Method 2.6.2

Determination of different forms of nitrogen in fertilisers containing nitrogen only as nitric, ammoniacal and urea nitrogen

1. **Object**

The purpose of the present document is to specify a simplified method for the determination of different forms of nitrogen in fertilisers containing nitrogen only as nitric, ammoniacal and urea nitrogen.

2. **Field of application**

The present method can be used for all fertilisers mentioned in Annex I which contain exclusively nitric, ammoniacal or urea nitrogen.

3. **Principle**

The following determinations are made on different portions of a single sample solution:

3.1. *Total soluble nitrogen:*

3.1.1. in the absence of nitrates, by direct Kjeldahl digestion of the solution,

3.1.2. in the presence of nitrates, by Kjeldahl digestion of a portion of the solution after reduction by the Ulsch method; ammonia is determined in both cases as described in Method 2.1;

3.2. total soluble nitrogen except nitric nitrogen, by Kjeldahl digestion after elimination of nitric nitrogen in acid medium by means of ferrous sulphate; ammonia is determined as described in Method 2.1;

3.3. nitric nitrogen, by the difference between 3.1.2 and 3.2 or between total soluble nitrogen (3.1.2) and the sum of ammoniacal and urea nitrogen (3.4 + 3.5);

3.4. ammoniacal nitrogen, by cold distillation after slight alkalinisation; the ammonia is obtained in a solution of sulphuric acid and determined as in Method 2.1;

3.5. *urea nitrogen, either:*

3.5.1. by transformation using urease, into ammonia, which is determined by titration with a standard solution of hydrochloric acid,

3.5.2. by gravimetry using xanhydrol: co-precipitated biuret can be taken with urea nitrogen with little error; its level is usually of small absolute value in compound fertilisers,

3.5.3. by difference, following the table:

Case	Nitrate nitrogen	Ammoniacal nitrogen	Difference
1	Absent	Present	(3.1.1) — (3.4)
2	Present	Present	(3.2) — (3.4)

4. **Reagents**

Distilled or demineralised water.

4.1. Potassium sulphate for analysis

4.2. Iron for analysis, hydrogen reduced (the specified amount of iron must be able to reduce at least 50 mg of nitrate nitric N)

4.3. Potassium nitrate for analysis

4.4. Ammonium sulphate for analysis

4.5. Urea for analysis

4.6. Sulphuric acid solution: 0,2 mol/l

4.7. Concentrated sodium hydroxide solution: approximately 30 % (W/V) aqueous solution of NaOH, free of ammonia

4.8. Sodium or potassium hydroxide solution: 0,2 mol/l, free of carbonates

4.9. Sulphuric acid density (d_{20} = 1,84 g/ml)

4.10. Dilute hydrochloric acid: one volume of hydrochloric acid (d_{20} = 1,18 g/ml) plus one volume of water

4.11. Acetic acid: 96 to 100 %

4.12. Sulphuric acid solution containing approximately 30 % H_2SO_4 (W/V), free of ammonia

4.13. Ferrous sulphate: crystalline, $FeSO_4 . 7H_2O$

4.14. Titrated sulphuric acid solution: 0,1 mol/l

4.15. Octyl alcohol

4.16. Saturated potassium carbonate solution

4.17. Sodium or potassium hydroxide: 0,1 mol/l

4.18. Saturated barium hydroxide solution

4.19. Sodium carbonate solution: 10 % (W/V)

4.20. Hydrochloric acid: 2 mol/l

4.21. Hydrochloric acid solution: 0,1 mol/l

4.22. *Urease solution*

Make a suspension of 0,5 g of active urease in 100 ml of distilled water, using 0,1 mol/l hydrochloric acid (4.21), adjust the pH to 5,4, measured with pH meter.

4.23. *Xanthydrol*

5 % solution in ethanol or methanol (4.28) (do not use products giving a high proportion of insoluble material); the solution can be kept for three months in a carefully stoppered bottle in darkness.

4.24. *Catalyst*

Copper oxide (CuO): 0,3 to 0,4 g per determination, or an equivalent amount of copper sulphate pentahydrate ($CuSO_4 . 5H_2O$) of 0,95 to 1,25 g determination.

