2021

SFRI-GUIDE FROM SAMPLE RECEIVING TO ISSUANCE OF TEST RESULTS

Compiled and edited by Dr. Muhammad Akram Qazi

Director, Soil Fertility Research Institute, Punjab, Lahore

with the assistance of the editors

Muhammad Salik Ali Khan

and

Dr. Faraz Ahmed

Funded by the Soil Fertility Research Institute, Punjab, Lahore

---

Recommended Citation

M. A. Qazi, M. S. A. Khan, F. Ahmed (2021), *SFRI-Guide from sample receiving to issuance of test results.* Directorate of Soil Fertility Research Institute, Department of Agriculture, Punjab, Lahore

Important Disclaimer

A reasonable care was taken to make the information in this SFRI-Guide accurate and up-to-date and in accordance with the ISO/IEC 17025:2017 international standard and Standard Operating Procedures (SOP) issued by the Directorate, Soil Fertility Research Institute, Punjab, Lahore from time to time. However, authors would appreciate any comments and suggestions for further improvement of this SFRI-Guide.

Authors accept no liability whatsoever, by reason of negligence or otherwise, arising from any use or release of information in, or referred to in, this guide, or any error, inaccuracy, or omission in the information.

The analysis methods described in this guide need verification to ensure that the laboratory is capable of meeting the test method performance specifications. Verification of a test method demonstrates that the laboratory has met the test method's performance specifications and must be completed before the method is used for routine testing.

■ FOREWORD

During visit to Soil and Water Testing Laboratories across the Province Punjab, it was observed that different work strategies and methods for analysis of fertilizer samples were adopted in laboratories working under administrative control of Soil Fertility Research Institute, Punjab, Lahore. It is noteworthy that different analysis methods produce different results and hence separate interpretation is required. A need was identified to develop a guide to harmonize the work strategy and analysis methods among the laboratories.

This SFRI-Guide should be followed in all soil and water testing laboratories across province Punjab and will help to reduce the reporting of contradictory analytical results between the laboratories.

I hope you will find this SFRI-Guide useful and welcome any comments that may help for improvement.

Director

Soil Fertility Research Institute (SFRI), Punjab, Lahore

■ ACKNOWLEDGEMENT

Dr. Asad Rehman Gillani, Secretary Agriculture, Punjab deserves special acknowledgement for his inspiration and commitment in enabling us to produce a document harmonizing the analysis work performed in all SWT Laboratories of Agriculture Department.

FUTURE WORK STRATEGY OF SFRI

1. To improve the efficiency of service delivery of all divisional and district labs.
2. In all labs, all laboratory tests will be performed in accordance with uniform standard test methods.
3. To improve the handling of fertilizer samples collected as part of an anti-adulteration campaign for accuracy, repeatability, and reliability.
4. By implementing reforms in the field wing, the institute's original mandate of formulating fertilizer recommendations will be made more efficient and authenticated. In Sha Allah

■ CONTENTS

List of appendices

■ Introduction

The Soil Fertility Research Institute, Lahore oversees 37 soil and water testing laboratories, one in each of Punjab's districts. Nine (9) laboratories are part of the provincial system including Provincial Reference Fertilizer Testing Laboratory, Raiwind, while 28 are part of the district system. These laboratories provide advice or services to farmers and other stakeholders, such as the fertilizer industry, on the quality of soil, water, plants, fertilizers, amendments, etc. as well as how to make the best use of their soil and water resources.

Nine state-of-the-art laboratories functioning under provincial jurisdiction are ISO-17025:2017 accredited. These laboratories have the capability of analyzing soil, water, plant, and fertilizer samples with high-tech instruments such as ICP-OES, HPLC, GC, AAS, and FTIR. These laboratories also provide analytical services to the research institutes in addition to their routine activities. More significantly, these laboratories are monitoring the quality of fertilizers sold in Punjab as part of the province's anti-adulteration drive, which is governed by the Fertilizer Control Order 1973.

■ Guidelines for handling of FCO fertilizer samples

All the divisional (Primary) Soil and Water Testing Laboratories across Punjab should strictly adhere to the ISO-17025:2017 standard clause 4 which emphasizes the implementation of "Impartiality" and "Confidentiality" in all regulatory laboratories. The following instructions, in addition to the ISO standard requirements, are advised to be followed in this regard:

The principal scientist shall notify a committee with a detailed description of their TORs on the subject of receiving, handling, storage, coding, decoding, and reporting results of FCO fertilizer samples. For the sake of strict safety and security, two officers will be nominated as custodians of all forms of samples, especially in court cases and result discrepancy cases. Moreover, any member of this committee will not be given the task of analyzing FCO samples for the sake of confidentiality and impartiality.

■ TORs : Sample receiving

1. At the time of receiving of the fertilizer samples, the committee will check the following information.

- **a.** Cross match the name and CNIC # of the special messenger with his original CNIC as authorized samples carrier mentioned in forwarding letter of the Controller (Assistant / deputy Director, Agriculture (Extension).
- **b.** The sample seal must be intact, readable, reliable, and match with the imprint on the outer covering of the cloth bag and Form-1.
- **c.** Check Form-1, label, and outer covering of the fertilizer samples for following needful requirements.
  - i. Form-1 shall have standard format.
  - ii. Ensure that there are at least 10 working days left in the registration's expiry date, counted from the next day of bringing the sample into the lab.

iii. All boxes on this form must be filled with relevant information, and the entries must be compared to those on the sample label, the outer covering of the cloth bag, and the forwarding letter.

iv. Compare the name, address, and signature of the dealer on Form-1 with the name, address, and signature on the outer covering cloth bag.

v. Crossmatch the brand name, manufacturer, registration, and batch listed on form-1 with the outer covering cloth bag and fertilizer sample label.

vi. Ingredients with symbolic formulas such as K_2O, P_2O_5, and concentrations in percentages are written on this Form-1, which must crossmatch with the fertilizer sample label.

vii. Check the signatures of the Controller, Dealer, and Witnesses, and compare them to the signatures on the outer covering of cloth bag.

2. The receipt for receiving the samples will be given to the special messenger for his records.

■ **TORs: Sample coding**

1. The following details of fertilizer samples shall be entered in the Data Record Register by the sample opening committee.

a. Serial number

b. Date of opening the sample.

c. Registration number/Code

d. The controller and his letter No. and date.

e. Manufacturer of fertilizer and his address

f. Dealer and his address

g. Trade name of fertilizer

h. Ingredients to be measured in terms of concentration. The nutrients in liquid fertilizer samples, in particular, are only measured in terms of weight / volume (W/V %). The controller will need to correct a liquid sample that is labeled as W/W %.

i. Detailed description of the physical conditions of the samples (shape, size, colour, moisture or dryness of sample and texture).

j. Assign a special code for the order of analysis and should never be disclosed to anyone.

k. Analyst, who is engaged in fertilizer analysis, should not be included, neither in this committee nor in fertilizer receiving, storing, coding, decoding, and issuing results of these samples.

l. Each member of the committee shall have equal rights.

m. After completing the receiving formalities, the receiving committee must sign off on each sample entered in the data record register.

■ **TORs: Sample storage**

1. The fertilizer samples must be kept in chronological order in a safe place that is double locked with keys. Liquid fertilizer samples should be placed on the lower shelves of the cabinet to prevent contamination of other samples in the case of a leak or spill.

2. All previously taken over and fresh samples should be placed in the control sample room for the placement of samples in secure custody. The room should have two different locks meant for a separate member of the committee. The door of the sample control room shall not be opened by any single member, both of which will operate the sample room door jointly with the permission of chief scientist.

3. No duplicate key shall be allowed to any other staff member and not even chief scientist or Principal Scientist shall be allowed to open this lock of door and the record of opening the sample control room door shall be well documented.

4. Committee shall maintain the database of each sample placed in the sample room.

5. Each member of the committee shall have equal rights.

6. Strict adherence to the above TORs shall be observed.

■ **TORs: Sample movement within lab.**

1. The committee shall transfer a little part of each received sample in a separate washed, cleaned, and dry container after marking respective code on it. Along-with this container, the committee will prepare samples analysis order sheet containing only the following information.

a. A secret code

b. Detailed description of the physical conditions of the samples (shape, size, color, and texture).

c. Name of the parameter to be tested, without disclosing the company claim or any other identity.

d. Separate analysis order sheet must also be given to each analyst.

■ **TORs: Sample analysis**

1. The fertilizer sample, along with an "Analysis order sheet/Flow sheet" (see Appendix-3) and a transcript of the sample's physical conditions as noted by the sample opening committee, should be sent to the relevant analyst.

2. The analysis order sheet should be filled out in such a way that it aids in the construction of a timeline of events that begins with the receipt of the sample and ends with the delivery of the test results.

3. The analyst will receive the samples after cross-checking the physical conditions written on the analysis order sheet with those of the actual samples in the containers.

4. The analyst will follow the standard test methods (STM) mentioned in this SFRI-Guide, however, all laboratories must verify that they can perform the methods (STM) and achieve desired results. As part of the ISO 17025:2017 standard, a record of the verification must be kept. Verification must be validated if the STM is amended by the issuing authority.

5. The STMs / flow chart must be displayed near the test site, preferably at the sample analysis workplace.

6. All regulatory fertilizer samples should be analyzed in three replicates, and if necessary, a second analyst should be engaged to confirm the results.

7. To ensure the validity of test results, the analyst must use reference materials/reference standards or quality control materials in addition to the fertilizer sample. Each batch of analysis should include these reference standards as a sample. As a reference standards,

you can use samples received from the Proficiency Testing Program or samples from the Inter laboratory Comparison Program, or analytical grade salts of the element under test.

8. The recovery of these reference standards should not be less than 95%. Use correction factor in case test results of reference standards differ from its true value up to the extent of 5%. If deviation is more than 5%, repeat the analysis.
9. The analyst should keep track of all sources of verification that can be used to confirm the recorded results. All such records are classified as technical records under the ISO 17025:2017 standard (Clause 7.5) and should be maintained in perfect shape. This record may include:
    a. Logbooks of the instruments
    b. Readouts from instruments and titrations readings
    c. All sort of calculations
    d. Test repeatability
    e. Graphs or its equation with fitness of the curve
    f. Crossmatch sample results to those of a reference sample on a regular basis to ensure their authenticity.
10. Do not use lead pencil while recording the sources of verifications.
11. When an analyst seeks supervisory assistance from one of his or her senior officers, he or she should document it in his or her lab notebook. To avoid lost papers or sheets, lab notebooks should be in bind format.
12. Every page of the lab notebooks should be a control document, page numbering and certification on first page by the Lab Supervisor.
13. Analysts must sign and document the results reporting date at the end of their shift on that working day.
14. The analysts will submit the results with his / her signature on analysis order sheet to the supervisor.
15. The analysts will submit the analysis result within ten working days whereas, intelligence-based raided samples within two working days.

