu, SFe, SMn Suggested Weight	
% Guarantee	Weight (g)
<0.008	5
0.008 - 0.08	0.04 / % Guarantee
0.08 - 0.8	0.2 / % Guarantee
>0.8	0.25

SZn Suggested Weight	
% Guarantee	Weight (g)
<0.008	5
0.008 - 0.16	0.04 / % Guarantee
>0.16	0.25

Where,

weight factor = (target ppm) (extraction vol) / (% conversion factor)

0.04 = (2 ppm) (200 mL) / (10000)

0.2 = (10 ppm) (200 mL) / (10000)

10.1.3. Samples are weighed into 200mL volumetric flasks.

10.1.4. Weigh 0.5g SRM695 (NIST) to be used as spike sample.

10.1.5. The batch size should not exceed the limit specified in PREP 453, Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.1.6. Collect batch samples from the properly identified bin(s).

10.2. Extraction of Samples

10.2.1. Using a graduated cylinder, add 100mL of the appropriate buffer to the weighed samples:

10.2.1.1. Soluble Iron Buffer, for Fe samples
10.2.1.2. Soluble Manganese Buffer Solution (0.05M KHP), for Mn samples
10.2.1.3. Soluble Zn/Cu Buffer Solution (0.24M BIS-TRIS), for Soluble Copper\Zinc Samples

10.2.2. Bring to volume with DI water.

10.2.3. Shake the flask for a minimum of 30 seconds, vigorously, and allow the sample to stand for a least 45 minutes.

10.2.4. Filter all cloudy samples or liquid samples containing "free-floating" particles and undissolved particles using an aqueous 25mm, 0.45µm non-sterile Syringe Filter .

10.2.5. Verify pH of buffer solution.

10.3. Transfer of Samples

10.3.1. Assemble a set of 15mL Centrifuge Tubes, one per sample.

10.3.2. Place the sample tubes on a suitable test tube rack, in a numerical sequence.

10.3.3. Samples can be centrifuged, or syringe filtered.

10.3.4. Label sample tubes with the sample ID number.

10.3.5. Pour approximately 14mL of each sample into the test tubes. This allows for a gap between the top of the tube and the sample surface that prevents spills.

10.3.6. Cover the samples with parafilm and make a seal to avoid spills or cross contamination.

10.3.7. Samples must be analyzed on the same day as extracted or must be filtered immediately following preparation. Samples extracted for SZn must be analyzed within one hour or filtered immediately following preparation.

10.4. Data Entry into Laboratory Information Management System (LIMS)

10.4.1. Log into Laboratory Information Management System (LIMS).

10.4.2. Double click the "Results" folder and single click "Results Entry".

10.4.3. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names for each metal:

10.4.3.1. Soluble Iron: FERT_AA_SOLUBLE_IRON
10.4.3.2. Soluble Manganese: FERT_AA_SOLUBLE_MANGANESE
10.4.3.3. Soluble Copper: FERT_AA_SOLUBLE_COPPER
10.4.3.4. Soluble Zinc: FERT_AA_SOLUBLE_ZINC

10.4.4. For each sample, enter the flask volume and save.

10.4.5. Reopen the template to verify that the data was saved.

10.4.6. Print and close the template.

10.4.7. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names. For example: select the:

10.4.7.1. Soluble Iron: FERT_PRINT_AA_SFE
10.4.7.2. Soluble Manganese: FERT_PRINT_SMN
10.4.7.3. Soluble Copper: FERT_PRINT_SCU
10.4.7.4. Soluble Zinc: FERT_PRINT_SZN

10.4.8. Print and close the template.

10.4.9. Indicate the QC sample number on the printouts, initial and date.

10.5. Delivery

10.5.1. Deliver all samples and paperwork to the designated area, using a laboratory-approved sample carrier.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Excess Extracts	Dispose down drain while flushing
Excess Buffers	Dispose down drain while flushing

12. REFERENCES

12.1. Soluble Iron FM-832 - QC Corporation – Baltimore, M.D. (Method # AWWA B402-90) FSFL-SOP-508
12.2. Soluble Manganese FM-830 – AOAC 16th Edition, Method 972.03 FSFL-SOP-508
12.3. Soluble Copper FM-831 – FSFL SOP-508
12.4. Soluble Zinc FM-833 – AOAC 16th Edition, Method 972.03 FSFL SOP-508
12.5. Preparation and Analysis of Soluble and Chelated Micro Nutrient Samples By AA FSFL-SOP 509.

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	11.01.2019	Original Version	Jack Andreu Christian Amason	
2.0	02.11.2021 02.22.2023	Added section 5.1. Added section 6.15. Expanded upon instructions in section 8.4.2. Added recommended sample weight table to section 10.1.2. Section 10.1.2.3. corrected buffer to samples Section 10.2.4. expanded upon criteria for filtering samples. Section 10.3.3 updated step to align with currently laboratory activities. Section 10.3.7. added instructions for samples extracted for SZn. Added section 12.5.		Zachary Tower Patrizia Lemma

14. SIGNATURE HISTORY

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PREP 464

Sample preparation for Chelated Fe, Mn, Cu, Zn [FM 840] [FM 841]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of samples for the analysis of Chelated Iron, Manganese, Copper and Zinc. These samples subsequently undergo Atomic Absorption analysis.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer that is analyzed in the Bureau of Agricultural Environmental Laboratories (BAEL).

2.2. In this method, a sodium acetate buffer solution is added to samples to adjust their pH between 3 and 4. The acidic extraction solution compensates for the basic condition brought about when large amounts of dolomite or limestone are used as fillers. It also prevents irreversible ligand exchange reactions of the iron chelates with copper, manganese, zinc and other metal ions, and formation of insoluble iron salts. In the absence of a complexing agent, Ferric (Fe^{+3}) iron forms insoluble salts at pH > 3. The chelated metal is quantitated by atomic absorption spectroscopy analysis.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Collection of Weighed Samples
4.2. 10.2 Extraction of Samples
4.3. 10.3 Transfer of Samples
4.4. 10.4 Data Entry into Laboratory Information Management System (LIMS)
4.5. 10.5 Delivery

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Graduated Polystyrene Centrifuge Tube, 15mL
6.2. Syringe with Luer-Lok tips (without needle), 20mL
6.3. Analytical Balance (accuracy to 0.0001g)
6.4. Aqueous 0.45µm nonsterile Syringe Filters, 25mm disc
6.5. Bin to accommodate up to 25 200mLvolumetric flasks

6.6. Glass pipets (Class A)
6.7. Green rubber stoppers, size 3
6.8. Magnetic Stir Bar, approximately 3 inches
6.9. Nalgene Container, 20L
6.10. Parafilm
6.11. pH meter (accuracy to 0.01 pH units)
6.12. Volumetric flasks Class A, 100, 200, and 1000mL

7. REAGENTS and MEDIA

7.1. Diammonium phosphate (DAP), $(NH_4)_2HPO_4$, solid
7.2. Glacial Acetic Acid (CH_3COOH), **Caution:** Avoid breathing vapors and skin contact. Use in a fume hood and wear protective equipment.
7.3. Sodium Acetate ($C_2H_3NaO_2$)
7.4. 5% Sodium hypochlorite solution, (NaClO)

7.5. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. DAP Solution

8.1.1. Directions: Dissolve 10g of DAP in approximately 500mL of DI Water in a 1000mL volumetric flask, and bring up to volume with DI water; mix well.

8.2. 0.1% Sodium Hypochlorite Solution

8.2.1. Directions: Add 20.0mL of 5% sodium hypochlorite to a 1000mL volumetric flask containing approximately 500mL of DI water, and bring up to volume; mix well.

8.3. Chelate Buffer

8.3.1. Preparation of 1L: Dissolve 1g of sodium acetate and 25mL of glacial acetic acid in 700mL of DI water; bring to a final volume of 1L with DI water.

8.3.2. Preparation of 20L: Add 20g of sodium acetate and 500mL of acetic acid to a calibrated container; bring up to a final volume of 20L with DI water; mix well.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation, unless otherwise specified.

8.7. Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates
9.2. F00015, Reagent Preparation
9.3. L003, Reagent
9.4. PREP 453, Weighing of Fertilizer Sample
9.5. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Samples are weighed according to PREP 453, Weighing of Fertilizer Sample.

10.1.2. Follow Laboratory Information Management System (LIMS) weight recommendations.

10.1.3. Weigh samples into 200mL volumetric flasks per the table below:

Guarantee (Guar)	Weight
<0.008%	5g
0.008-0.08%	0.4/Guar
0.08-0.8%	0.2/Guar
>8%	0.25g

10.1.4. The batch size should not exceed the limit specified in Prep 453, Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.1.5. Collect batch samples from the properly identified bin(s).

10.1.6. Follow Laboratory Information Management System (LIMS) weight recommendations.

10.2. Extraction of Samples

10.2.1. Add 100mL of Chelate Buffer to the samples and bring to volume with DI water.

10.2.2. Shake sample flasks for a minimum of 30 seconds, vigorously, and allow samples to stand for at least 45 minutes.

10.2.3. Filter liquid samples containing “free floating” particles and undissolved particles using a screen column.

10.2.4. Filter cloudy samples using an aqueous 25mm, 0.45µm non-sterile syringe filter.

10.2.5. If Chelated Iron results are excessively high (% found > 3x % guarantee), samples must be re-prepared and analyzed.

10.2.5.1. Weigh sample in a 200mL volumetric flask. Add 100mL of Chelate Buffer, 15mL Diammonium phosphate (DAP) Solution and 15mL Sodium Hypochlorite Solution. Bring up to a 200mL final volume and shake for 30 seconds. Allow sample to sit 5-10 minutes to ensure precipitation reaction goes to completion.

10.3. Transfer of Samples

10.3.1. Assemble a set of 15mL Graduated Polystyrene Centrifuge Tubes, one per sample.

10.3.2. Place sample tubes on a suitable test tube rack, in a numerical sequence.

10.3.3. Label sample tubes with the sample ID number.

10.3.4. Pour approximately 14mL of each sample into the test tube. This allows a gap between the top of the tube and sample surface that prevents spills.

10.3.5. Cover samples with parafilm and make a seal to avoid spills or cross contamination.

10.4. Data Entry into Laboratory Information Management System (LIMS)

10.4.1. Log onto Laboratory Information Management System (LIMS).

10.4.2. Double click the “Results” folder and single click “Results Entry”.

10.4.3. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names for each metal:

10.4.3.1. Chelate Iron: FERT_AA_CHELATE_IRON
10.4.3.2. Chelate Manganese: FERT_AA_CHELATE_MANGANESE
10.4.3.3. Chelate Copper: FERT_AA_CHELATE_COPPER
10.4.3.4. Chelate Zinc: FERT_AA_CHELATE_ZINC

10.4.4. For each sample, enter the flask volume and save.

10.4.5. Reopen template to verify that the data was saved.

10.4.6. Print and close the template.

10.4.7. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names. For example: select the:

10.4.7.1. Chelate Iron: FERT_PRINT_AA_CHELATE_FE
10.4.7.2. Chelate Manganese: FERT_PRINT_AA_MN
10.4.7.3. Chelate Copper: FERT_PRINT_AA_CU
10.4.7.4. Chelate Zinc: FERT_PRINT_AA_ZN

10.4.8. Print and close the template.

10.4.9. Indicate the QC sample number on the printouts, initial and date.

10.5. Delivery

10.5.1. Deliver all samples and paperwork to the designated area, using a laboratory-approved sample carrier.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Excess extracts	Dispose down drain while flushing
Excess buffer and reagents	Dispose down drain while flushing

12. REFERENCES

12.1. AOAC 16th Edition, Method 938.03

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	11.01.2019	Original Version	Jack Andreu Christian Amason	
2.0	10.16.2023	Removed reference to filter screen column throughout. Section 10.1.3. added the guarantee/ weight determining table.		Taleya Williams

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation of samples for the individual nitrogen break-down components. These samples subsequently undergo flow injection analysis.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples in the Bureau of Agriculture Environmental Laboratories (BAEL). This preparation is for the analysis of Nitrate nitrogen, Urea or other water soluble nitrogen, and Ammoniacal nitrogen through the usage of a Flow Injection Analyzer. Fertilizer samples that only contain Nitrate nitrogen, Urea or other water soluble nitrogen, and Ammoniacal nitrogen will be labeled as 430 NAU. Fertilizer samples that contain some or all the analytes in NAU as well as Water Insoluble Nitrogen will be labeled 430 KJL.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Collection of Weighed Samples
4.2. 10.2 Extraction of Samples
4.3. 10.3 Transfer of Samples
4.4. 10.4 Data Entry into Laboratory Information Management
4.5. 10.5 Delivery

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Bin
6.2. Centrifuge tube (15-50mL)
6.3. Flask (250mL)
6.4. Parafilm
6.5. Screening column
6.6. Stopper/cork
6.7. Test tube, Glass (13-15mL)

7. REAGENTS and MEDIA

7.1. Methanol, Optima or equivalent
7.2. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. N/A

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates.
9.2. PREP 453, Weighing of Fertilizer Sample
9.3. PREP 453 Attachment A, Fertilizer Laboratory Standard and Batch Size

10. SPECIFIC PROCEDURES

10.1. Collection of Weighed Samples

10.1.1. Samples are weighed according to PREP 453, Weighing of Fertilizer Sample.

10.1.2. The batch size should not exceed the limit specified in Prep 453, Attachment A, Fertilizer Laboratory Standard and Batch Size.

10.1.3. Collect batch samples from the properly identified bin(s).

10.2. Extraction of Samples

10.2.1. For samples 430 KJL, which contain Water Insoluble Nitrogen (WIN), it may be necessary to wet sample with 1 to 2mL of methanol, to prevent clumping before adding water.

10.2.2. Bring the samples to volume (i.e. 250mL) using deionized water.

10.2.3. Stopper and mix contents by inverting and vigorously shaking the flask and allow to settle.

10.3. Transfer of Samples

10.3.1. Assemble two sets of tubes. Every sample should have a glass test tube and a centrifuge tube.

10.3.2. Place glass test tubes on numbered rack, starting with the laboratory control sample (LS) then in sample number ascending order.

10.3.3. Put screening columns into the glass test tubes, ensuring that there is a filter in the columns.

10.3.4. Remove the labels from the flasks and place them on the respective glass test tubes.

10.3.5. Make a duplicate label for the centrifuge tubes.

10.3.6. Pour approximately 9mL of each sample into the respective test tube in order to leave headspace.

10.3.7. Pour sample into centrifuge tube to approximately 15mL.

10.3.8. Place parafilm over the test tubes and cap the centrifuge tubes.

10.4. Data Entry into Laboratory Information Management System

10.4.1. Log onto Laboratory Information Management System (LIMS).

10.4.2. Double click the “Results” folder and single click “Results Entry”.

10.4.3. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names. For example: select the FERT_PREP_NAU_SHORT for 430 NAU samples or FERT_PREP_NAU_KJL for 430 KJL samples.

10.4.4. For each sample, enter the flask volume and save.

10.4.5. Reopen template to verify that the data was saved.

10.4.6. Print and close the template.

10.4.7. Refer to ADMIN 023, Attachment AA, Fertilizer QC Loc Code and Result Entry Templates, for specific result entry template names. For example: select the template FERT_PRINT_NAUS for 430 NAU samples or FERT_PRINT_NAUL for 430 KJL samples.

10.4.8. Print and close the template.

10.4.9. Indicate the QC sample number on the printouts, initial and date the printouts.

10.5. Delivery

10.5.1. Deliver all samples and paperwork to the designated area, using a laboratory approved sample carrier.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Screening Column	Discard in trash
Excess Extract	Discard down drain while flushing

12. REFERENCES

12.1. Preparation for Breakdown Nitrogen Analysis, FSFL-SOP 491 Rev.:1.0 (2006/01/17)

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	03.29.2017	Original Version	Ian McCoy	
2.0	09.20.2019	Update Outline Format Changed Ian Linton to Ian McCoy, Same individual last name was changed		Taleya Williams

14. SIGNATURE HISTORY

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Wet Chemistry and Preparation Manager / Editor

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Ian McCoy
Author

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METHOD 500
Sample Preparation and Analysis of Direct Water Soluble Nitrogen
[FM 425, FM 440]

1. PURPOSE

This standard operating procedure (SOP) provides instruction for determination of water-soluble nitrogen.

2. SCOPE

The procedure delineated below is applicable to use for the Kjeldahl method in determining water soluble nitrogen by converting nitrate into nitrate gas using ferrous sulfate and distilling the by-product into an acid trap and titrating the excess.

Samples that contain water insoluble nitrogen are associated with the term “440” and sample lacking ammoniacal and water insoluble nitrogen are associated with the term “425”.

3. HEALTH and SAFETY

A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE required for this procedure includes face shield and acid resistant apron.

4. OUTLINE of PROCEDURE

4.1 10.1 Weighing
4.2 10.2 Filtration
4.3 10.3 Digestion
4.4 10.4 Acid Trap Setup
4.5 10.5 Distillation
4.6 10.6 Titration
4.7 10.7 Result Entry

5. INTERFERENCE

N/A

6. APPARATUS and MATERIAL

6.1 Auto titration system
6.2 Beaker, 300-ml
6.3 Filter paper
6.4 Flask, 250-ml
6.5 Flask, round bottom 800-ml
6.6 Fume hood
6.7 Kjeldahl digestion and distillation unit

6.8 Stopper
6.9 Weigh container

7. REAGENTS and MEDIA

7.1 Ammonium sulfate
7.2 Boiling stones, granular
7.3 Bromocresol green
7.4 Ferrous sulfate
7.5 Methanol
7.6 Methyl red
7.7 Sodium hydroxide, pellets
7.8 Sodium hydroxide, 50%
7.9 SRM-695
7.10 Sulfuric acid, concentrated reagent grade
7.11 Tris-hydroxymethyl aminomethane
7.12 Water, deionized
7.13 Water, tap

8. REAGENT PREPARATION GUIDELINES

8.1 0.5N Sulfuric Acid (Standard Acid)

8.1.1 Directions: Add approximately 20L of DI Water into a 50L plastic carboy marked at 41L. Slowly add 580mL of sulfuric acid, bring to 41L with DI water. Bubble air through this solution until thoroughly mixed.

8.1.2 Titration of Sulfuric Acid Solution (Option 1): Dry tris-hydroxymethyl aminomethane (THAM) in oven at 105°C for at least 2 hours. Cool it down in the desiccator. Weigh 0.4500g of THAM into each of the six 125mL Erlenmeyer flasks. Add 5 drops of Bromocresol Green to each flask. Add approximately 50mL of DI water to each flask. The color of the solution should be blue. If not blue, add more bromocresol green a drop at time to the flask. Add 0.5N sulfuric acid solution to 25mL dispensing burette using a pipet. Slowly titrate THAM with sulfuric acid to bromocresol green end point (pH 4.7). Discard the highest and lowest results and average the remaining four results.

8.1.3 Determine the concentration (normality) of each of the six sulfuric acid solutions using the formula:

$$\boldsymbol{Acid\ Normality} = \frac{[\boldsymbol{THAM\ Weight\ (g)}]\ \boldsymbol{x}\ \mathbf{8.2549}}{\boldsymbol{Acid\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE}\mathbf{: 8.2549} = \frac{\mathbf{1000}\boldsymbol{mL/L\ x\ 2(Conversion\ from\ Molarity\ to\ Normality)}}{\mathbf{121.14}\boldsymbol{g/mol\ x\ 2(Stoichiometric\ Ratio)}}$$

8.1.4 Titration of Sulfuric Acid Solution (Option 2): Add 4mL of 0.5N Sulfuric Acid (Standard Acid) to seven (7) 300mL beakers then fill them to approximately 250mL with water. Fill a separate 300mL beaker with 200mL of certified 0.5N NaOH; place the line of the titrator into the beaker and purge the syringe of the

titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.1.4.1 Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Acid 1 – Acid 6.

8.1.5 Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$A_N = \frac{B_N \, x \, B_V}{A_V}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.1.6 Discard the highest and lowest results and average the remaining results

8.2 18.75N Stock Sodium Hydroxide Solution

8.2.1 Directions: Add 1 liter of deionized (DI) water into a 2L volumetric flask with stir bar in it on a stirring plate. Add 1500g of sodium hydroxide pellets in small (~100g) portions with continuous mixing. Bring solution volume to 2L with DI water.

8.3 0.2N Sodium Hydroxide (Standard Base)

8.3.1 Directions (Option A): Fill a beaker with 1000ml of DI water. Add stir bar and start mixing. Add 1000ml of 50% Sodium Hydroxide and let it mix for 30 minutes.

Directions (Option B): Fill a 20L glass carboy, marked at 16L, half full of DI water. Add stir bar and start mixing. Add 300mL of stock sodium hydroxide solution to carboy while continuously mixing. Bring solution to a volume of 16L with DI water. Stir solution for approximately 30 minutes.

8.3.2 Titration of Sodium Hydroxide Solution (Option 1): Dry potassium acid phthalate (PAP) in oven at 105°C for at least 2 hours and then allow it to cool in desiccators. Take six 300mL beakers and weigh approximately 0.8000g of PAP into each. Add 150mL of DI water into each of the six beakers. Allow PAP to dissolve. Titrate PAP solution on the QC_Titrate System.

8.3.3 Titration of PAP Solution on the QC-Titrator: Click on PAP icon. Type set name and click OK. Type the exact weights of PAP. Highlight the first line on time-table and click start. When titration is done, a hard copy is printed automatically.

8.3.4 Calculation of Sodium Hydroxide: Determine the concentration (normality) of the six sodium hydroxide solutions using the formula:

$$Base\ Normality = \frac{[PAP\ Weight\ (g)]\ x\ 4.8964}{Base\ Volume\ (mL)}$$

$$NOTE: 4.8964 = \frac{1000mL/L\ x\ 1(Conversion\ from\ Molarity\ to\ Normality)}{204.23g/mol\ x\ 1(Stoichiometric\ Ratio)}$$

8.3.5 Titration of Sodium Hydroxide Solution (Option 2): Add 4mL of 0.35N Sodium Hydroxide to seven (7) 300mL beakers. Fill a separate 300mL beaker with 200mL of the normalized solution from 8.1; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.3.5.1 Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Base 1 – Base 6.

8.3.6 Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$B_N = \frac{A_N\ x\ A_V}{B_V}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.3.7 Discard the highest and lowest results and average the remaining four results. Change base normality on Kjeldahl and Direct Excel spreadsheets for LIMS.

8.4 Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.5 The specific ingredient amounts can vary to accommodate the batch size.

8.6 Expiration Date: 12 months from date of preparation unless otherwise specified.

8.7 Store at room temperature

9 ASSOCIATED DOCUMENTS

9.1 ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template

9.2 Paradigm 3, Document Control Software, Improvement Module

10 SPECIFIC PROCEDURES

10.1 Weighing

10.1.1 Using ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template, create a laboratory identification number for the laboratory control sample (LS).

10.1.2 Weigh approximately 0.5 g of the applicable laboratory control sample.

10.1.2.1 SRM-695 for "440"

10.1.2.2 Ammonium sulfate for “425”.

10.1.3 Weigh samples to approximately 0.9 grams except liquid samples that have been diluted. Diluted liquid samples are approximately doubled in weight (i.e. approximately 1.8 g).

10.1.4 Enter weight into Laboratory Information Management System (LIMS) using the template FERT_Nitrogen_KJL. *Be aware of the given acid trap volume because it will be used later in the process*.

10.2 Filtration

10.2.1 Only samples that contain water insoluble nitrogen (“440” sample) require filtration.

10.2.2 Fold the filter paper and place it into funnel that drains into 250-ml flask.

10.2.3 Wet the sides of the filter paper with deionized water to create a seal for the filter.

10.2.4 Pour the sample into filter paper.

10.2.5 Using deionized water, rinse the weigh container clean of sample into filter paper.

10.2.6 If the sample is clumping, use 2 to 5 mL of methanol to break up clumping.

10.2.7 Fill the filter paper with deionized water and allow to drain completely. Repeat the washing and draining process twice, for a total of three times.

10.2.8 Bring the filtrate, in the flask, up to 250-mL mark with deionized water.

10.2.9 After the filtrate is brought to volume, stopper in flask to prevent evaporation.

10.3 Digestion

10.3.1 Add 4 to 8 granular boiling stones to each 800-ml round bottom flask.

10.3.2 Add ¼ teaspoon of ferrous sulfate to each 800-ml round bottom flask.

10.3.3 This part of the process differs depending on whether the sample lacks ammoniacal and water insoluble nitrogen or the sample contains water insoluble nitrogen.

10.3.3.1 For sample lacking ammoniacal and water insoluble nitrogen (“425” sample):

10.3.3.1.1 Wash sample into round bottom flask using deionized water.

10.3.3.1.2 Wash weigh container, at least 3 times, until sample is completely transferred.

10.3.3.2 For sample containing water insoluble nitrogen (“440” sample):

10.3.3.2.1 Mix the contents of the 250-mL flask. To ensure that the filtrated sample is thoroughly mix, shake and invert at least 7 times.

10.3.3.2.2 Add 100 mL of filtrate to each flask using either graduated cylinder or pipette.

10.3.4 Add 20 mL of concentrated sulfuric acid to each flask. Swirl flask to mix solution.

10.3.4.1 Turn on fume hood and rack ventilation systems.

10.3.4.2 Digest sample on the digestion rack.
10.3.4.2.1 Approximately 60 minutes for “425” samples.
10.3.4.2.2Approximately 75 minutes for “440” samples.
10.3.4.3 If any viscous black\brown material forms on top of round bottom flask, rotate flask on digestion rack to facilitate the digestion of material.
10.3.4.4 After digestion is complete, allow sample to cool to room temperature.
10.3.4.5 Once samples have reached room temperature, fill round bottom flask to approximately half way with deionized water.

10.4 Acid Trap Setup

10.4.1 The preparation of the acid trap, using the acid dispenser, differ depending on the specific sample.
10.4.1.1 For Blank:
10.4.1.1.1 Dispense 4 mL of standard acid into 300-ml beaker.
10.4.1.1.2 Bring to the 250-mL mark with deionized water.
10.4.1.1.3 Placed beaker, with sample, in slot 1 on the auto titrator.
10.4.1.2 For Laboratory Control Sample (used in “440” preparation):
10.4.1.2.1 Dispense 4 mL of standard acid into 300-mL beaker.
10.4.1.2.2 Bring to 50-mL mark with deionized water.
10.4.1.2.3 Add 1-4 drops of methyl red indicator.
10.4.1.3 For Laboratory Control Sample (used in “425” preparation):
10.4.1.3.1 Dispense 18 mL of standard acid into 300-ml beaker.
10.4.1.3.2 Bring to 50-mL mark with deionized water.
10.4.1.3.3 Add 1-4 drops of methyl red indicator.
10.4.1.4 For Sample:
10.4.1.4.1 Dispense the applicable volume of standard acid into the 300-ml beaker. *This volume should be based upon the acid trap volume that was calculated in LIMS earlier in the process*.
10.4.1.4.2 Bring to the 50-mL mark with deionized water.
10.4.1.4.3 Add 1-4 drops of methyl red indicator.
10.4.2 Make extra 4-ml aliquots of standard acid. *This standard acid can be added to distillation acid trap if indicator starts to turn orange/yellow*.

10.5 Distillation

10.5.1 Place acid trap into its corresponding spot on the distillation rack with the tip of the distillation tube visibly submerged in the liquid.
10.5.2 Turn on cooling jackets and fume hood.
10.5.3 Set heaters on high and allow to heat up.
10.5.4 Swirl round bottom flask vigorously ensuring sample is mixed thoroughly.
10.5.5 Slowly add 80 mL of 50% sodium hydroxide to each flask.

10.5.6 Immediately after sodium hydroxide is added, attach flask to the distillation column and place on heating rack.
10.5.7 Allow distillation to proceed until acid trap volume reaches 250-mL mark.
10.5.8 Remove the acid trap from the distillation column.
10.5.9 Turn off heating racks and fume hood.
10.5.10 Once samples have stopped boiling, turn off cooling jackets.

10.6 Titration

10.6.1 Start Pc titrate program.
10.6.2 Once program opens, load “Ammonium” template. NOTE: The icon is two test tubes.
10.6.3 Delete any extra spaces from the template. The amount of spaces needed in the template is one more than the number of samples. If there are 11 samples there should be 12 spaces in template.
10.6.4 Enter sample identification numbers into template.
10.6.5 The blank should be in position 1. Label position 1 with the date and user initials.
10.6.6 The laboratory control sample should be in position 2.
10.6.7 Place acid trap into corresponding locations on auto titration rack.
10.6.8 Select position 1 in the template. NOTE: This is where the auto titrator will start the titration process.
10.6.9 Make sure all acid traps correspond to their location on the template.
10.6.10 Click start and ensure that the titration process begins.
10.6.11 Sign, date and file the instrument’s print out of titration results.

10.7 Result Entry

10.7.1 Record the results (from the print out) into the FERT_NITROGEN_KJL template in the LIMS. A laboratory notebook may be used as an intermediate location for the results.
10.7.2 Any additional acid used during the distillation must be added to the acid trap value in the LIMS. This updated value will be used in calculations.
10.7.3 The raw data, from the auto titrator, is uploaded to the folder on the “\\tlhaeschrome” drive.

11 Waste Management

Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Boiling stones	Trash
Residue (from filtration process)	Trash
Sample extract, excess	Stored in waste container until neutralized

Neutralized sample extract, excess	Flushed down the sink with water

12 REFERENCES

1. E00151, Calibration and use of QC-Titrate™ Titration system, FSFL-SOP 494
2. E00149, Kjeldahl Digestion of Fertilizer Samples, FSFL-SOP 492
3. E00150, Kjeldahl Distillation of Fertilizer Samples, FSFL-SOP 493

13 VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	2017.05.04	Original Version	Zachary Tower	
2.0	01.16.2024	Updated SOP to new format Added reagents. Remove signature page Minor grammatical fixes Added external document numbers		Amy Bryant Zachary Tower

METHOD 501

Sample Preparation and Analysis of Water Insoluble Nitrogen

[FM 445]

1. PURPOSE

This standard operating procedure (SOP) provides instruction for the determination of water insoluble nitrogen in fertilizer.

2. SCOPE

The procedure delineated below is applicable to use for the Kjeldahl method for the determination of water insoluble nitrogen in fertilizer samples first by digestion using sulfuric acid and Kjeldahl tablets, then distilling the by-product into an acid trap using a standard acid, and finally titrating the excess using a standard base.

This method does not give the degree to which the water insoluble nitrogen is available.

3. HEALTH and SAFETY

A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE required for this procedure includes face shield and acid resistant apron.

4. OUTLINE of PROCEDURE

4.1 10.1 Weighing
4.2 10.2 Filtration
4.3 10.3 Digestion
4.4 10.4 Acid Trap Setup
4.5 10.5 Distillation
4.6 10.6 Titration
4.7 10.7 Data Evaluation
4.8 10.8 Result Entry
0.5 N Sulfuric Acid (Standard Acid)
18.75N Stock Sodium Hydroxide
0.25 N Sodium Hydroxide (Standard Base)

5. INTERFERENCE

N/A

6. APPARATUS and MATERIAL

1. Auto titration system
2. Beaker, 300-ml
3. Filter paper
4. Flask, 250-ml
5. Flask, round bottom flasks 800-ml

6. Fume hood
7. Funnels
8. Kjeldahl digestion and distillation unit
9. Stoppers
10. Weigh container
11. Acid dispenser
12. Class A pipet

7. REAGENTS and MEDIA

1. Boiling stones, granular
2. Hollow Glass Beads
3. Bromocresol green
4. FisherTab, Kjeldahl tablets (Kel Tabs)
5. Methanol
6. 0.5% Methyl Red indicator
7. Sodium hydroxide, 50%
8. Sodium hydroxide, 0.5N, certified
9. SRM-695
10. Sulfuric Acid, concentrated reagent grade
11. Water, deionized (type II)
12. Water (Tap)
13. Sodium Hydroxide Pellets NF/FCC

8. REAGENT PREPARATION GUIDELINES

8.1 0.5N Sulfuric Acid (Standard Acid)

8.1.1 Directions: Add approximately 20L of DI Water into a 50L plastic carboy marked at 41L. Slowly add 580mL of sulfuric acid, bring to 41L with DI water. Bubble air through this solution until thoroughly mixed.

8.1.2 Titration of sulfuric acid (option1): Dry tris-hydroxymethyl aminomethane (THAM) in oven for at least 2 hours. Cool it down in the desiccator. Weigh 0.4500g of THAM into each of the six 125ml Erlenmeyer flask. Add 5 drops of bromocresol green to each flask. Add approximately 50ml of DI water to each flask. The color of the solution should be blue. If not blue, add more bromocresol green, one drop at a time to the flask. Add 0.5N sulfuric acid to 25ml dispensing burette using a pipet. Slowly titrate THAM with sulfuric acid to bromocresol green end point (ph7). Discard the highest and lowest results and average the remaining results. Determine the concentration (normality) of each of the six sulfuric acid solutions using the formula:

$$\text{Acid Normality} = \frac{[\text{THAM weight(g)}]\times 8.2549}{\text{Acid volume}}$$

$$NOTE: 8.2549 = \frac{1000mL/L\ x\ 2(Conversion\ from\ Molarity\ to\ Normality)}{121.14g/mol\ x\ 2(Stoichiometric\ Ratio)}$$

8.1.3 Titration of Sulfuric Acid Solution (option 2): Add 4mL of Reagent 01 to seven (7) 300mL beakers then fill them to approximately 250mL with water. Fill a separate 300mL beaker with 200mL of certified 0.5N NaOH; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.1.3 Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Acid 1 – Acid 6.