4.25. Pumice stone granules washed with hydrochloric acid and calcined

4.26. *Indicator solutions*

4.26.1. Mixed indicator

Solution A: Dissolve 1 g of methyl red in 37 ml of 0,1 mol/l sodium hydroxide solution and make up to one litre with water.

Solution B: Dissolve 1 g of methylene blue in water and make up to one litre.

Mix one volume of A with two volumes of B.

This indicator is violet in acid solution, grey in neutral solution and green in alkaline solution. Use 0,5 ml (10 drops) of this indicator.

4.26.2. Methyl red indicator solution

Dissolve 0,1 g of methyl red in 50 ml of 95 % ethanol. Make up to 100 ml with water and filter if necessary. Four or five drops of this indicator can be used instead of the previous one.

4.27. *Indicator papers*

Litmus, bromothymol blue (or other papers sensitive to pH 6 to 8).

4.28. Ethanol or methanol: 95 % (W/V)

5. **Apparatus**

5.1. *Distillation apparatus*

See Method 2.1.

5.2. *Apparatus for determination of ammoniacal nitrogen (7.5.1)*

See Method 2.6.1 and Figure 6.

5.3. *Apparatus for determination of urea nitrogen by the urease technique (7.6.1)*

See Method 2.6.1 and Figure 7.

5.4. Rotary shaker (35 to 40 turns per minute)

5.5. A pH meter

5.6. *Glassware:*

precision pipettes of 2, 5, 10, 20, 25, 50 and 100 ml,

long-necked Kjeldahl flasks of 300 and 500 ml,

graduated flasks of 100, 250, 500 and 1 000 ml,

crucibles of sintered glass, pore diameter, 5 to 15 μm,

mortar.

6. **Preparation of the sample**

See Method 1.

7. **Methods**

7.1. *Preparation of solution for analysis*

Weigh out, to an accuracy of 1 mg, 10 g of sample, and transfer to a 500-ml graduated flask. Add 50 ml water and then 20 ml dilute hydrochloric acid (4.10). Shake. Allow to rest until any CO_2 release comes to an end. Add 400 ml of water; shake for half an hour (5.4); make up to volume with water, homogenise, filter on a dry filter into a dry container.

7.2. *Total nitrogen*

7.2.1. In absence of nitrates

Pipette into a 300-ml Kjeldahl flask a portion of the filtrate (7.1), containing a maximum of 100 mg N. Add 15 ml of concentrated sulphuric acid (4.9), 0,4 g of copper oxide or 1,25 g of copper sulphate (4.24), and a few glass beads to control boiling. Heat moderately at first in order to initiate the reaction, then more strongly until the liquid becomes colourless or slightly greenish and white fumes unmistakably appear. After cooling, transfer the solution into the distillation flask, dilute to approximately 500 ml with water and add a few granules of pumice stone (4.25). Connect the flask to the distillation apparatus (5.1) and carry out the determination as described in 7.1.1.2, Method 2.6.1.

7.2.2. In the presence of nitrates

Pipette into 500-ml Erlenmeyer a portion of the filtrate (7.1) containing not more than 40 mg of nitric N. At this stage of the analysis, the total quantity of N is unimportant. Add 10 ml of 30 % sulphuric acid (4.12), 5 g of reduced iron (4.2), and immediately cover the Erlenmeyer with a watch glass. Heat gently until the reaction becomes strong but not violent. Stop heating and leave for at least three hours at ambient temperature. Transfer the liquid quantitatively to a 250-ml graduated flask, ignoring undissolved iron. Make up to the mark with water. Homogenise carefully. Pipette a portion containing a maximum of 100 mg N into a 300-ml Kjeldahl flask. Add 15 ml of concentrated sulphuric acid (4.9), 0,4 g of copper oxide or 1,25 g of copper sulphate (4.24), and some glass beads for the control of boiling. Heat moderately at first in order to initiate the reaction, then more strongly until the liquid becomes colourless or slightly greenish and white fumes unmistakably appear. After cooling, transfer the solution quantitatively to the distillation flask, dilute to approximately 500 ml with water, and add a few granules of pumice stone (4.25). Connect the flask to the distillation apparatus (5.1) and continue the determination as described in 7.1.1.2, Method 2.6.1.

7.2.3. Blank test

Carry out a blank test (omitting the sample) under the same conditions, and use the result in calculating the final result.