■ **TORs: Issuance of test results**

1. The Assistant Agricultural Chemist shall check the analysis results and verify the sources of verification. (See Appendix-3) and after satisfaction, he / she shall put signature on sources of verification and analysis order sheet and will send the analysis order sheet/flow sheet to the Agricultural Chemist.
2. The Assistant Agricultural Chemist should ensure that all lab activities related to fertilizer samples are tracked in a continuous chain from sample receiving to lab results issuance.
3. It is important to obtain a second analyst's opinion before reporting the results for confirmation.
4. The Agricultural Chemist will review analysis work that has been reported by the analyst on a random basis.
5. The Agricultural Chemist will approve the results and allow for issuance of results.
6. After approval, Assistant Agricultural Chemist will finalize the report on prescribed format and issue the report after getting signatures.

■ **Fertilizer samples disposal committee**

Agricultural Chemist will notify the fertilizer disposal committee after getting approval from Additional Secretary (Task Force),

- This committee should comprise of three officers including one convener and will be responsible for disposal of 1st portion of fertilizer samples.
- A list will be prepared for the following categories.
    - Fit samples
    - Unfit samples
    - Court/appeal cases
    - Unfit samples from reference lab after retesting of 30% fit-declared samples.
- Except fit samples, all samples will be retained till the finality of the decision.
- List will be prepared as following format.

Sr. No.	Product Name	Lab. Code	Composition

- Disposal committee will get the final approval from Agricultural Chemist for disposal.
- Disposal committee will also be responsible for handing-over the stock of samples to the respective/nearest research institute/station.
- All the record of handing over and receiving will be maintained properly.

■ Fertilizer Testing

ESTIMATION OF UREIC, AMMONIACAL AND NITRIC NITROGEN IN FERTILIZER BY KJELDAHL METHOD

■ Principle

The NH_3 in fertilizer sample or digested fertilizer sample is distilled in alkaline medium and absorbed in standard acid. Absorbing solution is an aqueous boric acid [$B(OH)_3$] solution of 2-4% concentration. The ammonia is quantitatively captured by the boric acid solution forming solvated ammonium ions. When using the boric acid solution as absorbing solution, titration is performed using standard solutions of sulfuric acid (concentrations in the range of 0.01N to 0.5N) and the amount of ammonia (as N) is calculated from the volume of standard acid consumed.

■ Total N

Total Nitrogen includes all forms of inorganic-N like NH_4-N, NO_3-N, Urea-N, and also compounds like protein, amino acids, and other derivatives. Depending upon the form of N present in particular sample, specific methods are adopted to get the Total Nitrogen.

■ Ureic N

Organic nitrogenous materials when digested with H_2SO_4 are oxidized to CO_2 and H_2O and their inorganic N is released. During digestion part of H_2SO_4 is reduced to SO_2 which in turn reduces nitrogenous materials to ammonia (NH_3). Ammonia combines with H_2SO_4 and form $(NH_4)_2SO_4$ at the end of digestion.

■ Nitrate-N

The procedure for the determination of nitrate and ammoniacal nitrogen with reduction is done by using Devarda's alloy. When a solution of nitrate ions and Devarda's alloy is mixed with aqueous sodium hydroxide, the mixture gently liberates ammonia gas. This conversion under the form of ammonia, requires some minutes. The method is applicable to all nitrogenous fertilizers, including compound fertilizers, in which nitrogen is found exclusively in nitrate form or in ammoniacal-N and nitrate form.

■ Equipment

- Kjeldhal's distillation apparatus
- Digestion block
- Digestion tubes
- Conical flasks, 500ml
- Pipette,1ml, 5ml, 10 ml (Bulb type)
- Cylinder, 50ml
- Beaker glass 500ml, 1000ml
- Wash bottle
- Burette

■ Reagents

- **H_2SO_4 (0.1N):** Prepare standard 0.1N solution (dissolve 2.8ml of H_2SO_4 when the specific gravity is 1.84 and purity is 95.0% and make volume one liter) and standardize against 0.1N NaOH.
- **NaOH (0.1N):** (Prepare 0.1 N NaOH by dissolving 4.0 g NaOH in distilled water and make volume 1 liter and standardize it against 0.1 N potassium hydrogen phthalate (dissolve 20.423g of potassium hydrogen phthalate and make volume one liter).
- **Sodium hydroxide (NaOH) 40% solution:** Dissolve 400 g solid NaOH in distilled water and dilute to one liter.
- **Boric acid 4%solution:** Dissolves 40g boric acid in distilled water and dilute to one liter.
- **Bromocresol green indicator:** Weigh 0.5g bromocresol green and 0.1g methyl red indicator and make volume 100ml with 95% ethyl alcohol.
- **Devarda's alloy:** (50% Cu, 45% Al, 5% Zn)
- **Digestion Mixture:** (9:1, K_2SO_4: $CuSO_4$)

■ Procedure

1. Ureic nitrogen

Weigh accurately 0.5g, ground and homogenized sample and transfer to the digestion tube, take 1ml of filtered sample in case of liquid. Add 1.0g of digestion mixture Add 10-12 ml of concentrated sulfuric acid to the digestion tube. Place the tube in the digestion block. Continue

heating for at least 2 hours at 400 ^{o}C until the contents of tube changes from black to light green or straw yellow or water white. Remove the digestion tube from digestion block and cool. Now sample is ready for distillation apparatus.

Place tube on distillation unit add 50ml of 40% NaOH and 10ml of distilled water in digestion tube and start distillation process. Place 250ml conical flask on receiver containing 40ml of 4% boric acid and few drops of bromocresol green indicator in such a way that outlet of receiver properly dipped in the boric acid. Nitrogen will be collected in the receiver containing 4% boric acid. Titrate against 0.1N standardized sulfuric acid from golden yellow to purple end point.

2. Ammoniacal-N and nitrate-N

Weigh accurately 0.5 g ground and homogenized fertilizer sample in the digestion tube. Place the tube on distillation apparatus. If NO_3-Nitrogen and NH_4-Nitrogen are to be determined, add 3.0 g Devarda's alloy along with the fertilizer sample but if only NH_4-Nitrogen is to be determined then just take 0.5 g of sample in the digestion tube and distillate (without Devarda's alloy). Distillate on distillation unit. Nitrogen will be collected in the receiver containing 4% boric acid. Titrate against 0.1N standardized sulfuric acid from golden yellow to purple end point.

■ Conditions for automatic Kjeldahl distillation unit

- Volume of 40% NaOH: 80 ml
- Volume of 4% Boric Acid: 40 ml
- Volume of distilled water: 10 ml
- Steam Flow: 100 %
- Distillation time: 4 minutes

■ Calculations

1 ml of 1 N H_2SO_4 = 0.0141g N.

1ml of 0.1 N H_2SO_4 = 0.00141 g N

If

0.1 N H_2SO_4 used in back titration = C ml

Then

C ml of 0.1 N H_2SO_4 contains N = 0.00141 g N x C

Therefore

% N = C x 0.00141 X 100/w (A-B) = C

Whereas

A = ml of 0.1N H_2SO_4 used for sample titration

B = ml of 0.1 N H_2SO_4 used for blank

W = weight in grams of fertilizer sample used.

■ Reference / Related Documents

- Tandon HLS (Ed.) 2009. Methods of Analysis of Soils, Plants, Waters, Fertilizer and Organic Manures Fertilizer Development and Consultation Organization, New Delhi. Pp 161-162
- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.4.03 (AOAC Official Method 955.04), Fertilizers Chapter 2 Page 14-15.
- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.4.10 (AOAC Official Method 892.01), Fertilizers Chapter 2 Page 15.
- VELP SCIENTIFICA®. APPLICATION NOTE-F&F-K-004-2018/A1. APPLICATION NOTEF&F-K-004-2. Nitrate and Ammonia Determination in Fertilizers according to the Devarda's Method Reference: UNIENISO 15476:2009

ESTIMATION OF PHOSPHORUS IN PHOSPHATIC FERTILIZER BY TITRIMETRIC METHOD

■ Principle

The available-P in phosphate containing fertilizer is extracted with weak acid (citric acid solution). Extracted phosphorous as orthophosphate can be determined as ammonium phosphomolybdate after precipitation with ammonium molybdate solution. Precipitates are filtered, washed to acid free and dissolved in standardized 0.1N sodium hydroxide. The excess of NaOH is titrated against standardized 0.1N Sulphuric acid to determinate amount of P in fertilizer sample.

■ Equipment

- Weighing balance
- Volumetric flask-100 ml, 500ml, 1000ml
- Beaker-100 ml, 500ml, 100ml
- Bulb type pipette 1ml, 5ml,10ml
- Conical flask-250ml, 500ml
- Water bath
- Wash Bottle
- Filter paper Whatman No.42
- Funnel with stand
- Blue Litmus paper
- Filter paper sheet

■ Reagents

- **Concentrated Nitric Acid**

- **Citric Acid Solution 2%:** Dissolve 20g of citric acid 1-hydrate salt ($C_6H_8O_7.H_2O$, 210.14g/mol, Analytical grade) in distilled water and make volume one liter.
- **Ammonium Molybdate Solution, (3%):** Dissolve 30-gram Ammonium Molybdate tetra hydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$, Analytical grade) salt in 1000ml distilled water.
- **Ammonium Nitrate Solution, (50 %):** Dissolve 500-gram Ammonium Nitrate (NH_4NO_3, 80.04g/mol, Analytical grade) salt in1000ml distilled water.
- **Phenolphthalein indicator:** Dissolve 01-gram phenolphthalein indicator in 100ml 50% ethanol (for 50% ethanol take 50ml ethanol and add 50ml distilled water)
- **Standardized 0.1 N Sulphuric Acid:** Dissolve 2.8ml of concentrated H_2SO_4 of AR grade with specific gravity 1.84 and purity 95.0% and make volume one liter with distilled water and standardize against 0.1N NaOH.
- **NaOH 0.1N:** Prepare 0.1 N NaOH by dissolving 4.0 g NaOH in distilled water and make volume 1 liter and standardize it against 0.1 N potassium hydrogen phthalate.
- **Potassium Hydrogen Phthalate Solution, 0.1N:** Dissolve 20.423gram Potassium Hydrogen Phthalate ($C_8H_5KO_4$, 204.23g/mol Analytical grade) salt in 1000 ml DI water.