8.1.4 Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$A_N = \frac{B_N\ x\ B_V}{A_V}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.1.5 Discard the highest and lowest results and average the remaining results

8.1.6 Change acid normality on the Kjeldahl and Direct Excel spreadsheets. The new value for acid must be changed in LIMS.

8.2 18.75N Stock Sodium Hydroxide

8.2.1 Directions: Add 1L of deionized (DI) water into a 2L volumetric flask with a stir bar in it, on a stir plate. Add 1500g of sodium hydroxide pellets in small (~100g) portions with continuous mixing. Bring solution volume to 2L with DI water.

8.2.2 0.25N Sodium Hydroxide (Standard Base)

Directions (Option A): Fill a beaker with 1000ml of DI water. Add stir bar and start mixing. Add 1000ml of 50% Sodium Hydroxide and let it mix for 30 minutes.

Directions (Option B): Fill a 20L glass carboy, marked at 16L half full of DI water. Add stir bar and start mixing. Add 300ml of stock sodium hydroxide solution to carboy while continuously mixing. Bring solution to a volume of 16L with DI water. Stir solution for approximately 30 minutes.

8.2.3 Titration of Sodium Hydroxide Solution (Option 1): Dry potassium acid phthalate (PAP) in oven at 105°C for at least 2 hours and then allow it to cool in desiccators. Take six 300mL beakers and weigh approximately 0.8000g of PAP into each. Add 150mL of DI water into each of the six beakers. Allow PAP to dissolve. Titrate PAP solution on the QC Titrate System.

8.2.4 Titration of PAP Solution on the QC-Titrator: Click on PAP icon. Type set name and click OK. Type the exact weights of PAP. Highlight the first line on time-table and click start. When titration is done, a hard copy is printed automatically.

8.2.5 Calculation of Sodium Hydroxide: Determine the concentration (normality) of the six sodium hydroxide solutions using the formula:

$$\boldsymbol{Base\ Normality} = \frac{[\boldsymbol{PAP\ Weight\ (g)}]\ \boldsymbol{x\ 4.8964}}{\boldsymbol{Base\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE: 4.8964} = \frac{\boldsymbol{1000 mL/L\ x\ 1(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{204.23 g/mol\ x\ 1(Stoichiometric\ Ratio)}}$$

8.2.6 Titration of Sodium Hydroxide Solution (Option 2): Add 4mL of Reagent 03 to seven (7) 300mL beakers. Fill a separate 300mL beaker with 200mL of the normalized solution from 8.1; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.2.6.1 Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Base 1 – Base 6.

8.2.7 Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{B_N} = \frac{\boldsymbol{A_N\ x\ A_V}}{\boldsymbol{B_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.2.8 Discard the highest and lowest results and average the remaining results. Change base normality on Kjeldahl and Direct Excel spreadsheets for LIMS.

8.3 Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.4 The specific ingredient amounts can vary to accommodate the batch size.

8.5 Expiration Date: 12 months from date of preparation unless otherwise specified.

8.6 Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1 ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.2 DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs
9.3 Paradigm 3, Document Control Software, Improvement Module

9.4 Labop 140 at J, Maintenance, Operation, and Procedure of QC Titrator System

10. SPECIFIC PROCEDURES

10.1 Weighing

10.1.1 Using ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template, create a laboratory identification number for the laboratory control sample (LS).
13.1.2 Weigh approximately 0.5 g of LS, SRM-695
10.1.3 Weigh samples to approximately 0.9 grams except liquid samples that have been diluted. Diluted liquid samples are approximately doubled in weight (i.e. approximately 1.8 g).
10.1.4 Enter weight into Laboratory Information Management System (LIMS) using the template FERT_Nitrogen_KJL. *Be aware of the given acid trap volume because it will be used later in the process*.

13.2 Filtration

10.2.1 Fold filter paper and place into funnel that drains into 250-ml flask.
10.2.2 Wet sides of the filter paper with deionized water to create a seal for the filter.
10.2.3 Pour sample into filter paper.
10.2.4 Using deionized water, rinse the weigh container clean of sample into filter paper.
10.2.5 If sample is clumping, use 2 to 5 mL of methanol to break up clumping
10.2.6 Fill the filter paper up with deionized water and allow to drain completely. Repeat the washing and draining process twice, for a total of three times.
10.2.7 Bring the filtrate, in the flask, up to 250-mL mark with deionized water.
10.2.8 After the filtrate is brought to volume, put stopper in flask to prevent evaporation.

10.3 Digestion

10.3.1 Add 4 to 8 granular boiling stones or glass beads, to each 800 ml round bottoms flask.
10.3.2 Add 2 Kjeldahl tablets to each round bottom flask.
10.3.3 Add filter paper to each round bottom flask making sure that the filter paper reaches the bottom of each flask.
10.3.4 Add 40 ml of concentrated sulfuric acid to each flask. Swirl flask until all filter paper is thoroughly dissolved.
10.3.5 Turn on fume hood and rack ventilation systems.
10.3.6 Digest samples on the digestion rack for approximately 75 minutes.
10.3.7 If any viscous black\brown material forms on top of round bottom flask, rotate flask on digestion rack to facilitate the digestion of material.
10.3.8 After digestion is complete allow samples to cool to room temperature.

10.3.9 Once samples have reached room temperature, fill round bottom flasks half to approximately half way with water from the hose adjacent to digestion rack.

10.4 Acid Trap setup

10.4.1 The preparation of the acid trap, using the acid dispenser or class A pipet, differ depending on the specific sample.

10.4.2 For Instrument Blank:

10.4.2.1 Dispense 4 ml of standard acid into 300 ml beaker labeled blank.
10.4.2.2 Bring to the 250-ml mark with deionized water.
10.4.2.3 Place the beaker, with sample, in slot 1 on the auto titrator.

10.4.3 For Laboratory Control Sample

10.4.3.1 Dispense 4 mL of standard acid in 300-mL beaker.
10.4.3.2 Bring to the 50ml mark with deionized water.
10.4.3.3 Add 1-2 drops of Methyl Red indicator. This is the LS.

10.4.4 For Sample:

10.4.4.1 Dispense the applicable volume of standard acid into the 300-ml beaker. *This volume should be based upon the acid trap volume that was calculated in LIMS earlier in the process*.
12.4.4.2 Bring to the 50-mL mark with deionized water.
12.4.4.3 Add 1-2 drops of methyl red indicator.
12.4.4.4 Make extra 4-ml aliquots of standard acid. *This standard acid can be added to distillation acid trap if indicator starts to turn orange/yellow*.

10.5 Distillation

10.5.1 Place acid traps into its corresponding spots on the distillation rack with the tip of the distillation tube visibly submerged in the liquid.
10.5.2 Turn on cooling jackets and fume hood.
10.5.3 Set heaters on high and allow to heat up.
10.5.4 Swirl round bottom flasks vigorously to ensuring sample is mixed thoroughly.
10.5.5 Slowly add 80mL of 50% sodium hydroxide to each flask
10.5.6 Immediately after sodium hydroxide is added, attach flask to the distillation column and place on heating rack.
10.5.7 Allow distillation to proceed until acid trap volume reaches 250-mL mark. If acid trap starts to turn yellow/orange, add extra 4ml aliquot of standard acid. Record how much acid was added in notebook.
10.5.8 Remove the acid trap from the distillation column.
10.5.9 Turn off heating racks
10.5.10 Once samples have stopped boiling, turn off cooling jackets and fume hood.

10.6 Titration

10.6.1 Start Pc titrate program.

10.6.2 Once program opens, load “Ammonium” template. NOTE: The icon is two test tubes.
10.6.3 Delete any extra spaces from the template. The amount of spaces needed in the template is one more than the number of samples. If there are 11 samples there should be 12 spaces in template.
10.6.4 Enter sample identification numbers into template.
10.7 The blank should be in position 1. Label position 1 with the date and user initials.
10.8 The laboratory control sample should be in position 2.
10.9 Place acid trap into corresponding locations on auto titration rack.
10.10 Select position 1 in the template. NOTE: This is where the auto titrator will start the titration process.
10.11 Make sure all acid traps correspond to their location on the template.
10.12 Click start and ensure that the titration process begins.
10.13 Sign, date and file the instrument’s print out of titration results.

10.14 Data Evaluation
10.14.1 Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.
10.14.2 Evaluate the Lab Control sample results.
10.14.3 To determine if it is within acceptable limits refer to Data 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits.

10.15 Result Entry
10.15.1 Record the results (from the print out) into the FERT_NITROGEN_KJL template in the LIMS. A laboratory notebook may be used as an intermediate location for the results.
10.15.2 Any additional acid used during the distillation must be added to the acid trap value in the LIMS. This updated value will be used in calculations.
10.15.3 The raw data, from the auto titrator, is uploaded to the folder on the “\\tlhaeschrome” drive.

11 Waste Management

11.1 Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Boiling stones	Trash
Residue (from the filtration process)	Trash
Sample extract, excess	Add to KJL waste, neutralize with acid, and flushed down the sink with

	running water.

12 REFERENCES

12.1 E00151, Calibration and use of QC-Titrate Titration system, FSFL-SOP 494
12.2 E00150, Kjeldahl Distillation of Fertilizer Samples, FSFL-SOP 493
12.3 E00152, Water Insoluble Nitrogen - Kjeldahl, FM-445
12.4 E00153, Breakdown Nitrogen Preparation. FM-430
12.5 E00149, Kjeldahl Digestion of Fertilizer Samples, FSFL-SOP 492

13 VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	2017.11.16	Original Version	Zachary Tower	
2.0	01.16.2024	Added reagents Updated formatting Minor clarifications throughout Added external document numbers Removed signature page		Amy Bryant Katherine Malik

METHOD 503
Analysis of Total Nitrogen using LECO Combustion Analyzer [FM 411]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for determination of total nitrogen content in fertilizer samples by the LECO Total Nitrogen Analyzer.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples in the Bureau of Agricultural Environmental Laboratories (BAEL), requiring the analysis of total nitrogen.

2.2. Nitrogen is released from a fertilizer through combustion at high temperature utilizing ultra-high purity (99.99%) oxygen. The nitrogen is quantitatively measured by a thermal conductivity detector and converted to w/w percent nitrogen in the sample using the appropriate mechanism.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 9.1 Sample Handling
4.2. 9.2 Calibration Standards
4.3. 9.3 Instrument Startup
4.4. 9.4 Sample Analysis
4.5. 9.5 Troubleshooting the Instrument
4.6. 9.6 Exporting Data
4.7. 9.7 Instrument Shutdown
4.8. 9.8 Data Reduction and Reporting
4.9. 9.9 Quality Control

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Analytical Instrument
  6.1.1. LECO TruSpec N Nitrogen Determinator
  6.1.2. Sample Carousel
  6.1.3. Balance
6.2. Tin Foil Cups
6.3. Tin Capsules

7. REAGENTS and MEDIA

7.1. EDTA (Nitrogen standard)
7.2. Sucrose
7.3. SRM695
7.4. Helium, Ultra Pure (Carrier)
7.5. Oxygen (Combustion)
7.6. Air, Compressed

8. ASSOCIATED DOCUMENTS

8.1. E00059, TruSpecN: Nitrogen Determinator Instruction Manual (March 2006)

8.2. LABOP 140 Attachment G, Maintenance, Operation and Procedure of The LECO Total Combustion Analyzer
8.3. Data Evaluation for Feed, Fertilizer, and Pesticide Residue Programs, DATA 051.

9. SPECIFIC PROCEDURES

9.1. Sample Handling

Weigh ~0.1000g of sample to tin foil cup and add ~0.2000g of sucrose to each. Double the weight for 2X liquid dilutions. Different weights may be necessary for high and low guarantees.

9.2. Calibration Standards

9.2.1. Recommended Standard Levels:

9.2.1.1. CAL 5, 0.3950-0.4050g of EDTA
9.2.1.2. CAL 4, 0.2950-0.3050g of EDTA
9.2.1.3. CAL 3, 0.1950-0.2050g of EDTA
9.2.1.4. CAL 2, 0.0950-0.1050g of EDTA
9.2.1.5. CAL 1, 0.0450-0.0550g of EDTA

9.2.2. Make sure that the purity percent is updated in the standard if there is a change of purity in the new standard.

9.3. Instrument Startup

9.3.1. Instrument is normally left on. However, when needed, turn power on and make sure computer is on.

9.3.2. Once powered up, open software and allow it to connect to instrument.

9.3.3. Check temperature setpoints and allow to come to temperature. Could take up to 12 hours to stabilize.

9.3.4. Clean the sample carousal and replace the furnace filter

9.3.5. In software, run System Check for leaks.

9.3.6. Analysis Parameters:

9.3.6.1. Combustion Furnace Temperature 950º C
9.3.6.2. Afterburner Temperature 850º C

9.3.7. Element Parameters: Nitrogen

9.3.7.1. Minimal Analysis Time 30 seconds
9.3.7.2. Comparator Level 1.00
9.3.7.3. Endline Time 1 second
9.3.7.4. Conversion Factor 1.00
9.3.7.5. Significant Digits 5
9.3.7.6. TC Baseline Delay Time 5 seconds
9.3.7.7. TC Baseline Time 2 seconds

9.3.8. Burn Profile:

9.3.8.1. Burn Steps 1
9.3.8.2. Time 60 seconds
9.3.8.3. Furnace Flow High

9.3.9. Macro Ballast Parameters: Ballast

9.3.9.1. Equilibrate Time 30 seconds

9.3.9.2. Not Filled Timeout 300 seconds

9.3.10. Aliquot Loop

9.3.10.1. Fill Time 20 seconds

9.3.10.2. Equilibrate Pressure Time 4 seconds

9.4. Sample Analysis

9.4.1. Place the standards and samples (tin foil cup or tin capsule) into sample carousel following the sequence

9.4.1.1. Five blanks must be injected at the beginning of a sample sequence to ensure that the system is free of contaminants.

9.4.1.2. Analyze the 5 point calibration curve.

9.4.1.3. In lieu of full calibration, a drift correction may be used to adjust the original calibration response to match the current instrument condition and response.

9.4.1.4. See DATA 051 for further information on sample sequence, initial calibration, and CCV requirements.

9.4.2. Samples require the proper laboratory information management system (LIMS) codes to allow required uploading.

9.4.2.1. Example; AA12345 411-0

Where: 0 corresponds to the sample weight (0, 1, 2)

9.4.3. Continuing Calibration Check Standard:

9.4.3.1. A CCV must be analyzed after every 10 samples and at the end of the sequence.

9.4.3.2. The CCV may consist of any level used in the initial calibration. Levels near the middle of the curve are recommended.

9.4.3.3. If a CCV response is not within the +/-2% limits, all samples analyzed before and after it must be reanalyzed.

9.4.3.4. If the area reading of any sample exceeds the calibration range, then weigh less sample and reanalyze.

9.5. Troubleshooting the Instrument

9.5.1. If instrument maintenance or troubleshooting is required, the following documents can be used for guidance.

9.5.1.1. E00059, TruSpecN: Nitrogen Determinator Instruction Manual (March 2006)

9.5.1.2. LABOP 140 Attachment G, Maintenance, Operation and Procedure of The LECO Total Combustion Analyzer

9.6. Exporting Data

9.6.1. Highlight the data and export to the flash drive.

9.6.2. Transfer the data from flash drive to the network drive TLHAESCHROME.

9.6.3. Upload the data from the network drive to the LIMS.

9.7. Instrument Shutdown

9.7.1 Refer to: LABOP 140 Attachment G, Maintenance, Operation and Procedure of The LECO Total Combustion Analyzer.

9.8. Data Reduction and Reporting

9.8.1. The Laboratory Control Sample must be evaluated first and if it fails, the associated samples in the batch are not uploaded.

9.8.2. Data for all passing batches is uploaded into LIMS and evaluated. Refer to Feed, Fertilizer, and Pesticide Residue Programs to evaluate the data against the established criteria.

9.9. Quality Control

9.9.1. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples. Guidelines that supersede those of QUALITY 112 are addressed in this document.

10. WASTE MANAGEMENT

10.1. Samples can be discarded in regular waste receptacles.

11. REFERENCES

11.1. Nitrogen Analysis. FM-410 (01/29/2003)
11.2. Hazardous Waste Disposal. PLS_SOP 009, Rev. 1.0 (02/09/2006)
11.3. Laboratory Safety, SAFETY 001
11.4. Using the Laboratory Information Management System. ADMIN 023
11.5. E00060, Nitrogen Determination in Fertilizers, LECO Corporation, 06/09
11.6. AOAC 933.13, Nitrogen (total) in Fertilizer – Combustion Method

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	12/28/2018	Original Version	Carl Huang	
2.0	12/02/2020	Changed CCV frequency and changed Shutdown procedures to refer to LABOP 140 attch G Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Christian Amason

13. SIGNATURE HISTORY

_____
Patricia Lucas
Chief, Technical Director

Date_____

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Teresa Rygiel
Laboratory Director

Date_____

_____
Quentin Cooper
Quality Assurance Officer

Date_____

_____
Christian Amason
Instrumental Manager/Editor

Date_____

METHOD 504

Sample Preparation and Analysis of Direct Ammoniacal Nitrogen for Sample with Urea Absent [FM 420]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of direct ammoniacal nitrogen in fertilizer that does not contain urea, water soluble organic nitrogen, or water insoluble nitrogen using the Kjeldahl method. This is a direct method and does not undergo digestion.

2. SCOPE

2.1. The analytical procedure delineated below is applicable to the direct determination of ammoniacal nitrogen in fertilizer samples that do not contain urea or water insoluble nitrogen by making the sample basic, distilling into an acid trap and titrating excess acid with a standard base.

2.2. Refer to BAEL METHOD 530 for an alternative sample preparation and analysis of direct ammoniacal nitrogen with urea absent using the Buchi.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE that may be used for this procedure includes face shield and acid resistant apron.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weighing
4.2. 10.2 Acid Trap Setup
4.3. 10.3 Distillation
4.4. 10.4 Titration
4.5. 10.5 Data Evaluation
4.6. 10.6 Result Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Balance
6.2. Beaker, 300mL
6.3. Acid Dispenser
6.4. Class A Pipettes
6.5. Auto Titration System
6.6. Computer
6.7. Kjeldahl Distillation Unit
6.8. Flask, round bottom 800mL
6.9. Weigh containers

7. REAGENTS and MEDIA

7.1. Ammonium sulfate
7.2. Bromocresol Green, indicator
7.3. Boiling stones
7.4. Hallow Glass Beads
7.5. 0.5% Methyl Red indicator
7.6. Sodium hydroxide, 50%
7.7. Sodium hydroxide, 0.5N, Certified
7.8. Water, Deionized (DI), Type II

8. REAGENT PREPARATION GUIDELINES

8.1. Reagent 01: 0.5N Sulfuric Acid (Standard Acid)

8.1.1. Directions: Add approximately 20L of DI Water into a 50L plastic carboy marked at 41L. Slowly add 580mL of sulfuric acid, bring to 41L with DI water. Bubble air through this solution until thoroughly mixed.

8.1.2. Titration of Sulfuric Acid Solution (Option 1): Dry tris-hydroxymethyl aminomethane (THAM) in oven at 105°C for at least 2 hours. Cool it down in the desiccator. Weigh 0.4500g of THAM into each of the six 125mL Erlenmeyer flasks. Add 5 drops of Bromocresol Green to each flask. Add approximately 50mL of DI water to each flask. The color of the solution should be blue. If not blue, add more bromocresol green a drop at time to the flask. Add 0.5N sulfuric acid solution to 25mL dispensing burette using a pipet. Slowly titrate THAM with sulfuric acid to bromocresol green end point (pH 4.7). Discard the highest and lowest results and average the remaining four results.

8.1.3. Determine the concentration (normality) of each of the six sulfuric acid solutions using the formula:

$$\boldsymbol{Acid\ Normality} = \frac{[\boldsymbol{THAM\ Weight\ (g)}]\ \boldsymbol{x\ 8.2549}}{\boldsymbol{Acid\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE{:}\ 8.2549} = \frac{\boldsymbol{1000mL/L\ x\ 2(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{121.14g/mol\ x\ 2(Stoichiometric\ Ratio)}}$$

8.1.4. Titration of Sulfuric Acid Solution (Option 2): Add 4mL of Reagent 01 to seven (7) 300mL beakers then fill them to approximately 250mL with water. Fill a separate 300mL beaker with 200mL of certified 0.5N NaOH; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.1.4.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Acid 1 – Acid 6.

8.1.5. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{A_N} = \frac{\boldsymbol{B_N\ x\ B_V}}{\boldsymbol{A_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.1.6. Discard the highest and lowest results and average the remaining results.

8.2. Reagent 02: 18.75N Stock Sodium Hydroxide Solution

8.2.1. Directions: Add 1 liter of deionized (DI) water into a 2L volumetric flask with stir bar in it on a stirring plate. Add 1500g of sodium hydroxide pellets in small (~100g) portions with continuous mixing. Bring solution volume to 2L with DI water.

8.3. Reagent 03: 0.35N Sodium Hydroxide (Standard Base)

8.3.1. Directions: Fill a 20L glass carboy, marked at 16L, half full of DI water. Add stir bar and start mixing. Add 300mL of stock sodium hydroxide solution to carboy while continuously mixing. Bring solution to a volume of 16L with DI water. Stir solution for approximately 30 minutes.

8.3.2. Titration of Sodium Hydroxide Solution (Option 1): Dry potassium acid phthalate (PAP) in oven at 105°C for at least 2 hours and then allow it to cool in desiccators. Take six 300mL beakers

and weigh approximately 0.8000g of PAP into each. Add 150mL of DI water into each of the six beakers. Allow PAP to dissolve. Titrate PAP solution on the QC_Titrate System.

8.3.3. Titration of PAP Solution on the QC-Titrator: Click on PAP icon. Type set name and click OK. Type the exact weights of PAP. Highlight the first line on time-table and click start. When titration is done, a hard copy is printed automatically.

8.3.4. Calculation of Sodium Hydroxide: Determine the concentration (normality) of the six sodium hydroxide solutions using the formula:

$$\boldsymbol{Base\ Normality} = \frac{[\boldsymbol{PAP\ Weight\ (g)}]\ \boldsymbol{x\ 4.8964}}{\boldsymbol{Base\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE}\text{: } \boldsymbol{4.8964} = \frac{\boldsymbol{1000mL/L\ x\ 1(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{204.23g/mol\ x\ 1(Stoichiometric\ Ratio)}}$$

8.3.5. Titration of Sodium Hydroxide Solution (Option 2): Add 4mL of Reagent 03 to seven (7) 300mL beakers. Fill a separate 300mL beaker with 200mL of the normalized solution from 8.1; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.3.5.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Base 1 – Base 6.

8.3.6. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{B_N} = \frac{\boldsymbol{A_N\ x\ A_V}}{\boldsymbol{B_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.3.7. Discard the highest and lowest results and average the remaining four results. Change base normality on Kjeldahl and Direct Excel spreadsheets for LIMS.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.7. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.2. DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs

10. SPECIFIC PROCEDURES

10.1. Weighing

10.1.1. Using ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template, create a laboratory identification number for the laboratory control sample (LS).

10.1.2. Weigh approximately 0.5g of the laboratory control standard (LS), ammonium sulfate.

10.1.3. Weigh samples to approximately 0.5 grams as well except liquid samples that have been diluted. Diluted liquid samples are approximately doubled in weight (i.e. approximately 1.00g).

10.1.4. Enter weight into Laboratory Information Management System (LIMS) using the template FERT_Nitrogen_Direct. *Be aware of the given acid trap volume because it will be used later in the process*.

10.1.5. Add 4 to 8 boiling stones or glass beads to each round bottom flask.

10.1.6. Rinse sample with deionized water into round bottom flask from weight container a minimum of 3 times. If sample is still present in weight container, continue to rinse.

10.1.7. Fill round bottom flasks half to approximately half way with tap water.

10.1.8. Turn on the fume hood and both sides of the cooling racks.

10.1.9. Preheat each distillation unit on high.

10.2. Acid Trap Setup

10.2.1. Measure approximately 10mL of 50% sodium hydroxide and set aside for each sample.

10.2.2. The preparation of the acid trap, using the acid dispenser or a class A pipette, differ depending on the specific sample.

10.2.2.1. For Instrument Blank:

10.2.2.1.1. Dispense 4mL of standard acid into 300mL beaker labeled blank.

10.2.2.1.2. Bring to the 250mL mark with deionized water.

10.2.2.1.3. Place the beaker, with sample, in slot 1 on the auto titrator.

10.2.2.2. For Laboratory Control Sample

10.2.2.2.1. Dispense 18mL of standard acid in 300mL beaker.

10.2.2.2.2. Bring to the 50mL mark with deionized water.

10.2.2.2.3. Add 1-2 drops of Methyl Red indicator. This is the LS.

10.2.2.3. For Sample:

10.2.2.3.1. Dispense the applicable volume of standard acid into the 300mL beaker. *This volume should be based upon the acid trap volume that was calculated in LIMS earlier in the process*.

10.2.2.3.2. Bring to the 50mL mark with deionized water.

10.2.2.3.3. Add 1-2 drops of methyl red indicator.

10.2.3. Make extra 4mL aliquots of standard acid. This standard acid can be added to distillation acid trap if indicator starts to turn orange/yellow.

10.3. Distillation

10.3.1. Place acid traps into its corresponding spots on the distillation rack with the tip of the distillation tube visibly submerged in the liquid.

10.3.2. Turn on cooling jackets and fume hood.

10.3.3. Set heaters on high and allow to heat up.

10.3.4. Swirl round bottom flasks vigorously to ensuring sample is mixed thoroughly.

10.3.5. Quickly add sodium hydroxide to each flask and immediately attach flask to the distillation column and place on heating rack.

10.3.6. Allow distillation to proceed until acid trap volume reaches 250mL.

NOTE: If the color starts to change to yellow/orange, add 4mL of the standard acid, write down how much extra acid was added to sample in notebook.

10.3.7. Remove the acid trap from the distillation column.

10.3.8. Turn off heating racks and fume hood once sample stop boiling.

10.3.9. Once samples have stopped boiling, turn off cooling jackets.

10.4. Titration

10.4.1. Start Pc titrate program.

10.4.2. Once program opens, load "Ammonium" template.

NOTE: The icon is two test tubes.

10.4.3. Delete any extra spaces from the template. The amount of spaces needed in the template is one more than the number of samples. If there are 11 samples there should be 12 spaces in template.

10.4.4. Enter sample identification numbers into template.

10.4.4.1. The blank should be in position 1. Label position 1 with the date and user initials.

10.4.4.2. The laboratory control sample should be in position 2.

10.4.5. Place acid trap into corresponding locations on auto titration rack.

10.4.6. Select position 1 in the template.

NOTE: This is where the auto titrator will start the titration process.

10.4.7. Make sure all acid traps correspond to their location on the template.

10.4.8. Click start and ensure that the titration process begins.

10.4.9. Initial, date and file the instrument's print out of titration results.

10.5. Data Evaluation

10.5.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.5.1.1. Evaluate the Lab Control sample results.

10.5.2. To determine if it is within acceptable limits refer to Data 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits.

10.6. Result Entry

10.6.1. Record the results (from the print out) into the FERT_NITROGEN_Direct template in the LIMS. A laboratory notebook may be used as an intermediate location for the results.

10.6.2. Any additional acid used during the distillation must be added to the acid trap value in the LIMS. This updated value will be used in calculations.

10.6.3. The raw data, from the auto titrator, is uploaded to the folder on the "\\tlhaeschrome" drive.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Boiling stones	Trash
Residue (from the filtration process)	Trash
Sample extract, excess	Pour waste into KJL waste container, then neutralize with acid. Flush neutralized waste down drain with running water.

12. REFERENCES

12.1. Calibration and use of QC-Titrate™ Titration system. FSFL-SOP 494, (2006/01/17)
12.2. Nitrogen (urea not present)-Direct FM-420, (2003/02/05)
12.3. Distillation. SOP-493, (2006/01/17)

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	11.16.2017	Original Version	Zachary Tower	
2.0	09.30/2020	Added section 8.0 Reagent Preparation Guide Updated Scope Updated Apparatus and Materials Updated Reagents and Media Minor clarifications throughout Updated disposal mechanism for sample extract		Katherine Malik

14. SIGNATURE HISTORY

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Wet Chemistry and Preparation Manager

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Katherine Malik
Editor

Date_____

METHOD 505

Sample Preparation and Analysis of Direct Ammoniacal Nitrogen for Sample with Urea Present [FM 415]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of direct ammoniacal nitrogen for samples that may contain urea or water soluble organic nitrogen using the Kjeldahl method. This is a direct method and does not undergo digestion.

2. SCOPE

2.1. The procedure delineated below is applicable to the direct determination of ammoniacal nitrogen in fertilizer samples that contain urea nitrogen by making the sample basic, distilling into an acid trap and titrating excess acid with a standard base.

2.2. Refer to BAEL METHOD 530 for an alternative sample preparation and analysis of direct ammoniacal nitrogen with urea present using the Buchi.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE that may be used for this procedure includes face shield and acid resistant apron.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weighing
4.2. 10.2 Acid Trap Setup
4.3. 10.3 Distillation
4.4. 10.4 Titration
4.5. 10.5 Data Evaluation
4.6. 10.6 Result Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Acid Dispenser
6.2. Auto Titration system
6.3. Balance
6.4. Beaker, 300mL
6.5. Class A Pipettes
6.6. Computer
6.7. Kjeldahl Distillation unit
6.8. Round bottom flask, 800mL
6.9. Weigh containers

7. REAGENTS and MEDIA

7.1. Ammonium sulfate
7.2. Bromocresol Green, indicator
7.3. Boiling stones
7.4. Hallow Glass Beads
7.5. Magnesium Oxide
7.6. 0.5% Methyl Red, indicator
7.7. Sodium hydroxide, 50%
7.8. Sodium hydroxide, 0.5N, Certified

7.9. Sulfuric Acid Reagent Grade
7.10. Water, Deionized (DI) Type II

8. REAGENT PREPARATION GUIDELINES

8.1. Reagent 01: 0.5N Sulfuric Acid (Standard Acid)

8.1.1. Directions: Add approximately 20L of DI Water into a 50L plastic carboy marked at 41L. Slowly add 580mL of sulfuric acid, bring to 41L with DI water. Bubble air through this solution until thoroughly mixed.

8.1.2. Titration of Sulfuric Acid Solution (Option 1): Dry tris-hydroxymethyl aminomethane (THAM) in oven at 105°C for at least 2 hours. Cool it down in the desiccator. Weigh 0.4500g of THAM into each of the six 125mL Erlenmeyer flasks. Add 5 drops of Bromocresol Green to each flask. Add approximately 50mL of DI water to each flask. The color of the solution should be blue. If not blue, add more bromocresol green a drop at time to the flask. Add 0.5N sulfuric acid solution to 25mL dispensing burette using a pipet. Slowly titrate THAM with sulfuric acid to bromocresol green end point (pH 4.7). Discard the highest and lowest results and average the remaining four results.

8.1.3. Determine the concentration (normality) of each of the six sulfuric acid solutions using the formula:

$$\boldsymbol{Acid\ Normality} = \frac{[\boldsymbol{THAM\ Weight\ (g)}]\ \boldsymbol{x\ 8.2549}}{\boldsymbol{Acid\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE: 8.2549} = \frac{\boldsymbol{1000mL/L\ x\ 2(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{121.14g/mol\ x\ 2(Stoichiometric\ Ratio)}}$$

8.1.4. Titration of Sulfuric Acid Solution (Option 2): Add 4mL of Reagent 01 to seven (7) 300mL beakers then fill them to approximately 250mL with water. Fill a separate 300mL beaker with 200mL of certified 0.5N NaOH; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.1.4.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Acid 1 – Acid 6.

8.1.5. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{A_N} = \frac{\boldsymbol{B_N\ x\ B_V}}{\boldsymbol{A_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.1.6. Discard the highest and lowest results and average the remaining results.

8.2. Reagent 02: 18.75N Stock Sodium Hydroxide Solution

8.2.1. Directions: Add 1 liter of deionized (DI) water into a 2L volumetric flask with stir bar in it on a stirring plate. Add 1500g of sodium hydroxide pellets in small (~100g) portions with continuous mixing. Bring solution volume to 2L with DI water.

8.3. Reagent 03: 0.35N Sodium Hydroxide (Standard Base)

8.3.1. Directions: Fill a 20L glass carboy, marked at 16L, half full of DI water. Add stir bar and start mixing. Add 300mL of stock sodium hydroxide solution to carboy while continuously mixing. Bring solution to a volume of 16L with DI water. Stir solution for approximately 30 minutes.

8.3.2. Titration of Sodium Hydroxide Solution (Option 1): Dry potassium acid phthalate (PAP) in oven at 105°C for at least 2 hours and then allow it to cool in desiccators. Take six 300mL beakers and weigh approximately 0.8000g of PAP into each. Add 150mL of DI water into each of the six beakers. Allow PAP to dissolve. Titrate PAP solution on the QC_Titrate System.

8.3.3. Titration of PAP Solution on the QC-Titrator: Click on PAP icon. Type set name and click OK. Type the exact weights of PAP. Highlight the first line on time-table and click start. When titration is done, a hard copy is printed automatically.

8.3.4. Calculation of Sodium Hydroxide: Determine the concentration (normality) of the six sodium hydroxide solutions using the formula:

$$\boldsymbol{Base\ Normality} = \frac{[\boldsymbol{PAP\ Weight\ (g)}]\ \boldsymbol{x}\ \boldsymbol{4.8964}}{\boldsymbol{Base\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE}\colon \boldsymbol{4.8964} = \frac{\boldsymbol{1000mL/L\ x\ 1(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{204.23g/mol\ x\ 1(Stoichiometric\ Ratio)}}$$

8.3.5. Titration of Sodium Hydroxide Solution (Option 2): Add 4mL of Reagent 03 to seven (7) 300mL beakers. Fill a separate 300mL beaker with 200mL of the normalized solution from 8.1; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the “Ammonium” template. When titration is complete initial, date, and file results.