7.2.4. Expression of the result

$$\% \text{ N (total)} = \frac{(a - A) \times 0{,}28}{M}$$

where

a = ml of titrated 0,2 mol/l sodium or potassium hydroxide solution (4.8), used in the blank test, carried out by placing 50 ml of titrated 0,2 mol/l sulphuric acid solution into the receiver of the apparatus (4.6),

A = ml of titrated 0,2 mol/l sodium or potassium hydroxide solution (4.8), used for the analysis,

M = mass of the test sample, in grams, present in the aliquot (7.2.1 or 7.2.2).

7.3. *Total nitrogen excluding nitric N*

7.3.1. Analysis

Pipette into a 300-ml Kjeldahl flask an aliquot of filtrate (7.1) containing not more than 50 mg N to be determined. Dilute to 100 ml with water, add 5 g of ferrous sulphate (4.13), 20 ml of concentrated sulphuric acid (4.9), and a few glass beads to control boiling. Heat moderately at first, then more strongly until white fumes appear. Continue the reaction for 15 minutes. Stop heating, introduce 0,4 g of copper oxide or 1,25 g of copper sulphate (4.24) as catalyst. Resume heating and maintain production of white fumes for 10 to 15 minutes. After cooling, transfer the contents of the Kjeldahl flask quantitatively to the distillation flask (5.1). Dilute to approximately 500 ml with water, and add a few granules of pumice stone (4.25). Connect the flask to the distillation apparatus and continue the determination as in 7.1.1.2, Method 2.6.1.

7.3.2. Blank test

See 7.2.3.

7.3.3. Expression of the result

$$\% \text{ N (total)} = \frac{(a - A) \times 0{,}28}{M}$$

where

a = ml of titrated 0,2 mol/l sodium or potassium hydroxide solution (4.8), used in the blank test, carried out by pipetting 50 ml of titrated 0,2 mol/l sulphuric acid solution (4.6) into the receiver of the apparatus,

A = ml of titrated 0,2 mol/l sodium or potassium hydroxide solution, used for the analysis,

M = mass of the sample, expressed in grams, present in the aliquot used in the determination.

7.4. *Nitric nitrogen*

Is obtained by the difference between:

7.2.4 — (7.5.3 + 7.6.3)

or

7.2.4 — (7.5.3 + 7.6.5)

or

7.2.4 — (7.5.3 + 7.6.6)

7.5. *Ammoniacal nitrogen*

7.5.1. Analysis

Pipette into the dry flask of the apparatus (5.2) a portion of filtrate (7.1) containing a maximum of 20 mg of ammoniacal N. Connect up the apparatus. Pipette into the 300-ml Erlenmeyer exactly 50 ml of titrated 0,1 mol/l sulphuric acid solution (4.14) and an amount of distilled water so that the level of the liquid is approximately 5 cm above the opening of the intake tube. Introduce through the side neck of the reaction flask, distilled water so as to bring the volume to approximately 50 ml. Shake. In order to avoid the formation of froth on the introduction of the gaseous flow, add several drops of octyl alcohol (4.15). Add 50 ml of saturated potassium carbonate solution (4.16), and immediately begin to expel the ammonia thus released from the cold suspension. The intense air flow required for this purpose (flow rate of approximately three litres per minute) is previously purified by passage through washing flasks containing dilute sulphuric acid and dilute sodium hydroxide. Instead of using air under pressure, a vacuum may be used (water suction pump) provided that the connections between the apparatus are air tight.

The elimination of ammonia is generally completed after three hours.

However, it is desirable to make certain of this by changing the Erlenmeyer. When the process is finished, separate the Erlenmeyer from the apparatus, rinse the end of the intake tube and the Erlenmeyer walls with a little distilled water, and titrate the excess acid against a standard 0,1 mol/l sodium hydroxide solution (4.17).

7.5.2. Blank test

See 7.2.3.

7.5.3. Expression of the result

$$\% \text{ N (ammoniacal)} = \frac{(a - A) \times 0{,}14}{M}$$

where

a = ml of titrated 0,1 mol/l sodium or potassium hydroxide solution (4.17), used in the blank test, carried out by pipetting into the 300-ml Erlenmeyer of the apparatus (5.2) 50 ml of titrated 0,1 mol/l sulphuric acid solution (4.14),

A = ml of titrated 0,1 mol/l sodium or potassium hydroxide solution, used for the analysis (4.17),

M = mass of the sample, expressed in grams, present in the aliquot used for the analysis.