■ Procedure

Weigh accurately 0.5 g homogenized phosphatic fertilizer sample previously grinded and sieved through 40 mesh sieves, in 100 ml volumetric flask, add citric acid solution (2%) approximately 50ml, raise the temperature up to 65^oC in water bath and shake for 60 minutes on mechanical shaker @ 270rpm. After shaking make volume up to mark with citric acid (2%) solution. Filter and take 10 ml solution from filtrate, in a 250 ml conical flask. Add 5 ml, concentrated nitric acid and 15 ml, ammonium nitrate solution (50 %). Heat the contents gently in water bath at 65^oC and then add gradually 50 ml of ammonium molybdate solution (3%). Shake the conical flask during ammonium molybdate solution addition.

Yellow precipitate of ammonium phosphomolybdate will form depending on the concentration of phosphorus present in the given fertilizer sample. Stay for one night. Next day filter the yellow precipitates using Whatman No. 42 and wash with ice cold distilled water till the filtrate does not turn blue litmus to red. This indicates that precipitates are now acid free. Now transfer the acid free precipitates along with filter paper into the same conical flask. Care should be taken that the same conical flask should also be acid free. Dissolve the precipitates completely in 0.1 N sodium hydroxide by adding 10 ml each time until the yellow color disappears. Note the amount of alkali used. Now add 2-3 drops of phenolphthalein indicator. Pink color will develop. Titrate against 0.1 N Sulphuric acid with continuous shaking till colourless end point. Note the volume of Sulphuric acid used.

■ CALCULATIONS

$$\% P_2O_5 = \frac{0.000309x\ X\text{-}Y\ x\ 100x100}{10x0.5}$$

Whereas

X = 0.1N NaOH used to dissolve precipitate

Y = 0.1N H_2SO_4 used for back titration.

OR

$\%P_2O_5$ = (x-y) x 0.618

If 0.5gm fertilizer sample is used

■ DETAIL OF CULCULATIONS

During chemical reaction out of 23 molecules of NaOH only one molecule of Na is used to form Na (NH_4) HPO_4 which contain one molecule of P.

Therefore, ammonium phosphomolybdate precipitate contains Na and P in the ratio of 1:1.

i .e, normal solution of NaOH (23gm Na/L) =1 N solution of P (31g P / L).

So 31/ 23 =1.3478g P/ L

1 N NaOH =1.3478x2.29g P_2O_5/ L (for P to P_2O_5 use 2.29)

-do- =3.0864g P_2O_5 / liter

-do- =0.003086g P_2O_5/ml

1 ml of 0.1 N NaOH =0.0003086g P_2O_5/ml

R ml of 0.1 N NaOH =0.0003086x R g P_2O_5 /ml (R = reading)

10ml of 0.1 sample contains = 0.000386 x R If 10 ml aliquot is used.

1 ml of sample contains =0.0003086 x R/10

100ml of sample contains=0.0003086 x R x 100/10

0.5 gm fertilizer contain P_2O_5=0.0003086 x Rx 100/10

1 gm fertilizer contain P_2O_5 =0.0003086 x R x 100/10 x 0.5

100gm fertilizer contain P_2O_5=0.0003086 x R x 100 x 100/10 x 0.5

So

P_2O_5 (%) =0.618 x R (Where sample (solid) taken is 0.5g)

For liquid samples

P_2O_5 (%) =0.0618 x R

Whereas

Sample (liquid) taken is 5ml.

■ Precautions

This method gives erroneous results in case of liquid samples containing P_2O_5 contents more than 10%. In such case, first make appropriate dilutions then proceed further.

■ Reference / Related Documents:

- Pakistan standard for Single Super Phosphate (2nd edition) PS: 67-1996. PSQCA. Karachi

ESTIMATION OF TOTAL PHOSPHORUS FROM BIO-ORGANIC FERTILIZERS

■ Principle

This testing method is applicable to fertilizers containing organic matters. Test sample is pretreated with nitric acid to convert total phosphorus into phosphate ions. Converted phosphate ions can be determined as ammonium phosphomolybdate after precipitation with ammonium molybdate solution. Precipitates are filtered, washed to acid free and dissolved in standardized 0.1N sodium hydroxide. The excess of which is back titrated against standardized 0.1N Sulphuric acid to determine amount of phosphorus in fertilizer sample.

■ Equipment

- Weighing balance
- Volumetric flask-100 ml
- Volumetric flask 1000 ml
- Beaker-100 ml
- Bulb type pipette-10 ml
- Graduated pipette 10 ml
- Conical flask-250 ml
- Wash Bottle
- Whatman No.42 filter paper
- Funnel with stand
- Blue Litmus paper
- Burette 100 ml

■ Reagents

- **Concentrated Nitric Acid**
- **Ammonium Molybdate Solution (3%):** Dissolve 30-gram Ammonium Molybdate tetra hydrate ($(NH_4)Mo_7O_{24}.4H_2O$, Analytical grade) salt in 1000ml volumetric flask and make up volume by DI water up to the mark.
- **Ammonium Nitrate Solution, (50 %):** Dissolve 500-gram Ammonium Nitrate (NH_4NO_3, 80.04g/mol, Analytical grade) salt in 1000ml volume metric flask and make up the volume with DI water up to the mark.(d)Phenolphthalein indicator(e)
- **Standardized 0.1 N Sulphuric Acid**
- **Standardized 0.1 N Sodium Hydroxide**
- **Potassium Hydrogen Phthalate Solution, (0.1 N):** Dissolve 20.423gram Potassium Hydrogen Phthalate ($C_8H_5KO_4$, 204.23g/mol Analytical grade) salt in 1000 ml volumetric flask and make up volume with DI water up to the mark.

■ Procedure

Weigh accurately 0.5 g homogenized BOP fertilizer sample previously grinded and sieved through 40 mesh sieves in 100 ml volumetric flask, add 5 ml concentrated nitric acid, add about 50 ml distilled water place the sample on hot plate and raise the temperature up to 65^{o}C on hot plate. Shake for 60 minutes on mechanical shaker. After shaking make volume up to mark distilled water. Filter and take 10 ml solution from filtrate, in a 250 ml conical flask. Add 5 ml, concentrated nitric acid and 15 ml, ammonium nitrate solution (50 %). Heat the contents gently on a hot plate at 65^{o}C and then add gradually 50 ml ammonium molybdate solution (3%). Shake the conical flask during ammonium molybdate solution addition. Yellow precipitate of ammonium phosphomolybdate will form depending on the concentration of phosphorus present in the given BOP sample. Stay for one night. Next day filter the yellow precipitates using Whatman No. 42 and wash with ice cold distilled water till the filtrate does not turn blue litmus to red. This indicates that precipitates are now acid free. Now transfer the acid free precipitates along with filter paper into the same conical flask. Care should be taken that the same conical flask should also be acid free. Dissolve the precipitates completely in 0.1 N sodium hydroxide by adding 10 ml each time. Note the amount of alkali used. Now add 2-3 drops of phenolphthalein indicator. Pink color will develop. Titrate against 0.1 N Sulphuric acid with continuous shaking till colorless end point. Note the volume of Sulphuric acid used.

■ Calculations

% P2O5=0.000309 x X -Y x 100 x 100 / 10 x 0.5
Where, X = 0.1N NaOH used to dissolve precipitate
Y = 0.1N H2SO4 used for back titration.
OR %P2O5= (x-y) x 0.618
If 0.5 gm fertilizer sample is used

■ **Detail of Calculation**

During chemical reaction out of 23 molecules of NaOH only one molecule of Na is used to form Na (NH4) HPO4 which contain one molecule of P.

Therefore, ammonium phosphomolybdate precipitate contains Na and P in the ratio of 1:1. i .e, normal solution of NaOH (23gm Na/L) =1 N solution of P (31g P / L).

So, 31/ 23 =1.3478g P/ L
1 N NaOH =1.3478x2.29g P2O5/ L (for P to P_2O_5 use 2.29)
-do-=3.0864g P_2O_5/ liter
-do-=0.003086g P_2O_5/ml
1 ml of 0.1 N NaOH =0.0003086g P_2O_5/ml
R ml of 0.1 N NaOH =0.0003086x R g P_2O_5 / ml (R = reading)
10ml of 0.1 sample contains = 0.000386 x R g P_2O_5(If 10 ml aliquot is used).
1 ml of sample contains =0.0003086 x R/10
100ml of sample contains=0.0003086 x R x 100/10
0.5 gm BOP contain P_2O_5=0.0003086 x Rx 100/10 (where sample taken is 0.5 g)
1 gm BOP contain P_2O_5 =0.0003086 x R x 100/10 x 0.5
100gm BOP contain P_2O_5=0.0003086 x R x 100 x 100/10 x 0.5
So
P_2O_5 (%) =0.618 x R (Where sample taken is 0.5g)

■ **Reference**

- Pakistan standard for BOP.PS:5295/2017 (2ndRev.), PSQCA. Karachi.

ESTIMATION OF POTASSIUM IN POTASH FERTILIZER BY FLAME PHOTOMETER METHOD

■ **Principle**

This test method is applicable to fertilizers containing potassium salts. Extract by adding water to an analytical sample and determine the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify water-soluble potassium (W-K_2O) in an analytical sample.

■ **Equipment**

- Flame photometer
- Analytical balance
- Volumetric flask-100 ml, 500ml, 1000ml
- Beaker glass 100 ml, 500ml, 100ml
- Bulb type pipette 1ml, 5ml,10ml
- Wash bottle

- Funnel with stand
- Filter paper sheet

■ **Reagents**

Potassium chloride: Use 1000 ppm Certified Reference Material (CRM) of potassium Chloride

Prepare a sub stock solution by taking 10 ml from 1000 ppm stoke solution and dilute to 100ml. Series of working standard solutions from the sub-stock solution as follows:
Dilute 5, 10, 15-, 20-, and 25-mL sub-stock solution to 100-mL final volume of each by adding DI water. These solutions contain 5, 10, 15, 20 and 25 ppm K, respectively.

■ **Procedure**

Dissolve 2.5 g ground potash fertilizer material in 200 ml distilled water in 500ml beaker. Cover the beaker with a watch glass and heat on a hot plate to boil for about 15 minutes. After standing to cool, transfer to a 250-mL volumetric flask with water. Give washings to the beaker with distilled water and make volume of volumetric flask up to the marked line. Make further dilutions if necessary.