8.3.5.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Base 1 – Base 6.

8.3.6. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{B_N} = \frac{\boldsymbol{A_N\ x\ A_V}}{\boldsymbol{B_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.3.7. Discard the highest and lowest results and average the remaining four results. Change base normality on Kjeldahl and Direct Excel spreadsheets for LIMS.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.7. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.2. DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs

10. SPECIFIC PROCEDURES

10.1. Weighing

10.1.1. Using ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template, create a laboratory identification number for the laboratory control sample (LS).

10.1.2. Weigh approximately 0.5g of laboratory control standard (LS), ammonium sulfate.

10.1.3. Weigh samples to approximately 0.5 grams except liquid samples that have been diluted. Diluted liquid samples are approximately doubled in weight (i.e. approximately 1.00g).

10.1.4. Enter weight into Laboratory Information Management System (LIMS) using the template FERT_Nitrogen_Direct. *Be aware of the given acid trap volume because it will be used later in the process*.

10.1.5. Add 4 to 8 boiling stones or glass beads to each round bottom flask

10.1.6. Rinse sample with DI water into round bottom flask from weigh container a minimum of 3 times. If sample is still present in weigh container, continue to rinse.

10.1.7. With the water hose adjacent to the distillation unit fill each round bottom to approximately half way.

10.1.8. Turn on the fume hood and both sides of the cooling racks.

10.1.9. Preheat each distillation unit on high.

10.2. Acid Trap Setup

10.2.1. Weigh approximately 6.5 grams of magnesium Oxide and set aside for each sample.

10.2.2. The preparation of the acid trap, using the acid dispenser or class A pipette, differ depending on the specific sample.

10.2.2.1. For Instrument Blank:

10.2.2.1.1. Dispense 4mL of standard acid into 300mL beaker labeled blank.

10.2.2.1.2. Bring to the 250mL mark with deionized water.

10.2.2.1.3. Place the beaker, with sample, in slot 1 on the auto titrator.

10.2.2.2. For Laboratory Control Sample

10.2.2.2.1. Dispense 18mL of standard acid in 300mL beaker.

10.2.2.2.2. Bring to the 50mL mark with deionized water.

10.2.2.2.3. Add 1-2 drops of Methyl Red indicator. This is the LS.

10.2.2.3. For Sample:

10.2.2.3.1. Dispense the applicable volume of standard acid into the 300-ml beaker. *This volume should be based upon the acid trap volume that was calculated in LIMS earlier in the process*.

10.2.2.3.2. Bring to the 50mL mark with deionized water.

10.2.2.3.3. Add 1-2 drops of methyl red indicator.

10.2.3. Make extra 4mL aliquots of standard acid. This standard acid can be added to distillation acid trap if indicator starts to turn orange/yellow.

10.3. Distillation

10.3.1. Place acid traps into their corresponding spots on the distillation rack with the tip of the distillation tube visibly submerged in the liquid.

10.3.2. Swirl round bottom flasks vigorously to ensure sample is mixed thoroughly.

10.3.3. Quickly add magnesium oxide to each flask and immediately attach flask to the distillation column and place on heating rack.

NOTE: Step must be performed quickly to avoid loss of ammonium gas.

10.3.4. Allow distillation to proceed until acid trap volume reaches 250mL.

NOTE: If the color starts to change to yellow/orange, add 4mL of the standard acid. Record how much extra acid is added in notebook.

10.3.5. Remove the acid trap from the distillation column and turn off heating racks, and once samples stop boiling, turn off fume hood and cooling jackets.

10.4. Titration

10.4.1. Start Pc titrate program.

10.4.2. Once program opens, load “Ammonium” template.

NOTE: The icon is two test tubes.

10.4.3. Delete any extra spaces from the template. The amount of spaces needed in the template is one more than the number of samples. If there are 11 samples there should be 12 spaces in template.

10.4.4. Enter sample identification numbers into template.

10.4.4.1. The blank should be in position 1. Label position 1 with the date and user initials.

10.4.4.2. The laboratory control sample should be in position 2.

10.4.5. Place acid trap into corresponding locations on auto titration rack.

10.4.6. Select position 1 in the template.

NOTE: This is where the auto titrator will start the titration process.

10.4.7. Make sure all acid traps correspond to their location on the template.

10.4.8. Click start and ensure that the titration process begins.

10.4.9. Initial, date and file the instrument’s print out of titration results.

10.5. Data Evaluation

10.5.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.5.1.1. Evaluate the Lab Control sample results.

10.5.2. To determine if it is within acceptable limits refer to Data 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits.

10.6. Result Entry

10.6.1. Record the results (from the print out) into the FERT_NITROGEN_Direct template in the LIMS. A laboratory notebook may be used as an intermediate location for the results.

10.6.2. Any additional acid used during the distillation must be added to the acid trap value in the LIMS. This updated value will be used in calculations.

10.6.3. The raw data, from the auto titrator, is uploaded to the folder on the “\\tlhaeschrome” drive.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Boiling stones	Trash
Residue (from the filtration process)	Trash
Sample extract, excess	Pour waste into KJL waste container. Flush waste down drain with running water.

12. REFERENCES

12.1. Calibration and use of QC-Titrate™ Titration system. FSFL-SOP 494, (2006/01/17)
12.2. Breakdown Nitrogen Preparation. FM-430, (2001/11/12)
12.3. Ammoniacal Nitrogen (urea present)-Direct. FM-415

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	11.16.2017	Original Version	Zachary Tower	
2.0	09.30.2020	Added section 8.0 Reagent Preparation Guide Updated Scope Updated Apparatus and Materials Updated Reagents and Media Minor clarifications throughout Updated disposal mechanism for sample extract Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Katherine Malik

14. SIGNATURE HISTORY

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Teresa Rygiel
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Taleya Williams
Wet Chemistry and Preparation Manager

Date_____

_____
Katherine Malik
Editor

Date_____

METHOD 506

Sample Preparation and Analysis of Direct Ammoniacal Nitrogen for Sample with Urea and Water Insoluble Nitrogen Present [FM 435]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the preparation and analysis of direct ammoniacal nitrogen using the Kjeldahl method. This is a direct method and does not undergo digestion.

2. SCOPE

2.1. The analytical procedure delineated below is applicable to the determination of direct ammoniacal nitrogen in fertilizer samples that contain urea and water insoluble nitrogen by making the sample basic, distilling into an acid trap and titrating excess acid with a standard base.

2.2. Refer to BAEL METHOD 530 for an alternative sample preparation and analysis of direct ammoniacal nitrogen with urea and water insoluble present using the Buchi.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE which can be used for this procedure includes face shield and acid resistant apron.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weighing and Filtering
4.2. 10.2 Acid Trap Setup
4.3. 10.3 Distillation
4.4. 10.4 Titration
4.5. 10.5 Data Evaluation
4.6. 10.6 Result Entry

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Acid Dispenser
6.2. Auto Titration system
6.3. Balance
6.4. Beaker, 300mL
6.5. Class A pipettes
6.6. Computer
6.7. Flask, round bottom 800mL
6.8. Flask, 250mL
6.9. Filter paper, 125mm
6.10. Funnel
6.11. Kjeldahl Distillation unit
6.12. Pipette or Graduated cylinder, 100mL
6.13. Stoppers
6.14. Weigh containers

7. REAGENTS and MEDIA

7.1. Granular Boiling stones
7.2. Hallow glass beads

7.3. Magnesium Oxide
7.4. Methanol
7.5. 0.5% Methyl Red indicator
7.6. Sodium hydroxide, 50%
7.7. Sodium hydroxide, 0.5N Certified
7.8. SRM-695
7.9. Sulfuric Acid Reagent Grade
7.10. Water, Deionized (DI) Type II

8. REAGENT PREPARATION GUIDELINES

8.1. Reagent 01: 0.5N Sulfuric Acid (Standard Acid)

8.1.1. Directions: Add approximately 20L of DI Water into a 50L plastic carboy marked at 41L. Slowly add 580mL of sulfuric acid, bring to 41L with DI water. Bubble air through this solution until thoroughly mixed.

8.1.2. Titration of Sulfuric Acid Solution (Option 1): Dry tris-hydroxymethyl aminomethane (THAM) in oven at 105°C for at least 2 hours. Cool it down in the desiccator. Weigh 0.4500g of THAM into each of the six 125mL Erlenmeyer flasks. Add 5 drops of Bromocresol Green to each flask. Add approximately 50mL of DI water to each flask. The color of the solution should be blue. If not blue, add more bromocresol green a drop at time to the flask. Add 0.5N sulfuric acid solution to 25mL dispensing burette using a pipet. Slowly titrate THAM with sulfuric acid to bromocresol green end point (pH 4.7). Discard the highest and lowest results and average the remaining four results.

8.1.3. Determine the concentration (normality) of each of the six sulfuric acid solutions using the formula:

$$\boldsymbol{Acid\ Normality} = \frac{[\boldsymbol{THAM\ Weight\ (g)}]\ \boldsymbol{x\ 8.2549}}{\boldsymbol{Acid\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE}\text{: } \boldsymbol{8.2549} = \frac{\boldsymbol{1000mL/L\ x\ 2(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{121.14g/mol\ x\ 2(Stoichiometric\ Ratio)}}$$

8.1.4. Titration of Sulfuric Acid Solution (Option 2): Add 4mL of Reagent 01 to seven (7) 300mL beakers then fill them to approximately 250mL with water. Fill a separate 300mL beaker with 200mL of certified 0.5N NaOH; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the "Ammonium" template. When titration is complete initial, date, and file results.

8.1.4.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Acid 1 – Acid 6.

8.1.5. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{A_N} = \frac{\boldsymbol{B_N\ x\ B_V}}{\boldsymbol{A_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.1.6. Discard the highest and lowest results and average the remaining results.

8.2. Reagent 02: 18.75N Stock Sodium Hydroxide Solution

8.2.1. Directions: Add 1 liter of deionized (DI) water into a 2L volumetric flask with stir bar in it on a stirring plate. Add 1500g of sodium hydroxide pellets in small (~100g) portions with continuous mixing. Bring solution volume to 2L with DI water.

8.3. Reagent 03: 0.25N Sodium Hydroxide (Standard Base)

8.3.1. Directions: Fill a 20L glass carboy, marked at 16L, half full of DI water. Add stir bar and start mixing. Add 300mL of stock sodium hydroxide solution to carboy while continuously mixing. Bring solution to a volume of 16L with DI water. Stir solution for approximately 30 minutes.

8.3.2. Titration of Sodium Hydroxide Solution (Option 1): Dry potassium acid phthalate (PAP) in oven at 105°C for at least 2 hours and then allow it to cool in desiccators. Take six 300mL beakers and weigh approximately 0.8000g of PAP into each. Add 150mL of DI water into each of the six beakers. Allow PAP to dissolve. Titrate PAP solution on the QC_Titrate System.

8.3.3. Titration of PAP Solution on the QC-Titrator: Click on PAP icon. Type set name and click OK. Type the exact weights of PAP. Highlight the first line on time-table and click start. When titration is done, a hard copy is printed automatically.

8.3.4. Calculation of Sodium Hydroxide: Determine the concentration (normality) of the six sodium hydroxide solutions using the formula:

$$\boldsymbol{Base\ Normality} = \frac{[\boldsymbol{PAP\ Weight\ (g)}]\ \boldsymbol{x\ 4.8964}}{\boldsymbol{Base\ Volume\ (mL)}}$$

$$\boldsymbol{NOTE}\text{: } \boldsymbol{4.8964} = \frac{\boldsymbol{1000mL/L\ x\ 1(Conversion\ from\ Molarity\ to\ Normality)}}{\boldsymbol{204.23g/mol\ x\ 1(Stoichiometric\ Ratio)}}$$

8.3.5. Titration of Sodium Hydroxide Solution (Option 2): Add 4mL of Reagent 03 to seven (7) 300mL beakers. Fill a separate 300mL beaker with 200mL of the normalized solution from 8.1; place the line of the titrator into the beaker and purge the syringe of the titrator a minimum of two times into a waste container. Load and run the “Ammonium” template. When titration is complete initial, date, and file results.

8.3.5.1. Enter the sample identification numbers into the template. The Blank will always be in Position 1. The rest of the beakers should be labeled Base 1 – Base 6.

8.3.6. Determine the concentration (normality) of each of the seven sulfuric acid solutions using the formula:

$$\boldsymbol{B_N} = \frac{\boldsymbol{A_N\ x\ A_V}}{\boldsymbol{B_V}}$$

Where: A_N=Acid Normality
A_V=Acid Volume
B_N=Base Normality
B_V=Base Volume

8.3.7. Discard the highest and lowest results and average the remaining four results. Change base normality on Kjeldahl and Direct Excel spreadsheets for LIMS.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.7. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Templates
9.2. DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs

10. SPECIFIC PROCEDURES

10.1. Weighing and Filtering

10.1.1. Label weigh containers making the first container the laboratory control sample. Ensure that the beakers, flask, distillation and auto titration sample slots are labeled corresponding to the weigh containers.

10.1.2. Using ADMIN 023 Attachment AA, Fertilizer QC Loc Codes and Result Entry Template, create a laboratory identification number for the laboratory control sample (LS).

10.1.3. Weigh approximately 0.5g of laboratory control standard (LS), SRM-695. Measure all solid samples to approximately 0.9 grams. Liquid samples should be doubled in weight to approximately 1.8 grams if they have been diluted.

10.1.4. Enter weight into Laboratory Information management system (LIMS) using the template FERT_Nitrogen_KJL and record the given acid trap volume in the laboratory notebook to use later.

10.1.5. Fold filters and place into funnels which drain into 250mL volumetric flasks. Wet sides of the filter paper with deionized water to create a seal for the filter.

10.1.6. Pour weighed samples into filter paper using deionized water to rinse the weight container clean of sample into filter paper.

10.1.7. If sample is clumping, use 2 to 5mL of methanol to break up clumping

10.1.8. Fill the filter paper up with DI water and allow to drain completely between washings. Repeat washing step 2 more times for a total of 3 washes.

10.1.9. Bring the filtrate receiving flask up to 250mL volume mark with DI water.

10.1.10. Put stopper in after flask is brought to volume to prevent evaporation.

10.1.11. Add 4 to 8 boiling stones or glass beads, to each round bottom flask

10.1.12. With filtrate from the filtering process measure out approximately 100mL of filtrate into a clean 100mL graduated cylinder or pipette and place into 800mL round bottom. (Make sure to invert filtrate bottle at least 7 times and shake thoroughly to insure sample is uniform before measuring).

10.1.13. With the water hose adjacent to the distillation unit, fill each round bottom to approximately half way.

10.1.14. Turn on the fume hood and both side of the cooling racks.

10.1.15. Preheat each distillation unit on high.

10.2. Acid Trap Setup

10.2.1. Measure approximately 6.5 grams of magnesium oxide and set aside for each sample.

10.2.2. The preparation of the acid trap, using the acid dispenser or class A pipette, differ depending on the specific sample.

10.2.2.1. For Instrument Blank:

10.2.2.1.1. Dispense 4mL of standard acid into 300mL beaker labeled blank.

10.2.2.1.2. Bring to the 250mL mark with deionized water.

10.2.2.1.3. Place the beaker, with sample, in slot 1 on the auto titrator.

10.2.2.2. For Laboratory Control Sample

10.2.2.2.1. Dispense 4mL of standard acid in 300mL beaker.

10.2.2.2.2. Bring to the 50mL mark with deionized water.

10.2.2.2.3. Add 1-2 drops of Methyl Red indicator. This is the LS.

10.2.2.3. For Sample:

10.2.2.3.1. Dispense the applicable volume of standard acid into the 300mL beaker.

NOTE: This volume should be based upon the acid trap volume that was calculated in LIMS earlier in the process.

10.2.2.3.2. Bring to the 50mL mark with deionized water.

10.2.2.3.3. Add 1-2 drops of methyl red indicator.

10.2.3. Make extra 4mL aliquots of standard acid. This standard acid can be added to distillation acid trap if indicator starts to turn orange/yellow.

10.3. Distillation

10.3.1. Place acid traps into their corresponding spots on the distillation rack with the tip of the distillation tube visibly submerged in the liquid.

10.3.2. Make sure both cooling jackets are turned on and working and exhaust fan is turned on.

10.3.3. Set heaters on high and allow time to heat up.

10.3.4. Swirl round bottom flasks vigorously insuring sample is mixed thoroughly and then add magnesium oxide to each flask.

10.3.5. Immediately after magnesium oxide is added attach flask to the distillation column and place on heating rack.

10.3.6. Allow distillation to proceed until acid trap volume reaches 250mL. Once volume is reached remove the acid trap for the distillation column and turn off heating racks. When samples stop boiling turn off exhaust vent and cooling jackets.

10.3.7. If acid trap starts to turn orange/yellow add extra 4mL of acid. Record how much extra acid is added in notebook.

10.4. Titration

10.4.1. Start “Pc titrate” program.

10.4.2. Once program opens, load “Ammonium” template.

NOTE: Icon is two test tubes.

10.4.3. In template, ensure there are only the amount of spaces plus one loaded in the template (e.g. if you have 11 samples there should be 12 spaces in template). Delete any extra spaces.

10.4.4. Position number 1 should always be the instrument blank. In the “Ammonium” template, label position 1 with the date and initials.

10.4.5. Enter LS in position 2 of the template.

10.4.6. Enter sample ID numbers into template.

10.4.7. Place acid trap beakers into corresponding locations on auto titration rack.

10.4.8. Select position 1 in the template (The selected position should be highlighted blue. This is where the auto titrator will start titration).

10.4.9. Click start and ensure that the titration proceed. Initial, date, and file readout.

10.5. Data Evaluation

10.5.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.5.1.1. Evaluate the Lab Control sample results.

10.5.2. To determine if it is within acceptable limits refer to DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits.

10.6. Result Entry

10.6.1. Record the results (from the print out) into the FERT_NITROGEN_KJL template in the LIMS. A laboratory notebook may be used as an intermediate location for the results.

10.6.2. Any additional acid used during the distillation must be added to the acid trap value in the LIMS. This updated value will be used in calculations.

10.6.3. The raw data, from the auto titrator, is uploaded to the folder on the "\\tlhaeschrome" drive.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Boiling stones	Trash
Residue (from the filtration process)	Trash
Sample extract, excess	Pour waste into KJL waste container. Flush waste down drain with running water

REFERENCES

11.2. FSFL-SOP 494 Calibration and use of QC-Titrate™ Titration system (2006/01/17)
11.3. FM-430 Breakdown Nitrogen Preparation (2001/11/12)
11.4. FM-435 Ammoniacal nitrogen (urea Present) Kjeldahl 02/04/2003

12. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	11.16.2017	Original Version	Zachary Tower	
2.0	09.30.2020	Added section 8.0 Reagent Preparation Guide Updated Scope Updated Apparatus and Materials Updated Reagents and Media Minor clarifications throughout Updated disposal mechanism for sample extract		Katherine Malik

13. SIGNATURE HISTORY

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Patricia Lucas
Bureau Chief, Technical Director

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1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for determination of acid soluble metals including total micronutrients and heavy metals (Aluminum, Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc) in mixed or pure material fertilizer samples in both liquid and dry matrices by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES).

2. SCOPE

2.1. The procedure delineated below is applicable to ICP-OES determination of acid soluble total micronutrients and heavy metals (Aluminum, Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc) in fertilizer. It is achieved by measuring the intensity of the electromagnetic radiation produced by the analyte in the inductively coupled plasma. A quantitative determination of the amount of analyte present can be made at the specific wavelength by measuring the excited atoms and ions emitted. The intensity of this emission is indicative of the concentration of the analyte within the sample. Acid match standards solutions are used to calibrate the ICP-OES. Cesium is used as an ionization buffer to minimize easily ionized element effects.

2.2. This method is restricted to use by or under the supervision of a trained analyst. Each analyst must demonstrate the ability to generate acceptable results with this method.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. Torch and interface regions of the ICP can be very hot and caution is needed when working around these areas if the ICP has recently been running. The ICP generates very high voltages. Specific safeties are in place on the instrument to protect the operator from accidental exposure to electrical shock; these safeties should not be subverted. Extreme caution should be used when performing any maintenance that would expose the operator to high voltages. Protective covers shall remain in place at all times except when special maintenance and/or repairs require their removal.

3.3. Exhaust from combustion fumes and vapors from the ICP torch housing can produce toxic vapors and may pose a health hazard if not adequately vented to the outside.

4. OUTLINE of PROCEDURE

4.1. 10.1 Standards
4.2. 10.2 Instrument and Computer Startup
4.3. 10.3 Recommended Instrument Parameters
4.4. 10.4 Sample Handling
4.5. 10.5 Sample Analysis
4.6. 10.6 Data Evaluation
4.7. 10.7 Quality Control
4.8. 10.8 Export Data
4.9. 10.9 Instrument Shutdown

5. INTERFERENCE

5.1. Spectral interferences are caused by background emissions from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, ionization interferences, or unresolved overlap of molecular band spectra. Interferences may be overcome by dilution of the digestate, application of interelement correction

factors, use of alternate wavelengths, or addition of cesium chloride as an ionization buffer to minimize easily ionized element effects. A target of at least 2200mg/kg cesium chloride solution must be presented at the nebulizer.

5.2. Yttrium is found in low levels in some phosphate ore sources and should not be used as an internal standard.

5.3. Samples containing Fe will interfere with the following wavelengths: As 193.696, Cd 214.439, Cd 226.502, Pb 220.353, Pb 217.000 and Se 196.026. Cr can interfere with As 188.980. Spectral deconvolution must be utilized if Fe and Cr are present in samples at levels of interference for the forementioned elements. Multicomponent Spectral Fitting (MSF) is utilized in this method according to the procedure below:

5.3.1. **MSF Modeling:** Spectra must be collected from a matrix blank (B), a standard of the analyte of interest (A), and a standard of the interfering analyte(s) (I) using the same analysis method that will be used to normally analyze samples.

Note: The Peak Algorithm must be set Peak Area or Peak Height within the analysis method. The MSF algorithm is not used during this time.

5.3.2. Spectral profile is independent of concentration, however, to optimize the MSF model, the concentration of the analyte should be approximately 100 times its detection limit at the affected wavelength and the concentration of the interfering analyte should approximate maximum concentration that could be present.

E.g., ~100 times the detection limit of Pb would be approximately 10 ppm. In this example, 10 ppm Pb standard was selected. A 1000 ppm Fe standard was selected to represent the maximum concentration of the interfering Fe (10% Fe in the sample).

Note: All Analytes must be in the correct matrix (same acid ratio as the extracts).

5.3.3. After the analysis of the blank, analyte, and interferent, build the MSF model using the Examine MSF window. Label the blank (B), analyte (A) and interferent (I) on the wavelength of interest (e.g., 213.617 nm). Once the MSF file is saved, Select MSF as the Peak Algorithm within the analysis method and specify which MSF file to be used on the Spectral Corrections tab.

5.3.4. Confirm the new MSF model is function as intended by reanalyzing the interferent and a known sample or standard such as a LS. The resulting interferent should not be detected at the wavelength of interest.

5.3.5. If a wavelength calibration is performed, the MSF must be recreated.

Note: Wavelength calibration is performed during the annual PM.

6. APPARATUS and MATERIAL

6.1. Analytical Instruments:
- 6.1.1. Dual view ICP-OES, Perkin Elmer Optima 5300 DV and 8300 DV or equivalent.
- 6.1.2. Autosamplers; SC4 DX and SC2 DX or equivalent
- 6.1.3. Peristaltic Pump capable of handling three introduction lines
- 6.1.4. Chiller: PolyScience or equivalent

6.2. Computer system capable of operating integration software which interfaces with the ICP and acquires and stores all the required data.

6.3. Pump and tubings: black/black, grey/grey, orange/green, red/red

6.4. Volumetric flasks
6.5. Volumetric pipettes

7. REAGENTS and MEDIA

7.1. Cesium Chloride (CsCl), Trace metal grade
7.2. Hydrochloric Acid (HCl) concentrated, Trace metal grade
7.3. Nitric Acid (HNO_3) concentrated, Trace metal grade
7.4. Triton X-100
7.5. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Carrier Solution

8.1.1. Directions: Add 200mL deionized water in 1L volumetric flask, add 90mL of HNO_3 and 30mL HCl and swirl to mix. Fill the flask with deionized water to the mark, invert to mix.

Optional: 1-2 drops of Triton X-100 may be added. (Triton X -100 may cause beading in some spray chambers and should be omitted in these cases.)

8.2. Wash Solution

8.2.1. Directions: Add approximately 5L DI water to a 15L carboy, add 307mL HCl, swirl to mix. Add DI water to the 15L mark, cap the carboy, and shake to mix.

Optional: 7.5mL of Triton X-100 may be added. (Triton X -100 may cause beading in some spray chambers and should be omitted in these cases.)

8.3. Internal Standard / Ionization Buffer

8.3.1. Directions: Add approximately 1500mL DI water to a 2000mL volumetric flask. Add 24g CsCl and swirl to mix. Add 10 mL of 1000 ppm Scandium standard solution and 40mL of 1000 ppm Beryllium standard solution (the final concentration of the Internal Standards are 5.0 ppm Sc and 20 ppm Be). Add 80mL of HNO_3 and swirl. Cap and invert several times to mix.

Optional: 1-2 drops of Triton X-100 may be added. (Triton X -100 may cause beading in some spray chambers and should be omitted in these cases.)

8.3.2. Expiration is 6 months from preparation date.

8.4. 9% Nitric Acid: 3% Hydrochloric Acid

8.4.1. Directions: Add 200mL deionized water in 1L volumetric flask, add 90mL of HNO_3 and 30mL HCl and swirl to mix. Fill the flask with deionized water to the mark, invert to mix.

8.5. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.6. The specific ingredient amounts can vary to accommodate the batch size.

8.7. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.8. Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Using the Laboratory Information Management System
9.2. ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer
9.3. ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer
9.4. DATA 050, Data Control
9.5. DATA 051, Data Evaluation for Chemical Analysis
9.6. DATA 051 Attachment C, Fertilizer Continuing Calibration Verification Acceptance Limits
9.7. DATA 051 Attachment A, Fertilizer Analysis Requirements
9.8. LABOP 140 Maintenance, Operation, and Program of ICPOES, Attachment I
9.9. QUALITY 112, Quality Control

10. SPECIFIC PROCEDURES

10.1. Standards

10.1.1. Primary standards and internal standards (1000ppm, 5000ppm or 10,000ppm) shall be prepared from neat material or purchased through certified vendors that are NIST traceable.

10.1.2. Working standards are to be used for ICP calibration and unknown sample identification and quantification. They are made by diluting with 9% HNO_3: 3% HCl to obtain the suggested concentrations.

10.1.3. Recommended Standard Levels:

10.1.3.1. Calibration Standards: The tables below contain the recommended calibration curve levels. Other levels may be used providing they are within the instrument's linear range.

NOTE: Continuing Calibration Verification Standards (CCV's) are evaluated for each curve separately.

10.1.3.1.1. Recommended Standards Levels:

	Standard Level (ppm) Low Curve				
Analyte	1	2	3	4	5
As	0.10	0.5	5.0	10	20
Ca	0.10	0.5	5.0	10	20
Cd	0.10	0.5	5.0	10	20
Co	0.10	0.5	5.0	10	20
Cr	0.10	0.5	5.0	10	20
Cu	0.10	0.5	5.0	10	20
Fe	0.10	0.5	5.0	10	20
Mg	0.10	0.5	5.0	10	20
Mn	0.10	0.5	5.0	10	20
Mo	0.10	0.5	5.0	10	20
Ni	0.10	0.5	5.0	10	20
Pb	0.10	0.5	5.0	10	20
Se	0.10	0.5	5.0	10	20
Zn	0.10	0.5	5.0	10	20

Standard Level (ppm) High Curve					
Analyte	6	7	8	9	10
Ca	50	100	300	500	600
Fe	50	100	300	500	600
Mg	50	100	300	500	600
Mn	50	100	300	500	600
Zn	50	100	300	500	600

10.2. Instrument and Computer Startup

10.2.1. See LABOP 140 Maintenance, Operation, and Program of ICPOES, Attachment I for details.

10.2.2. Turn on the plasma

10.2.2.1. Allow the system to stabilize for a minimum of 30 minutes.

10.3. Recommended Instrument Parameters

10.3.1. The following parameters may vary for different ICP makes and models and therefore are listed as recommended and may require minor modifications in order to optimize performance.

10.3.2. For Perkin Elmer Optima 5300 DV

Plasma Flow	15 L/min
Auxiliary Flow	0.2 L/min
Nebulizer Flow	0.65 L/min
RF Power	1450 watts
Autosampler Flow Rate	0.8 mL/min
Carrier Tubing	Black/Black
Internal Std. Tubing	Orange/Green
Waste Tubing	Red/Red
Wash	0.8 mL/min
Spray Chamber	Cyclonic
Loop Size	1.0 mL
Replicate Reads	3

10.3.3. For Perkin Elmer Optima 8300 DV

Plasma Flow	15 L/min
Auxiliary Flow	0.2 L/min
Nebulizer Flow	0.65 L/min
RF Power	1450 watts
Autosampler Flow Rate	0.44 mL/min
Carrier Tubing	Black/Black
Internal Std. Tubing	Orange/Green
Waste Tubing	Grey/Grey
Wash	0.44 mL/min
Spray Chamber	Cyclonic
Loop Size	1.5 mL
Replicate Reads	3

10.3.4 Recommended Wavelengths and Plasma View:

Element	Wavelength nm	Plasma View
As	188.979*	Axial
As	193.696	Axial
Be	313.107	Axial/Radial
Be	234.861	Axial/Radial
Ca	317.933*	Radial
Ca	315.887	Radial
Cd	228.802*	Axial
Cd	214.44	Axial

Co	228.616*	Axial
Co	238.892	Axial
Cr	267.716*	Axial
Cr	205.56	Axial
Cu	327.393*	Radial
Cu	324.752	Radial
Fe	238.204*	Radial
Fe	239.562	Radial
Mg	285.213*	Radial
Mg	279.077	Radial
Mn	257.610*	Radial
Mn	259.372	Radial
Mo	202.031*	Axial
Mo	203.845	Axial
Ni	231.604*	Axial
Ni	221.648	Axial
Pb	220.353*	Axial
Pb	217	Axial
Sc	361.383	Axial/Radial
Se	196.026*	Axial
Se	203.985	Axial
Zn	206.200*	Radial
Zn	213.857	Radial
Y	371.029*	Axial

10.4. Sample Handling

10.4.1. Sample extracts are filtered during the preparation procedure. Extracts that contain excessive particulates or cloudy extracts, should be re-filtered or centrifuged before analysis.

10.5. Sample Analysis

10.5.1. Use the Sample Information File (SIF) to create the sequence.

10.5.2. An instrument blank must be injected at the beginning of a sample sequence to ensure that the system is free of contaminants.

10.5.3. After the initial calibration, there must be a continuing calibration check standard analyzed after every 10 samples and at the end of the run.

10.5.4. The Laboratory Control Samples (LCS) should be analyzed with their associated samples, not grouped together at the beginning of the sequence.

10.5.5. The sequence requirements are found in, DATA 051 Attachment A, Fertilizer Analysis Requirements.

10.5.6. The sequence must end with a CCV.

10.5.7. Samples require the proper laboratory information management system (LIMS) codes to allow required uploading. Refer to ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer, for additional information.

10.5.7.1. Example: AA99999-0AT

10.5.7.1.1. 0 corresponds to the sample weight (0, 1, 2.)
10.5.7.1.2. A corresponds to the different injections due to dilutions (A=1st injection, B=2nd injection, C=3rd injection.)
10.5.7.1.3. T or H corresponds to analysis type (T=Totals and H=Heavy Metals)

10.5.8. Dilution factors must be entered into the instrument sequence.

10.6. Data Evaluation

10.6.1. Refer to DATA 051, Data Evaluation for Chemical Analysis, to evaluate the data against the established criteria.

10.7. Quality Control

10.7.1. Initial Calibration:

10.7.1.1. The calibration is based on plotting the ratio of the analyte response to the internal standard response as a function of the analyte concentration of the standards.

10.7.1.2. The correlation coefficient of the calibration curve R value must be ≥0.999 or the R^2 value must be ≥0.998.

10.7.1.3. Refer to DATA 051, Data Evaluation for Chemical Analysis; for additional calibration procedures.

10.7.2. Continuing Calibration Check Standard:

10.7.2.1. Once the initial calibration has passed, a continuing calibration check standard must be analyzed after every 10 samples and at the end of the sequence. CCVs must be run for each separate curve.

10.7.2.2. CCV acceptance criteria are listed below. Addition information may be found in DATA 051 Attachment C, Fertilizer Continuing Calibration Verification Acceptance Limits.

Analyte	CCV Limit (%)
As	±10
Ca	±4
Cd	±10
Co	±10
Cr	±10
Cu	±8
Fe	±8
Mg	±4
Mn	±8
Mo	±4
Ni	±10
Pb	±10
Se	±10
Zn	±8

10.7.2.3. The CCV may consist of any concentration level used in the initial calibration. Levels near the middle of the curve are recommended.

10.7.2.4. If a CCV response is not within the limits, all samples analyzed before and after it must be reanalyzed.

10.7.2.5. If upon reanalysis of the CCV, the response is still not within the above acceptance criteria, then a new initial calibration must be performed. It is recommended that routine instrument maintenance be performed prior to the new curve analysis.

10.7.3. If the ppm reading exceeds the calibration range, then the sample must be diluted and reanalyzed prior to quantification. The sample should be diluted with 9% HNO_3 and 3% HCl to maintain the appropriate acid proportion.