7.6. *Urea nitrogen*

7.6.1. Urease method

Pipette into a 500-ml graduated flask a portion of filtrate (7.1) containing not more than 250 mg of urea nitrogen. To precipitate phosphates, add a suitable quantity of saturated barium hydroxide solution (4.18) until further addition does not cause the production of more precipitate. Eliminate excess barium ions (and any dissolved calcium ions) by means of 10 % sodium carbonate solution (4.19). Allow to settle and check whether precipitation is complete. Make up to the mark, homogenise, and filter through a folder filter. Pipette 50 ml of filtrate into the 300-ml Erlenmeyer of the apparatus (5.3). Acidify with 2 mol/l hydrochloric acid (4.20) to pH 3,0, measured by means of pH meter. Raise the pH to 5,4 by means of 0,1 mol/l sodium hydroxide (4.17). To avoid ammonia losses when hydrolysis by urease occurs, close the Erlenmeyer by means of a stopper attached to a dropping funnel and a small protective container holding exactly 2 ml of 0,1 mol/l hydrochloric acid solution (4.21). By way of the dropping funnel, introduce 20 ml of urease solution (4.22). Leave for one hour at 20 to 25 °C. Pipette 25 ml of the standard 0,1 mol/l hydrochloric acid solution (4.21) into the dropping funnel, allow to run into the solution, then rinse with a little water. Also transfer quantitatively the contents of the protective container to the solution held in the Erlenmeyer. Titrate the excess acid using the standard 0,1 mol/l sodium hydroxide solution (4.17), until a pH of 5,4 is obtained, measured on the pH meter.

Remarks

1. After precipitation by the barium hydroxide and sodium carbonate solutions, make up to the mark, filter, and neutralise as quickly as possible.

2. The titration may also be assessed using the indicator (4.26), although the change of colour is more difficult to observe.

7.6.2. Blank test

See 7.2.3.

7.6.3. Expression of the result

$$\% \text{ N (urea)} = \frac{(a - A) \times 0{,}14}{M}$$

where

a = ml of titrated 0,1 mol/l sodium or potassium hydroxide solution (4.17), used in the blank test, carried out in exactly the same conditions as the analysis,

A = ml of titrated 0,1 mol/l sodium or potassium hydroxide solution (4.17), used in the analysis,

M = sample mass, expressed in grams, present in the aliquot used for the analysis.

7.6.4. Gravimetric method with xanthydrol

Pipette into a 100-ml beaker a portion of filtrate (7.1) containing not more than 20 mg of urea. Add 40 ml of acetic acid (4.11). Stir with a glass rod for one minute. Allow any precipitate to settle for five minutes. Filter, wash with a few millilitres of acetic acid (4.11). Add 10 ml of xanthydrol to the filtrate drop by drop (4.23), stirring continually with a glass rod. Leave it to settle until the precipitate appears, and at that juncture stir again for one to two minutes. Leave for one and a half hours. Filter on a glass filtration crucible, previously dried and weigh, using a slight reduction of pressure; wash three times with 5 ml of ethanol (4.28), without aiming to eliminate all the acetic acid. Transfer to oven and maintain at 130 °C for one hour (do not exceed 145 °C). Allow to cool in a desiccator and weigh.

7.6.5. Expression of the result

$$\% \text{ N (urea)} = \frac{6{,}67 \times m}{M}$$

where

m = mass of the precipitate obtained, in grams,

M = mass of the sample, in grams, present in the aliquot used in the determination.

Make the corrections for the blank. Biuret can generally be taken with urea nitrogen without large error, its level being of small absolute value in compound fertilisers.

7.6.6. Difference method

Urea N can also be calculated as indicated in the following table:

Case	Nitric N	Ammoniacal N	Urea N
1	Absent	Present	(7.2.4) — (7.5.3)
2	Present	Present	(7.3.3) — (7.5.3)

8. **Verification of the result**

Before each analysis, check the functioning of the apparatus and the correct application of the methods used with a standard solution containing the different forms of nitrogen in proportions similar to those in the sample. This standard solution is prepared from titrated solutions of potassium nitrate (4.3), ammonium sulphate (4.4) and urea (4.5).