■ **Standard calibration curve**

Optimize the instrument by adjusting the fuel and air flow. Adjust instrument read-out to zero for blank and 100 for 25 ppm standard. Now take readings of 5, 10, 15, 20 and 25 ppm standards and develop calibration curve accordingly.

Take readings of the sample filtrate and calculate the K concentration from standard calibration curve.

■ **Calculations**

$$K_2O\% = \frac{K\ (ppm) \times dilution\ Factor \times 1.2046}{10000}$$

■ **Reference / Related Documents**

- Testing Methods for Fertilizers (2016). Incorporated Administrative Agency. Food and Agricultural Materials Inspection Center. Japan. Section: 4.3.3.a
- Standard operating manual of instrument.

ESTIMATION OF WATER-SOLUBLE ZINC IN FERTILIZER BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle.** This test method is applicable to fertilizers that contain zinc contents as zinc sulphate. Extraction is done by boiling test sample in distilled water. Zinc contents are determined

by flame atomic absorption spectrometry (FAAS) in which monochromatic radiation, characteristic for the metal in question, is passed through a long, thin flame, into which the sample solution is sprayed. A flame is produced in a pre-mix type burner with a 10cm long slit. The burner head is aligned so that the radiations will pass exactly over this slit. These conditions result in absorption of radiation that is selective for element. The extent of absorption will be proportional to the number of ground state atoms present in the flame.

■ Reagents

- Certified Reference Material (CRM) of Zinc (1000 ± 5ppm Zn, stock solution). It should be NIST traceable and manufactured by ISO certified company.
- Prepare stock solution of 100ppm in Deionized water and can be stored for 15 days. Electrical conductivity (EC) of water should not be more than 20µS/cm.
- Prepare at least 3 working standards between 0 to 3ppm. Use graduated pipette for preparation of working standards. Read the lower meniscus for colorless liquids keeping mark on pipette at eye-level.

■ Equipment

- Analytical balance (with readout up to 3 decimals minimum)
- Hot Plate calibrated at 150 C^0.
- Atomic Absorption Spectrophotometer
- Glass Beaker 150 mL (Tall form) and use glass lid while boiling.
- Measuring cylinder 100mL
- Volumetric flask 100mL, 500mL, 1000mL, 2000 mL
- Glass Funnel
- Filter Paper (Whatman No.42)
- Graduated and calibrated pipette 2ml, 5ml.
- All the glassware should be Pyrex, Class-A type.
- Sieve Mesh No. 40
- Mortar and pestle

■ Procedure

Sample preparation: Grind almost 100g of sample as it is, (do not dry or desiccate) and pass all the ground sample from Mesh No. 40 sieve. Place 1.00 g well-ground test portion (2ml for liquid sample) in 150 mL glass beaker (tall form). Add 75mL de-ionized water and boil for 30 minutes (count time after boiling begins) on hot plate at 150 C^0. Put watch-glass on beaker. Wash the watch-glass into filtrate to avoid sample loss. Wash the beaker, watch-glass into 1 Liter volumetric flask. Make the volume up to the mark with distilled water. Shake well and filter through Whatman No. 42 filter paper. Re dilute if necessary.

Measurements: Optimize Atomic Absorption Spectrophotometer (AAS) parameters i.e., fuel flow, lamp energy current, lamp orientation and burner height. Calibrate the AAS between 0 and 3ppm standards. R^2 value of calibration curve should be 0.998 or higher otherwise recheck or repeat. Measure readings between 0.3 to 2.8ppm. In case, reading is above or below this limit,

dilute or concentrate the sample accordingly. Determine concentration of element in solution (ppm) from calibration curve or digital concentration readout following the standard operating parameters. Run the blank (D.I. water) between working standards and samples. Run the reference salt as check sample. This is optional.

Element	Wavelength (nm)	Flame	Range (ppm)
Zinc	213.8	Air-C_2H_2	0 - 3.0

■ **Calculations**

Zinc (%) = Reading x dilution /10,000.

■ **References / Related Documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Volume-1. Method No. 2.6.01 (AOAC Official Method 965.09) Fertilizers, Chapter 2, Page 29-30
- Operating manual of Atomic Absorption Spectrophotometer

ESTIMATION OF ACID-SOLUBLE ZINC COPPER IRON AND MANGANESE IN INORGANIC MATERIALS AND MIXED FERTILIZERS BY ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle**

This test method is applicable to in-organic mixed fertilizers containing zinc , copper, iron, and manganese. The process involves wet digestion with acid to release and solubilize the nutrients and flame atomic absorption spectrometry technique is used for determination. Atomic absorption spectrophotometry involves monochromatic radiation, characteristic for the metal in question, is passed through a long, thin flame, into which the sample solution is sprayed. A flame is produced in a pre-mix type burner with a 10cm long slit. The burner head is aligned so that the radiations will pass exactly over this slit. These conditions result in absorption of radiation that is selective for element. The extent of absorption will be proportional to the number of ground state atoms present in the flame.

■ **Reagents**

- **Concentrated HCl (Purity 37%)**
- **2M HCL solution**: Dissolve 165.8 ml of 37% pure HCl in 1 Liter volumetric flask and make up to 1 L.
- **0.5M HCl Solution**: Dissolve 82.89 ml of 37% pure HCl in 2 Liter volumetric flask and make-up to 2 L.
- **Certified Reference Material** (CRM) of Zinc, Copper, Iron and Manganese (1000 ± 5ppm, stock solution). CRM should be NIST traceable and manufactured by ISO certified company.

- Prepare stock solution of 100ppm in D.I. water and store for 15 days. Electrical Conductivity of water should not be more than 20µS/cm.
- Prepare at least 3 working standards considering the detection range of respective element given in table below. Use graduated pipette for preparation of working standards. Read the lower meniscus for colorless liquids keeping mark on pipette at eye-level.

■ Equipment

- Analytical balance (with readout up to 3 decimals minimum)
- Hot Plate
- Atomic Absorption Spectrophotometer
- Glass Beaker 150 or 250 mL (Tall form) and use glass lid while boiling.
- Measuring cylinder 100ml
- Volumetric flasks 100mL, 250mL
- Glass Funnel
- Filter Paper (Whatman No.1)
- Graduated and calibrated pipette 2ml, 5ml, 10mL.
- All the glassware should be Pyrex, Class-A type.
- Sieve Mesh No. 40
- Mortar and pestle

■ Procedure

Grind almost 100g of sample as it is, (do not dry or desiccate) and pass all the ground sample from Mesh No. 40 sieve. Weigh 1g well-ground, homogenized test portion or 2ml liquid sample into 100 mL glass beaker. Add 10 mL concentrated 37% pure HCl. Boil and evaporate solution nearly to dryness on hot plate. Do not bake residue. Re-dissolve residue in 20 mL 2M HCl, boiling gently if necessary. Wash the beaker, watch-glass into 100 ml volumetric flask. Make the volume up to the mark with distilled water. Shake well and filter through Whatman No. 41 filter paper. Measure absorbance of respective micronutrient directly or dilute with 0.5M HCl to obtain solution within range of calibration curve by using instrument conditions for each element. Determine concentration of element in solution (mg/L) from calibration curve or digital concentration readout following the standard operating parameters.

Element	Wavelength, A	Flame	Range µg/ml
Zinc	2138	Air-C_2H_2	0.5 to 5.0
Copper	3247	Air-C_2H_2	2 to 20
Iron	2483	Air-C_2H_2	2 to 20
Manganese	2795	Air-C_2H_2	2 to 20

■ Calculations:

Element % = Reading x dilution /10000.

■ **Reference / Related Documents:**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.6.01-C (AOAC Official Method 965.09), Fertilizers Chapter 2, Subchapter 6, Page 29-30
- Operating manual of instrument Atomic Absorption Spectrophotometer

ESTIMATION OF WATER-SOLUBLE FRACTION OF MICRONUTRIENTS (ZINC, COPPER, IRON AND MANGANESE) BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle**

This method is applicable to in-organic (synthetic) fertilizers containing salts of zinc, copper, iron, and manganese. The process involves water extraction to solubilize the nutrients and atomic absorption spectrophotometric method is applied for determination.

Flame atomic absorption spectrometry involves monochromatic radiation, characteristic for the metal in question, is passed through a long, thin flame, into which the sample solution is sprayed. A flame is produced in a pre-mix type burner with a 10cm long slit. The burner head is aligned so that the radiations will pass exactly over this slit. These conditions result in absorption of radiation that is selective for element. The extent of absorption will be proportional to the number of ground state atoms present in the flame.

■ **Reagents**

- **Certified Reference Material** (CRM) of Zinc, Copper, Iron and Manganese (1000 ± 5ppm, stock solution). CRM should be NIST traceable and manufactured by ISO certified company.
- Prepare stock solution of 100ppm in D.I. water and store for 15 days. Electrical Conductivity of water should not be more than 20µS/cm.

■ **Equipment**

- Analytical balance
- Hot Plate
- Atomic Absorption Spectrophotometer
- Volumetric flask
- Funnel
- Filter Paper (Whatman No.1)
- Pipette

■ **Procedure**

Place 1.00g test portion into 100 mL glass beaker. Add 75mL D.I water and boil 30 minutes. Filter in 1-liter volumetric flask, washing filter with D.I water. Make the volume up-to the mark with D.I water. Re-dilute if necessary. Determine concentration of element in solution (mg/L) from calibration curve or digital concentration readout following the standard operating parameters.

Element	Wavelength nm	Flame	Range µg/ml
Zinc	213.8	Air-C2H2	0.5-5
Copper	324.7	Air-C2H2	2-20
Iron	248.3	Air-C2H2	2-20
Manganese	279.5	Air-C2H2	2-20

■ Calculations

$$\% \text{ element} = (\mu g/ml) \times \text{dilution} \times 10^{-4}$$

■ Reference / Related Documents:

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.6.01-C(e1). (AOAC Official Method 965.09), Fertilizers Chapter 2, Sub Chapter-6. Page 29-30
- Operating manual of instrument Atomic Absorption Spectrophotometer

ESTIMATION OF CHARRED/ASHED FRACTION OF MULTI-MICROS (ZINC, COPPER, IRON AND MANGANESE) BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ Principle

Method is applicable to organic fertilizers containing multi-micros nutrients (minors). The process involves furnace ashing of fertilizer to release/solubilize the nutrients and Atomic absorption spectrophotometric method is applied for determination.