10.7.4. Internal Standard criteria for samples is 80% - 120% recovery.

10.7.5. %RSD for the sample replicates shall not exceed 10%.

10.7.6. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples and for a description of quality control (QC) samples required in the batch.

10.8. Export Data

10.8.1. Data Manager exports data to the P:Drive. Refer to DATA 050, Data Control.

10.8.2. Upload data from the P:Drive to the LIMS. Refer to ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer.

10.9. Instrument Shutdown

10.9.1. See LABOP 140 Attachment I: Maintenance, Operation, and Program of ICPOES, for details.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Sample digests	Sample digests are good to analyze for up to 6 months and may be disposed of after analysis unless there is limited sample volume; then they may need to be kept for up to 90 days after laboratory report has been issued. Non-hazardous sample digests are disposed of down a sink while flushing with water.
Instrument Waste	Disposed of down a sink while flushing with water.
Non-hazardous, calibration standards, tune standards, stock standards and internal standards.	Disposed of down a sink while flushing with water.
Samples or standards containing antimony, arsenic, cadmium, chromium, molybdenum, lead, thallium above 100 ppm	Labeled as hazardous waste and are disposed of into hazardous waste container labeled for metals.

12. REFERENCES

12.1. Preparation and Analysis of Acid Soluble (Total) Micro Nutrients by ICP-OES, FSFL-SOP 504 (04/15/2009).
12.2. U.S. EPA. 1994. "Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4.4. Cincinnati, OH **External 00131**
12.3. Analysis of Water-Soluble Mg and Potash, BAEL_SOP 201 FSFL-SOP 518 (04/15/2009).
12.4. Operation and Maintenance of Perkin Elmer Inductively Coupled Plasma – Optical Emission Spectrometry 3300 Dual View System, FSFL-SOP 525 (16/07/2007).
12.5. *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, Method **2017.02,** External 00135.

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	04.04.2017	Original Version	Naser Tassobi	
2.0	05.17.2019	Updated Outline Format, Updated Title, Updated Outline, Added Section 8, Added required documents to Associated Documents, Added section 10.6 Updated Curve Tables, Verified and Updated wavelength table, Moved maintenance, operation, and procedure of the instrument to LABOP 140 attachment I Added Section 11, Waste Managements		Christian Amason .
3.0	09.30.2020	Updated Purpose, Scope and Health and Safety Updated Interference Section Updated Curve Tables Updated Reagents and Media Updated Reagent Preparation Guidelines Updated title for DATA 051 through out Updated Instrument and Computer Startup Updated Recommended Instrument Parameters Updated Recommended Wavelengths and Plasma View Updated Quality Control Updated References		William Meeks Jr.
4.0	10.16.2023	Added external document number to external references Added section 5.3 to document interferences.		William Meeks Jr.

METHOD 510
Sample Preparation and Analysis of Chloride in Fertilizer
[FM 611]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of chloride in fertilizer using an Automatic Titrator.

2. SCOPE

2.1. The procedure delineated below is applicable to determining chlorine in fertilizer samples. Chloride is extracted from the fertilizer by boiling in water. The sample is then titrated to an end point with silver nitrate. The end point of the titration is reached when all of the chloride ions are precipitated.

$$AgNO_3\,(aq) + Cl^-\,(aq) \rightarrow AgCl\,(s)$$

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Sample Preparation
4.2. 10.2 Standardization of Silver Nitrate
4.3. 10.3 Analysis
4.4. 10.4 Calculations

5. INTERFERENCE

5.1. AgCl precipitate will deposit on the electrode. The electrode's response will slow down and may become erratic. Care must be taken to clean the electrode by wiping the electrode with a wet paper tissue and/or by using a 0.1% NH_3 wash. Silver, in the presence of an ammonia solution will become $Ag[(NH_3)_2]^+$, silver(I)diammine ion, assisting with the AgCl cleaning.

6. APPARATUS and MATERIAL

6.1. Mettler Toledo Excellence Titrator T5 or equivalent
6.2. Mettler Toledo DMi141-SC silver ring electrode or equivalent
6.3. 100mL plastic beakers
6.4. 1L glass (amber) container
6.5. 50mL digestion tube
6.6. Eppendorf pipette or equivalent

7. REAGENTS and MEDIA

7.1. Type I-DI water
7.2. 0.1% Ammonia wash solution
7.3. Electrolyte Solution KNO_3 1 mol/L (potassium nitrate)
7.4. Silver Nitrate Solution N/10 (protect from light)
7.5. Standard 0.1M NaCl, or KCl

8. REAGENT PREPARATION GUIDELINES

8.1. 0.1% Ammonia wash solution
  8.1.1. ~1mL Ammonia Hydroxide to 1L DI water.

8.2. Purchased Electrolyte Solution KNO_3 1 mol/L (potassium nitrate)

8.3. Purchased Silver Nitrate Solution N/10 (protect from light)

8.4. Standard 0.1M NaCl, or 0.1M KCl Standard Solution (Purchased or made from salt)

8.4.1. 0.1M KCl Standard Solution

8.4.1.1. Dry KCl at 106°C for approximately 4 hours, weigh 7.455g of dried KCl into 1L volumetric flask, bring to volume with DI water.

8.4.2. 0.1M NaCl Standard Solution

8.4.2.1. Dry NaCl at 106°C for approximately 4 hours, weigh 5.844g of dried NaCl into 1L volumetric flask, bring to volume with DI water.

8.5. Label the containers appropriately, label L003 indicates required information. Add relevant information to Reagent Preparation, form F00015

8.6. The specific ingredient amounts can vary to accommodate the batch size.

8.7. Expiration Date: 12 months from date of preparation, unless specifically stated in the reagent directions or from manufacturer.

8.8. Store at room temperature, unless specifically stated in the reagent directions

9. ASSOCIATED DOCUMENTS

9.1. LABOP 121, Maintenance, Operation and Program of Balance
9.2. Label L003, Reagent Label
9.3. Form F00015, Reagent Preparation Form

10. SPECIFIC PROCEDURES

10.1. Sample Preparation

10.1.1. Weighing sample

10.1.1.1. Label 50mL tubes with the sample number

10.1.1.2. Weigh 0.20g of sample in labeled 50mL digestion tube.

10.1.1.3. Add approximately 30mL of DI water (Type I DI water is required)

10.1.1.4. Digest 60 minutes on hot block at ~106 C

10.1.1.5. Let cool to room temperature

10.1.1.6. Transfer the sample from the 50mL digestion tube to a 100mL beaker. Ensure that the sample has been completely transferred, by rinsing the digestion tube 3 times with deionized water to the beaker.
NOTE: Use of small rinse volumes is recommended, so that the total volume does not exceed 60mL.

10.2. Standardization of Silver Nitrate

10.2.1. Pipette three 2mL aliquots of the 0.1M NaCl (or 0.1M KCl) standard solution into separate 100mL beakers.

10.2.2. Bring each to approximately 40mL with DI water.

10.2.3. Prime the $AgNO_3$ burette.

10.2.4. Open the Mettler LabX Software.

10.2.5. Titrate with 0.1M $AgNO_3$, using the instrument method titled “Standardization 0.1M $AgNO_3$ with Cl Standard”

10.2.6. Enter the volume of the NaCl (or KCl) standard solution into the corresponding sample position.

10.2.7. At the end of the run, the 0.1M $AgNO_3$ titrant is automatically standardized using the titer factor found above.

10.3. Analysis

10.3.1. Prime the $AgNO_3$ burette.

10.3.2. Open the Mettler LabX Software.

10.3.3. Titrate samples with 0.1M $AgNO_3$ using the instrument method titled “BAEL Method 510”.

10.3.4. Enter the sample ID and weight into the corresponding sample position.

10.3.5. Enter % found into the LIMS.

10.4. Calculations

10.4.1. Standardization of $AgNO_3$

10.4.1.1. Determination of Titer with Liquid Cl

$$\text{Titer} = \frac{c_{(Cl)} \times V_{(Cl)}}{c_{(AgNO3)} \times V_{EQ}}$$

Symbol	Definition	Mettler Symbol
$c_{(Cl)}$	Concentration of Cl standard (mol/L)	cst
$V_{(Cl)}$	Volume of Cl standard (mL)	m
$c_{(AgNO3)}$	Concentration of $AgNO_3$ titrant (mol/L)	c
V_{EQ}	Volume of titrant consumed (mL)	VEQ

10.4.1.2. Determination of Titer with Solid KCl*

$$\text{Titer} = \frac{m_{(KCl)}}{c_{(AgNO3)} \times V_{EQ} \times MW_{(KCl)}}$$

Symbol	Definition	Mettler Symbol
$m_{(KCl)}$	mass of KCl standard (mg)	m
$c_{(AgNO3)}$	Concentration of $AgNO_3$ titrant (mol/L)	c
V_{EQ}	Volume of titrant consumed (mL)	VEQ
$MW_{(KCl)}$	molecular weight of KCl (g/mol)	M

*If using other solid standards, substitute the appropriate standard in place of KCl. e.g. using a solid NaCl standard, replace KCl concentration and molecular weight for that of NaCl.

10.4.2. % Cl Sample Calculations

$$\% \text{ Cl} = \frac{c_{(AgNO3)} \times V_{EQ} \times \text{Titer} \times MW_{(Cl)} \times 100}{m_{(sample)} \times 1000}$$

Symbol	Definition	Mettler Symbol
$c_{(AgNO3)}$	Concentration of AgNO3 titrant (mol/L)	c
V_{EQ}	Volume of titrant consumed (mL)	VEQ
$m_{(sample)}$	mass of sample (g)*	m
	*When aliquot of a sample extract is used $m_{(sample)} = \frac{\text{weight}_{(g)} \times \text{aliqout}_{(mL)}}{\text{extraction vol}_{(mL)}}$	
Titer	Titer correction factor from standardization	TITER
$MW_{(Cl)}$	molecular weight of Cl (35.4527g/mol)	M

10.4.3. Mettler Titrator Formula

$$\% \text{ Cl} = \frac{Q \times C}{m}$$

Mettler Symbol	Definition
Q	mmol of titrant consumed (VEQ*c*TITER)
C	constant (M/10)

11. WASTE MANAGEMENT

Sample waste from the titration will contain silver chloride precipitate along with some free silver ions. NaCl can be added to this waste at a concentration of 0.1% (1g NaCl / 1 L waste, then invert bottle to ensure reaction), to precipitate any remaining silver in solution. The AgCl precipitate can then be filtered and submitted to hazardous waste. The aqueous portion may be disposed of as non-hazardous waste.

12. REFERENCES

12.1. E00154, *Chlorine (water soluble) in Fertilizer*. Method 928.02A, AOAC 15th Edition.
12.2. E00165, Potash Analysis, Chlorine. FM-611, December 9, 2009
12.3. E00166, Operation and Maintenance of Mettler Titrator. FSFL-SOP 528, Rev 1.0

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	10.17.2018	Original Version	William Meeks	
2.0	12.09.2020	Corrected typo in section 1 Corrected numbering in section 4 Replaced Elizabeth Schneider with Quentin Cooper in Signature History		Taleya Williams
3.0	02/08/2024	Added option to purchase KCl standard solution Added instructions for creating NaCl standard solution		Wiliiam Meeks Jr. Patrizia Lemma, Ph. D.

METHOD 513

Analysis of Water Soluble Potash, Water Soluble Magnesium, and Water Soluble Boron using Inductively Coupled Plasma Optical Emission Spectrometry [FM604, FM803]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of water soluble potash (K_2O), water soluble Magnesium (Mg), and water soluble Boron (B) in mixed or pure material fertilizer samples in both liquid and dry matrices by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES).

2. SCOPE

2.1. The procedure delineated below is applicable to an ICP-OES determination of water soluble potash, water soluble magnesium, and water soluble boron in fertilizer and is achieved by measuring the intensity of the electromagnetic radiation produced by the analyte in the inductively coupled plasma. A quantitative determination of the amount of analyte present can be made at the specific wavelength by measuring the excited atoms and ions emitted. The intensity of this emission is indicative of the concentration of the analyte within the sample. The determined amount of potassium (K) is converted to potash (K_2O equivalent) and reported as such.

2.2. Fertilizer samples analyzed for Mg and K_2O are prepared by digesting the sample according to PREP 454, Sample Preparation for Water Soluble Potash and Magnesium Analysis.

2.3. Fertilizer samples analyzed for B are prepared by digesting the sample according to PREP 455, Sample Preparation for Water Soluble Boron Analysis.

2.4. This method is restricted to use by or under the supervision of a trained analyst. Each analyst must demonstrate the ability to generate acceptable results with this method. Refer to ADMIN 016, Training and Competency, for demonstration of competence procedures.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. 10.1 Sample Handling
4.2. 10.2 Standards
4.3. 10.3 Instrument Startup
4.4. 10.4 Sample Analysis
4.5. 10.5 Export Data
4.6. 10.6 Instrument Shutdown
4.7. 10.7 Data Reduction and Reporting
4.8. 10.8 Quality Control
4.9. 10.9 Instrument Blank

5. INTERFERENCE

5.1. Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, ionization interferences (for K), or unresolved overlap of molecular band spectra. Interferences may be overcome by dilution of the digestate, application of interelement correction factors, use of alternate wavelengths, or addition of cesium chloride ionization buffer (for K). A target of at least 2200mg/kg cesium chloride solution must be presented at the nebulizer.

6. APPARATUS and MATERIAL

6.1. Analytical Instruments

6.1.1. Dual view ICP-OES
6.1.2. Autosampler
6.1.3. Peristaltic Pump capable of handling three introduction lines
6.1.4. Chiller
6.1.5. Integration software

6.2. ICP autosampler tubes
6.3. Pump tubing: black/black, orange/green, red/red
6.4. Volumetric flasks
6.5. Volumetric pipettes
6.6. 0.45µm Syringe Filters

7. REAGENTS and MEDIA

7.1. Cesium Chloride (CsCl), Trace metal grade, solid
7.2. Hydrochloric Acid (HCl) concentrated, Trace meal grade
7.3. Nitric Acid (HNO_3) concentrated, Trace metal grade
7.4. Triton X-100
7.5. Scandium Solution, 1000 ppm
7.6. Water, Deionized (DI), Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Carrier Solution

8.1.1. Directions: Fill a 1L volumetric flask almost to volume with DI water. Add 1-2 drops Triton X-100, then fill to the mark with DI water. Some spray chambers do not work with the Triton X. It may be left out in those cases. Invert flask several times to mix.

8.2. Wash Solution

8.2.1. Directions: Fill the 15L carboy with approximately 14 liters of DI water. Add approximately 7.5mL Triton X-100 (optional) and 307mL HCl and fill to the mark with DI water. Some spray chambers do not work with the Triton X. It may be left out in those cases. Swirl to mix.

8.3. Internal Standard / Ionization Buffer

8.3.1. Directions: Add approximately 1500mL DI water to a 2000mL volumetric flask. Add 24g CsCl and swirl to mix. Add 10mL of 1000ppm Scandium Internal Standard Solution (the final concentration of scandium Internal Standard is 5.0ppm). Add 80mL of HNO_3 and swirl to mix. Add 1-2 drops Triton X-100 (optional). Some spray chambers do not work with the Triton X. It may be left out in those cases. Fill to volume with DI water. Cap and invert to mix.

8.3.2. Expiration is 6 months from preparation date.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation unless otherwise indicated.

8.7. Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment M Instrument Sequence Generation for Fertilizer
9.2. ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer

9.3. DATA 051, Data Evaluation for Feed, Fertilizer, and Pesticide Programs
9.4. DATA 051 Attachment A, Fertilizer Analysis Requirements
9.5. DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptance Limits
9.6. DATA 051 Attachment C, Continuing Calibration Verification Acceptance Limits for AA and ICP
9.7. LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES
9.8. PREP 454, Sample Preparation for Water Soluble Potash and Magnesium Analysis
9.9. PREP 455, Sample Preparation for Water Soluble Boron Analysis
9.10. QUALITY 112, Quality Control
9.11. SAFETY 002, Waste Management

10. SPECIFIC PROCEDURES

10.1. Sample Handling

10.1.1. Sample extracts containing particulates should be filtered before analysis.

10.1.2. Aqueous sample digestates may be held for up to two weeks after digestion.

10.2. Standards

10.2.1. Primary standards and internal standards (1000ppm or 10,000ppm) should be prepared from neat material or purchased through certified vendors that are NIST traceable.

10.2.2. Working standards are to be used for ICP calibration and unknown sample identification and quantification. They are made by diluting into DI water to obtain the following concentrations.

10.2.3. Recommended Standard Levels:

Standard Level	Concentration (ppm) K	Concentration (ppm) Mg	Concentration (ppm) B
Low	**Curve**		
1	-	0.5	0.5
2	5	2.5	2.5
3	10	5	5
4	20	10	10
5	50	-	-
High	**Curve**		
4	-	10	10
5	50	25	25
6	100	50	50
7	200	100	100
8	400	200	200
9	600	-	-

10.3. Instrument Startup

10.3.1. See LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES

10.3.2.

Element	Wavelength nm	Plasma View
K	766.490*	Radial
K	404.721	Radial
B	249.677*	Axial
B	249.772	Axial
Mg	285.213*	Axial/Radial
Mg	279.077	Axial/ Radial
Sc	361.383*	Axial/Radial
*Primary Wavelength		

10.3.3.

	PE Optima 5300	PE Optima 8300
Plasma Flow	15 L/min	12 L/min
Auxiliary Flow	0.2 L/min	0.2 L/min
Nebulizer Flow	0.65 L/min	0.68 L/min
RF Power	1450 watts	1450 watts
Peristaltic Pump Flow Rate	0.8 mL/min	0.44 mL/min
Carrier Tubing	Black/Black	Black/Black
Internal Std. Tubing	Orange/Green	Orange/Green
Waste Tubing	Red/Red	Red/Red

10.4. Sample Analysis

10.4.1. Use the Sample Information File (SIF) to create the sequence.

10.4.2. An instrument blank must be injected at the beginning of a sample sequence to ensure that the system is free of contaminants.

10.4.3. After the initial calibration, there must be a continuing calibration check standard analyzed after every ten samples and at the end of the run.

10.4.4. The Laboratory Control Samples (LCS) must be analyzed with their associated samples, not grouped together at the beginning of the sequence.

10.4.5. The sequence requirements are found in, DATA 051 Attachment A, Fertilizer Analysis Requirements.

10.4.6. The sequence must end with a CCV. The ending CCV should be at a different concentration than the mid-sequence CCVs.

10.4.7. Samples require the proper laboratory information management system (LIMS) codes to allow required uploading. Refer to ADMIN 023 Attachment M Instrument Sequence Generation for Fertilizer for additional information.

10.4.7.1. Example: AA99999-0AS

10.4.7.1.1. 0 corresponds to the sample weight (0, 1, 2.)
10.4.7.1.2. A corresponds to the different injections due to dilutions (A=1st injection, B=2nd injection, C=3rd injection.)
10.4.7.1.3. S corresponds to analysis type (S=soluble)

10.4.8. Dilution factors must be entered into the instrument sequence.

10.4.9. Initial Calibration:

10.4.9.1. The calibration curve is based on response area versus concentration in parts per million (ppm) of analyte.

10.4.9.2. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs; for calibration procedures and acceptance criteria.

10.4.9.3. %RSD for the sample replicates shall not exceed 10%.

10.4.10. Continuing Calibration Check Standard:

10.4.10.1. Refer to Data 051 Attachment C, Fertilizer Continuing Calibration Verification Acceptance Limits, for acceptance criteria.

10.4.10.2. Once the initial calibration has passed, a continuing calibration check standard must be analyzed after every 10 samples and at the end of the sequence. CCVs must be run for each separate curve.

10.4.10.3. The CCV may consist of any concentration level used in the initial calibration. Levels near the middle of the curve are recommended.

10.4.10.4. If a CCV response is not within the limits listed in DATA 051 Attachment C, Continuing Calibration Verification Acceptance Limits for AA and ICP, all samples analyzed before and after it must be reanalyzed.

10.4.10.5. If upon reanalysis of the CCV, the response is still not within the above acceptance criteria, then a new initial calibration must be performed. It is recommended that routine instrument maintenance be performed prior to the new curve analysis.

10.4.10.6. The Internal Standard for CCVs shall not exceed 80%-120% recovery.

10.4.10.7. %RSD for the sample replicates shall not exceed 10%.

10.4.11. If the ppm reading exceeds the calibration range, then the sample will be diluted and reanalyzed prior to quantification. Depending upon whether the ppm reading is below or above the calibration curve, a higher dilution of the sample or a larger amount weighed for the sample will be required.

10.4.12. Internal Standard criteria for samples is 80%-120% recovery.

10.4.13. %RSD for the sample replicates shall not exceed 10%.

10.5. Export Data

10.5.1. Data Manager exports data to the network drive TLHAESCHROME.

10.5.2. Upload data from the network drive to the LIMS.

10.6. Instrument Shutdown

10.6.1. See LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES

10.7. Data Reduction and Reporting

10.7.1. The Laboratory Control Sample must be evaluated first and if it fails, the associated samples in the batch are not uploaded.

10.7.2. Data for all passing batches is uploaded into LIMS and evaluated. Refer to DATA 051, Data Evaluation for Feed, Fertilizer, and Pesticide Programs and ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer for additional information.

10.8. Quality Control

10.8.1. Laboratory Control Sample

10.8.1.1. This standard is weighed with a set of samples and is processed in the same manner as the samples. The percent found must be within the limits listed in DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptance Limits.

10.9. Instrument Blank

10.9.1. This blank consists of DI water and is used to ensure the instrument is free from contamination. The response of the instrument blank must be below the Method Detection Limit (MDL). Refer to QUALITY 112, Quality Control for additional information.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Aqueous extracts	Disposed of by dumping down the sink and flushing with water
Instrument waste	Disposed of by dumping down the sink and flushing with water.

12. REFERENCES

12.1. Analysis of Water Soluble Mg and Potash. FSFL-SOP 518, Rev. 3.0 (04/15/2009)
12.2. Hazardous Waste Disposal. PLS_SOP 009, Rev. 1.0 (02/09/2006)
12.3. Laboratory Safety. BAEL_SOP 200, Rev. 1.0 (09/12/2014)
12.4. Method for the Preparation of Water Soluble Magnesium and Potash. F_SOP 204, Draft (09/01/2013)
12.5. Operation and Maintenance of Perkin Elmer Inductively Coupled Plasma–Optical Emission Spectrometry 5300 Dual View System. FSFL-SOP 526, Draft (01/14/2009)
12.6. Potash Analysis AA. FM-601, Rev. 1.0 (04/03/2002)
12.7. Using the Laboratory Information Management System. ADMIN 023, Rev. 1.0 (09/11/2015)
12.8. Determination of Metals and Trace Metals in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry. EPA Method 200.7, Rev. 4.4
12.9. Determination of Trace Elements in Drinking Water by Axially Viewed Inductively Coupled Plasma-Atomic Emission Spectrometry. EPA Method 200.5, Rev. 4.2
12.10. U.S. Code of Federal Regulations. 40 CFR, Parts 405-471.

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	01.13.2016	Original Version	William Meeks Naser Tassobi	
2.0	03.29.2019	Updated Outline Format Moved troubleshooting and maintenance information to LABOP 140 Attachment I Added tables for method specific instrument parameters Added Section 8, reagent information, Added 10.1.2 Added Section 11 Waste Management Added required associated documents, in section 9 and in section 10 of the document		Christian Amason
3.0	09.10.2019	Added soluble boron references to SOP and title. Clarified 8.2.1 and 8.3.1 Added 9.8, 9.9, 9.11, 10.4.9.3, 10.4.12, 10.4.10.6, 10.4.10.7, 10.4.13 Minor grammatical fixes		Christian Amason
4.0	03.11.2021	Edited to reflect QC requirements spelled out in data 051		Christian Amason

14. SIGNATURE HISTORY

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Patricia Lucas
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Quality Assurance Officer

Date_____

_____
Christian Amason
Instrumental Manager / Editor

Date_____

METHOD 514
Fertilizer Preparation and Analysis of Potash by Volumetric Sodium Tetraphenylboron using an Autotitrator

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of potash in fertilizer by the volumetric sodium tetraphenylboron (STPB) method, using an Automatic Titrator.

2. SCOPE

2.1. The procedure delineated below is applicable to determining potassium in fertilizer samples. Potassium is extracted from the fertilizer with water and ammonium oxalate. STPB is added to the extract in excess. As potassium reacts with the STPB, it forms a precipitate. The excess STPB is then back-titrated with benzalkonium chloride (BAC). Clayton yellow is used as the end-point indicator.

$$NaB(C_6H_5)_{4\ (aq)} + K^+_{(aq)} \rightarrow KB(C_6H_5)_{4\ (s)} + Na^+$$

2.2. The end-point is detected using an autotitrator with a DP5 phototrode set at 555nm. Due to the particulates generated during this titration, the mirror must be removed from the DP5 to allow for the reflected light to be detected from the particulates. As the amount of particulates increases, the amount of reflected light leads to an increase in signal. Once the end-point is reached, the sample turns pink, no longer reflects light, and produces a drop in signal.

2.3. This method is not suitable for samples that contain high levels of organic matter.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. A respirator or dust mask should be used when handling Aluminum Hydroxide. Samples must be boiled under the fume hood. Formaldehyde should be used under the fume hood.

4. OUTLINE of PROCEDURE

4.1. 10.1 Standardization of BAC solution
4.2. 10.2 Standardization of STPB solution
4.3. 10.3 Sample Preparation
4.4. 10.4 QC Preparation
4.5. 10.5 STPB Reaction
4.6. 10.6 Autotitration
4.7. 10.7 Post Titration Cleanup
4.8. 10.8 Calculations
4.9. 10.9 Sample Determination of $\%K_2O$

5. INTERFERENCE

5.1. Ammonia interference during the potassium precipitation is avoided by complexing the ammonia with formaldehyde under alkaline conditions before the potassium is precipitated.

5.2. Build-up of precipitate on the phototrode must be removed periodically, after every analyzed batch or every 9 analyzed samples.

5.3. High organic content causes interference.

6. APPARATUS and MATERIAL

6.1. Mettler Toledo Excellence Titrator T5 or equivalent
6.2. Mettler Toledo DP5 phototrode or equivalent
6.3. 100mL plastic beaker for titrator autosampler, per sample
6.4. 120mL amber container

6.5. 50mL volumetric flask, per sample
6.6. 250mL volumetric flask, per sample
6.7. 250mL Erlenmeyer flask, per sample
6.8. Glass funnel, per sample
6.9. Filter paper, folded, Whatman no. 2V, 150 mm, per sample
6.10. Filter paper, Whatman no. 4 and no. 42
6.11. Funnel rack
6.12. Mechanical pipette or Class A Volumetric pipette
6.13. Hot plate
6.14. Smooth boiling stones, glass rods or equivalent (per sample)
6.15. Transfer pipette, per sample
6.16. Beaker
6.17. 3 reagent reservoirs
6.18. 2L beaker
6.19. 2L filtration flask
6.20. Stir bars

7. REAGENTS and MEDIA

7.1. Water, Deionized (DI) Type I
7.2. Triton X-100
7.3. Formaldehyde 37% - HCHO
7.4. Sodium hydroxide 20%
7.5. Aluminum Hydroxide - $Al(OH)_3$
7.6. Ammonium Oxalate - $(NH_4)_2C_2O_4$
7.7. Potassium phosphate monobasic – KH_2PO_4
7.7.1. Dry KH_2PO_4 at 106°C for approximately 4 hours
7.8. Sodium tetraphenylboron (STPB)
7.9. 17% Benzalkonium chloride (BAC)

8. REAGENT PREPARATION GUIDELINES

8.1. 4% Ammonium Oxalate

8.1.1. Weigh 360g of Ammonium Oxalate into a beaker. Transfer content of beaker into the Ammonium Oxalate reservoir. Add DI water to the 9L mark and stir solution overnight.

8.2. ~0.1% Triton X-100 wash solution

8.2.1. Dilute ~ 2mL of Triton X-100 to a volume of ~2L with DI water, and stir.

8.3. 0.04% Clayton yellow indicator solution

8.3.1. Dissolve 0.04g of Clayton yellow in 100mL DI water.

8.3.2. Transfer solution to an amber jar.

8.4. ~0.625% BAC Solution

8.4.1. Add 266mL 17% BAC to the BAC reservoir, fill the reservoir with DI water to the 7L mark, and stir.

8.4.2. Standardize with STPB, section 10.1.

8.5. ~1.2% STPB Solution

8.5.1. Weigh 60g of STPB and transfer to a 2L beaker, add about 1500mL of DI water and stir to dissolve. Add 30g of $Al(OH)_3$ and stir.

Note: Aluminum hydroxide is added as a preservative. Since it is added in excess, it will not completely dissolve.

8.5.2. Filter the solution with rinses using a no. 4 Whatman filter and transfer the filtrate into 2L container. Filter again using a no. 42 Whatman filter. If the solution is still cloudy, re-filter using another no. 42 filter. Add 10mL of 20%NaOH, transfer the filtrate into the STPB reservoir, and fill with DI water to the 5L mark. Stir solution with a stir bar, overnight. Allow solution to stand for 48 hours.

8.5.3. Standardize with KH_2PO_4, section 10.2.

8.6. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.7. The specific ingredient amounts can vary to accommodate the batch size.

8.8. Expiration Date: 12 months from date of preparation

8.9. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. LABOP 121, Maintenance, Operation and Program of Balance
9.2. LABEL L003, Reagent
9.3. Form F00015, Reagent Preparation

10. SPECIFIC PROCEDURES

10.1. Standardization of BAC solution

10.1.1. Add exactly 4mL STPB solution to a minimum of 5 100mL plastic beakers.

10.1.2. Add ~20mL of DI water.

10.1.3. Add 2.5mL of 4% ammonium oxalate.

10.1.4. Add 2mL of 20% NaOH.

10.1.5. Add 5mL of 37% formaldehyde.

10.1.6. Add 6 drops or approximately 0.9mL of 0.04% Clayton yellow indicator solution into each 100mL beaker.

10.1.7. Bring to approximately 50mL final volume with DI water.

10.1.8. Prime the BAC burette.

10.1.9. Set DP5 phototrode to 555nm.

10.1.10. Open the Mettler LabX Software.

10.1.11. Titrate STPB with BAC using the instrument method titled “BAC Standardization”.

10.1.12. Select “Start,” then select “T5 Titrator 2.”

10.1.13. Enter the STPB reagent ID and STPB volume into the corresponding sample positions.

10.1.14. BAC Standardization factor will be automatically stored as an “auxiliary value” for BAC in the Mettler LabX software.

10.1.15. The BAC solution is standardized upon preparation. It is re-standardized whenever a new STPB solution is prepared.

10.2. Standardization of STPB solution

10.2.1. Weigh 1.0g Potassium Phosphate Monobasic (KH_2PO_4) standard into a minimum of 5 250mL volumetric flasks.

NOTE: KH_2PO_4 standard must be dried at 100°C, for at least 2 hours, before being weighed. Leftover can be stored in a desiccator.

10.2.2. Add 50mL of 4% Ammonium Oxalate Solution to the 250-mL flasks.

10.2.3. Bring the Potassium standards to volume with DI water. Cap and invert/shake standards to mix.

10.2.4. Aliquot exactly 10.0mL of each standard into a labeled 50mL volumetric flask.

10.2.5. Add 2mL of 20% NaOH to each 50mL volumetric flask.

10.2.6. Under a fume hood, add 5mL of Formaldehyde to each 50mL volumetric flask.

10.2.7. Add exactly 15.0mL STPB solution into each 50mL volumetric flask.

10.2.8. Bring standards to volume with DI water. Cap and invert/shake standards to mix.

10.2.9. Let standards settle for approximately 15 minutes.

10.2.10. Place 2V folded filter paper into the funnels on the funnel apparatus and place labeled flasks on the funnel apparatus to collect filtrate.

10.2.11. Pour each standard into the funnels, allowing the standard to filter into the collection flasks.

10.2.12. Aliquot exactly 25.0mL of each standard into 100mL plastic beakers.

10.2.13. Add 6 drops or approximately 0.9mL of 0.04% Clayton yellow indicator solution into each 100mL beaker.

10.2.14. Bring to approximately 50mL volume.

10.2.15. Prime the BAC burette.

10.2.16. Set DP5 phototrode to 555nm.

10.2.17. Open the Mettler LabX Software.

10.2.18. Titrate standards with BAC using the instrument method titled “STPB Standardization”.

10.2.19. Select “Start,” then select “T5 Titrator 2.”

10.2.20. Enter the standard ID, weight and STPB volume (“Correction Factor”) into the corresponding sample position.

10.2.21. STPB factor will be automatically stored as an “auxiliary value” for STPB in the Mettler LabX software.

10.2.22. The STPB solution is standardized upon preparation. It is re-standardized whenever a new BAC solution is prepared, immediately after the standardization of BAC.

10.3. Sample Preparation

10.3.1. Samples with guarantees of ≥6% K_2O

10.3.1.1. Weigh sample into labeled 250mL volumetric flask according to the suggested weight code, in the LIMS.

10.3.1.1.1. A weight tolerance of ±0.05g is recommended.

10.3.1.2. Add 50mL of 4% Ammonium Oxalate Solution to the 250mL flask containing the sample.

10.3.2. Samples with guarantees of <6% K_2O

10.3.2.1. Weigh sample into labeled 50mL volumetric flask according to the suggested weight code, in the LIMS.

10.3.2.2. Add 4mL of 4% Ammonium Oxalate Solution to the 50mL flask containing the sample.

10.3.3. Add 2-3 drops of tributyl citrate.

10.3.4. Add boiling rod (or boiling stones) and boil for 20 minutes using a hot plate.

10.3.5. Note: DI water may be added to foaming samples.

10.3.6. Let samples cool to room temperature

10.3.7. Bring samples to volume with DI water. Cap and invert/shake samples to mix.

10.3.8. Let samples stand for 2 hours to settle. (sample solutions may be left overnight)

10.4. QC Preparation

10.4.1. Weigh 1.0 g KH_2PO_4 into a 250mL volumetric flask.

10.4.2. If a liquid stock standard is available, an aliquot of the standard equivalent to 0.04g of KH_2PO_4 (or 1.0 g x 10/250) can be added into the 50mL volumetric flask, together with 2.5mL of 4% Ammonium Oxalate (step 10.5.1.1.).

10.4.3. QC samples do not require boiling and are carried through the rest of the procedure.

10.5. STPB Reaction

10.5.1. Samples with guarantees of ≥6% K_2O

10.5.1.1. Aliquot exactly 10.0mL of sample extract into a labeled 50mL volumetric flask.

10.5.2. Samples with guarantees of <6% K_2O

10.5.2.1. Aliquot exactly 25.0mL of sample extract into a labeled 50mL volumetric flask.

10.5.3. Add 2mL of 20% NaOH to the 50-mL volumetric flask.

10.5.4. Under a fume hood, add 5mL of Formaldehyde to the 50mL volumetric flask.

10.5.5. Add exactly 15.0mL STPB solution into the 50mL volumetric flask**.**

10.5.6. Bring samples to volume with DI water. Cap and invert/shake samples to mix.

10.5.7. Let samples settle for approximately 15 minutes.

10.5.8. Place 2V folded filter paper into the funnels on the funnel apparatus and place labeled flasks on the funnel apparatus to collect filtrate.

10.5.9. Pour the sample into the funnel, allowing the sample to filter into the collection flask.

10.5.10. Aliquot exactly 25.0mL of sample into a 100mL plastic beaker.

10.5.11. Add 6 drops or approximately 0.9mL of 0.04% Clayton yellow indicator solution into the 100mL beaker.

10.5.12. Bring to approximately 50mL volume.

10.6. Autotitration

10.6.1. Prime the BAC burette.

10.6.2. Set DP5 phototrode to 555nm.

10.6.3. Open the Mettler LabX Software.

10.6.4. If analyzing samples with guarantees ≥6%, titrate samples using the instrument method titled “Potassium in Fertilizer by Volumetric STPB”. If analyzing samples with guarantees <6%, use instrument method titled “Potassium in Fertilizer by Volumetric STPB_low percent.”

10.6.5. Select “Start,” then select “T5 Titrator 2.”

10.6.6. In “Task Name,” enter the batch name. The batch name includes the date (yymmdd), analyst’s initials, and number of batch run for the day. Example: 181205jc01.

10.6.7. Enter the sample ID, weight and STPB volume (“Correction Factor”) into the corresponding sample position. Start titration.

10.6.8. ~0.1% Triton X-100 may be used as a rinse.

10.6.9. Enter % found into the LIMS.

10.7. Post Titration Cleanup

10.7.1. Carefully wipe the phototrode, stirrer and dispenser tube with a Kimwipe to remove particulates accumulated during the titration.

10.7.2. Stirring at high speeds in ~0.1% Triton X-100 will aid in the removal of remaining particulates.

10.8. Calculations

10.8.1. Sample weight determination

10.8.1.1. Samples with K_2O guarantees ≥6%:

$$\boldsymbol{Sample\ Wt\ (g) = \frac{0.3\ g\ K2O * 100\%}{\%\ K2O\ guaranteed\ in\ sample}}$$

10.8.1.2. Samples with K_2O guarantees <6%:

$$\boldsymbol{Sample\ Wt\ (g) = \frac{0.024\ g\ K2O * 100\%}{\%\ K2O\ guaranteed\ in\ sample}}$$

10.8.2. Standardization of BAC

$$\boldsymbol{BAC\ Factor = \frac{V_{(STPB)}}{V_{(BAC)}}}$$

Formula Term	Definition	Mettler Symbol
$V_{(STPB)}$	Volume of STPB (mL)	m
$V_{(BAC)}$	Volume of BAC titrant consumed (mL)	VEQ
BAC Factor	Ratio of STPB to BAC	H[BAC]

10.8.3. Standardization of STPB

$$\boldsymbol{STPB_{(EX)} = \frac{V_{(BAC)}\ x\ H_{(BAC)}\ x\ 50mL}{25mL}}$$

$$\boldsymbol{STPB\ Factor = \frac{34.61\%\ x\ m_{(KH_2PO_4)}\ x\ 10mL\ x\ 25mL\ x\ 1000}{(V_{(STPB)} - STPB_{(EX)})250mL\ x\ 50mL\ x\ 100}}$$

Formula Term	Definition	Mettler Symbol
34.61%	% K_2O of KH_2PO_4	-
$m_{(KH2PO4)}$	Weight (g) of KH_2PO_4	m
250mL	Final volume from extraction step	-
10mL	Aliquot from extraction step	-
50mL	Final Volume after STPB addition	-
25mL	Aliquot from STPB addition step	-
100	Conversion from % to decimal	-
1000	Conversion from g to mg	-
$V_{(STPB)}$	Volume of STPB (mL)	f
$V_{(BAC)}$	Volume of BAC titrant consumed (mL)	VEQ
$STPB_{(Ex)}$	mL of STPB in excess	C
$H_{(BAC)}$	Ratio of STPB to BAC	H[BAC]
STPB Factor	mg K_2O / mL STPB	H[STPB]

10.9. Sample Determination of $\%K_2O$

10.9.1. Samples with K_2O guarantees ≥6%

$$STPB_{(EX)} = \frac{V_{(BAC)}\ x\ H_{(BAC)}\ x\ 50mL}{25mL}$$

$$\%K_2O = \frac{(V_{(STPB)} - STPB_{(EX)})250mL\ x\ 50mL\ x\ F\ x\ 100}{m_{(Sample)}\ x\ 10mL\ x\ 25mL\ x\ 1000}$$

10.9.2. Samples with K_2O guarantees <6%

$$STPB_{(EX)} = \frac{V_{(BAC)}\ x\ H_{(BAC)}\ x\ 50mL}{25mL}$$

$$\%K_2O = \frac{(V_{(STPB)} - STPB_{(EX)})50mL\ x\ 50mL\ x\ F\ x\ 100}{m_{(Sample)}\ x\ 25mL\ x\ 25mL\ x\ 1000}$$

Formula Term	Definition	Mettler Symbol
$m_{(sample)}$	Weight (g) of sample	m
250mL / 50mL	Final volume from extraction step	-
10mL / 25mL	Aliquot from extraction step	-
50mL	Final Volume after STPB addition	-
25mL	Aliquot from STPB addition step	-
100	Conversion from decimal to %	-
1000	Conversion from mg to g	-
$V_{(STPB)}$	Volume of STPB (mL)	f
$V_{(BAC)}$	Volume of BAC titrant consumed (mL)	VEQ
$STPB_{(Ex)}$	mL of STPB in excess	C
$H_{(BAC)}$	Ratio of STPB to BAC	H[BAC]
F	mg K_2O / mL STPB	H[STPB]

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism

Material	Disposal Mechanism
Contents of flask after initial ammonium oxalate boiling step	Hazardous waste
Contents of titration cups and leftover reagents	Hazardous waste
Used Filters	Dry in hood, place in box and submit as Hazardous Waste

12. REFERENCES

12.1. *Potassium in Fertilizer, Volumetric Sodium Tetraphenylboron Method I*. Method 958.02, AOAC 15th Edition, External 00138.
12.2. Volumetric Sodium Tetraphenylboron Method I. University of Kentucky, College of Agriculture Division of Regulatory Services, June 26, 2008, External 00137.
12.3. Ingram, C.W., Bryant, M., Terry, D. (2008, June). *Comparison of Manual and Automatic Titrametric Analysis of Potash in Fertilizer using AOACI Method 958.02*. Presentation at ASPFCO, New Orleans, LA, External 00136.

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	03.01.2019	Original Version	William Meeks / Dr. Patrizia Lemma	
2.0	12.05.2023	Updater reference section to add external document numbers. Removed signature page Reformatted section 8 to align with current practices.		William Meeks, Jr.

METHOD 515
Analysis of NAU using Flow Injection Analyzer
[FM452, FM472, FM462]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the analysis of Nitrate (NO_3), Ammonia (NH_4^+) and Urea in fertilizer samples by utilizing a Flow Injection Analyzer (FIA).

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples that are analyzed in the Bureau of Agricultural Environmental Laboratories (BAEL). The method is for the determination of Nitrate-Nitrogen [FM 452], Ammoniacal-Nitrogen [FM 472] and Urea-Nitrogen [FM 462] in DI Water extracts of fertilizer samples.

2.2. NO3 -Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

2.3. NH4 -This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration/

2.4. Urea -This is a two channel, low flow method, which measures ammonia on one channel (FM-471), and the sum of ammonia and urea on a second channel. The sample is injected into a urease solution. The result on the ammonia channel (FM-471) consists of only the ammonia present in the sample. Urease cleaves urea (CH4N2O) to NH3 + CO2 on the second channel. The result consists of the ammonia initially present in the sample, plus the ammonia produced by the action of urease on urea. When ammonia is heated with salicylate and hypochlorite in an alkaline phosphate buffer, a colored product results. Absorbance of the colored product at 660 nm is directly proportional to the ammonia concentration. The color is intensified by the addition of sodium nitroprusside. The method detection limit is 1 mg N/L as NH4. At the end of the analysis, the ammonia channel (FM-471) result is subtracted from the urea + ammonia result to give the percent urea N present in the sample. An ammonia standard is used to calibrate both channels. Urea stock solutions are analyzed to determine the percent conversion of urea to ammonia. Percent conversions of urea to ammonia are generally in the range of 93-98%.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. Preparation of Reagent
4.2. Preparation of Standard
4.3. Lachat Operation
4.4. Initial Calibration
4.5. Laboratory Control Sample
4.6. Continuing Calibration Verification
4.7. Run Sequence
4.8. Printing and Reporting Data
4.9. Instrument Shutdown
4.10. Quality Control
4.11. Attachment A, Nitrate Parameters and Manifold Diagram

4.12. Attachment B, Ammonia Parameters and Manifold Diagram
4.13. Attachment C, Urea/Ammonia Parameters and Manifold Diagram

5. INTERFERENCE

5.1. Residual chlorine can interfere by oxidizing the reductor column.
5.2. Low results for Nitrate would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA can be added to the buffer to reduce this interference.
5.3. Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA may be added to the sample in-line in order to prevent this problem
5.4. Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH-dependent. Likewise, ensure that pH of standard ammonia solutions approximates that of samples.
5.5. Non-volatile amines such as cysteine, ethanolamine and ethylenediamine may cause a decrease in ammonia sensitivity.
5.6. Nitrogen oxides must be eliminated before color is formed to prevent interference.
5.7. Iron and dimeric silicates may cause high results, if present.
5.8. Sample turbidity, color and particulates must be removed by filtration, prior to analysis.

6. APPARATUS and MATERIAL

6.1. Autosampler
6.2. Autosampler rack, 15 x 6
6.3. Balance, analytical minimum capacity 0.001g
6.4. Balance, top loading minimum capacity 0.1g
6.5. Beaker
6.6. Filter,13mm IC Acrodisc with 0.45µm syringe filter, or equivalent
6.7. Flask, class A volumetric
6.8. Flow Injection Analyzer
6.9. pH paper
6.10. Pipette, Eppendorf or equivalent
6.11. Pipette, class A volumetric
6.12. Pipette tip
6.13. Stir Bar
6.14. Syringe, plastic disposable
6.15. Test tube, borosilicate culture 13 x100mm or equivalent
6.16. Weighing boat, plastic disposable
6.17. Dialysis membrane
6.18. Cadmium column

7. REAGENTS and MEDIA

7.1. Sodium phosphate heptahydrate
7.2. Sodium hydroxide
7.3. Potassium sodium tartrate tetrahydrate
7.4. Sodium salicylate
7.5. Sodium nitroprusside
7.6. Brij-35 (>95%); Other concentrations may be used.
7.7. Urease
7.8. Sodium hypochlorite
7.9. Ammonium chloride
7.10. Hydrochloric acid, concentrated (32%)
7.11. Sulfanilamide
7.12. N-(1-napthyl)ethylenediamine dihydrochloride (NED)

7.13. Phosphoric acid, concentrated (85%)
7.14. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. 10% w/v Sodium Hydroxide

8.1.1. In a 100mL flask, add approximately 50mL of deionized water and add 10g of sodium hydroxide (NaOH). Mix thoroughly to dissolve solids and dilute to volume.

Caution: the solution will get very hot!

8.2. 20% v/v Hydrochloric Acid

8.2.1. In a 100mL flask, add approximately 60mL of deionized water, add 20mL of concentrated hydrochloric acid (HCl). Dilute to volume and mix by inversion.

Caution: the solution will get very hot!

8.3. Ammonium Chloride Buffer-Acceptor

8.3.1. In a 2000mL flask, add approximately 1600mL of deionized water, and add 42.5g of ammonium chloride (NH_4Cl). Adjust pH to 5.5-6.0 with 10% sodium hydroxide. Dilute to volume and mix with a magnetic stirrer.

8.4. Ammonium Chloride Buffer-Acceptor

8.4.1. In a 2000mL flask, add approximately 1600mL of deionized water. Add 127.5 grams of ammonium chloride (NH_4Cl). Adjust pH to 5.5-6.0 with 10% sodium hydroxide. Dilute to volume mix with a magnetic stirrer.

8.5. Sulfanilamide color reagent

8.5.1. In a 2000mL flask, add approximately 1200mL of deionized water. Add 80.0g of sulfanilamide [$C_6H_8N_2O_2S$] and 2.0g N-(1-napthyl)ethylenediamine dihydrochloride (NED), stir with a magnetic stirrer. Add 200mL concentrated phosphoric acid (H_8PO_4) and dilute to volume.

8.6. Buffer Solution

8.6.1. In a 2000mL flask, add approximately 1200mL of deionized water. Add 40.0g of sodium hydroxide (NaOH), 84.0 g sodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) and 100.0 g sodium potassium tartrate tetrahydrate ($NaKC_4H_4O_8 \cdot 4H_2O$), stir with a magnetic stirrer and dilute to volume.

8.7. Nitroprusside/Salicylate Color Reagent

8.7.1. In a 2000mL flask, add approximately 1200mL of deionized water. Add 300.0g of sodium salicylate, 2.0g sodium nitroprusside and 0.8g Brij-35 (>95%), stir with a magnetic stirrer, and dilute to volume. If the Brij-35 is a lower concentration, a larger weight will be needed to meet the necessary final concentration. For example: Brij-35(30%w/v) would need 2.67g weighed.

8.8. Hypochlorite Solution

8.8.1. In a 1000mL flask, add approximately 800mL of deionized water. Add 100mL of approximately 5% sodium hypochlorite, stir with a magnetic stirrer and dilute to volume.

Note: If full peaks are not observed at the higher curve concentrations, add additional hypochlorite.

8.8.2. Expires 5 days after preparation; store in refrigerator

8.9. Urease Solution

8.9.1. In a 500mL flask, add approximately 400mL of deionized water. Add 0.260g urease, stir with a magnetic stirrer and dilute to volume.

8.9.2. Expires 5 days after preparation; store in refrigerator

8.10. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.11. The specific ingredient amounts can vary to accommodate the batch size.

8.12. Expiration Date: 1 month from date of preparation unless otherwise specified.

8.13. Store at room temperature unless otherwise specified.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer
9.2. ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer
9.3. DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs
9.4. DATA 051 Attachment A, Fertilizer Analysis Requirements
9.5. DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptance Limits
9.6. DATA 051 Attachment C, Fertilizer Continuing Calibration Verification Acceptance Limits
9.7. Form F00015, Reagent Preparation
9.8. Label L003, Reagent
9.9. LABOP 140 Attachment F, Maintenance and Operation of Flow Injection Analyzer
9.10. QUALITY 112, Quality Control

10. SPECIFIC PROCEDURES

10.1. Preparation of Reagent

10.1.1. Reagents are prepared according to the applicable instructions in section 8.

10.2. Preparation of Standard

10.2.1. Stock Standard 2000ppm NH_4-N and 1000ppm NO_3-N in DI Water. In a 500mL volumetric flask, dissolve 4.7170g ammonia sulfate $(NH_4)_2SO_4$ and 3.6110g Potassium Nitrate (KNO_3) in 100 ml of DI Water. Dilute to mark with DI Water and invert to mix.

NOTE: Stock standard solutions are stable for 1 month. Purchased stock standards should adhere to manufacturer's expiration date.

10.2.2. Intermediate Standards
- 10.2.2.1. Standard A (600mg NH_4-N/L, 300mg NO_3-N/L)
- 10.2.2.2. Fill a 100mL flask with 50mL of DI Water
- 10.2.2.3. Using pipette, add 30mL of 2000/1000ppm Stock Solution
- 10.2.2.4. Dilute to 100mL with DI water
- 10.2.2.5. Invert to mix

10.2.3. Standard B (300mg NH_4-N/L, 150mg NO_3-N/L)
- 10.2.3.1. Fill a 200mL flask with 100mL of DI Water
- 10.2.3.2. Using pipette, add 30mL of 2000/1000ppm Stock Solution
- 10.2.3.3. Dilute to 200mL with DI Water
- 10.2.3.4. Invert to mix

10.2.4. Standard C (150mg NH_4-N/L, 75mg NO_3-N/L)
- 10.2.4.1. Fill a 200mL flask with 100mL of DI Water
- 10.2.4.2. Using pipette, add 15mL of 2000/1000ppm Stock Solution
- 10.2.4.3. Dilute to 200mL with DI Water
- 10.2.4.4. Invert to mix

10.2.5. Standard D (60mg NH_4-N/L, 30mg NO_3-N/L)

10.2.5.1. Fill a 100mL flask with 50mL of DI Water

10.2.5.2. Using pipette, add 3mL of 2000/1000ppm Stock Solution

10.2.5.3. Dilute to 100mL with DI Water

10.2.5.4. Invert to mix

10.2.6. Standard E (30mg NH_4-N/L, 15mg NO_3-N/L)

10.2.6.1. Fill a 200mL flask with 100mL of DI Water

10.2.6.2. Using pipette, add 3mL of 2000/1000ppm Stock Solution

10.2.6.3. Dilute to 200mL with DI Water

10.2.6.4. Invert to mix

10.3. Lachat Operation

10.3.1. Set up manifolds as shown in Attachment A, Nitrate Manifold Diagram.

10.3.2. Set up manifolds as shown in Attachment B, Ammonia Manifold Diagram.

10.3.3. Set up manifolds as shown in Attachment C, Urea/Ammonia Manifold Diagram.

10.3.4. Place all the lines in DI and switch the pump on to check for any leaks.

10.3.5. Stop the pump.

10.3.6. Take the lines out from DI water and place in the respective reagent containers.

10.3.7. Restart the pump.

10.3.8. Allow 20 to 30 minutes for instrument warm-up time. Monitor the base line until a stable baseline is obtained.

10.4. Initial Calibration

10.4.1. A minimum of five calibration standards must be injected. For analytes that require a second order fit, a minimum of six calibration curve standards is required. One of the standards should be near the Method Detection Limit (MDL) concentration. There should be at least one order of magnitude difference in the curve range.

10.4.2. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs; for calibration procedures.

10.5. Laboratory Control Sample

10.5.1. Refer to Data 051 Attachment A, Fertilizer Analysis Requirements, for acceptance criteria.

10.5.2. Reference material used for the Lab Standard is defined in PREP 453 attachment A.

10.6. Continuing Calibration Verification

10.6.1. Refer to Data 051 Attachment B, Fertilizer Continuing Calibration Verification Acceptance Limits, for acceptance criteria.

10.7. Run Sequence

10.7.1. Refer to Data 051 Attachment C, Fertilizer Analysis Requirements, for sequence generation and ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer, for sample nomenclature.

10.8. Printing and Reporting Data

10.8.1. Click on ‘Tool’ button and select ‘Custom Report’

10.8.2. Print the Report

10.8.3. Be sure the report includes the following

10.8.3.1. Cup number
10.8.3.2. Sample identification (ID)
10.8.3.3. Final concentration
10.8.3.4. Dilution factor
10.8.3.5. Calibration charts
10.8.3.6. Detection date and time

10.8.4. Upload data according to ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer

10.9. Instrument Shutdown

10.9.1. Clean the equipment, auto sampler, sample racks, etc. with a damp cloth.

10.9.2. Refer to LABOP 140 Attachment F, Maintenance and Operation of Flow Injection Analyzer, for shutdown procedures and additional information on maintenance and troubleshooting.

10.10. Quality Control

10.10.1. Refer to DATA 051 Attachments B, Fertilizer Laboratory Control Sample Acceptance Limits, for acceptance criteria.

10.10.2. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Instrument Effluent	Neutralize with NaOH and dispose down drain
Excess filtered samples	Dispose down drain
Unused Reagents	Neutralize with NaOH and dispose down drain
Cd Column	Submit to Hazardous Waste

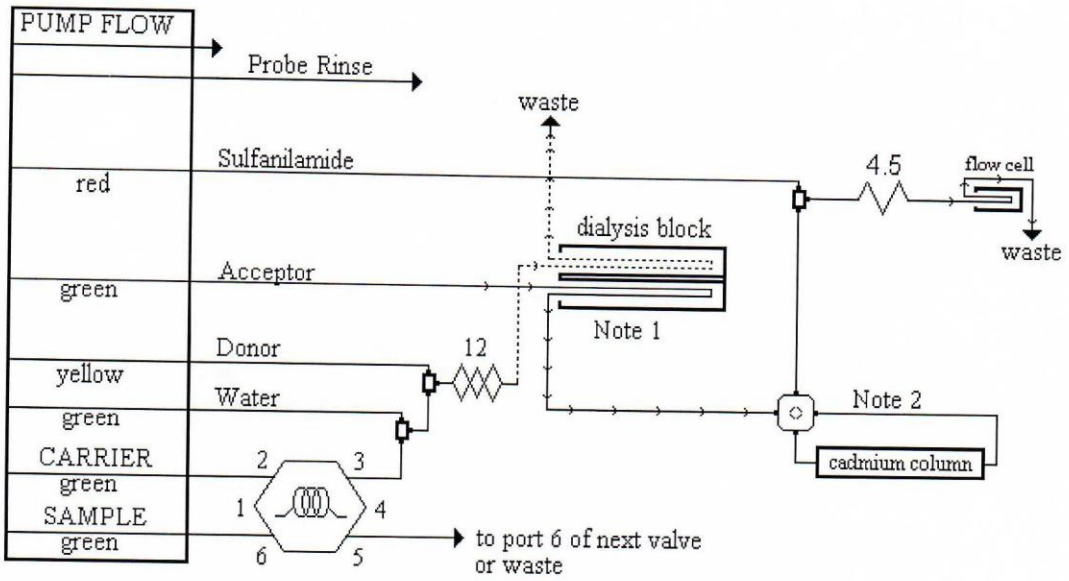
12. REFERENCES

12.1. Determination of Nitrate in Fertilizers by Flow Injection Analysis Colorimetry. QuickChem Method 14-107-04-1-B.
12.2. Determination of Ammonia in Fertilizers by Flow Injection Analysis Colorimetry. QuickChem Method 14-107-06-2-C
12.3. Determination of Urea in Fertilizers by Flow Injection Analysis Colorimetry. QuickChem Method 14-206-00-2-A
12.4. Lachat QC FIA + 8000 Flow Injection Analyzer Manual

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	03.29.2019	Original Version	Carl Huang	
2.0	05.21.2021	Updated formatting throughout Updated reagent expiration information in sections 8.8.2. and 8.9.2. Updated QAO signature block to replace Elizabeth Schneider with Quentin Cooper. Updated storage specifications in section 8.13.		Steven Daley
3.0	07.21.2022	Section 8.0 adjusted volume of sodium hypochlorite and added note. Section 12.0 Updated References added 12.2 and 12.3.		Christian Amason
4.0	03.31.2023	Updated sections 7.6. and 8.7.1. to add allowance for different Brij concentrations. Removed signature page		Christian Amason

11.3. NITRATE MANIFOLD DIAGRAM

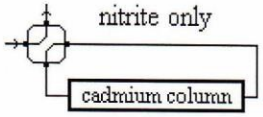


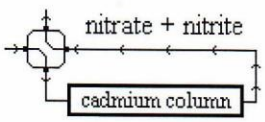
Carrier: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 8 cm
QC8000 Sample Loop: 13.5 cm
Interference Filter: 520 cm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

4.5: **70** cm of tubing on a 4.5 cm coil support
12: **255** cm of tubing on a 12 cm alternating coil support

Note 1: 6 inch pathlength dialysis block and a Type C membrane.
Note 2: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.

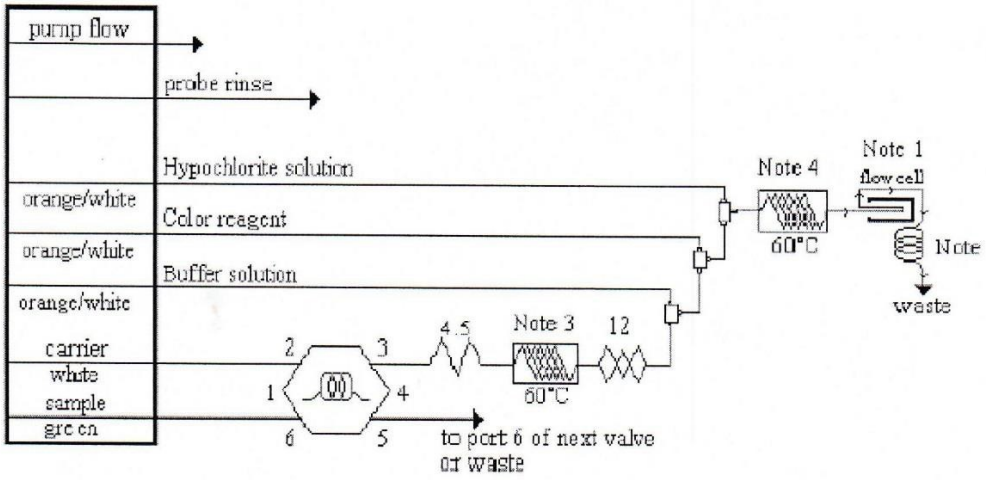




METHOD 515 Attachment B
Ammonia Manifold Diagram

Version: 1.0	Effective Date: 03/29/2019	Page 1 of 1

AMMONIA MANIFOLD DIAGRAM



Carrier:	**D.I. water**
Manifold Tubing:	**0.5 mm (0.022 in) i.d.** This is **5.2 µL/cm**.
QC8000 Sample Loop:	13 cm
Interference Filter:	660 nm
Apparatus:	An injection valve, a 1 mm path length flow cell, and a colorimetric detector module is required
4.5:	**70** cm of tubing on a 4.5 cm coil support
12:	**255** cm of tubing on a 12 cm alternating coil support
Note 1:	The flow cell is **1 mm** path length.
Note 2:	50 cm back pressure loop, 0.52 mm (0.022 in) i.d.
Note 3:	175 cm of tubing on the heater block.
Note 4:	650 cm of tubing on the heater block.
Note 5:	The same heating module is used for both (see notes 3 and 4) heaters.

FM-462

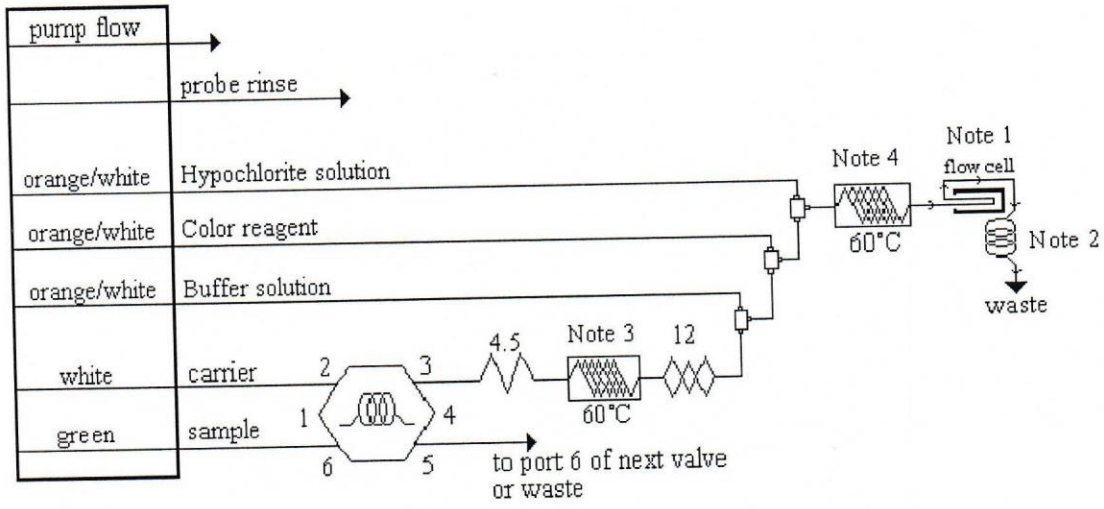
Urea Nitrogen – Flow Injecti

Probe in Sample Period: 35 s

Valve Timing:

Load Time: 0 s
Load Period: 25 s
Inject Period: 65 s

UREA/AMMONIA MANIFOLD DIAGRAM



Carrier: **Urease Solution** (Reagent 3)
Manifold Tubing: **0.5 mm (0.022 in) i.d.** This is **2.5 µL/cm**.
AE Sample Loop: 7.5 cm x 0.5 mm i.d.
QC8000 Sample Loop: 13 cm x 0.5 mm i.d.
Interference Filter: 660 nm

Apparatus: An injection valve, a 1 mm path length flow cell, and a colorimetric detector module is required. The  shows xxx cm (see notes below) of tubing wrapped around the heater block at the specified temperature.

4.5: **70** cm of tubing on a 4.5 cm coil support
12: **255** cm of tubing on a 12 cm alternating coil support

Note 1: The flow cell is **1 mm** path length
Note 2: 50 cm backpressure loop x 0.5 mm i.d.
Note 3: 175 cm of tubing on the heater block
Note 4: 650 cm of tubing on the heater block
Note 5: The same heating module is used for both heaters (see notes 3 and 4)

METHOD 516

Analysis of Available Phosphorous and Soluble Potash in Fertilizers using ICP-OES

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of available phosphorous (P_2O_5) and soluble potash (K_2O) in mixed or pure material fertilizer samples, in both liquid and dry matrices by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES).

2. SCOPE

2.1. The procedure delineated below is applicable to an ICP-OES determination of available phosphorous (APA) and soluble potash in fertilizer. Fertilizer samples are extracted with a citrate-EDTA solution (as per PREP 461, *Sample Preparation for Available Phosphorus and Soluble Potash in Fertilizer*) and analyzed by ICP-OES. The analysis is achieved by measuring the intensity of the electromagnetic radiation produced by the analyte in the inductively coupled plasma. A quantitative determination of the amount of analyte present can be made at the specific wavelength by measuring the excited atoms and ions emitted. The intensity of this emission is indicative of the concentration of the analyte within the sample. The determined amount of potassium (K) is converted to potash (K_2O equivalent) and reported as such. The determined amount of phosphorous (P) is converted to APA (P_2O_5 equivalent) and reported as such.

2.2. Standards solutions used to calibrate the ICP-OES are made to match the matrix of the final sample extracts. Cesium is used as an ionization buffer to minimize easily ionized element effects.

2.3. This method is adapted from AOAC Method 2015.18. The method described in this SOP deviates from Method 2015.18 by bringing the samples to a final volume of 500 mL instead of 250 mL. Thus, the citrate-EDTA of the sample is diluted prior to analysis, eliminating the need to dilute the sample inline by increasing the tubing size of the buffer/internal standard peristaltic tube and reducing the tubing size of the sample peristaltic tube. Sample weight was increased from 0.5 g to 0.75 g to achieve required detection limit.

2.4. This method is restricted to use by or under the supervision of a trained analyst. Each analyst must demonstrate the ability to generate acceptable results with this method. Refer to ADMIN 016, Training and Competency, for demonstration of competence procedures.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. Torch and interface regions of the ICP can be very hot and caution is needed when working around these areas if the ICP has recently been running. The ICP generates very high voltages. Specific safeties are in place on the instrument to protect the operator from accidental exposure to electrical shock; these safeties should not be subverted. Extreme caution should be used when performing any maintenance that would expose the operator to high voltages. Protective covers shall remain in place at all times, except when special maintenance and/or repairs require their removal.

3.3. Exhaust from combustion fumes and vapors from the ICP torch housing can produce toxic vapors and may pose a health hazard if not adequately vented to the outside.

4. OUTLINE of PROCEDURE

10.1 ample Handling
10.2 tandards
10.3 Instrument Startup
10.4 Instrument Parameters

5. INTERFERENCE

5.1. Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, ionization interferences (for K), or unresolved overlap of molecular band spectra. Interferences may be overcome by dilution of the digestate, application of interelement correction factors, use of alternate wavelengths, or addition of cesium chloride ionization buffer (for K). A target of at least 2200 mg/kg cesium chloride solution must be presented at the nebulizer.

5.2. Plasma efficiency can be reduced by the carbon in the citrate and EDTA. Therefore, samples must be diluted prior to analysis. This can be achieved by increasing the peristaltic tubing for the buffer/internal standard and decreasing the peristaltic tubing for the sample tubing or diluting the sample to a larger final volume as per PREP 461.