■ Reagents

- **Certified Reference Material** (CRM) of Zinc, Copper, Iron and Manganese (1000 ± 5ppm, stock solution). CRM should be NIST traceable and manufactured by ISO certified company.
- Prepare stock solution of 100ppm in D.I. water and store for 15 days. Electrical Conductivity of water should not be more than 20µS/cm.

■ Equipment

- Analytical balance
- Hot Plate
- Atomic Absorption Spectrophotometer
- Volumetric flask
- Funnel
- Filter Paper (Whatman No.42)
- Pipette

■ Procedure

Place 1 .00 g sample in 150 mL beaker (Pyrex, or equivalent). Char on hot plate and ignite 1-hour at 500° with muffle door propped open to allow free access of air. Break up cake with stirring rod

and dissolve in 10 mL HC1. Boil and evaporate solution nearly to dryness on hot plate while covering the beaker with watch-glass. Do not bake residue. Re-dissolve residue in 20 mL 2M HC1, boiling gently, if necessary. Wash the beaker, watch-glass into 100 ml volumetric flask. Make the volume up to the mark with distilled water. Shake well and filter through Whatman No. 41 filter paper.

Measure absorption of solution directly or dilute with 0.5M HC1 to obtain solutions within ranges of instrument.

Element	Wavelength nm	Flame	Range µg/ml
Zinc	213.8	Air-C_2H_2	0.5-5
Copper	324.7	Air-C_2H_2	2-20
Iron	248.3	Air-C_2H_2	0.2-2
Manganese	279.5	Air-C_2H_2	2-20

■ **Calculation**

$$\% \text{ element} = (\mu g/ml) \text{ X dilution x } 10^{-4}$$

■ **Reference / Related Documents:**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.6.01-C(b). (AOAC Official Method 965.09), Fertilizers Chapter 2, Sub Chapter-6. Page 29-30 .
- Operating manual of instrument Atomic Absorption Spectrophotometer.

ESTIMATION OF CHELATED ZINC IN CHELATED ZINC FERTILIZER BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle**

This method is applicable to fertilizers containing chelated zinc. Mineral-Zn fraction is masked by Na_2CO_3 solution and filtrate is directly run for analysis of chelated fraction through atomic absorption spectrophotometer.

$$\textbf{Zn-EDTA} + \textbf{ZnSO}_4 + \underset{\textbf{(0.1 Molar. pH 8.9)}}{\textbf{Na}_2\textbf{CO}_3} \longrightarrow \textbf{Zn-EDTA} + \textbf{ZnCO}_3 + \textbf{Na}_2\textbf{SO}_4$$

$$\textbf{Zn-EDTA} \quad \text{Acid Hydrolysis} \longrightarrow \textbf{Total chelated Zinc}$$

■ **Equipment**

● Volumetric flask

- Pipette
- Analytical balance
- Atomic absorption spectrophotometer

■ Reagents

- **Certified Reference Material** (CRM) of Zinc (1000 ± 5ppm Zn, stock solution). CRM should be NIST traceable with valid certificate of analysis and manufactured by ISO certified company.
- Prepare stock solution of 100ppm Zn in D.I. water and store for 15 days. Electrical Conductivity of water should not be more than 20µS/cm.
- Prepare working standards at least 3 and between 0 to 3ppm. Use graduated pipette for preparation of working standards. Read the lower meniscus for colorless liquids keeping mark on pipette at eye-level.
- **Na_2CO_3 (0.1M):** Dissolve 21.2g Na_2CO_3 in 2-liter deionized water
- **H_2SO_4** AR grade

■ Procedure

Take 1.25 g homogenized sample previously ground and sieved through Mesh No. 40 (or 2ml filtered liquid sample) in 250ml flask. Add 100 ml Na_2CO_3 (0.1M) solution and shake well. Make volume with Na_2CO_3 (0.1M) solution up to the mark. Let stay sample for 10 minutes. Non-chelated zinc will precipitate, filter the solution through Whatman filter paper No.42. Take 1ml of filtrate in 100mL volumetric flask and add 5ml conc. H_2SO_4 and make volume 100ml using distilled water. Take reading on Atomic Absorption Spectrophotometer.

■ Calculation

Zinc (%) = Reading x dilution /10,000

Whereas

For solid $\frac{250}{1.25} \times \frac{100}{1} = \frac{20000}{10000}$ = Dilution Factor 2

For Liquid $\frac{250}{2ml} \times \frac{100}{1} = \frac{5000}{10000} = \frac{1}{2}$ = Dilution Factor 1.25

■ References/Related Documents

- Vogel's Textbook of quantitative chemical Analysis, Sixth Edition. J Mendham, R C Denney, J D Barnes, M J K Thomas
- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.6.01 (AOAC Official Method 965.09), Fertilizers Chapter 2, Page 29-30
- M. S. A. Khan, M. A. Qazi, S.M. Mian, M. Akram, Comparison of Three Analytical Methods for Separation of Mineral and Chelated Fraction from an Adulterated Zn-EDTA Fertilizer, *Journal of Chemical Society of Pakistan*, 35, 2 (2013).

ESTIMATION OF CHELATED IRON IN CHELATED FERTILIZER BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ Principle

This method is applicable to fertilizers containing chelated-Fe. Test portion is dissolved in water and non-chelated Fe is precipitated as $Fe(OH)_3$, at pH 8.5 and removed. Chelated Fe is determined by atomic absorption spectrophotometer using standard solutions containing Na_2H_2EDTA.

■ Equipment

- Analytical balance (with readout up to 3 decimals minimum)
- Atomic Absorption Spectrophotometer
- Glass Beaker 150 or 250 mL (Tall form).
- Measuring cylinder 100ml
- Volumetric flasks 100mL, 250mL, 1L
- Glass Funnel
- Filter Paper (Whatman No.42)
- Graduated and calibrated pipette 2ml, 5ml, 10mL.
- Sieve Mesh No. 40
- Mortar and pestle

■ Reagents

- **Sodium Hydroxide Solution**: 0.5M, Dissolve 20g NaOH in H_2O and dilute to 1L.
- **Disodium EDTA solution**: 0.66%, Dissolve 0.73g $Na_2H_2EDTA.\ 2H_2O$ in H_2O and dilute to 100mL.
- **0.5M HCl Solution**: Dissolve 82.89 ml of 37% pure HCl in 2 Liter volumetric flask and make up to 2 L.
- **Iron Stock Solution**: **Certified Reference Material** (CRM) of Iron (1000 ppm Fe). CRM should be manufactured by ISO certified company and NIST traceable.
- **Intermediate Solution**: 100µg Fe/100mL, Pipet 10mL Fe stock solution and 10mL $Na_2H_2EDTA.\ 2H_2O$ solution into 100mL volumetric flask and dilute to volume with water.
- **Working Solutions**: Dilute aliquots of intermediate solutions with 0.5M HCl to make ≥ 4 standard solutions within range of determination (2-20 µg Fe/mL)

■ **Procedure**

Weigh 1g well-ground, sieved through Mesh No. 40 and homogenized test portion or 2ml liquid sample into 250mL tall-form beaker. Wet with 2-3 drops of ethanol and dissolve in 100 mL H_2O. Add 4 drops of 30% H_2O_2, mix and adjust pH of solution to 8.5 with 0.5M NaOH. If pH drifts above 8.8, discard solution and repeat analysis. Transfer solution to 250mL volumetric flask. Dilute to volume with water and mix. Filter solution through quantitative paper. Pipet 1mL filtrate into 100mL volumetric flask and dilute to volume with 0.5M HCl. Determine concentration of iron in solution (mg/L) from calibration curve or digital concentration readout following the standard operating parameters of atomic absorption spectrometer.

Element	Wavelength, A	Flame	Range µg/ml
Iron	2483	Air-C_2H_2	2 to 20

■ **Calculation**

Chelated Fe % = Reading x dilution factor/10000.

■ **References / Related Documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No. 2.6.16 (AOAC Official Method 983.03), Fertilizers Chapter 2, Page 35

ESTIMATION OF CHELATED COPPER AND MANGANESE IN CHELATED FERTILIZER BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle**

Sample is dissolved in water and pH of the solution is adjusted at 8.5-8.8. Non-chelated mineral fraction is precipitated and removed. Chelated fraction is detected by Atomic Absorption Spectrophotometer.

■ **Equipment**

- Volumetric flask ,
- Pipette, Beakers
- Analytical balance
- Atomic absorption spectrophotometer

■ **Reagents**

- **Sodium hydroxide solution. 0.5N:** . Dissolve 20 g NaOH in distilled water and dilute to 1 liter.
- **Hydrogen peroxide solution:** 30%

■ **Procedure**

Weigh 0.5 g sample (ca 40 mg) into 200 mL tall-form beaker. Wet with 2-3 drops of alcohol and dissolve in 100 mL DW. Add 4 drops of 30% H_2O_2, mix and adjust pH of solution to 8.5-8.8 with 0.5N NaOH. If pH drifts above 8.8, discard solution and repeat analysis. Transfer solution to 250 mL vol. flask, dilute to volume with DW, and mix. Filter solution using Whatman 42 filter paper. Dilute further, where necessary. Use air-acetylene flame and detect concentration from either calibration curve or digital readout. In same manner, run blank on all reagents used and take reading on atomic absorption spectrophotometer following standard operating procedure.

Element	Wavelength nm	Flame	Range µg/ml
Copper	324.7	Air-C2H2	2-20
Manganese	279.5	Air-C2H2	2-20

■ **Calculations**

$$\% \text{ element} = (\mu g/ml) \text{ X dilution x } 10^{-4}$$

■ **References / Related Documents**

- María Villén, Juan José Lucena, M. Carmen Cartagena, Raquel Bravo, Josemaría García-Mina, and M. Ignacia Martín de la Hinojosa, 2007.Comparison of Two Analytical Methods for the Evaluation of the Complexed Metal in Fertilizers and the Complexing Capacity of Complexing Agents. J. Agric. Food Chem. 2007, 55, 14, 5746–5753.
- Modified method based on AOAC method 983.03.

ESTIMATION OF ACID-SOLUBLE FRACTION OF CALCIUM AND MAGNESIUM BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

■ **Principle**

This method is applicable to in-organic fertilizers. The process involves wet digestion with acid to release and solubilize the nutrients and atomic absorption spectrophotometric method is applied for determination.