5.3. Samples containing copper (Cu) will interfere with the P wavelengths of 213.617 nm and 214.914 nm due to Cu emission intensity at the wavelengths of 213.598 nm and 214.898 nm. Spectral deconvolution must be utilized if reporting P from the affected wavelengths. Multicomponent Spectral Fitting (MSF) is utilized in this method according to the procedure below:

5.3.1. **MSF Modeling:** Spectra must be collected from a matrix blank (B), a standard of the analyte of interest (A), and a standard of the interfering analyte(s) (I) using the same analysis method that will be used to normally analyze samples.

Note: The Peak Algorithm must be set Peak Area or Peak Height within the analysis method. The MSF algorithm is not used during this time.

5.3.2. Spectral profile is independent of concentration, however, to optimize the MSF model, the concentration of the analyte should be approximately 100 times its detection limit at the affected wavelength and the concentration of the interfering analyte should approximate maximum concentration that could be present.

E.g., ~100 times the detection limit of P would be approximately 500 ppm. In this example, 400 ppm P standard was selected. A 40 ppm Cu standard was selected to represent the maximum concentration of the interfering Cu (2 or 3% Cu in the sample).

Note: All Analytes must be in the same matrix as the samples (final buffer).

5.3.3. After the analysis of the blank, analyte, and interferent, build the MSF model using the Examine MSF window. Label the blank (B), analyte (A) and interferent (I) on the wavelength of interest (e.g., 213.617 nm). Once the MSF file is saved, Select MSF as the Peak Algorithm within the analysis method and specify which MSF file to be used on the Spectral Corrections tab.

5.3.4. Confirm the new MSF model is function as intended by reanalyzing the interferent and a known sample or standard such as a LS. The resulting interferent should not be detected at the wavelength of interest.

5.3.5. If a wavelength calibration is performed, the MSF must be recreated.

Note: Wavelength calibration is performed during the annual PM.

5.4. Fertilizers containing phosphite will also be detected by this method.

5.5. Due to the susceptibility of the citrate-EDTA solution to microbial degradation, the citrate-EDTA solution has a 2-week expiration when stored in the dark and a 16-hour expiration for sample extracts. An inhouse longevity study demonstrated microbial degradation could be mitigated by utilizing refrigeration. The expiration dates found in this SOP have been extended based on this study.

5.6. For most fertilizer samples, Scandium (Sc) and Beryllium (Be) can be used as the internal standard for P. Polyphosphates present additional challenges to the plasma that are not detected by Sc; in this case, Be may be used as internal standard for P.

6. APPARATUS and MATERIAL

6.1. Analytical Instruments

6.1.1. Dual view ICP-OES
6.1.2. Autosampler
6.1.3. Peristaltic Pump capable of handling three introduction lines
6.1.4. Chiller
6.1.5. Integration software

6.2. ICP autosampler tubes
6.3. Pump tubing: black/black, orange/green, red/red
6.4. Volumetric flasks
6.5. Volumetric pipettes
6.6. 0.45µm Syringe Filters

7. REAGENTS and MEDIA

7.1. Ammonium citrate dibasic, ACS grade or equivalent
7.2. Ammonium hydroxide, ACS grade or equivalent
7.3. Beryllium Solution, 1000 ppm
7.4. Cesium Chloride (CsCl), Trace metal grade, solid
7.5. EDTA, disodium salt, dihydrate, >99%
7.6. Hydrochloric Acid (HCl) concentrated, Trace meal grade
7.7. Nitric Acid (HNO_3) concentrated, Trace metal grade
7.8. Scandium Solution, 1000 ppm
7.9. Triton X-100
7.10. Water, Deionized (DI), Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Citrate-EDTA Solution

8.1.1. Directions: Add 25 g disodium EDTA and 50 g dibasic ammonium citrate to a 2-L volumetric flask containing ~1500 mL DI water. Adjust to near neutral by adding 30 mL of a solution of ammonium hydroxide-water (1+1, v/v), under a hood. (Volumes are scalable as needed.)

8.1.2. While stirring and using a pH electrode, adjust the final pH to 7.00 by adding the ammonium hydroxide-water (1+1, v/v) solution dropwise. After a stable pH of 7.00 ±0.02 has been attained, dilute to volume with DI and mix.

8.1.3. Citrate-EDTA solution has a **2-months expiration** when stored refrigerated.

8.2. Carrier Solution

8.2.1. Directions: Fill a 1L volumetric flask almost to volume with DI water. Add 1-2 drops Triton X-100, then fill to the mark with DI water. Triton X – 100 causes beading in some spray chambers, therefore, it may be left out in those cases. Invert the flask several times to mix.

8.3. Wash Solution

8.3.1. Directions: Add approximately 5L DI water to a 15-L carboy, add 307 mL HCl, swirl to mix. Add DI water to the 15-L mark, cap the carboy, and shake to mix.

Optional: 7.5 mL of Triton X-100 may be added. (Triton X – 100 causes beading in some spray chambers, therefore, it may be left out in those cases.).

8.4. Internal Standard/Ionization Buffer

Directions: Add approximately 1500 mL DI water to a 2000-mL volumetric flask. Add 24 g CsCl and swirl to mix. Add 10 mL of 1000 ppm Scandium standard solution (40 mL of 1000 ppm Beryllium standard solution may also be added as needed). The final concentration of the Internal Standards are 5.0 ppm Sc (20 ppm Be). Add 80 mL of HNO_3, cap and invert multiple times to mix.

Optional: 1-2 drops of Triton X-100 may be added. (Triton X – 100 causes beading in some spray chambers, therefore, it may be left out in those cases.).

8.4.1. Expiration is **6 months** from preparation date.

8.5. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015.

8.6. The specific ingredient amounts can vary to accommodate the batch size.

8.7. Expiration Date: 12 months from date of preparation unless otherwise indicated.

8.8. Store at room temperature.

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment M Instrument Sequence Generation for Fertilizer
9.2. ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer
9.3. DATA 051, Data Evaluation for Feed, Fertilizer, and Pesticide Programs
9.4. DATA 051 Attachment A, Fertilizer Analysis Requirements
9.5. DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptance Limits
9.6. DATA 051 Attachment C, Continuing Calibration Verification Acceptance Limits for AA and ICP
9.7. LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES
9.8. PREP 461, Sample Preparation for Available Phosphorus and Water-Soluble Potash in Fertilizer
9.9. QUALITY 112, Quality Control
9.10. SAFETY 002, Waste Management

10. SPECIFIC PROCEDURES

10.1. Sample Handling

10.1.1. Sample extracts containing particulates should be filtered or centrifuged before analysis.

10.1.2. Extracts must be stored refrigerated and are to be analyzed within **3 days**.

10.2. Standards

10.2.1. Primary standards and internal standards (1000ppm or 10,000ppm) should be prepared from neat material or purchased through certified vendors that are NIST traceable.

Note: Purchased standards preserved with acid should not be utilized since the acid will change the pH and matrix of the calibration standards.

10.2.2. Working standards are to be used for ICP calibration and unknown sample identification and quantification. They are made by diluting with a solution of citrate-EDTA (that matches the sample extracts) to obtain the desired concentrations.

10.2.3. Working Standards have expiration **2 months** when stored refrigerated.

10.2.4. Recommended Standard Levels:

Standard Level	Stock (ppm)	Stock (mL)	Citrate-EDTA Solution (mL)	Final Volume (mL)	K (ppm)	P (ppm)
Intermediate Standard	10,000	100	-	1000	1,000	1,000
0	-	-	200	1000	-	-
1	1,000	5	200	1000	5	5
2	1,000	10	200	1000	10	10
3	1,000	20	200	1000	20	20
4	10,000	5	200	1000	50	50
5	10,000	10	200	1000	100	100
6	10,000	20	200	1000	200	200
7	10,000	40	200	1000	400	400
8	10,000	60	200	1000	600	600

10.3. Instrument Startup

10.3.1. See LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES

10.3.2. Turn on the plasma and peristaltic pump.

10.3.2.1. Allow the system to stabilize for a minimum of 30 minutes.

10.4. Recommended Instrument Parameters

10.4.1. The following parameters may vary for different ICP makes and models and therefore require minor modifications in order to optimize performance.

	PE Optima 5300	PE Optima 8300
Plasma Flow	15 L/min	15 L/min
Auxiliary Flow	0.2 L/min	1.5 L/min
Nebulizer Flow	0.65 L/min	0.65 L/min
RF Power	1450 watts	1450 watts
Peristaltic Pump Flow Rate	0.8 mL/min	0.44 mL/min
Carrier Tubing	Black/Black	Black/Black
Internal Std. Tubing	Orange/Green	Orange/Green
Waste Tubing	Red/Red	Red/Red
Wash	0.8 mL/min	0.44 mL/min

Spray Chamber	Cyclonic	Cyclonic
Loop Size	1.0 mL	1.5 mL
Replicate Reads	3	3

10.4.2. Recommended Wavelengths and Plasma View:

Element	Wavelength nm	Plasma View
K	766.490*	Radial
K	404.721	Axial
P	177.434	Axial
P	178.222	Axial
P	213.617*	Axial
P	214.914	Axial
Sc	361.383*	Axial/Radial
Be	313.107*	Axial/Radial
*Primary Wavelength		

10.5. Sample Analysis

10.5.1. Use the Sample Information File (SIF) to create the sequence.

10.5.2. A calibration blank is injected with the calibration standards to correct for background.

10.5.3. After the initial calibration, there must be a continuing calibration check standard analyzed after every 10 samples and at the end of the run.

10.5.4. The Laboratory Control Samples (LCS) must be analyzed with their associated samples, not grouped together at the beginning of the sequence.

10.5.5. The sequence requirements are found in, DATA 051 Attachment A, Fertilizer Analysis Requirements.

10.5.6. The sequence must end with a CCV. The ending CCV should be at a different concentration than the mid-sequence CCVs.

10.5.7. Samples require the proper laboratory information management system (LIMS) codes to allow required uploading. Refer to ADMIN 023 Attachment M Instrument Sequence Generation for Fertilizer for additional information.

10.5.7.1. Example: AA99999-0AP

10.5.7.1.1. 0 corresponds to the sample weight (0, 1, 2.)
10.5.7.1.2. A corresponds to the different injections due to dilutions (A=1st injection, B=2nd injection, C=3rd injection.)
10.5.7.1.3. P corresponds to analysis type (P= P&K Method)

10.5.8. Dilution factors must be entered into the instrument sequence.

10.6. Export Data

10.6.1. Data Manager exports data to the network drive TLHAESCHROME.

10.6.2. Upload data from the network drive to the LIMS.

10.7. Instrument Shutdown

10.7.1. See LABOP 140 Attachment I: Maintenance, Operation and Program of ICPOES

10.8. Quality Control

10.8.1. Initial Calibration:

10.8.1.1. The calibration is based on plotting the ratio of the analyte response to the internal standard response as a function of the analyte concentration of the standards.

10.8.1.2. The correlation coefficient of the calibration curve R value must be ≥0.999 or the R^2 value must be ≥0.998.

10.8.1.3. The internal standard RSD value in the calibration blank must be less than 2%. If internal standard RSD is greater than 2%, reanalyze the calibration blank until 2% is achieved.

10.8.1.4. Refer to DATA 051, Data Evaluation for Chemical Analysis; for additional calibration procedures.

10.8.2. Continuing Calibration Check Standard:

10.8.2.1. Once the initial calibration has passed, a continuing calibration check standard must be analyzed after every 10 samples and at the end of the sequence. CCVs must be run for each separate curve.

10.8.2.2. CCV acceptance criteria for P and K are ±6%. Addition information may be found in DATA 051 Attachment C, Fertilizer Continuing Calibration Verification Acceptance Limits.

10.8.2.3. The CCV may consist of any concentration level used in the initial calibration. Levels near the middle of the curve are recommended.

10.8.2.4. If a CCV response is not within the limits, all samples analyzed before and after it must be reanalyzed.

10.8.2.5. If upon reanalysis of the CCV, the response is still not within the above acceptance criteria, then a new initial calibration must be performed. It is recommended that routine instrument maintenance be performed prior to the new curve analysis.

10.8.3. When using the suggested sample weight and volume in PREP 461, any sample reading exceeding the calibration range would exceed the sample's theoretical maximum threshold and must be reanalyzed or re-prepped.

10.8.4. Internal Standard criteria for samples is 80% - 120% recovery.

10.8.5. %RSD for the sample replicates shall not exceed 5%.

10.8.6. Laboratory Control Sample

10.8.6.1. This standard is weighed with a set of samples and is processed in the same manner as the samples. The percent found must be within the limits listed in DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptance Limits.

10.8.7. Instrument Blank

10.8.7.1. This blank matches the citrate-EDTA extract matrix (standard 0) and is used to ensure the instrument is free from contamination. The response of the instrument blank must be below the Method Detection Limit (MDL). Refer to QUALITY 112, Quality Control for additional information.

10.9. Data Reduction and Reporting

10.9.1. Calculations for P_2O_5:

$$P_2O_5\% = \frac{P(\mu g/mL) \times 500(mL) \times 141.95}{Weight(g) \times 30.97 \times 2 \times 10{,}000}$$

Where:

$$P_2O_5\% = \frac{P(\mu g/mL) \times \text{Final Vol.}(mL) \times P_2O_5(MW)}{\text{Weight}(g) \times P(MW) \times (\text{mole ratio of } P_2O_5 \text{ to } P) \times (\text{ppm to } \%)}$$

10.9.2. Calculations for K_2O:

$$K_2O\% = \frac{K(\mu g/mL) \times 500(mL) \times 94.2}{\text{Weight}(g) \times 39.09 \times 2 \times 10{,}000}$$

Where:

$$K_2O\% = \frac{K(\mu g/mL) \times \text{Final Vol.}(mL) \times K_2O(MW)}{\text{Weight}(g) \times P(MW) \times (\text{mole ratio of } K_2O \text{ to } K) \times (\text{ppm to } \%)}$$

10.9.3. The Laboratory Control Sample must be evaluated first and if it fails, the associated samples in the batch are not uploaded.

10.9.4. Data for all passing batches is uploaded into LIMS and evaluated. Refer to DATA 051, Data Evaluation for Feed, Fertilizer, and Pesticide Programs and ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer for additional information.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Aqueous extracts	Disposed of by dumping down the sink and flushing with water
Instrument waste	Disposed of by dumping down the sink and flushing with water.

12. REFERENCES

12.1. *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, **Method 2015.18**, ww.eoma.aoac.org, External Document 00135.

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	09.17.2021	Original Version	William Meeks	
2.0	10.16.2023	Added external document number external reference. Section 5.3 added Multicomponent Spectral Fitting (MSF) procedure to SOP.		William Meeks

METHOD 520
Sample Preparation and Analysis of Combined Sulfur
[FM805]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for preparation and analysis of combined sulfur by gravimetric method in fertilizer samples.

2. SCOPE

2.1. The procedure delineated below is applicable to the proper protocol to be followed by all staff in analyzing combined sulfur in fertilizer samples.

2.2. The combined sulfur result is found through a gravimetric method in which the sulfate is precipitated as barium sulfate by the addition of 10% barium chloride in excess. The final precipitate is expressed as percent of combined sulfur (%S).

2.3. The manufacturer's tag should state or specify "combined sulfur."

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required. Additional PPE is included such as oven gloves.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weighing and LIMS Entry
4.2. 10.2 Digestion for Solid Samples
4.3. 10.3 Digestion for Liquid Samples
4.4. 10.4 Barium Sulfate Precipitation for both Solid and Liquid samples
4.5. 10.5 Calculation
4.6. 10.6 Data Evaluation

5. INTERFERENCE

5.1. N/A

6. APPARATUS and MATERIAL

6.1. Balance, accuracy to 0.001g
6.2. Beaker, 250mL
6.3. Bottle, 20L Nalgene plastic or equivalent
6.4. Filter paper, Whatman (125mm) 2V folded filter paper or equivalent
6.5. Fume hood
6.6. Glass fiber pads 2.4cm Whatman 934-AH or equivalent
6.7. Glass funnel
6.8. Gooch crucibles (40mL) or equivalent
6.9. Hotplate
6.10. Muffle furnace
6.11. Vacuum trap apparatus
6.12. Volumetric flasks, 500mL and 1L (class "A")

7. REAGENTS and MEDIA

7.1. Ammonium Sulfate, certified American Chemical Society (A.C.S.) grade or equivalent
7.2. Barium chloride·$2H_2O$, certified American Chemical Society (A.C.S.) grade or equivalent
7.3. Calcium thiosulfate, pure, 30-60% solution in water, or equivalent
7.4. Hydrochloric acid, certified A.C.S. grade or equivalent
7.5. Hydrogen peroxide, 30% certified A.C.S. grade or equivalent
7.6. Sodium hydroxide, NF/FCC Pellets, or equivalent

7.7. Standard Reference Material (SRM) 695 or equivalent
7.8. Water, Deionized (DI) water

8. REAGENT PREPARATION GUIDELINES

8.1. 10% Barium Chloride

8.1.1. Add 1173g of barium chloride ($BaCl_2 \cdot 2H_2O$) to approximately 8L of deionized water in a 10L Nalgene plastic bottle. Bring to a final volume of 10L with deionized water and mix.

8.2. 50% Sodium Hydroxide

8.2.1. Slowly add 500g of sodium hydroxide while mixing to approximately 450mL of deionized water. Bring to a final volume of 1L with deionized water and mix.

8.3. 1:1 Hydrochloric Acid

8.3.1 Add 50mL of concentrated hydrochloric acid to approximately 40 mL of deionized water in a 100mL flask and mix. Bring to a final volume of 100mLwith deionized water and mix.

8.4. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.5. The specific ingredient amounts can vary to accommodate the batch size.

8.6. Expiration Date: 12 months from date of preparation

8.7. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Attachment P, Manual Result Entry
9.2. Data 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs
9.3. DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits
9.4. F00015, Reagent Preparation
9.5. L003, Reagent
9.6. METHOD 196, Determination of Percent Moisture for Fertilizer
9.7. PREP 453, Weighing of Fertilizer Sample

10. PROCEDURE

10.1. Weighing and LIMS Entry

10.1.1. Create a sample list per the Laboratory Information Management System (LIMS).

10.1.2. Locate samples and gather the amount of gooch crucibles and glass fiber pads that is needed to correspond with samples. Add a glass fiber pad to the crucible and dry the gooch crucibles with glass fiber pads at 250°C. Allow them to cool and take them to the weighing room.

10.1.3. Set the crucibles to correspond with the number you assigned to the sample order.

10.1.4. Open the Laboratory Information Management System (LIMS) and enter or scan sample ID numbers in to the FERT_SULFUR_COMBINED template. Further LIMS instruction can be found in ADMIN 023 Attachment P, Manual Result Entry.

10.1.5. Gypsum samples being analyzed for combined sulfur must be dried according to METHOD 196.

10.1.6. Using the suggested weight below, weigh directly into a 250mL beaker and enter the weight into the 805_WT code. Refer to PREP 453, Weighing of Fertilizer Sample, for further instruction.

Guarantee	Weight
<2%	1.5g
2-10%	1g
10-12%	0.5g
>12%	0.4g

10.1.6.1. When weighing liquid samples, weigh double the suggested weight.

10.1.7. For solid samples weigh 0.40g of the ammonium sulfate laboratory control sample directly into a 250mL beaker and enter the weight into the 805_WT code. For liquid samples included in the set also weigh 1.00g of calcium thiosulfate for the laboratory control sample.

10.1.8. Tare the scale and weigh the crucible with the glass pad and record the weight in the LIMS code 805_TARE_WT.

10.2. Digestion for Solid Samples

10.2.1. Digestion for samples must be completed in a hood.

10.2.2. Add 150mL of deionized water then 10mL of concentrated hydrochloric acid (HCl) to beaker.

10.2.3. Gently boil the sample on a hotplate for approximately 20 minutes.

10.2.4. Filter through Whatman 2V filter paper into another 250mL beaker and wash with hot DI water to obtain a volume of 150mL. Discard filter paper.

10.3. Digestion for Liquid Samples

10.3.1. Digestion for samples must be performed in a hood.

10.3.2. Add 50mL of deionized (DI) water, followed by 2mL of 50% sodium hydroxide (NaOH) and then 2mL of 30% hydrogen peroxide (H_2O_2).

10.3.3. Cover with watch glass and reflux the sample on a hotplate for approximately 60 minutes.

10.3.4. Slowly add 5mL of 30% H_2O_2 in 1mL increments into sample.

Note: Caution, add 30% H_2O_2 very slowly to beaker. Reaction is extremely exothermic!

10.3.5. Rinse watch glass into sample and dilute to approximately 175mL with hot DI water.

10.3.6. Add 10mL 1:1 HCl:DI water, boil 5 minutes and filter.

10.4. Barium Sulfate Precipitation for both Solid and Liquid samples

10.4.1. Bring filtrate back to a slow boil.

10.4.2. Carefully add 2mL of 10% barium chloride solution, in small increments to beaker to avoid a violent reaction.

10.4.3. Add an additional 15mL of 10% barium chloride solution to beaker, adjust hotplate so that solution is just below boiling for approximately 60 minutes.

10.4.4. A white precipitate (barium sulfate) will form in the beaker after a few minutes

10.4.5. Allow the samples to cool to room temperature and let stand overnight.

10.4.6. Set up the gooch crucible with pad on vacuum apparatus.

10.4.7. Seal pad with water and vacuum filter sample through crucible with hot DI water.

CAUTION: Pad seal must not be allowed to leak!

10.4.8. Rinse the beaker several times into crucible with hot DI water.

10.4.9. If necessary, use a glass rod to break up precipitant while rinsing.

10.4.10. Remove the crucible from vacuum apparatus.

10.4.11. Dry crucible at 250°C for one hour.

10.4.12. After drying, allow crucible to cool to room temperature.

10.4.13. Weigh the crucible and record the weight in the LIMS code 805_FINAL_WT.

10.5. Calculation:

$$\frac{[\boldsymbol{Final\ Weight_{BaSO4}}(\boldsymbol{g}) - \boldsymbol{Tare\ Weight_{Crucible\ \&\ Filter}}(\boldsymbol{g})](\mathbf{0.1374_{S/BaSO4}})}{\boldsymbol{Sample\ Weight}\ (\boldsymbol{g})}\boldsymbol{x100} = \%\ \boldsymbol{Combined\ Sulfur}$$

10.6. Data Evaluation

10.6.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.6.2. Evaluate the Lab Control sample results.

10.6.3. To determine if it is within acceptable limits refer to DATA 051 Attachment B, Fertilizer Laboratory Control Sample Acceptable Limits.

10.6.4. If there are multiple sample results, use the result closest to the guarantee if all the results are legal. Use the highest result if all the results are deficient. Refer to DATA 051.

10.6.5. Make sure that the found result is always completed.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Filters and Barium Sulfate	Disposed of in Trash
Filtrate	Disposed of down drain

12. REFERENCES

12.1. E00154, *Sulfur in Fertilizers, Gravimetric Method*. Method 980.02, AOAC 15th Edition
12.2. E00155, Combined Sulfur Analysis, FM 805
12.3 E00156, Analysis of Combined Sulfur, FSFL-SOP 520

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	04.14.2021	Original Version	William Meeks, Jr	
2.0	01/30/2024	Sections 6.3 and 6.8 added 'or equivalent'. Section 7.2 added ·2H2O to Barium Chloride. Sections 8.2 and 8.3 adjusted volume of water. Section 10.1.7 clarified which laboratory control sample to use for liquid and solid. samples. Section 10.2.2 and 10.3.2 clarified language in step. Section 10.4.3 clarified time for boiling. Added weight table Added external document numbers Removed signature page		Taleya Williams Chris Gismondi William Meeks, Jr

METHOD 521

Sample Preparation and Analysis of Free Sulfur [FM 806]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of free (elemental) sulfur in fertilizer samples by gravimetric method.

2. SCOPE

2.1. The procedure delineated below is applicable to fertilizer samples that contain free sulfur component. Free sulfur is extracted by Soxhlet using carbon disulfide (CS_2.) After solvent removal, the percent sulfur is determined after drying by weight difference.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. **Inhalation Hazard:** Carbon disulfide and carbon tetrachloride pose dangerous inhalation hazards and must be used with proper ventilation such as within a fume hood.

3.3. **Flammable:** Due to carbon disulfide's high flammability, care must be taken while working with or around heat. Do NOT store carbon disulfide next to heat sources.

3.4. **Carcinogenicity:** Carbon tetrachloride is classified as a suspected carcinogen. The use of PPE such as lab coat, gloves, and safety glasses are mandatory. Carbon tetrachloride shall only be used within a fume hood. Any spills must be cleaned immediately.

3.5. Ensure that a closed system is not created while setting up the distillation apparatus. Pressure build-up from heating organic solvents, such as carbon disulfide, can potentially result in an explosion.

4. OUTLINE of PROCEDURE

4.1. 10.1 Weighing and LIMS Entry
4.2. 10.2 Sample Washing to Remove Urea
4.3. 10.3 Drying samples
4.4. 10.4 Soxhlet Extraction
4.5. 10.5 Solvent Removal
4.6. 10.6 Final Weighing
4.7. 10.7 Calculation
4.8. 10.8 Data Evaluation
4.9. 10.9 Quality Control Sample

5. INTERFERENCE

5.1. Sulfur-coated urea tends to give elevated results in free sulfur analysis. It leaves a brownish, oily residue in the boiling flask after distillation. A hot water rinse of the sample is used to remove urea.

5.2. Moisture will interfere with the extraction process.

5.3. The use of stopcock grease may be dissolved by the carbon disulfide solution and may lead to positive bias interferences.

6. APPARATUS and MATERIAL

6.1. Analytical balance, minimum capacity 0.0001g
6.2. 125mL boiling flask with 24/40 glass joint
6.3. 30 mm Soxhlet extraction tube with 24/40 glass joint
6.4. 30 mm extraction condenser with 24/40 glass joint
6.5. Cellulose extraction thimbles (25 mm X 80 mm), Whatman Cat. # 2800258 or equivalent

6.6. Drying oven
6.7. Elbow joint with 24/40 glass joint
6.8. Fume hood
6.9. Water bath with Thermolyne hot plate or equivalent

7. REAGENTS and MEDIA

7.1. Water, Deionized
7.2. Carbon Disulfide CS_2, Reagent Grade A.C.S.
7.3. Carbon Tetrachloride CCl_4, 99% Anhydrous

8. REAGENT PREPARATION GUIDELINES

8.1. Carbon Disulfide Extraction Solution (CS_2:CCl_4, 60:40)

8.1.1. **Under a hood** and while wearing a respirator, add 400mL of carbon tetrachloride to 600mL of carbon disulfide. Mix well.

8.2. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.3. The specific ingredient amounts can vary to accommodate the batch size.

8.4. Expiration Date: 12 months from date of preparation

8.5. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. ADMIN 023 Attachment C, LIMS Sample Login for Multi Sample
9.2. DATA 051
9.3. F00015, Reagent Preparation
9.4. L003, Reagent
9.5. QUALITY 112
9.6. PREP 453, Weighing of Fertilizer Sample

10. SPECIFIC PROCEDURES

10.1. Weighing and LIMS Entry

10.1.1. Preparation

10.1.1.1. Create a sample list per backlog

10.1.1.2. Locate samples and gather all glassware needed. Check 125mL-boiling flasks and Soxhlet extraction tube for small damages and cracks.

10.1.1.3. Use LIMS, according to ADMIN 023 Attachment C, LIMS Sample Login for Multi Sample, to create a sample number for a lab standard.

10.1.1.4. Label all glassware to correspond to sample numbers. Set the thimbles to correspond with the sample numbers.

10.1.1.5. Reference PREP 453, Weighing of Fertilizer Sample for further instruction on preparation for weighing.

10.1.2. Weighing

10.1.2.1. Open the LIMS result entry template Fert_Sulfur_Free.

10.1.2.2. Place a cellulose extraction thimble in a holder (E.g., beaker or gooch crucible) and tare on the balance.

10.1.2.3. In LIMS each sample has a suggested weight.

10.1.2.4. Weigh the sample directly into the cellulose extraction thimble and record as the sample weight (SW) in LIMS. A holder (E.g., beaker, gooch crucible, etc.) may be used to assist in weighing.

10.1.2.5. Weigh the labeled 125mL-boiling flasks. Record this weight as the tare weight (TW) in LIMS.

10.1.2.6. Save and exit LIMS.

10.1.2.7. Return samples to sample storage area.

10.2. Sample Washing to Remove Urea

10.2.1. Place a gooch crucible in the vacuum apparatus.

10.2.2. Place the thimble with the sample in the gooch crucible.

10.2.3. Rinse the sample with approximately 20mL of hot DI water using vacuum. This should be done 2 more times for a total of 3 times.

Note: DI water should be brought to a boil and used immediately.

10.2.4. Remove the cellulous thimbles from the vacuum and place them in their respective holders.

10.3. Drying samples

10.3.1. Place all samples in the drying oven for 1- 2 hours at 105°C, or until cellulose thimble is dry.

Note: Moisture left in the cellulose thimble will interfere with extraction process.

10.3.2. Remove samples from oven and cool

10.4. Soxhlet Extraction

10.4.1. **The following steps of the Soxhlet extraction must take place in a fume hood.**

10.4.2. See METHOD 521 attachment A for the Soxhlet assembly setup. Ensure that the hood is on and in working order.

10.4.3. Add approximately 65mL of carbon disulfide extraction solution to the 125mL-boiling flask.

10.4.4. Attach the Soxhlet extraction tube to the 125mL-boiling flask and place the thimble with sample into the Soxhlet extraction tube.

10.4.5. Add approximately 15mL of carbon disulfide extraction solution to the Soxhlet extraction tube.

10.4.6. Attach the condenser to the Soxhlet extraction tube. Ensure that water is flowing through the condenser.

10.4.7. Turn the extraction heater or hot plate on high and reflux for 2 hours.

10.4.8. After refluxing is complete turn off heater\hot block.

10.4.9. **Allow flask and heating block to cool to room temperature.**

10.4.10. Remove the condenser assembly.

10.4.11. Use forceps to lift thimble and drain carbon disulfide-extraction solution. Allow thimble to drip dry in the Soxhlet extraction tube.

10.4.12. After draining is complete, remove cellulose thimble from Soxhlet extraction tube. Set it aside under the hood to allow CS_2 to completely evaporate before disposal.

10.4.13. Separate 125mL-boiling flask from the Soxhlet extraction tube.

10.4.14. Pour remaining carbon disulfide-extraction solution from Soxhlet extraction tube into the 125mL-boiling flask.

10.5. Solvent Removal

10.5.1. Attach a 125mL-boiling flask to a glass elbow.

10.5.2. Place 125mL-boiling flask into a hot water bath and attach the other end of the elbow to the cooling condenser.

10.5.3. Distill and collect carbon disulfide extraction solution for disposal.

10.5.4. Remove the 125mL-boiling flask from the hot water bath and disconnect the glass elbow.

10.5.5. Place flask in drying oven for 1 hour at approximately 105°C or until dry and cool to room temperature.

10.5.6. The yellowish residue in the flask is the free (elemental) sulfur.

10.6. Final Weighing

10.6.1. Open LIMS result entry template “Fert_Free_Sulfur.

10.6.2. Weigh the 125mL-boiling flask containing the residue (sulfur). This weight will be entered as the final weight (FW).

10.7. Calculation

10.7.1. Calculate the Free Sulfur result using the formula below:

$$\%S\ (\text{Free Sulfur}) = (FW - TW) / (SW) * 100$$

S = Free Sulfur
FW = Final weight of 125mL-boiling flask with sulfur residue
TW = Tare weight of 125mL-boiling flask
SW = Sample weight

10.8. Data Evaluation

10.8.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.9. Quality Control Sample

10.9.1. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples. Guidelines, that supersede those of QUALITY 112, are addressed in this document.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
CS_2:CCl_4	Dispose remainder in chlorinated waste container.
Extracted Fertilizer, Cellulose thimbles	Allow any residual solvent to evaporate in hood or vented oven and dispose in trash.
Gloves & kimwipes	Allow any residual solvent to evaporate in hood and dispose in trash.
Sulfur Waste	Dispose of in trash

12. REFERENCES

12.1. Sulfur in Fertilizers, Gravimetric Method. Method 980.02, AOAC 15th Edition.
12.2. FM-806, Secondary/Micronutrient, Sulfur – Free, 01/02/03 v.2
12.3. FSFL-SOP 521 Determination of Free (Elemental) Sulfur in Fertilizers by Gravimetric Method. 2006/01/17 v.1.0

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	04.11.2019	Original Version	William Meeks, Jr	

14. SIGNATURE HISTORY

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Date_____

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Teresa Rygiel
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William Meeks, Jr
Author

Date_____

METHOD 521 Attachment A

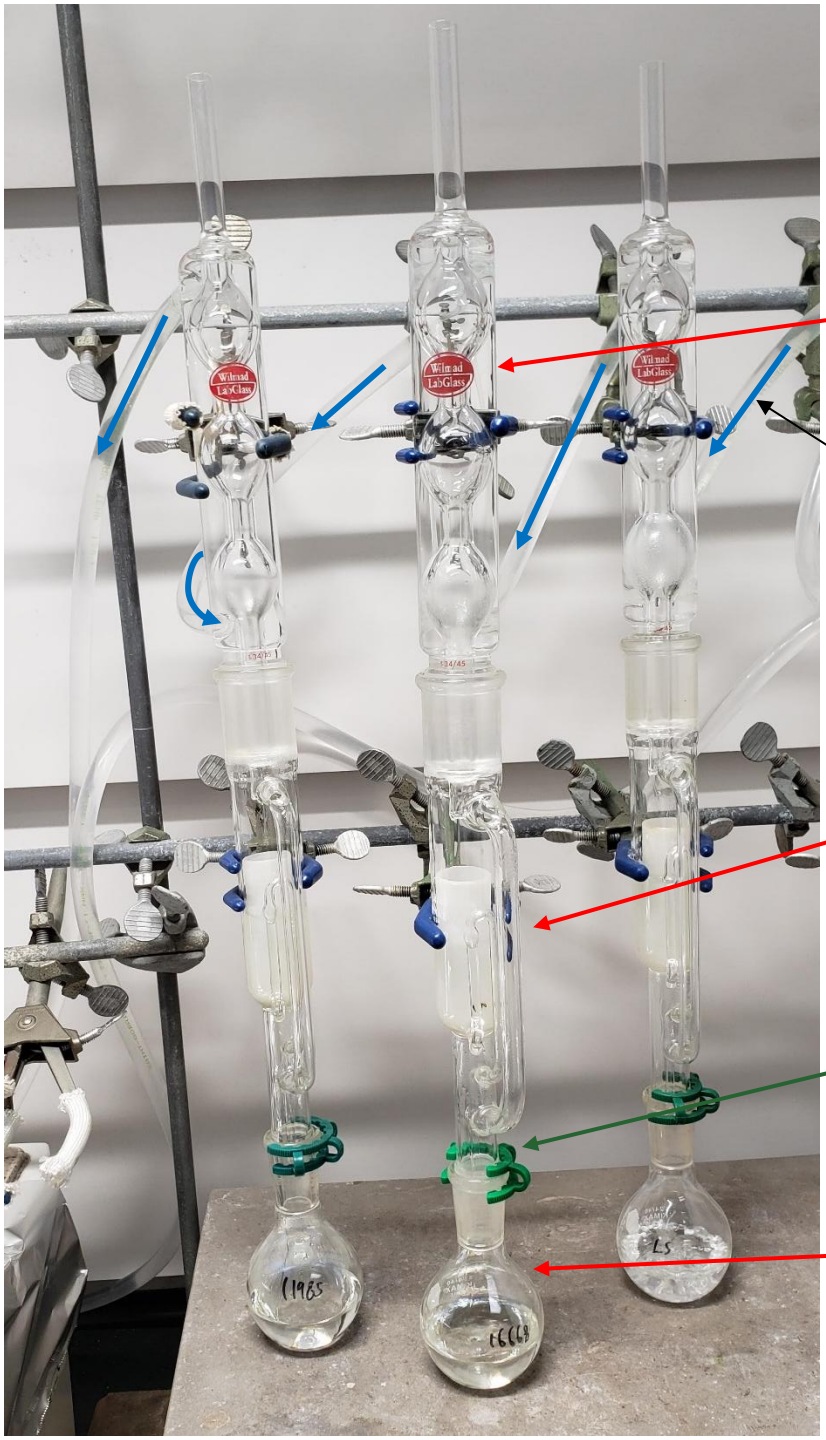
Soxhlet Apparatus Assembly

The following is a recommended setup of Soxhlet and Distillation apparatus. Variations of this setup are allowed in order to accommodate additional samples or variations in glassware or heating apparatus. All variations must be approved by a supervisor. Regardless of the configuration, care must be taken not to create a closed system.

Notes:

- The apparatus must be setup in a hood. Ensure the hood is in proper working condition.
- Do not create a closed system. Doing so may result in an explosion.
- Ensure the water is on and flowing from the bottom of the condenser to the top.
- Solvents such as Carbon Disulfide are extremely flammable. Ensure heating source is cool before removing/disassembling apparatus.
- Ensure that the receiver flask is large enough to accommodate total amount of solvent being distilled.
- Aluminum foil may be used to cover the water bath. This will help insulate the samples during distillation and maintain temperature for complete distillation.
- Store waste such as used cellulose thimbles, gloves, and Kimwipes in a box under the hood until residual solvent has evaporated.

Soxhlet Apparatus



Distillation Apparatus

Distilling Adapter, 105°, 24/40 glass joint, with Vacuum Take-Off

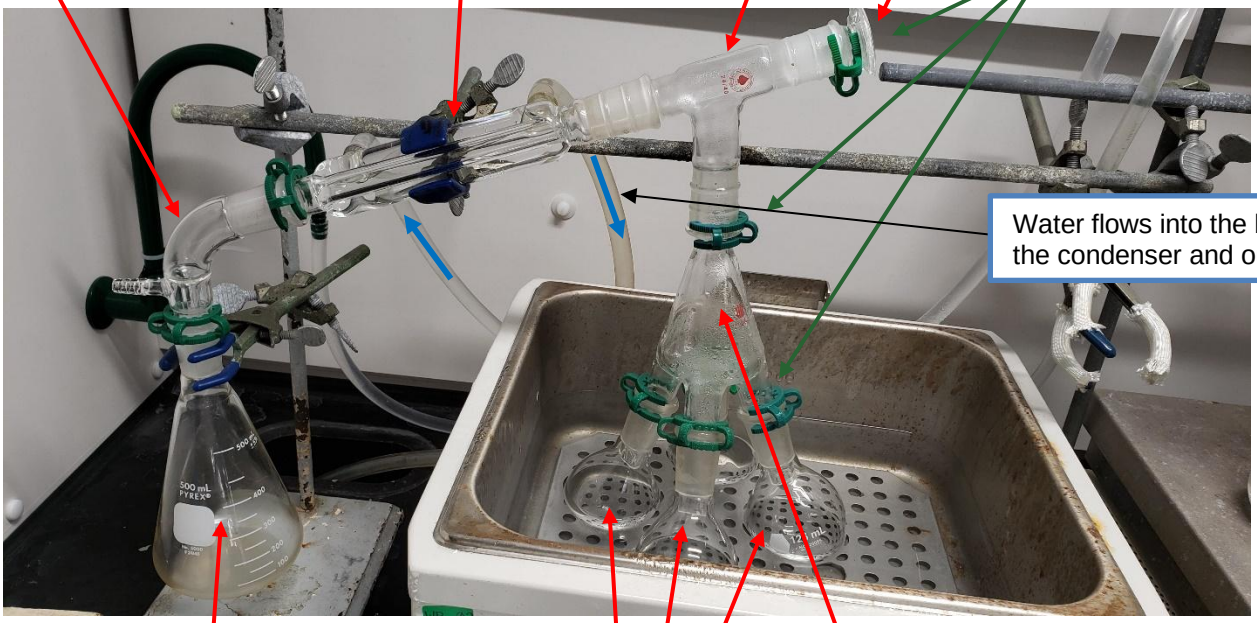
(**WARNING**: Vacuum Take-Off port must not be covered or closed. Doing so will make a closed system.)

Side Arm Adapter, 75°, 24/40 glass joint

30 mm extraction condenser, Liebig, 24/40 glass joint

Glass plug, 24/40 glass joint

Green Joint Clamp
(Use on all 24/40 joints)



Water flows into the bottom of the condenser and out of the

Receiver flask, 24/40 glass joint
(Large enough to receive total volume distilled.)

Glass Cow Distillation Receiver, 24/40 glass joint

125 mL boiling flask, 24/40 glass joint

METHOD 523

Analysis of Soluble and Chelate Metals by AA- [FM830, FM831, FM832, FM833, FM840, FM841]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for determination of soluble and chelated metals micronutrients: Iron, Manganese, Copper, and Zinc by Atomic Absorption Spectroscopy (AAS).

2. SCOPE

2.1. The procedure delineated below is applicable to Atomic Absorption Spectroscopy determination of soluble and chelated micronutrients: Iron, Manganese, Copper, and Zinc in fertilizer samples analyzed by the Bureau of Agricultural Environmental Laboratories. Quantitative determination of the amount of analyte present in the sample is achieved by measuring signal intensity at the wavelength of maximum absorption, which is specific to the element analyzed.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. Be careful with AAS Burner when handling it, it must be at room temperature.

3.3. Exhaust from combustion fumes and vapors from the AA flame housing can produce toxic vapors and may pose a health hazard if not adequately vented to the outside.

4. OUTLINE of PROCEDURE

4.1. 10.1 Sample Handling and Storage
4.2. 10.2 Standards
4.3. 10.3 Instrument Startup
4.4. 10.4 Sample Analysis
4.5. 10.5 Data Evaluation
4.6. 10.6 Quality Control Sample

5. INTERFERENCE

5.1. Polyammonium phosphate
5.2. Super phosphate

6. APPARATUS and MATERIAL

6.1. Perkin-Elmer PinAAcle 500 Atomic Absorption Spectrometer or equivalent
6.2. SC2-DX Autosampler
6.3. AA prep 2 Peristaltic Pump
6.4. Workstation Computer system capable of operating integration software which interfaces with the Atomic Absorption Spectrometer and acquires and stores all the required data.
6.5. Black-Black tubing (0.76mm ID, 0.86mm Wall, 72mm between Bridges Black\Black.
6.6. Volumetric flask Class A
6.7. Glass Pipettes Class A
6.8. Syringe with Luer lock Inlet at least for 20ml
6.9. Syringe Filter, 25mm 0.45µm aqueous base
6.10. 1000 ml Volumetric Flask, Class A

7 REAGENTS and MEDIA

7.1. Deionized Water (Type 1)
7.2 Isopropanol or Methanol
7.3 Nitric Acid, 69%

7.4 Triton X-100
7.5 Hydrochloric Acid

8 REAGENT PREPARATION GUIDELINES

8.1. 5% Nitric Acid

8.2.1 Directions: Add approximately 500 ml of DI Water (Type 2) in 1 Liter volumetric flask (class A). Add 7.4 ml of Nitric Acid (69%) concentrated. Gently swirl the volumetric. Bring up to volume with DI Water and mix well.

8.2. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.3. The specific ingredient amounts can vary to accommodate the batch size.

8.4. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.5. Store at room temperature

9 ASSOCIATED DOCUMENTS

9.1. ADMIN 023, Using the Laboratory Information Management System
9.2. ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer
9.3. ADMIN 023 Attachment O, Automatic Result Entry for Fertilizer
9.4. DATA 050, Data Control
9.5. DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs
9.6. DATA 051 Attachment C, Fertilizer Continuing Calibration Verification (CCV)
9.7. DATA 051 Attachment A, Fertilizer Analysis Requirements
9.8. LABOP 140 Maintenance Attachment k, Operation, and Program of Atomic Absorption Spectroscopy by Flame
9.9. QUALITY 112, Quality Control

10 SPECIFIC PROCEDURES

10.1. Sample Handling and Storage

10.1.1. All extracts may be filtered. Filtered extracts have no specified hold time.

10.1.2. Sample extracts containing particulates should be filtered before analysis**.** Use a Syringe Filter, 25mm 0.45µm aqueous base.

10.1.3. Unfiltered extracts should be prepared and analyzed according to the table below:

Micronutrient	Unfiltered Extract Hold Times
Copper (Cu)	Extracts can be held up to 24 hours
Iron (Fe)	not specified
Zinc (Zn)	Extracts are to be analyzed within an hour from when buffer is added
Manganese (Mn)	Extracts must be analyzed on the same day as extraction

10.2. Standards

10.2.1. Primary standards (Iron, Manganese, Copper and Zinc) 1,000 ppm should be prepared from neat material or purchased from certified vendors that are NIST traceable.

10.2.2. Working standards are to be used for AAS calibration. They are made by diluting into DI Water (Type 1 to obtain the following concentrations.

AAS Standard Mix 1 (0.5ppm)

Add to a 1000 ml volumetric flask 500 ml of DI Water (Type 1) approximately. Pipet 0.5 ml of Iron, Manganese, Copper and Zinc of the primary standard and 20.0 ml of HCl. Bring up to volume and mix well.

AAS Standard Mix 2 (5.0ppm Cu, Fe, Mn; 1.0ppm Zn)
Add to a 1000 ml volumetric flask 500 ml of DI Water (Type 1) approximately. Pipet 5.0 ml of Iron, Manganese and Copper. Pipet 1.0 ml of Zinc of the primary standard and 20.0 ml of HCl. Bring up to volume and mix well.

AAS Standard Mix 3 (8.0ppm Cu, Fe, Mn; 2.0ppm Zn)
Add to a 1000 ml volumetric flask 500 ml of DI Water (Type 1) approximately. Pipet 8.0 ml of Iron, Manganese and Copper. Pipet 2.0 ml of Zinc of the primary standard and 20.0 ml of HCl. Bring up to volume and mix well.

AAS Standard Mix 4 (10.0ppm Cu, Fe, Mn; 3.0ppm Zn)
Add to a 1000 ml volumetric flask 500 ml of DI Water (Type 1) approximately. Pipet 10.0 ml of Iron, Manganese and Copper. Pipet 3.0 ml of Zinc of the primary standard and 20.0 ml of HCl. Bring up to volume and mix well.

AAS Standard Mix 5 (20.0ppm Cu, Fe, Mn; 5.0ppm Zn)
Add to a 1000 ml volumetric flask 500 ml of DI Water (Type 1) approximately. Pipet 20.0 ml of Iron, Manganese and Copper. Pipet 5.0 ml of Zinc of the primary standard and 20.0 ml of HCl. Bring up to volume a mix well.

10.2.3. Recommended Standards Level:

Soluble and Chelate for Iron, Manganese and Copper

Standard Identification	**Concentration**
Standard 1	0.5 ppm
Standard 2	5.0 ppm
Standard 3	8.0 ppm
Standard 4	10.0 ppm
Standard 5	20.0 ppm

Soluble and Chelate Zinc

Standard Identification	**Concentration**
Standard 1	0.5 ppm
Standard 2	1.0 ppm
Standard 3	2.0 ppm
Standard 4	3.0 ppm
Standard 5	5.0 ppm

10.3. Instrument Startup

10.3.1. See LABOP 140 Maintenance, Operation and Program of AAS, Attachment K for details
10.3.2. Clean Burner with non-residual soap and water, do not scrape the slot flame burner exit, and dry. After inspecting, if there is still any residue, sonicate the burner for 20 minutes in a blend of methanol and acetone 50\50.
10.3.3. Set and align the burner into AAS analyzer
10.3.4. Turn on the AAS analyzer, pump and autosample
10.3.5. Turn on Fe, Mn, Cu and Zn multi lamp for 20 minutes
10.3.6. AAS spectrometer parameters used in soluble and chelate metal analysis:

AAS (AA-01) Perkin-Elmer AAnalyst 100	Burner Head Alignment	Wavelength (nm)	Slit Width (nm)	Acetylene Flow (L\Min)	Oxidant Flow (L\Min)
Iron	Straight	248.3	0.2	1.7	4.0
Manganese	Straight	279.5	0.2	1.7	4.0
Copper	Straight	324.8	0.7	1.7	4.0
Zinc	Straight	213.9	0.7	1.7	4.0

AAS (AA-02) Perkin-Elmer Pinnacle 500	Burner Head Alignment	Wavelength (nm)	Slit Width (nm)	Acetylene Flow (L\Min)	Oxidant Flow (L\Min)
Iron	Straight	248.3	0.2	10.0	2.50
Manganese	Straight	279.5	0.2	10.0	2.50
Copper	Straight	324.8	0.7	10.0	2.50
Zinc	Straight	213.9	0.7	10.0	2.50

10.4. Sample Analysis

10.4.1. Use the Sample Information File (Syngitix) to create the sequence.

10.4.2. An instrument blank must be injected at the beginning of a sample sequence to act as a correction.

10.4.3. After the initial calibration, there must be a continuing calibration check standard analyzed after every 10 samples and at the end of the sequence.

10.4.4. The Laboratory Control Samples (LCS) should be analyzed with their associated samples, not grouped together at the beginning of the sequence.

10.4.5. The sequence requirements are found in, DATA 051 Attachment A, Fertilizer Analysis Requirements.

10.4.6. Samples require the proper laboratory information management system (LIMS) codes to allow required uploading. Refer to ADMIN 023 Attachment M, Instrument Sequence Generation for Fertilizer, for additional information.

10.4.6.1. Example: AA99999-0AT

10.4.6.1.1. 0 corresponds to the sample weight (0, 1, 2.)
10.4.6.1.2. A corresponds to the different injections due to dilutions (A=1st injection, B=2nd injection, C=3rd injection.)
10.4.6.1.3. T or H corresponds to analysis type (T=Totals and H=Heavy Metals)

10.4.7. Dilution factors must be entered into the instrument sequence.

10.5. Data Evaluation

10.5.1. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.5.2. If the sample is excessive reprep using the excess method in PREP 462.

10.5.3. If liquid sample is deficient in Zinc, Manganese, or Copper and contains ammonium polyphosphate as a phosphate source, the sample should be re-prepped using the EDTA extraction in PREP 462.

10.6. Quality Control Sample

10.6.1. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples. Guidelines, that supersede those of QUALITY 112, are addressed in this document.

11 WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Aqueous extracts	Disposed of down sink while flushing water
AAS Instrument Waste	Disposed of down sink while flushing water

12 REFERENCES

12.1. PinAAcle 500 users guide, external document 00100
12.2. AA Instrument Maintenance Checklist and log, external document 00101
12.3. AA Fast Flame Manual, external document 00102
12.4. Manganese- Soluble AA. FM-830, Rev. 2.0 (01/03/2003)
12.5. Copper- Soluble AA. FM-831, Rev. 2.0 (01/03/2003)
12.6. Iron- Soluble AA. FM-832, Rev. 2.0 (01/03/2003)
12.7. Zinc- Soluble AA. FM-833, Rev. 2.0 (01/03/2003)
12.8. Iron Chelate – Pure Material- AA. FM-840 Rev. 2.0 (12/23/02)
12.9. Chelated Secondary/Micronutrient Elements – AA. FM-841 Rev 1.0 (11/12/2001)

13 VERSION HISTORY

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1.0	01.26.2022	Original Version	Jack Andreu	

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METHOD 530

Automated Direct Distillation of Ammoniacal Nitrogen in Fertilizer [FM415] [FM420] [FM435]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of ammoniacal nitrogen in fertilizer by direct ammonium distillation.

2. SCOPE

2.1. The procedure delineated below is applicable to the direct determination of ammoniacal nitrogen in fertilizer samples. Fertilizer samples are alkalized converting ammonium (NH_4^+) to the volatile ammonia (NH_3). The alkalization is followed by direct steam distillation into a pH-4.65 solution of 4% boric acid. The boric acid solution is then titrated back to pH 4.65 with sulfuric acid.

Distillation – $(NH_4)_2SO_4 + 2NaOH \rightleftharpoons 2NH_3 + Na_2SO_4 + 2H_2O$

Acid Trap – $B(OH)_3 + NH_3 + H_2O \rightleftharpoons NH_4^+ + B(OH)_4^-$

Titration – $2B(OH)_4^- + H_2SO_4 \rightleftharpoons SO_4^{2-} + 2B(OH)_3 + 2H_2O$

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

4. OUTLINE of PROCEDURE

4.1. System Startup
4.2. Ammoniacal Nitrogen Determination (FM420)
4.3. Ammoniacal Nitrogen Determination with Urea Present (FM415)
4.4. Ammoniacal Nitrogen Determination with Urea and Water-Insoluble Nitrogen Present (FM435)
4.5. Data Evaluation
4.6. Quality Control Sample

5. INTERFERENCE

5.1. Fertilizer samples containing urea can lead to false high results for ammoniacal nitrogen. The urea may breakdown to carbon dioxide and ammonia under alkaline conditions during steam distillation. This can be avoided by substituting the sodium hydroxide with an excess of magnesium oxide (MgO) to make the sample mildly alkaline.

5.2. Fertilizer samples containing water-insoluble nitrogen can lead to false high results for ammoniacal nitrogen. This can be avoided by filtering out the water-insoluble nitrogen after performing a water extraction.

6. APPARATUS and MATERIAL

6.1. Buchi KjelMaster K-375 with pH electrode
6.2. 300mL sample tube
6.3. 500mL sample tube
6.4. Glass funnel
6.5. 50mL centrifuge tube
6.6. Watman No. 42 Filter Paper, or equivalent
6.7. Geno/Grinder
6.8. Analytical balance

7. REAGENTS and MEDIA

7.1. Boric Acid (H_3BO_3), 4% w/v (Purchased)
7.2. Boric Acid (H_3BO_3), solid

7.3. Magnesium Oxide (MgO), solid, ACS grade
7.4. Sodium Hydroxide Solution (NaOH), 50% w/w for Kjeldahl Nitrogen Determination (Purchased)
7.5. Sulfuric Acid (H_2SO_4), concentrated, ACS grade
7.6. Sulfuric Acid, 0.5N certified
7.7. Water, Deionized (DI) Type I

8. REAGENT PREPARATION GUIDELINES

8.1. Reagent 01: 4% Boric Acid, pH 4.65

8.1.1. Directions: Dissolve 40g of boric acid in DI water to a final volume of 1000mL. (4% Boric Acid may also be purchased.)

8.1.2. Adjust the pH of the 4% boric acid while stirring with ~10% H_2SO_4 to a pH of 4.65 (use ~10% NaOH to adjust the pH). pH may be measured by selecting “System Preparation,” select “Measuring pH or mV.”

8.2. Reagent 02: ~10% H_2SO_4

8.2.1. Directions: Add approximately 5mL conc. H_2SO_4 to approximately 35mL of DI water. Bring to 50mL final volume.

8.3. Reagent 03: ~10% NaOH

8.3.1. Directions: Add approximately 2.5mL 50% NaOH to approximately 35mL of DI water. Bring to 50mL final volume.

8.4. Reagent 04: 32% NaOH

8.4.1. Directions: Add 1280mL of 50% NaOH to approximately 400mL of DI water. Mix and let cool. Bring to 2L final volume.

8.5. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.6. The specific ingredient amounts can vary to accommodate the batch size.

8.7. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.8. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. Form F00015, Reagent Preparation
9.2. Label L003, Reagent

10. SPECIFIC PROCEDURES

10.1. System Startup

10.1.1. Start chiller and allow to circulate for approximately 15 minutes prior to analysis.

10.1.2. Check waste reservoir level. Empty the waste into the Kjeldahl waste container for disposal.

10.1.3. Ensure the water reservoir, 4% Boric Acid reservoir, and H_2SO_4 titrant bottle have adequate volume for analysis.

10.1.4. Place instrument in Ready mode by selecting “Ready.”

10.1.5. Under “System Preparation,” select “Preheating.”

10.1.6. pH Probe Calibration

10.1.6.1. Select “System Preparation,” select “Calibration pH electrode.”

10.1.6.2. Calibrate the pH probe using pH 4 buffer and pH 7 buffer.

10.1.6.3. Acceptable slope is 95 to 105.

10.1.7. Priming

10.1.7.1. H_2SO_4 Buret

10.1.7.1.1. Remove the receiving vessel and place a beaker under the titrant line.

10.1.7.1.2. Under “System Preparation,” select “Buret functions” and “Start.”

10.1.7.1.3. Repeat to ensure that all bubbles are removed from the buret and tubing.

10.1.7.1.4. Replace receiving vessel.

10.1.7.2. Water, 4% Boric Acid, NaOH Priming

10.1.7.2.1. Under “System Preparation,” select “Priming”

Note: pH probe must be in the receiver while priming.

10.2. Ammoniacal Nitrogen Determination (FM420)

10.2.1. Blank

10.2.1.1. Add approximately 20mL of DI water to a 300mL sample tube.

10.2.1.2. Place the 300mL sample tube on the autosampler rack.

10.2.2. Lab Control Sample (LCS)

10.2.2.1. Weigh 0.2g of ammonium sulfate LCS into weighing vessel.

10.2.2.2. Transfer sample from the weighing vessel into a 300mL sample tube by rinsing the weighing vessel with DI water a minimum of 3 times.

10.2.2.3. Place the 300mL sample tube on the autosampler rack.

10.2.3. Samples

10.2.3.1. Weigh samples into weighing vessel using Table 1 as a guide.

Table 1

Ammoniacal N %	Sample Weight
<5%	1g
>5%	0.2g

10.2.3.1. Transfer sample from the weighing vessel into a 300mL sample tube by rinsing the weighing vessel with DI water a minimum of 3 times.

10.2.3.2. Place the 300mL sample tube on the autosampler rack.

10.2.4. Method

Table 2			
420 Ammoniacal			
H_2O volume	50 mL	Distillation mode	Fixed time
NaOH volume	30 mL	Distillation time	240 s
Reaction time	5 s	Determination mode	Standard
Titration type	Boric acid titration	Receiving solution volume	60 mL
Titration solution	H_2SO_4 0.25 mol/L	Molarity	0.25
Valence factor	2	Titer	1
Sensor type	Potentiometric	Titration mode	Standard
Measuring mode	Endpoint pH	Endpoint pH	4.65
Titration start volume	volume 0.000 mL	Titration algorithm	Normal
Aspiration sample tube	Yes	Aspiration receiving vessel	Yes

10.2.5. Sequence

10.2.5.1. Build sample sequence by selecting “Sequences” on the main home screen.

10.2.5.2. Name sequence.

10.2.5.3. “Preheating” and “Priming” steps may be added to the sequence as needed.

10.2.5.4. Add the appropriate sample rack to the sequence.

10.2.5.4.1. Select the rack position of blank. Choose “Blank” as the sample type. Enter the blank name and select “420 Ammoniacal” as the method.

Note: the blank is used for blank correction and must be run before the LCS and samples.

10.2.5.4.2. Select the rack position of the LCS. Choose “Reference substance” as the sample type and select the reference substance from the list. Enter the LCS name, weight, and method.

10.2.5.4.3. Select the rack position of the sample. Choose “Sample” as the sample type. Enter the sample name, weight, and method.

10.2.5.5. Add the “Cleaning” step to the sequence.

10.2.5.6. Add the “Dose H_3BO_3” step, unless the pH probe can be transferred to the probe storage solution immediately after the end of the sequence.

Note: The pH probe must not be stored dry.

10.3. Ammoniacal Nitrogen Determination with Urea Present (FM415)

10.3.1. Blank

10.3.1.1. Add approximately 20mL of DI water to a 300mL sample tube.

10.3.1.2. Place the 300mL sample tube on the sample rack.

10.3.2. Lab Control Sample (LCS)

10.3.2.1. Weigh 0.2g of ammonium sulfate LCS into weighing vessel.

10.3.2.2. Transfer sample from the weighing vessel into a 300mL sample tube by rinsing the weighing vessel with DI water a minimum of 3 times.

10.3.2.3. Place the 300mL sample tube on the sample rack.

10.3.3. Samples

10.3.3.1. Weigh samples into weighing vessel using Table 1 as a guide.

10.3.3.2. Transfer sample from the weighing vessel into a 300mL sample tube by rinsing the weighing vessel with DI water a minimum of 3 times.

10.3.3.3. Place the 300mL sample tube on the sample rack.

10.3.4. Method

Table 3			
415-435 Ammoniacal with Urea			
H_2O volume	50 mL	Distillation mode	Automatic-IntelliDist
NaOH volume	0 mL	Distillation time	240 s
Reaction time	5 s	Determination mode	Standard
Titration type	Boric acid titration	Receiving solution volume	60 mL
Titration solution	H_2SO_4 0.25 mol/L	Molarity	0.250
Valence factor	2	Titer	1.0000
Sensor type	Potentiometric	Titration mode	Standard
Measuring mode	Endpoint pH	Endpoint pH	4.65
Titration start volume	volume 0.000 mL	Titration algorithm	Normal
Aspiration sample tube	No	Aspiration receiving vessel	Yes

10.3.5. Sample List

10.3.5.1. Build sample list by selecting “Sample List” on the main home screen.

10.3.5.2. Name Sample List.

10.3.5.2.1. Choose “Blank” as the sample type. Enter the blank name and select “415-435 Ammoniacal with Urea” as the method.

Note: the blank is used for blank correction and must be run before the LCS and samples.

10.3.5.2.2. Choose “Reference substance” as the sample type and select the reference substance from the list. Enter the LCS name, weight, and method.

10.3.5.2.3. Choose “Sample” as the sample type. Enter the sample name, weight, and method.

10.3.6. Add approximately 4g MgO (~1 scoop) directly to the 300mL sample tube. Immediately place the sample tube on distillation unit.

Caution: Sample tube will be very hot after distillation. Use care when removing the sample tube, allow distillation line to completely drain into sample tube by gently tapping the distillation line.

Note: Samples using MgO must not be aspirated.

10.3.7. Shutdown

10.3.7.1. Transfer the pH probe to the probe storage solution immediately after the end of the sequence.

Note: The pH probe must not be stored dry.

10.3.7.2. Under “System Preparation,” select “Cleaning.”

10.4. Ammoniacal Nitrogen Determination with Urea and Water-Insoluble Nitrogen Present (FM435)

10.4.1. Blank

10.4.1.1. Add approximately 40mL of DI water to a 300mL sample tube.

10.4.1.2. Place the 300mL sample tube on the sample rack.

10.4.2. Lab Control Sample (LCS)

10.4.2.1. Weigh 0.2g of SRM 695 LCS into 50mL centrifuge tube.

10.4.2.2. Add approximately 40mL of DI water.

10.4.2.3. Agitate using Geno/Grinder for 1 minute at 1000rpm.

10.4.2.4. Filter sample into the 300mL sample tube using a folded filter placed in a glass funnel on the top of the 300mL sample tube. Wet with filter with DI water in insure a seal before adding the sample.

10.4.2.5. If sample is clumping, use 2 to 5mL of methanol to break up clumping.

10.4.2.6. Rinse the 50mL centrifuge tube a minimum of 3 times into the filter.

10.4.2.7. Remove filter paper and funnel from the 300mL sample tube.

10.4.3. Samples

10.4.3.1. Weigh samples into 50mL centrifuge tube using Table 1 as a guide.

10.4.3.2. Add approximately 40mL of DI water.

10.4.3.3. Agitate using Geno/Grinder for 1 minute at 1000rpm.

10.4.3.4. Filter sample into the 300mL sample tube using a folded filter placed in a glass funnel on the top of the 300mL sample tube. Wet with filter with DI water in insure a seal before adding the sample.

10.4.3.5. If sample is clumping, use 2 to 5mL of methanol to break up clumping.

10.4.3.6. Rinse the 50mL centrifuge tube a minimum of 3 times into the filter.

10.4.3.7. Remove filter paper and funnel from the 300mL sample tube.

10.4.4. Method

Table 3			
415-435 Ammoniacal with Urea			
H_2O volume	50 mL	Distillation mode	Automatic-IntelliDist
NaOH volume	0 mL	Distillation time	240 s
Reaction time	5 s	Determination mode	Standard
Titration type	Boric acid titration	Receiving solution volume	60 mL
Titration solution	H_2SO_4 0.25 mol/L	Molarity	0.250
Valence factor	2	Titer	1.0000
Sensor type	Potentiometric	Titration mode	Standard
Measuring mode	Endpoint pH	Endpoint pH	4.65
Titration start volume	volume 0.000 mL	Titration algorithm	Normal
Aspiration sample tube	No	Aspiration receiving vessel	Yes

10.4.5. Sample List

10.4.5.1. Build sample list by selecting “Sample List” on the main home screen.

10.4.5.2. Name Sample List.

10.4.5.2.1. Choose “Blank” as the sample type. Enter the blank name and select “415-435 Ammoniacal with Urea” as the method.

Note: the blank is used for blank correction and must be run before the LCS and samples.

10.4.5.2.2. Choose “Reference substance” as the sample type and select the reference substance from the list. Enter the LCS name, weight, and method.

10.4.5.2.3. Choose “Sample” as the sample type. Enter the sample name, weight, and method.

10.4.6. Add approximately 4g MgO (~1 scoop) directly to the 300mL sample tube. Immediately place the sample tube on distillation unit.

Caution: Sample tube will be very hot after distillation. Use care when removing the sample tube, allow distillation line to completely drain into sample tube by gently tapping the distillation line.

Note: Samples using MgO must not be aspirated.

10.4.7. Shutdown

10.4.7.1. Transfer the pH probe to the probe storage solution immediately after the end of the sequence.

Note: The pH probe must not be stored dry.

10.4.7.2. Under “System Preparation,” select “Cleaning.”

10.5. Data Evaluation

10.5.1. Calculation

$$\frac{[\text{mL of Titrant} - \text{mL of Titrant Blank}][0.25\text{mol of } H_2SO_4 \text{ Titrant}][2\text{mol } H_3BO_3][1\text{mol N}][14.0067\text{g N}][1\text{L } H_2SO_4]}{[\text{g sample weight}][1\text{ L of } H_2SO_4 \text{ Titrant}][1\text{mol } H_2SO_4][1\text{mol } H_3BO_3][1\text{mol N}][1000\text{ mL } H_2SO_4]} x100 = \%N$$

$$\frac{[\text{mL of Titrant} - \text{mL of Titrant for Blank}][0.5][1.40067]}{[\text{g sample weight}]} = \%N$$

10.5.2. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations, that supersede those of DATA 051, are addressed in this document.

10.6. Quality Control Sample

10.6.1. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples. Guidelines, that supersede those of QUALITY 112, are addressed in this document.

11. WASTE MANAGEMENT

11.1. Material, marked for disposal, is removed via the following mechanism:

Material	Disposal Mechanism
Waste Reservoir	Neutralize to 6-8 pH and dispose down sink

12. REFERENCES

12.1. Ammoniacal Nitrogen (Urea Present) – Direct. FM 415, January 29, 2003
12.2. Ammoniacal Nitrogen (Urea Not Present) – Direct. FM 420, February 5, 2003
12.3. Breakdown Nitrogen Preparation. FM 430, November 12, 2001
12.4. Ammoniacal Nitrogen (Urea Present) – Direct. FM 435, February 4, 2003
12.5. Selective nitrogen determination methods related to Kjeldahl, Buchi (Application Note No. 252/2016) November 2016.
12.6. AOAC, 920.03 *Nitrogen (Ammoniacal) in Fertilizer, Magnesium Oxide Method*, 2019

13. VERSION HISTORY

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METHOD 531

Automated Kjeldahl Digestion of WSON, WIN, and TN in Fertilizer

[FM425] [FM440] [FM445] [FM405]

1. PURPOSE

1.1. This standard operating procedure (SOP) provides instruction for the determination of urea and other water soluble organic nitrogen (WSON), water insoluble nitrogen (WIN), and total nitrogen (TN) in fertilizer by the Kjeldahl method. This SOP also includes instructions for the indirect determination of nitrate (NO_3).