■ **Reagents**

- **Certified Reference Material** (CRM) of Calcium and Magnesium (1000 ± 5ppm Ca/Mg, stock solution). CRM should be NIST traceable with valid certificate of analysis and manufactured by ISO certified company.
- Prepare stock solution of 100ppm of calcium and magnesium in D.I. water and store for 15 days. Electrical Conductivity of water should not be more than 20µS/cm.
- Prepare working standards at least 3 and between 0 to 3ppm. Use graduated pipette for preparation of working standards. Read the lower meniscus for colorless liquids keeping mark on pipette at eye-level.

■ **Equipment**

- Analytical balance
- Hot Plate
- Atomic Absorption Spectrophotometer
- Volumetric flask
- Funnel
- Filter Paper (Whatman No.42)
- Pipette

■ **Procedure**

Dissolve 1 .00 g well ground sample in 10 mL HC1 in 150 mL beaker. Boil and evaporate solution nearly to dryness on hot plate. Do not bake residue. Re-dissolve residue in 20 mL 2M HC1, boiling gently, if necessary. Filter through fast paper into 100 mL vol. flask, washing paper and residue thoroughly with H_2O. Measure absorption of solution directly or dilute with 0.5M HC1 to obtain solutions within ranges of instrument.
If Ca is to be determined add enough La stock solution to make final dilution 1% La.

Element	Wavelength nm	Flame	Range µg/ml
Calcium	422	Air-C2H2	2-10
Magnesium	285	Air-C2H2	0.2-2.0

■**Calculation**

$$\% \text{ element} = (\mu g/ml) \text{ X dilution x } 10^{-4}$$

■ **Reference / Related Documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016, Method No.2.6.01- C (e1). (AOAC Official Method 965.09), Fertilizers Chapter 2, Sub Chapter-6. Page 29-30
- Operating manual of instrument Atomic Absorption Spectrophotometer

ESTIMATION OF WATER-SOLUBLE BORON CONTENT IN FERTILIZER

■ **Principle**

This test method is applicable to fertilizers containing boron or borate. Boron is extracted by adding water to test samples and masking with ammonium acetate buffer solution. Extracted boron is measured spectrophotometrically, a technique that used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed or transmitted by the solution in a cuvette placed in the spectrophotometer.

■ **Equipment**

- Analytical balance (with readout up to 3 decimals minimum)
- Spectrophotometer
- Polypropylene flasks 500, 1000mL

- Volumetric flask (Pyrex) 100 mL, 500mL, and 1000mL flasks should be pretreated with HCl.
- Polypropylene tube with cap 15 ml
- Micropipette 100µL
- Glass Beaker
- Filter Paper Whatman No 42 or equivalent
- Funnel with stand
- Graduated and calibrated pipette 2ml, 5ml, 10mL.

■ **Reagents**

- **Certified Reference Material (CRM)** of Boron (1000 ± 5 mg B/L) with valid Certificate of Analysis (COA) can be used to prepare stock solution of 100 mg B/L. It should be NIST traceable and manufactured by ISO certified company.
- **Working solution: 0, 5, 10, 15, 20, 25, 30, 45 µg/ml.** Pipet 5, 10, 15, 20, 25, 30- and 45-ml from stock solution into 100 ml volumetric flask. Dilute to volume with water, mix well and transfer to plastic bottle. Electrical Conductivity of water should not be more than 20µS/cm.
- **Azomethine H color reagent:** Dissolve 0.9 g Azomethine H and 2.0 g ascorbic acid in100 ml water. Store in refrigerator and discard after 14 days**.**
- **Buffer masking solution:** Dissolve 140 g Ammonium Acetate, 10 g Potassium Acetate, 4 g Nitrilotriacetic acid, disodium salt 99+ %, 10 g (Etheline dinitrilo) Tetra acetic acid, and 350 ml 10 % Acetic acid in water and dilute to 1 liter with water. Solution is stable.
- **Color developing reagent**: Place 35 ml Azomethine H color reagent and 75 ml buffer masking solution into 250 ml volumetric flask and dilute to volume with water. Prepare fresh daily.

■ **Procedure**

Preparation of test solution: Weigh 2.0 g, ground, sieved test portion (2ml for filtered liquid sample) to 150 ml glass beaker add 50 ml water, and boil ca 10 minutes. Filter hot through Whatman No 40 or equivalent, into 500mL or 1000 mL volumetric flask. Wash precipitate with hot boiled water until volume in the flask is ca 495 ml or 995 ml. Cool and add 1.0 ml HCl, dilute to volume with water and mix. Transfer to plastic bottle immediately; dilute if required.

Determination: pipet 100 µl aliquots of 0, 5, 10, 15, 20, 25, 30 , 35 and 45µg B/ml standards and 100µl aliquots of test solution into separate 10 ml Polypropylene tube. Add 5.0 ml colour developing reagents by automatic pipette/dispenser and let stand for one hour at room temperature. Read Absorbance at 420 nm against water. Correct for reagent blank (0 mg B/ml). Construct standard curve by plotting Absorbance against µg/ml standard and read concentration (µg/ml) of test solution from standard curve.

■ **Calculation**

$$B\ \% = (\mu g/ml \text{ from standard curve}) \times \text{dilution factor} \times 10^{-4}$$

■ **Reference / Related Documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016. Method No. 2.6.04 (AOAC Official Method 982.01), Fertilizers Chapter 2, Subchapter 6, Page 31-32**.**
- Ryan, J., George Estefan, and Abdul Rashid. 2001. Soil and Plant Analysis Laboratory Manual. Second Edition. Jointly published by the International Center for Agricultural

Research in the Dry Areas (ICARDA) and the National Agricultural Research Center (NARC). Available from ICARDA, Aleppo, Syria.

- Standard operating manual of Spectrophotometer

GRAVIMETRIC METHOD FOR THE DETERMINATION OF HUMIC ACID CONTENT IN SOLID AND LIQUID FERTILIZERS

■ Principle

Humic Acid is dissolved in weak extraction solution. Particulate/Colloidal/inert matter in the sample is removed by subsequent filtration/centrifugation. Finally precipitates of actual humic acid are obtained by the addition of Nitric Acid ($HNO_{3)}$ and humic acid contents are calculated gravimetrically.

■ Equipment

- Weighing balance
- Mechanical shaker
- Oven
- Desiccator
- Centrifuge machine
- Volumetric flask100 ml, 1000ml
- Beaker 100 ml
- Wash Bottle
- Filter paper Whatman No.42
- Funnel with stand

■ Reagents

- Concentrated Nitric Acid
- Sodium hydroxide
- Ethanol
- Diethylenetriamine Penta acetic acid (DTPA)
- Humic Acid Standard (Aldrich)
- Extraction solution 0.05M (Dissolve 2g NaOH, 20ml Ethanol and 4ml of 0.001M DTPA in 1-liter deionized water)

■ Procedure

Grind humic acid sample and pass through 30 mesh sieve. Weigh accurately 0.5 g sample in 100 ml volumetric flask and in case of liquid sample take 5ml after filtration. Add 50ml extraction solution and shake the contents for one hour using mechanical shaker @ 270 rpm. Make volume up to mark with extraction solution. Filter/centrifuge (20 minutes @ 4000 RPM) the solution to

remove the colloidal/particulate/inert matter. Add concentrated Nitric Acid (HNO_3) in filtrate until the pH drops to 1.0. Keep the sample uninterrupted for 2 hours to complete the reaction. Humic acid will precipitate. Oven dries the filter paper Whatman No. 42 till constant weight and record the weight. Collect the precipitates by filtration through Whatman No. 42 filter paper or centrifuge (20 minutes @ 4000 RPM). Dry the precipitates in oven at 105^{o}C till constant dry weight. Finally record the weight of dry precipitates.

■ **Calculations**

$$\textbf{Humic Acid (\%)} = \frac{\textbf{Weight of oven dry precipitates}}{\textbf{Weight of sample taken}} \times \textbf{100}$$

Whereas:

Weight of dry precipitate = weight of oven dry precipitate with filter paper - weight of oven dry filter paper

■ **Reference / Related Documents**

- F.J. Stevenson, J. Environ. Quality, 1972, **1**, 333.
- A.K. Fataftah, PhD Thesis, Northeastern University, Boston, 1997.
- T. L. Senn and A. R. Kingman, A Review of Humus and Humic Acid Research,
- www.humates.com/methodology.html

ESTIMATION OF ORGANIC MATTER CONTENT IN COMPOST

■ **Principle**

This method is applicable to compost and other products containing organic matter. Organic matter is measured by loss of weight on ashing at 550 to 600 C^{o}.

■ **Equipment**

- Crucible
- Balance
- Oven
- Muffle furnace
- Desiccator

■ **Procedure**

Weigh clean dry crucible (W1). Add 5g of 2mm particle sized manure sample previously ground and sieved. Ovens dry at 105C^{o} for 4hrs. Weigh sample and crucible. This yields the oven dry

weight (W2). Place the same crucible in furnace at 550 to 600 C^o for 6 hrs. The crucible with ash is cooled in desiccator and weighed. This yields the ash weight (W3). Run a standard sample of known value with each group of samples.

■ **Calculations**

$$\text{Organic matter (\%)} = 100 - \text{Ash (\%)}$$

$$\text{Ash (\%)} = \frac{W3 - W1}{W2 - W1} \times 100$$

$$\text{Organic Carbon (\%)} = \frac{\text{Organic matter value}}{1.724}$$

Whereas

Factor 1.724 is derived from the ratio of Organic Carbon present in Organic matter. Organic matter contains 58% organic carbon.

■ **Precautions**

Muffle furnace temperature must not exceed 600 ^oC.

■ **Reference / Related Documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016. Method No. (AOAC Official Method 967.05), Fertilizers Chapter 2 Page 54
- Standard operating manual of oven/furnace.

DETERMINATION OF CATION EXCHANGE CAPACITY OF ORGANIC MATTER/ COMPOSTS

■ **Principle**

Cation exchange capacity is measure of total amount exchangeable cations that can be held by compost, expressed as milliequivalents/100g air-dried compost. Sample portion is shaken with 0.5M HCl to remove bases and to saturate sorption complex with H^+ . Excess acid is removed, absorbed H^+ is replaced with Ba^{+2} titrated with 0.1M NaOH, using phenolphthalein indicator.

■ **Equipment**

- Weighing balance
- Volumetric flask-100 ml
- Beaker-100 ml
- Bulb type pipette-10 ml
- Conical flask-250 ml
- Wash Bottle

- Whatman No.42 filter paper
- Funnel with stand 3.

■ **Reagent**

Dilute hydrochloric acid (0.5M) Dilute 42 ml HCl to 1L with H_2O
Barium acetate solution (0.25M) Dissolve 64g $Ba(CH_3 COO)_2$ in H2O and dilute to 1 Liter.
Sodium hydroxide standard solution (0.1M) Prepare and standardize.

■ **Method**

Place 2g of air-dried, ground, thoroughly mixed test sample in 300 mL Erlenmeyer flask. Add 100mL of 0.5M HCl solution. Stopper the flask and shake mechanically for 30 minutes. Filter and wash the test portion with 100 mL portion H_2O until 10mL wash shows no precipitation with 3ml of 1% $AgNO_3$. Discard filtrate. Immediately transfer moist sample to 300mL Erlenmeyer flask by puncturing apex of paper and forcing moist sample through funnel stem into flask, using spray bottle containing 100mL 0.25M $Ba(CH_3COO)_2$. Stopper flask and shake mechanically 15 minutes. Filter and wash with three 100mL portions H_2O. Titrate against 0.1M NaOH using 5 drops of phenolphthalein indicator to first pink end point.

■ **Calculation**

CEC Me/100g = NaOH used (mL) x molarity of NaOH x 100 / Sample taken (gm)

■ **Precautions / safety requirements**

- Analyze Certified Reference Material along with samples.
- Take ample quantity of sample (approximately 200g) for grinding. Care should be taken that all the sample taken for grinding must pass through sieve to obtain homogeneous sample.
- Standardize 0.1M NaOH against 0.1M Potassium Hydrogen Phthalate.

■ **Reference / Related documents**

- Official Methods of Analysis of AOAC International, 20th Edition, 2016. Method No. 2.7.13 (AOAC Official Method 973.09), Fertilizers Chapter 2, Subchapter 7, Page 56.

ESTIMATION OF SULPHUR IN SOP FERTILIZER SAMPLE

■ **Principle**

The testing sample is dissolved in deionized water and treated with Barium Chloride solution in the presence of concentrated HCl. White precipitates of Barium Sulphate are produced which are weighed to calculate the amount of Sulphur in sample.

■ **Apparatus**.

- Volumetric Flask 250 mL, 1 L
- Beaker 250mL, 500mL
- Pipette 2mL
- Muffle furnace
- Whatman No. 41
- Porcelain crucible

■ Reagent

- **Barium Chloride Solution:** Dissolve 122g $BaCl_2$ in deionized water and dilute to 1 Liter.
- **Hydrochloric Acid (HCl) concentrated**:

■ Procedure

1. Weigh 4g of Sulphate of Potash (SOP) fertilizer sample and dissolve in water to make volume 250mL. Mix well and filter through Whatman No. 41
2. Take 20mL of filtrate in 250 mL beaker and add 100mL deionized water and 2mL HCl (Conc.).
3. Boil the solution for 5 minutes.
4. Add 50mL $BaCl_2$ solution and keep boiling for further 5 minutes.
5. Cover beaker with cover glass and place in water bath for 1hr at 80 ^oC.
6. Filter the liquid through Whatman No.41
7. Wash precipitates on filter paper with distilled water until washings become chloride free (Check precipitates with $AgNO_3$ solution)
8. Place filter paper on crucible previously conditioned at 800 C^o and weigh "W_1"
9. Dry and Ignite for 1 hour at 800 C^o in furnace.
10. Cool in desiccator and weigh as "W_2"

■ Calculation

$$\textbf{Sulphur (\%)} = \frac{\textbf{W}_2\textbf{-W}_1 \textbf{ X 250 X 32 X 100}}{\textbf{Weight of SOP taken (4g) X 20 mL X 233}}$$

■ Precautions

- Run the analytical potassium sulfate (99% pure with certificate of analysis) in every batch along with sample to check the recovery of Sulphur. Use the correction factor according to the recovery of Sulphur in pure K_2SO_4.
- Run the blank

■ References/Related Documents

- Diagnosis and Improvement of Saline and Alkali soils, USDA, Handbook No. 60 pp146
- AOAC-2.6.28, Method 980.02, 17th edition, Determination of Sulphur in Fertilizer

- Pakistan Standard Specification for Potassium Sulphate fertilizer grade, 2nd Revision, PSQCA, Karachi, PS:1501-2011 (R) ICS: 65.080

ESTIMATION OF CHLORIDES IN SULPHATE OF POTASH FERTILIZER SAMPLE

■ Principle

The testing sample is dissolved in Deionized water and titrated against standardized silver nitrate solution. Silver reacts with chloride and forms white precipitates. As soon as all the chlorides are consumed, additional Silver reacts with chromate to form brick red colour precipitates. The endpoint for chloride analysis is the initiation of brick red colour. The amount of silver nitrate consumed, and its normality is used to quantify chlorides in the sample.

■ Equipment

- Conical Flask 250 mL
- Pipette 2mL, 10 mL
- Burette 25mL
- Cylinder 50mL, 100mL
- Volumetric Flask 1000mL

■ Reagent

- **Silver Nitrate 0.05N**: Dissolve 8.4935g $AgNO_3$ crystals in deionized water and dilute to 1 liter. Standardize against the 0.05 N NaCl solution using Potassium Chromate indicator till the endpoint of brick red colour.
- **Sodium Chloride Standard Solution 0.05N**: Dissolve 2.9225g NaCl (Previously dried at 105^{o}C for 1 hr.) and dilute to 1 liter .
- **Potassium Chromate indicator**: Dissolve 5g Potassium Chromate in 100mL deionized water and add AgNO3 until a slight red precipitate is appeared. Allow the solution to stand for 24 hrs. and then filter.
- **Phenolphthalein Indicator**: Dissolve 0.1g of solid Phenolphthalein in 100mL of 95% ethanol
- **Sulphuric Acid 0.02 N:** Dilute 200mL of 0.1N H_2SO_4 to 1 liter.
- **Sodium Hydroxide 0.02N**: Dilute 200mL of 0.1N NaOH to 1 liter.

■ Procedure

1. Weigh 1g ground sample of Sulphate of Potash (SOP) fertilizer in 1-liter volumetric flask. Add approximately 500 mL deionized water and shake on mechanical shaker for 30 minutes at 270 RPM. Make up the volume up to the mark and mix well. Filter if necessary.
2. Take 100mL filtered sample in conical flask using volumetric flask (100mL) and add 3-4 drops of phenolphthalein indicator. If solution turns pink, neutralize it with 0.02N H_2SO_4 but if solution remains colourless, first make alkaline with 0.02N NaOH and then neutralize with 0.02N H_2SO_4. (pH of the solution should be 7 to 8.3)

3. Add 1 mL Potassium Chromate indicator and shake. Titrate against 0.05N $AgNO_3$ until brick red end point appears. Note the volume of $AgNO_3$ used.

■ Calculation

The percentage of chloride is calculated as following:

$$\textbf{Chloride (\% as Cl)} = \frac{\textbf{(Vol of } AgNO_3 \textbf{ used - Blank) X 0.05 X 35.45 X1000 X 100}}{\textbf{Weight of SOP taken X mL of sample taken X 1000}}$$

■ Precautions

1. Always standardize $AgNO_3$ against NaCl before use.
2. Take aliquot/sample using volumetric flask (100mL)
3. Run the blank and subtract the $AgNO_3$ used from sample reading.
4. Run the NaCl (pure salt) in every batch along with sample to check the recovery of chloride. Chloride in NaCl should be 60.66%.
5. Use the correction factor according to the recovery of Cl in NaCl.
6. Titration should be stopped when first trace of red-brown colour is observed.
7. Keep the $AgNO_3$ in dark bottle as it is photosensitive.

■ References/Related Documents

- Diagnosis and Improvement of Saline and Alkali soils, USDA, Handbook No. 60 pp146
- AOAC-2.6.09, 17th edition, Method No. 928.02 Determination of chloride in Fertilizer
- Pakistan Standard Specification for Potassium Sulphate fertilizer grade, 2nd Revision, PSQCA, Karachi, PS:1501-2011 (R) ICS: 65.080

ESTIMATION OF PACLOBUTRAZOL IN PLANT GROWTH REGULATOR SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

■ Principle

The testing sample is dissolved in mobile phase Acetonitrile: water 50% :50%. Then the liquid chromatographic separation and determination of Paclobutrazol is conducted with UV detector.

■ Equipment

- High performance liquid chromatography with UV-225nm wavelength detector
- C-18 or equal grade
- Filter: 0.45um
- Sonicator

■ Reagent

- Acetonitrile: HPLC grade
- Water: HPLC Grade
- Certified Reference Material of Paclobutrazol with valid certificate of analysis and NIST traceable

■ Operating conditions

- Mobile phase: Acetonitrile: water = 50%: 50%
- Flow: 1.2 ml/min
- Detecting wavelength: 225 nm
- Injection-volume: 20ul
- Retention time: 8 minutes

■ Procedure

1. **Standard solution:** Weigh about 0.05g Paclobutrazol standard and put it into 50ml measuring flask. Add approximately 25ml mobile phase and sonicate for 5 minutes. Make volume up to the mark with mobile phase and shake it gently.
2. **Testing sample:** Dilute appropriate quantity of testing sample in 50ml measuring flask using mobile phase so that its concentration fell within the concentration range of standard solution. Sonicate for 5 minutes. Make the volume with mobile phase up to the mark and shake it gently.
3. **Determination:** Run the mobile phase for 10 minutes following prescribed operating conditions. Inject the standard solution and test sample when the base line becomes stable. Calculate the Peak area value of standard and test sample.

■ Calculation

The percentage of paclobutrazol is calculated as following:

$$\textbf{Paclobutrazole} = \frac{\textbf{Peak Area of Sample}}{\textbf{Peak Area of Standard}} \times \frac{\textbf{Weight of Standard}}{\textbf{Weight of Sample}} \times \textbf{Purity (\%)}$$

■ References/Related Document

- Sigma-Aldrich Laborchemikalien GmbH, Quality Management SA-LC. Certificate of Analysis

ESTIMATION OF NAPHTHYL ACETIC ACID (NAA) IN PLANT GROWTH REGULATOR SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

■ Principle

The testing sample is dissolved in mobile phase Acetonitrile: water 30% :70%. Then the liquid chromatographic separation and determination of NAA is conducted with UV detector

■ Equipment

- High performance liquid chromatography with UV-255nm wavelength detector
- Column C-18; 5µm or equal grade
- Filter: 0.45um
- Sonicator

■ Reagent

- Acetonitrile: HPLC grade
- Water: HPLC Grade
- Certified Reference Material of NAA with valid certificate of analysis and NIST traceable.