2. SCOPE

2.1. The procedure delineated below is applicable to the determination of urea and other water soluble organic nitrogen, water insoluble nitrogen, total nitrogen, and nitrate in fertilizer samples. Fertilizer samples are digested with a mixture of an inert salt, a catalyst, and sulfuric acid converting nitrogen to ammonium (NH_4^+). The ammonium is then alkalized to convert to the volatile ammonia (NH_3). The alkalization is followed by direct steam distillation into a pH-4.65 solution of 4% boric acid. The solution is then titrated back to pH 4.65 with sulfuric acid.

Digestion – Organic N + $H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$

Distillation – $(NH_4)_2SO_4 + 2NaOH \rightleftharpoons 2NH_3 + Na_2SO_4 + 2H_2O$

Acid Trap – $B(OH)_3 + NH_3 + H_2O \rightleftharpoons NH_4^+ + B(OH)_4^-$

Titration – $2B(OH)_4^- + H_2SO_4 \rightleftharpoons SO_4^{2-} + 2B(OH)_3 + 2H_2O$

2.2. Potassium sulfate is used during the digestion to increase the boiling point of sulfuric acid from approximately 335 °C to approximately 354 °C, thereby increasing the rate of the reaction. Optimal acid to salt ratio is 2:1 (2 mL H_2SO_4 to 1 g of potassium sulfate). A catalyst such as copper is utilized to increase the efficiency and speed of the digestion. Mercury is not used as a catalyst due to its toxicity.

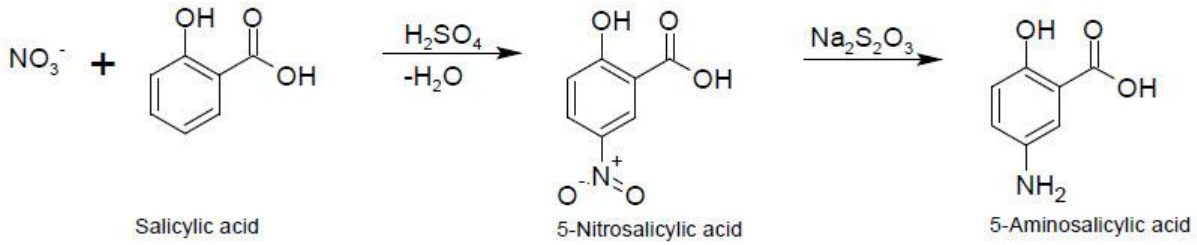
2.3. For urea determination, AOAC Method 955.04 was used as a basis for this procedure and was modified for the use of an automated Kjeldahl system utilizing steam distillation and a boric acid titration. Copper was substituted for mercury as the catalyst, and the acid to salt ratio was optimized. AOAC Method 930.1 and 930.2 were incorporated to liberate nitrate interference by the addition of ferrous sulfate to the digestion.

2.4. Since Kjeldahl nitrogen digestions include Ammoniacal nitrogen, the Ammoniacal nitrogen results must be subtracted to determine urea results.

2.5. Samples containing WIN are extracted with water and filtered prior to the digestion step. AOAC Method 945.01 was used as a basis for this procedure. Mechanical agitation and centrifugation were adapted. A finer grade of filter paper is also utilized (Whatman No. 42 was substituted for Whatman No. 2). The aqueous extract is then digested as above for urea analysis.

2.6. For WIN determination, the filter with the remaining insoluble residue is digested. AOAC Method 945.01 was used as a basis for this procedure and was modified for the use of an automated Kjeldahl system utilizing steam distillation and a boric acid titration. Copper was substituted for mercury as the catalyst, and the acid to salt ratio was optimized. Hydrogen peroxide was incorporated during the early stage of digestion to mitigate excess foam.

2.7. For total nitrogen determination, salicylic acid and sulfuric acid are used to convert nitrate to nitrosalicylic acid. Sodium thiosulfate is then used to reduce the nitrosalicylic acid to its amino form. AOAC Method 955.04 D. was used as a basis for this procedure and was modified for the use of an automated Kjeldahl system utilizing steam distillation and a boric acid titration. Copper was substituted for mercury as the catalyst, and the acid to salt ratio was optimized.



Salicylic acid — 5-Nitrosalicylic acid — 5-Aminosalicylic acid

2.8. Nitrate can be calculated from subtracting the total Kjeldahl nitrogen (TKN) from the Kjeldahl total nitrogen (TN). TKN consists of the sum of nitrogen from organic nitrogen and ammoniacal forms.

2.9. Nitrogen sources not detectable with Kjeldahl include azide, azine, azo, hydrazine, nitrile, nitro, nitroso, oxime, and semi-carbazone.

3. HEALTH and SAFETY

3.1. A reference file of Safety Data Sheet (SDS) is available to all personnel involved in the chemical analysis. All essential personal protective equipment (PPE) including safety glasses, gloves and laboratory coat is required.

3.2. There is a risk of acid fume inhalation during digestion. The use of a scrubber during digestion and cooling will assist in removing acid fumes. A fume hood must be used during the operation of the scrubber and digestor.

4. OUTLINE of PROCEDURE

10.1 Sample Preparation
- 10.1.1 Preparation for Urea (FM425)
- 10.1.2 Preparation for Urea (FM440) containing WIN (FM445)
- 10.1.3 Preparation for Total Nitrogen (FM405)

10.2 Digestion
- 10.2.1 Digestion of Urea (FM425/FM440)
- 10.2.2 Digestion of WIN (FM445)
- 10.2.3 Digestion of Total Nitrogen (FM405)

10.3 Distillation, and Titration
- 10.3.1 System Startup
- 10.3.2 Sequence
- 10.3.3 Distillation and Titration of Urea (FM425/440)
- 10.3.4 Distillation and Titration of WIN (FM445)
- 10.3.5 Distillation and Titration of Total Nitrogen (FM405)

10.4 Post-run Cleaning
10.5 Data Export and Upload
10.6 Data Evaluation
10.7 Quality Control Sample

5. INTERFERENCE

5.1. A digestion temperature must not exceed 380 °C. Temperatures above 390 °C lead to the possibility of nitrogen gas (N_2) formation resulting in low nitrogen results. The ratio of salt to sulfuric acid is critical in maintaining optimal temperature.

5.2. Interference for Urea Determination

5.2.1. Nitrate in fertilizer samples can cause low N recovery. Volatile N_2O can be produced when nitrates oxidize a portion of the ammonia released from the digestion. This can be avoided by the addition of ferrous sulfate with sulfuric acid to reduce nitrates present in the sample to nitric oxide (NO^-) gas.

$$3Fe^{2+} + NO_3^- + 4H^+ \rightarrow 3Fe^{3+} + NO^- + 2H_2O^+$$

5.2.2. WIN in fertilizer samples yields false high N results and must be filtered out for accurate determination of urea.

5.2.3. During digestion, black foam might form in the digestion tube. To achieve optimal reflux, efforts shall be made to keep the foam out of the condensation zone (5 cm below the constriction of the sample tube). This can be achieved by using antifoaming tablets and/or adjusting the temperature program to hold at a lower temperature, until the foaming has calmed, before continuing with the digestion ramp.

5.3. Interference for WIN Determination

5.3.1. It was found that, during sample digestion, the combination of water and a cellulose filter pad yields an excess of black foam that can rise into the suction module. To achieve optimal reflux, efforts shall be made to keep the foam out of the condensation zone (5 cm below the constriction of the sample tube). The addition of 30% H_2O_2 early in the digestion was found to mitigate the foam.

5.4. Interference for Total Nitrogen Determination

5.4.1. The reaction of nitrate to nitrosalicylic acid is inhibited by the presence of water. Liquid fertilizers may be analyzed up to 1 g, for reproducible results.

6. APPARATUS and MATERIAL

6.1. Buchi KjelDigester K-449
6.2. Buchi Scrubber K-415 Triple Scrub
6.3. Buchi KjelMaster K-375 with pH electrode
6.4. Buchi KjelSampler K-376
6.5. 300-mL sample tube
6.6. 500-mL sample tube
6.7. Glass funnel
6.8. 50-mL centrifuge tube
6.9. Geno/Grinder
6.10. Glass rods
6.11. Analytical balance
6.12. Plastic weigh boats
6.13. Nitrogen-free weigh boats

7. REAGENTS and MEDIA

7.1. Antifoaming Tablets
7.2. Boric Acid (H_3BO_3), 4% w/v (Purchased)
7.3. Bromothymol blue
7.4. Ferrous Sulfate ($FeSO_4$)

7.5. Hydrogen Peroxide (H_2O_2), 30%, ACS grade
7.6. Kjeldahl Tablets, CT-50 5 g K_2SO_4, 0.15 g $CuSO_4$
7.7. Salicylic Acid
7.8. Sodium carbonate (Na_2CO_3)
7.9. Sodium Hydroxide Solution (NaOH), 32% w/w for Kjeldahl Nitrogen Determination (Purchased)
7.10. Sodium Hydroxide Solution (NaOH), 50% w/w for Kjeldahl Nitrogen Determination (Purchased)
7.11. Sodium Thiosulfate
7.12. Sulfuric Acid (H_2SO_4), concentrated, ACS grade
7.13. Sulfuric Acid, 0.5N certified
7.14. Water, Deionized (DI), Type I

8. REAGENT PREPARATION GUIDELINES

8.1. **4% Boric Acid, pH 4.65**, Dissolve 40 g of boric acid in DI water to a final volume of 1000-mL. (4% Boric Acid may also be purchased.) Adjust the pH to 4.65 while stirring using ~10% H2SO4 or ~10% NaOH. pH may be measured using the Buchi pH meter, by selecting “System Preparation,” then“Measuring pH or mV.”

NOTE: The pH of 4% Boric Acid that has been purchased must be adjusted to 4.65.

8.2. **~10% H_2SO_4**, Add approximately 5 mL conc. H_2SO_4 to approximately 35 mL of DI water. Bring to 50-mL final volume.

8.3. **~10% NaOH,** Add approximately 10 mL 50% NaOH to approximately 35 mL of DI water. Bring to 50-mL final volume.

8.4. **32% NaOH**, Add 1280 mL of 50% NaOH to approximately 400 mL of DI water. Mix and let cool. Bring to 2-L final volume. (32% NaOH may be purchased)

8.5. **Scrubber Neutralization Solution,** Dissolve 600 g sodium carbonate in 3 L warm DI water. Add a spatula tip of Bromothymol blue as a color indicator.

Note: The fresh solution has a blue color while the neutralized or acidic solution has an orange yellowish color. The color transition occurs between pH 6.0 to 7.6.

8.6. Label the container appropriately, Label L003 indicates required information. Add relevant information to Reagent Preparation, Form F00015

8.7. The specific ingredient amounts can vary to accommodate the batch size.

8.8. Expiration Date: 12 months from date of preparation unless otherwise specified.

8.9. Store at room temperature

9. ASSOCIATED DOCUMENTS

9.1. LABOP 140 Attachment P, Maintenance, Operation, and Program for Büchi Kjeldahl Digestion Analyzer
9.2. Method 530, Automated Direct Distillation of Ammoniacal Nitrogen in Fertilizer [FN414] [FM420] [FM435]
9.3. External 00199 KjelDigester K-446/K-449 Operation Manual
9.4. External 00198 KjelMaster K-375 with KjelSampler K-376/K-377 Operation Manual

10. SPECIFIC PROCEDURES

10.1. Sample Preparation

10.1.1. Preparation for Urea (FM425)

10.1.1.1. Weigh the sample into a plastic weigh boat, using Table 1 as a guide.
10.1.1.2. A reagent blank and a laboratory control sample (LCS) should also be digested and analyzed with each prep batch. (The blank is prepared with the same acid volume, water volume, and chemicals as the sample.)

Table 1	
Urea & Ammoniacal N %	Sample Weight
<5%	1 g
>5%	0.2 g

10.1.1.3. Transfer the sample into a 300-mL sample tube by rinsing the weigh boat with DI water.
10.1.1.4. Add approximately 2 g (~¼ teaspoon) of ferrous sulfate to the 300-mL sample tube.
10.1.1.5. Add one antifoaming tablet to the 300-mL sample tube.
10.1.1.6. Bring to a volume of approximately 100 mL with deionized water and mix.
10.1.1.7. Add two Kjeldahl Tablets and 20 mL sulfuric acid to the 300-mL sample tube and mix.
10.1.1.8. Use a glass boiling rod in each tube to prevent bumping or boil overs during digestion.
10.1.1.9. Cover any open positions on the digestion rack with glass caps.
10.1.1.10. Digest the sample as in step **10.2.1 Urea (FM425/FM440) Digestion**

10.1.2. Preparation for Urea (FM440) containing WIN (FM445)

10.1.2.1. Weigh the sample into a 50-mL centrifuge tube using Table 2 as a guide.

10.1.2.2. A reagent blank and a laboratory control sample (LCS) should also be digested and analyzed with each prep batch. (The blank is prepared with the same acid volume, water volume, filter pad, and chemicals as the sample.)

Table 2 (use the higher weight)		
Sum of Urea & Ammoniacal N %	WIN %	Sample Weight
<5%	<5%	1 g
>5%	>5%	0.2 g

10.1.2.3. Add approximately 40 mL of DI water.

10.1.2.4. Agitate using Geno/Grinder for 1 minute at 1000rpm.

10.1.2.5. Centrifuge for 10 minutes at 4000 rcf.

10.1.2.6. Place a Whatman 42 filter in a glass funnel on the top of a 300-mL sample tube, for Urea **FM440**. Wet the filter with DI water to ensure a seal.

10.1.2.7. Decant the liquid portion of the sample through the filter into the 300-mL sample tube taking care not to disturb the solid sample pellet in the centrifuge tube.

Note: A few milliliters may be left in the tube so not to transfer more solid sample to the filter than necessary.

10.1.2.8. Wash the sample pellet in the centrifuge tube 3 times following the steps below:

10.1.2.8.1. Add approximately 20 mL DI water.

10.1.2.8.2. Agitate using Geno/Grinder for 1 minute at 1000rpm.

10.1.2.8.3. Centrifuge for 10 minutes at 4000 RCF.

10.1.2.8.4. Decant the liquid portion of the sample through the filter into the 300-mL sample tube taking care not to disturb the solid sample pellet in the centrifuge tube.

10.1.2.9. Transfer the filter paper to a new 300-mL sample tube for WIN **FM445** and keep the solid sample pellet in the centrifuge tube for later transfer.

10.1.2.10. To the 300-mL sample tube containing the filtered sample **FM440,** add approximately 2 g (~¼ teaspoon) of ferrous sulfate.

10.1.2.11. Add one antifoaming tablet to the 300-mL sample tube.

10.1.2.12. Add two Kjeldahl Tablets and 20 mL sulfuric acid to the 300-mL sample tube and mix.

10.1.2.13. Use a glass boiling rod in each tube to prevent bumping or boil overs during digestion.

10.1.2.14. Cover any open positions on the digestion rack with glass caps.

10.1.2.15. Digest urea samples as in step **10.2.1 Digestion of Urea (FM425/FM440)**.

10.1.2.16. Transfer and wash the sample pellet into the 300-mL sample tube for WIN **FM445** repeating 5 times the following the steps below:

10.1.2.16.1. Add approximately 5 mL DI water to the centrifuge tube.

10.1.2.16.2. Shake or, if needed, Vortex to break up the sample pellet.

10.1.2.16.3. Transfer into the 300-mL sample tube for WIN.

10.1.2.16.4. Wash the 50-mL centrifuge tube into the 300-mL sample tube for WIN 4 times with approximately 5 mL DI water. Do not exceed ~30 mL total volume.

10.1.2.17. Add one antifoaming tablet to the 300-mL sample tube.

10.1.2.18. Add two Kjeldahl Tablets and 20 mL sulfuric acid to the 300-mL sample tube and mix by swirling.

10.1.2.19. Use a glass boiling rod in each tube to prevent bumping or boil overs during digestion.

10.1.2.20. Cover any open positions on the digestion rack with glass caps.

10.1.2.21. Digest the sample as in step **10.2.2 Digestion of WIN (FM445).**

10.1.3. **Preparation for Total Nitrogen (FM405)**

10.1.3.1. Weigh the sample into a Nitrogen-free weigh boat, using Table 3 as a guide. Liquid fertilizer samples are weighed directly into a 300-mL sample tube.

10.1.3.2. A reagent blank and a laboratory control sample (LCS) should also be digested and analyzed with each prep batch. (The blank is prepared with the same acid volume, water volume, weigh boat, and chemicals as the sample.)

Table 3	
Total N %	Sample Weight
<5%	1 g
>5%	0.2 g

10.1.3.1. Transfer the weigh boat containing the sample into a 300-mL sample tube.
10.1.3.2. Add one antifoaming tablet to the 300-mL sample tube.
10.1.3.3. Add one Kjeldahl Tablets to the 300-mL sample tube, and mix.
10.1.3.4. Weigh approximately 1 g of salicylic acid in a plastic cup. Add 20 mL sulfuric acid and mix by swirling occasionally, until completely dissolved.
10.1.3.5. Add the salicylic acid/sulfuric acid mixture to the 300-mL sample tube. Swirl to mix.
10.1.3.6. Let stand for at least 30 min at room temperature, swirling occasionally.
10.1.3.7. Add approximately 2.5 g sodium thiosulfate pentahydrate, and mix by swirling.
10.1.3.8. Let stand for 5 min., at room temperature. Swirl to mix.
10.1.3.9. Use a glass boiling rod in each tube to prevent bumping or boil overs during digestion.
10.1.3.10. Cover any open positions on the digestion rack with glass caps.
10.1.3.11. Digest the sample as in step **10.2.3 Digestion of Total Nitrogen (FM405).**

10.2. **Digestion**

10.2.1. **Digestion of Urea (FM425/FM440)**
10.2.1.1. Ensure that the water is flowing through the condenser on the scrubber.
10.2.1.2. Ensure that the Scrubber Neutralization Solution is blue.
10.2.1.3. Digest the sample as in the table below:

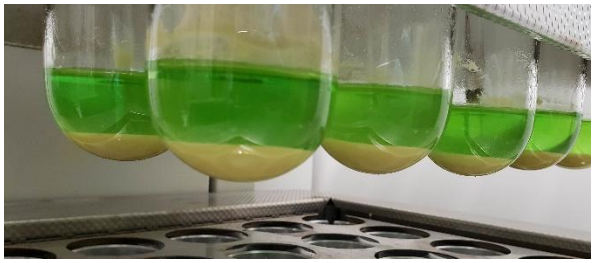
Note: The digestion block can be preheated at the desired temperature using Method 0.

Table 4

Urea Digestion (Method 1)		
Step	Temperature (°C)	Time (min)
1	250	5
2	280	20
3	330	30
4	420	65
Cooling	-	35

10.2.1.4. **Due to the volume of water in each sample, the CONDENSATION TRAP MAY NEED TO BE EMPTIED during the digestion, depending on the sample set size.**

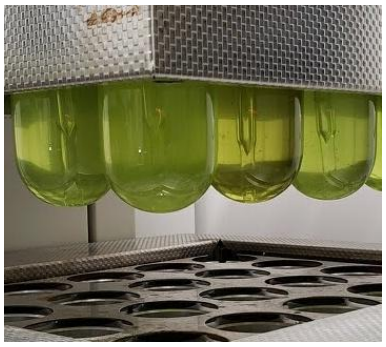
10.2.1.5. The digested sample is blue/green with a salt precipitate at the bottom. The salt must be re-dissolved into solution before analysis.



10.2.1.6. After the sample cools, add approximately 100 mL of deionized water to the 300-mL sample tube.
10.2.1.7. Heat the sample until the salt re-dissolves, as in the table below, and allow to cool:

Table 5

Re-dissolve (Method 2)		
Step	Temperature (°C)	Time (min)
1	280	0
2	380	10
Cooling	-	20



Note: If the above re-dissolve step was not preformed immediately after the digestion, precipitants may still be visually present. The sample should be immediately heated again with the above re-dissolve method.

10.2.1.8. Distill and titrate the sample as in step **10.3. Distillation and Titration,** using the appropriate instrument method.

10.2.2. **Digestion of WIN (FM445)**
10.2.2.1. Ensure that the water is flowing through the condenser on the scrubber.
10.2.2.2. Ensure that the Scrubber Neutralization Solution is blue.
10.2.2.3. Heat samples for 5 min as in the table below:

Note: The digestion block can be preheated at the desired temperature using Method 0.

Table 6

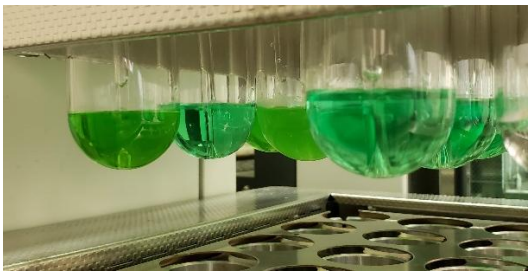
WIN Sample Heating (Method 3)		
Step	Temperature (°C)	Time (min)
1	300	5
Cooling	-	20

10.2.2.4. **CAUTION:** Slowly add ~10 mL of 30% hydrogen peroxide (H_2O_2) in increments of 2.5 mL, 2.5 mL, and 5 mL, allowing ~5 seconds between dosage. (Some samples may foam as the peroxide is added.)
10.2.2.5. Digest the sample as in the table below:

Table 7

WIN Digestion (Method 4)		
Step	Temperature (°C)	Time (min)
1	310	10
2	330	5
3	420	70
Cooling	-	35

10.2.2.6. The digested sample is blue/green.



10.2.2.7. If not distilling the sample immediately after digestion, add ~20 mL of water and swirl to mix, to prevent the sample from solidifying. If the sample has solidified, low heat may be applied to assistance in dissolving.

10.2.2.8. Distill and titrate the sample as in step **10.3. Distillation and Titration,** using the appropriate instrument method.

10.2.3. **Digestion of Total Nitrogen (FM405)**

10.2.3.1. Ensure that the water is flowing through the condenser on the scrubber.
10.2.3.2. Ensure that the Scrubber Neutralization Solution is blue.
10.2.3.3. Digest the sample as in the table below:

Note: The digestion block can be preheated at the desired temperature using Method 0.

Table 8

TN Digestion (Method 5)		
Step	Temperature (°C)	Time (min)
1	300	0
2	420	90
Cooling	-	35

10.2.3.4. If the sample is not clear blue/green, digest for an additional 15 minutes at 420°C.

10.2.3.5. If not distilling the sample immediately after digestion, add ~20 mL of water and swirl to mix, to prevent the sample from solidifying. If the sample has solidified, low heat may be applied to assistance in dissolving.

10.2.3.6. Distill and titrate the sample as in step **10.3. Distillation and Titration,** using the appropriate instrument method.

10.3. **Distillation and Titration**

10.3.1. System Startup

10.3.1.1. Start the chiller and allow to circulate for approximately 15 minutes prior to analysis.

10.3.1.2. Check the waste reservoir level. Empty the waste into the Kjeldahl waste container, for disposal.

10.3.1.3. Ensure that the water reservoir, the 4% Boric Acid reservoir, and the H_2SO_4 titrant bottle have adequate volume for analysis.

10.3.1.4. Place the instrument in Ready mode by selecting “Ready.”

10.3.1.5. Under “System Preparation,” select “Preheating.”

10.3.1.6. pH Probe Calibration

10.3.1.6.1. Select “System Preparation,” then “Calibration pH electrode.”

10.3.1.6.2. Calibrate the pH probe using the pH-4 buffer and the pH-7 buffer.

10.3.1.6.3. Acceptable slope is 95% to 105%.

10.3.1.7. Priming

10.3.1.7.1. H_2SO_4 Buret

10.3.1.7.1.1. Remove the receiving vessel and place a beaker under the titrant line.

10.3.1.7.1.2. Under “System Preparation,” select “Buret functions,” then“Start.”

10.3.1.7.1.3. Repeat to ensure that all bubbles are removed from the buret and the tubing.

10.3.1.7.1.4. Replace the receiving vessel.

10.3.1.7.2. Water, 4% Boric Acid, NaOH Priming

10.3.1.7.2.1. Under “System Preparation,” select “Priming”

Note: the pH probe must be in the receiver while priming.

10.3.2. Sequence

10.3.2.1. Build the sample sequence by selecting “Sequences” on the main home screen.

10.3.2.2. Name the sequence.

10.3.2.3. “Preheating” and “Priming” steps may be added to the sequence, as needed.

10.3.2.4. Add the appropriate sample rack to the sequence.

10.3.2.4.1. Select the rack position of the blank. Choose “Blank” as the sample type. Enter the blank name and select the appropriate method name (e.g., 425-440 Urea Kjeldahl, 445 WIN Kjeldahl, 405 TN Kjeldahl).

Note: the blank is used for blank correction and must be run before the LCS and samples.

10.3.2.4.2. Select the rack position of the LCS. Choose “Reference substance” as the sample type, and select the reference substance from the list. Enter the LCS name, weight, and method.

10.3.2.4.3. Select the rack position of the sample. Choose “Sample” as the sample type. Enter the sample name, weight, and method.

10.3.2.5. Add the “Cleaning” step to the sequence.

10.3.2.6. Add the “Dose H_3BO_3” step, unless the pH probe can be transferred to the probe storage solution immediately after the end of the sequence.

Note: The pH probe must not be stored dry.

10.3.3. **Distillation and Titration of Urea (FM425, FM440)**

Table 9

425-440 Urea Kjeldahl Distillation Parameters			
H_2O volume	0 mL	Distillation time	240 s
NaOH volume	90 mL	Stirring Speed	5
Reaction time	5 s	Steam Output	100%
Distillation mode	Fixed time		
Titration Parameters			
Titration type	Boric acid titration	Molarity	0.25
Titration solution	H_2SO_4 0.25 mol/L	Titration mode	Online
Sensor type	Potentiometric	Endpoint pH	4.65
Measuring mode	Endpoint pH	Stirring Speed	7
Receiving solution volume	60 mL	Titration algorithm	Normal
Titration start volume	volume 0.000 mL		
Determination Parameters			
Determination mode	Standard		
Aspiration sample tube	Yes	Aspiration receiving vessel	Yes

10.3.4. **Distillation and Titration of WIN (FM445)**

Table 10

445 WIN Kjeldahl Distillation Parameters			
H_2O volume	60 mL	Distillation time	240 s
NaOH volume	90 mL	Stirring Speed	5
Reaction time	5 s	Steam Output	100%
Distillation mode	Fixed time		
Titration Parameters			
Titration type	Boric acid titration	Molarity	0.25
Titration solution	H_2SO_4 0.25 mol/L	Titration mode	Online
Sensor type	Potentiometric	Endpoint pH	4.65
Measuring mode	Endpoint pH	Stirring Speed	7
Receiving solution volume	50 mL	Titration algorithm	Normal
Titration start volume	volume 0.000 mL		
Determination Parameters			
Determination mode	Standard		
Aspiration sample tube	Yes	Aspiration receiving vessel	Yes

10.3.1. **Distillation and Titration of Total Nitrogen (FM405)**

Table 11

405 TN Kjeldahl Distillation Parameters			
H_2O volume	60 mL	Distillation time	240 s
NaOH volume	90 mL	Stirring Speed	5
Reaction time	5 s	Steam Output	100%
Distillation mode	Fixed time		
Titration Parameters			
Titration type	Boric acid titration	Molarity	0.25
Titration solution	H_2SO_4 0.25 mol/L	Titration mode	Online
Sensor type	Potentiometric	Endpoint pH	4.65
Measuring mode	Endpoint pH	Stirring Speed	7
Receiving solution volume	50 mL	Titration algorithm	Normal
Titration start volume	volume 0.000 mL		
Determination Parameters			
Determination mode	Standard		
Aspiration sample tube	Yes	Aspiration receiving vessel	Yes

10.4. **Post-run Cleaning**

10.4.1. Wipe down any drips with a damp cloth.

10.4.2. Remove the suction module for cleaning. Place the suction module upside down in a sink, and flush with water. If major contaminates are visible, use a brush and detergent to clean the seals, the springs and the suction tubes. (The suction module may be cleaned in the dishwasher.)

10.4.3. Clean the drip tray with water.

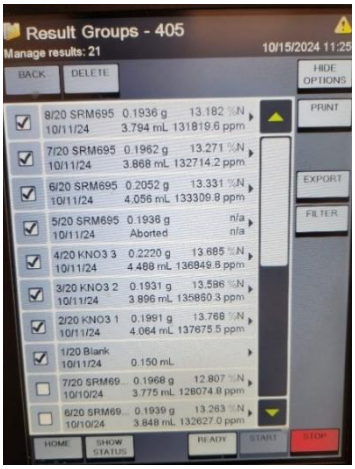
10.4.4. Wash the heating block cover as needed.

10.4.5. Empty and rinse the condensation trap with water.

10.4.6. See the K-449 manual for additional cleaning instructions.

10.4.7. After the sample tubes are cleaned, inspect the glassware for scratches or chips. Discard any scratched or chipped glassware.

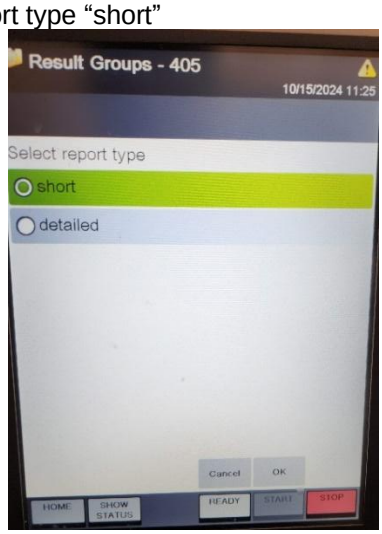
10.5. **Data Export and Upload**



10.5.1. Select the samples to be exported.

10.5.2. Select Export

10.5.3. Select Print, then select report type “short”



10.5.3.1. Copy the PDF report and the .kres files from the USB flash drive to P:\Pesticide Lab\BAEL_INSTRUMENT_DATA\AUTOMATED KJELDAHL
10.5.3.2. Open the file Buchi_File_Conversion.exe and follow the on-screen instructions to convert data to .csv file.

10.5.4. Upload the .csv file to the LIMS.

10.6. **Data Evaluation**

10.6.1. Titration Calculation

$$\frac{[\text{mL of Titrant} - \text{mL of Titrant Blank}][0.25\text{mol of } H_2SO_4 \text{ Titrant}][2\text{mol } H_3BO_3][1\text{mol N}][14.0067\text{g N}][1\text{L } H_2SO_4]}{[\text{g sample weight}][1\text{ L of } H_2SO_4 \text{ Titrant}][1\text{mol } H_2SO_4][1\text{mol } H_3BO_3][1\text{mol N}][1000\text{ mL } H_2SO_4]} x100 = \%N$$

$$\frac{[\text{mL of Titrant} - \text{mL of Titrant for Blank}][0.5][1.40067]}{[\text{g sample weight}]} = \%N$$

10.6.2. Urea Calculation

10.6.2.1. The Urea result can be calculated by subtracting the respective Ammoniacal (435 or 415 method) result from the respective Kjeldahl (440 or 425 method).

440 %N (Urea + Ammoniacal) - 435 %N (Ammoniacal) = Urea

or

425 %N (Urea + Ammoniacal) - 415 %N (Ammoniacal) = Urea

10.6.3. Nitrate Calculation

10.6.3.1. The Nitrate result can be calculated by subtracting the respective Kjeldahl (425 or 440 and 445 methods) result from the Total Kjeldahl (405 method).

405 %N (TN) - 425 %N = Nitrate

or

405 %N (TN) - 440 %N – 445 %N = Nitrate

10.6.4. Refer to DATA 051, Data Evaluation for Feed, Fertilizer and Pesticide Residue Programs, to evaluate the data against the established criteria. Evaluations that supersede those of DATA 051 are addressed in this document.

10.7. **Quality Control Sample**

10.7.1. Refer to QUALITY 112, Quality Control, to determine the outcome of Quality Control (QC) samples. Guidelines that supersede those of QUALITY 112 are addressed in this document.

11. WASTE MANAGEMENT

11.1. Material marked for disposal is removed via the following mechanism:

Material	Disposal Mechanism
Waste Reservoir	Neutralize to 6-8 pH and dispose down sink.

12. REFERENCES

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12.3. AOAC, 945.01 *Nitrogen (Water-Insoluble) in Fertilizer, Method I*, 2019
12.4. AOAC, 955.04 *Nitrogen (Total) in Fertilizer, Kjeldahl Method*, 2019
12.5. Buchi (2016). *Selective nitrogen determination methods related to Kjeldahl* (Application Note No. 252/2016). Retrieved from Buchi website: https://static1.buchi.com/sites/default/files/2016_252_AN_Selective_nitrogen_determination_0.pdf
12.6. FDACS (2001). *Water Insoluble Nitrogen – Kjeldahl*. FM 445, November 1, 2001.
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12.8. FDACS (2003). *Other Water Soluble Nitrogen B Kjeldahl – Direct*. FM 440, February 4, 2003.
12.9. FDACS (2003). *Other Water Soluble Organic Nitrogen – Direct.* FM 425, February 5, 2003.
12.10. Labconco (1998). *A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus* (2-41-9/98-JCON-5M-R3). Retrieved from Labconco website: https://www.labconco.com/resources/brochures

13. VERSION HISTORY

Version	Date	Description	Author	Editor
1.0	01.16.2025	Original Version	William Meeks, Jr., Patrizia Lemma, Ph.D.	
2.0	01.27.2025	On item 8.3, the volume of 2.5 mL of sodium hydroxide solution was changed to 10 mL		William Meeks Jr