■ Operating conditions

- Mobile phase: Acetonitrile: water = 30%: 70%
- Flow: 1.0 ml/min
- Detecting wavelength: 255 nm
- Injection-volume: 10ul
- Retention time: 2.5 minutes

■ Procedure

1. **Standard solution**
   Weigh about 0.01g NAA standard and put it into 25ml measuring flask. Add approximately 20ml mobile phase and sonicate for 5 minutes. Make volume up to the mark with mobile phase and shake it gently.
2. **Testing solution**
   Dilute appropriate quantity of testing sample in 25ml measuring flask using mobile phase so that its concentration fell within the concentration range of standard solution. Sonicate for 5 minutes. Make the volume with mobile phase up to the mark and shake it gently.
3. **Determination**
   Run the mobile phase for 10 minutes following prescribed operating conditions. Inject the standard solution and test sample when the base line becomes stable.

■ Calculation

The percentage of NAA is calculated as following:

$$\text{Naphthaleneacetic Acid} = \frac{\text{Peak Area of Sample}}{\text{Peak Area of Standard}} \times \frac{\text{Weight of Standard}}{\text{Weight of Sample}} \times \text{Purity (\%)}$$

■ References / Related Documents

Sigma-Aldrich Laborchemikalien GmbH, Quality Management SA-LC. Certificate of Analysis

ESTIMATION OF MEPIQUAT CHLORIDE IN PLANT GROWTH REGULATOR SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

■ Principle

The testing sample is dissolved in water (HPLC grade) and determination of Mepiquat chloride is carried out using UV detector.

■ Equipment

- High performance liquid chromatography with UV-191nm wavelength detector
- Column C-18 or equal grade
- Filter: 0.45um
- Sonicator

■ Reagent

- Acetonitrile: HPLC grade
- Water: HPLC Grade
- Certified Reference Material of Mepiquat Chloride with valid certificate of analysis and NIST traceable

■ Operating conditions

- Mobile phase: Acetonitrile 10 % in HPLC water
- Flow: 1.0 ml/min
- Detecting wavelength: 191 nm
- Injection-volume: 20µl
- Retention time: ~2.0 minutes

■ **Procedure**

1. **Standard solution**
   Weigh about 0.20g Mepiquat chloride standard and put it into 50ml measuring flask. Add approximately 25 mL water and sonicate for 5 minutes. Make volume up to the mark with water and shake it gently.
2. **Testing sample solution**
   Dilute appropriate quantity of testing sample in 50ml measuring flask using water so that its concentration fell within the concentration range of standard solution. Sonicate for 5 minutes. Make the volume with water up to the mark and shake it gently.
3. **Determination**
   Run the mobile phase for 10 minutes following prescribed operating conditions. Inject the standard solution and test sample when the base line becomes stable.

■ **Calculation**

The percentage of Mepiquat chloride is calculated as following:

$$\textbf{Mepiquat Chloride} = \frac{\textbf{Peak Area of Sample}}{\textbf{Peak Area of Standard}} \times \frac{\textbf{Weight of Standard}}{\textbf{Weight of Sample}} \times \textbf{Purity \%}$$

■ **References / Related Document**

- Sigma-Aldrich Laborchemikalien GmbH, Quality Management SA-LC. Certificate of Analysis
- Karasali, H. & Ioannou, S. 2009. HPLC determination of mepiquat chloride in commercial pesticide formulations. *Bulletin of environmental contamination and toxicology*, 83**,** 636.

ESTIMATION OF GIBBERELLIC ACID IN PLANT GROWTH REGULATOR SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

■ **Principle**

The testing sample is dissolved in mobile phase and determination of gibberellic acid is carried out using UV detector.

■ **Equipment**

- High performance liquid chromatography with UV-206nm wavelength detector
- Reversed Phase C-18
- Filter: 0.45µm
- Sample injector: 20µl

■ Reagent

- Acetonitrile: HPLC grade
- Certified Reference Material of gibberellic acid with valid certificate of analysis and NIST traceable

■Operating conditions

- Mobile phase: Acetonitrile and acidic water (0.01% H_3PO_4) in the ratio of 60:40
- Flow: 0.6 ml/min
- Detecting wavelength: 206 nm
- Injection-volume: 20µl
- Run time: 10 minutes.

■ Procedure

1. **Prepare standard solution.**
   Weigh about 0.10g Gibberellic standard and put it into 50ml measuring flask. Add approximately 25 mL Acetonitrile and sonicate for 5 minutes. Make volume up to the mark and shake it gently.
2. **Prepare testing sample.**
   Dilute appropriate quantity of testing sample in 50ml measuring flask so that its concentration fell within the concentration range of standard solution. Sonicate for 5 minutes. Make the volume up to the mark with acetonitrile and shake it gently.
3. **Determination**
   Run the mobile phase for 10 minutes following prescribed operating conditions. Inject the standard solution and test sample when the base line becomes stable.

■ Calculation

The percentage of Mepiquat chloride is calculated as following:

$$\textbf{Gibberellic Acid} = \frac{\textbf{Peak Area of Sample}}{\textbf{Peak Area of Standard}} \times \frac{\textbf{Weight of Standard}}{\textbf{Weight of Sample}} \times \textbf{Purity \%}$$

■ References/Related Documents

- K. Bhalla., R. Agarwal, Quantitative determination of gibberellins by high performance liquid chromatography from various gibberellins producing strains. *Environmental Monitoring and Assessment*. August 2009

APPENDIX-1

Analysis Fee Receipt (Template)

Agriculture Department, Government of the Punjab

Lab ID:	Soil & Water Testing Laboratory for Research, . Ph & Fax #, E-mail:
Document Title:	Analysis Fee Receipt

ANALYSIS FEE RECEIPT

Receipt No: _____ Dated: _____

Received with thanks from _____
_____

Sum of Rs. _____ (In words) _____

by Cash/Cheque/Draft No: _____

on account of: _____

Tentative Report Date: _____ Sample Received by:_____

_____
In charge Sample processing Lab

APPENDIX-2

Lab ID:	Soil & Water Testing Laboratory for Research, Ph & Fax #, E-mail:
Document Title:	**Analysis Request Form**

Agriculture Department, Government of the Punjab

Sr. No.	**Description**	
1.	Name of the Farmer	
2.	CNIC # (Optional)	
3.	Telephone No. (If available)	
4.	E-mail (If available)	
5.	Address: Village,	
6.	UC	
7.	Tehsil & District	
8.	Longitude (If available)	
9.	Latitude (If available)	
10.	Square/ Killa No/ Any other.	
11.	No of samples	
12.	Depth of soil sample	
13.	Crops Harvested/ Crops Planned:	
14.	Fees received	Rs._____,16%GST: _____ Total: _____
15.	Receipt Date:	
16.	Tentative Report Date:	

ANALYSIS REQUIREMENT:

SOIL ☐	**WATER** ☐	**FERTILIZER** ☐	**PLANT** ☐
No of Samples:	**No of Samples:**	**No of Samples:**	**No of Samples:**
PARAMETERS	**PARAMETERS**	**PARAMETERS**	**PARAMETERS**
Soil Texture ☐	Electrical Conductivity ☐	Nitrogen ☐	Nitrogen ☐
Electrical Conductivity ☐	Calcium and Magnesium ☐	Phosphorus ☐	Phosphorus ☐
Soil Reaction pH ☐	Sodium and Potassium ☐	Potassium ☐	Potassium ☐
Organic Matter ☐	Carbonates & ☐	Boron ☐	Micronutrients ☐
Extractable Phosphorus ☐	Bicarbonates ☐	Gypsum ☐	
Extractable Potassium ☐	Chlorides ☐	Micronutrients ☐	
Micronutrients ☐	SAR and RSC ☐		
Extractable boron ☐	Other: ☐	Other: ☐	Other ☐
Other: ☐	Depth of Bore ____		

Additional Information/Requirement/Purpose: _____
Signatures of Farmer /Representative: _____ Date: _____
Signatures: (In charge Sample Receipt):_____

(Appendix-3)

SOIL AND WATER TESTING LABORATORY FOR RESEARCH

QSP/QF/L4/055	**Order Sheet for Fertilizer Analysis**	Issue on	18.01.2021	Revision	3.4

ORDER SHEET FOR FERTILIZER ANALYSIS/ SAMPLES DELIVERY SHEET

Sent by: _____
Date: _____

Name of Analyst:_____

Sr No	**Sample(s) Code**	**Analyte (s) /Results**											**Remarks/ Physical Conditions**
		N (%)	**P_2O_5 (%)**	**K_2O (%)**	**Zn (%)**	**Cu (%)**	**Fe (%)**	**Mn (%)**	**B (%)**	**O.M (%)**	**CEC** (meq/100g)	**Others**	
1													
2													
3													

Received by: _____
Date: _____
Analyzed by:

Agricultural Officer/
Soil and Water Testing lab for Research
_____

Supervised, checked & verified
from sources of verification

Assistant Agricultural Chemist/
Soil and Water Testing lab for Research
Date:_____

Counter Signature

Agri. Chemist/Chief Scientist
Soil and Water Testing lab for Research
Date: _____

No: **Date:**

FERTILIZER ANALYSIS REPORT

1.	Sample reference:	Vide No. dated ……..			
2.	Name and Composition of the Fertilizer				
3.	Sample receiving date				
4.	Sample drawn from:				
5.	Name and address of company manufacture:				
6.	Batch No:				
7.	Physical status of the sample:	**Type of Fertilizer**	**Colour**	**Texture**	**Dry/ Moist**

8. **ANALYSIS DETAIL**

Lab. No.	**Nutrient**	**Company claim (%)**	**Results (%)**	**Remarks**

9. Method applied:

Note

i. This test was carried out at Temperature 25±5^{0}C and Humidity 30 to 75 %
ii. The analysis is good for the sample supplied at a confidence level of 95%; i.e., k=2 and pertaining to analyzed sample only.
iii. This test report cannot be reproduced except in full, without written approval of competent authority.
iv. The uncertainty of these parameters has been calculated by laboratory and will be reported on demand.
v. **Decision rule**: The 5% of claim value, including measurement uncertainty, will be considered as tolerance limit. The sample will be declared as "unfit" if the sum of test results and tolerance limit is less than the claim value.

Analyzed by ----------Agri. Officer (Lab.)/

Checked by ---------- Assistant Agri. Chemist/

Agricultural Chemist/ Chief or Principal Scientist
Soil and Water Testing laboratory for Research