SCHEDULE 2

METHODS OF ANALYSIS

PART I

8b.

DETERMINATION OF DIFFERENT FORMS OF NITROGEN IN THE SAME SAMPLE — IN THE ABSENCE OF CYANAMIDE NITROGEN

SCOPE

1. This method is for the determination of any one form of nitrogen in the presence of any other form, but in the absence of cyanamide nitrogen.

FIELD OF APPLICATION

2. This method is applicable to all fertilisers in Group 1(a) of Section A and Groups 1, 2 and 3 of Section B of the Table in Schedule 1 of the Fertilisers Regulations 1991 which contain exclusively nitric, ammoniacal or ureic nitrogen.

PRINCIPLE

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

Total soluble nitrogen

3.1.—(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.

3.2 *Total soluble nitrogen except nitric nitrogen*, by Kjeldahl digestion after elimination of nitric nitrogen in acid medium by means of ferrous sulfate; ammonia is determined as described in Method 2.

3.3 *Nitric nitrogen*, by difference: between 3.1.2 and 3.2 and/or between total soluble nitrogen (3.1.2) and the sum of ammoniacal and ureic nitrogen (3.4+3.5).

3.4 *Ammoniacal nitrogen*, by cold distillation of a weak alkaline solution; the ammonia is absorbed in a solution of sulfuric acid and determined as described in Method 2.

3.5 *Ureic nitrogen*, either:

(3.5.1) *By conversion using urease*, into ammonia, which is determined by titration with a standard solution of hydrochloric acid;

or,

(3.5.2) *By gravimetry using xanthydrol:* although biuret will also be precipitated by xanthydrol, this should not give rise to a significant error in the determination since its level is generally low in absolute value in compound fertilisers,

or,

(3.5.3) *By difference*, according to the following table:

Case	*Nitric nitrogen*	*Ammoniacal nitrogen*	*Difference*
1	Absent	Present	(3.1.1) – (3.4)
2	Present	Present	(3.2) – (3.4)

4 REAGENTS

4

4.1 Potassium sulfate.

4.2 Iron powder, reduced with hydrogen (the prescribed quantity of iron must be able to reduce at least 50 mg nitric nitrogen).

4.3 Potassium nitrate.

4.4 Ammonium sulfate.

4.5 Urea.

4.6 Sulfuric acid, 0.1 M solution.

4.7 Sodium hydroxide solution 30 g per 100 ml, ammonia free.

4.8 Sodium or potassium hydroxide, 0.2 M solution, free of carbonates.

4.9 Sulfuric acid (p=1.84 g/ml).

4.10 Hydrochloric acid solution: dilute an appropriate volume of hydrochloric acid (p=1.18 g/ml) with an equal volume of water.

4.11 Glacial acetic acid.

4.12 Sulfuric acid solution, approximately 30% (W/V) H2SO4.

4.13 Ferrous sulfate, crystalline FeSO4.7H2O.

4.14 Sulfuric acid, 0.05 M solution.

4.15 Octan-1-ol.

4.16 Potassium carbonate, saturated solution.

4.17 Sodium or potassium hydroxide, 0.1 M solution.

4.18 Barium hydroxide, saturated solution.

4.19 Sodium carbonate solution, 10 g per 100 ml.

4.20 Hydrochloric acid, 2 M solution.

4.21 Hydrochloric acid, 0.1 M solution.

4.22 Urease solution: suspend 0.5 g active urease in 100 ml distilled water. Using 0.1 M hydrochloric acid (4.21), adjust to pH 5.4, measured with pH meter.

4.23 Xanthydrol solution, 5 g per 100 ml in ethanol or methanol (4.28) (do not use products giving a high proportion of insoluble material). The solution can be kept for 3 months in a carefully stoppered bottle in the dark.

4.24 Catalyst: copper oxide (CuO), 0.3 to 0.4 g per determination, or an equivalent amount of copper sulfate pentahydrate, (0.95 to 1.25 g).

4.25 Anti-bump granules of pumice stone washed with hydrochloric acid and ignited.

4.26 Indicator solutions:

(4.26.1) Mixed indicator: Solution A: dissolve 1 g methyl red in 37 ml 0.1 M sodium hydroxide solution and make up to 1 litre with water. Solution B: dissolve 1 g methylene blue in water and make up to 1 litre. Mix 1 volume of solution A and 2 volumes of solution 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 methyl red in 50 ml 95% ethanol, make up to 100 ml with water and filter if necessary; 4 – 5 drops of this indicator can be used instead of the previous one.

4.27 Indicator papers: litmus, bromothymol blue (or other papers sensitive in the range pH 6 – 8).

4.28 Ethanol or methanol, 95% (V/V).

5 APPARATUS

5

5.1 Distillation apparatus. See Method 2.

5.2 Apparatus for determination of ammoniacal nitrogen. An example of the recommended apparatus is reproduced in Figure 6 in the Appendix.

5.3 Apparatus for determination of ureic nitrogen by the urease method (7.6.1). An example of the recommended apparatus is reproduced in Figure 7 in the Appendix.

5.4 Rotary shaker: 35 – 40 turns per minute.

5.5 pH meter.

5.6 Sintered glass crucibles, diameter of pores 5 to 15 microns.

6 PREPARATION OF SAMPLE

6

See Method 1.

7 PROCEDURE

7

Preparation of solution for analysis

7.1 Weigh to the nearest 0.001 g, 10 g of the prepared sample and transfer to a 500 ml graduated flask. Add 50 ml water and then 20 ml dilute hydrochloric acid (4.10) and mix. Allow to stand until the evolution of carbon dioxide ceases. Add 400 ml water, shake for half an hour; make up to volume with water, mix, filter through a dry filter into a dry container.

Total nitrogen

7.2.—(7.2.1) *In the absence of nitrates*

Transfer by pipette into a 300 ml Kjeldahl flask an aliquot portion of the filtrate (7.1) containing a maximum of 100 mg nitrogen. Add 15 ml concentrated sulfuric acid (4.9), 0.4 g copper oxide or 1.25 g copper sulfate (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 appear. After cooling, transfer the solution into the distillation flask, dilute to about 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 Method 8a, 7.1.1.2.

(7.2.2) *In the presence of nitrates*

Transfer by pipette into a 500 ml Erlenmeyer flask a aliquot portion of the filtrate (7.1) containing not more than 40 mg nitric nitrogen. At this stage of the analysis, the total quantity of nitrogen is unimportant. Add 10 ml of 30% sulfuric acid (4.12), 5 g reduced iron (4.2) and immediately cover the Erlenmeyer flask with a watch glass. Heat gently until the reaction becomes strong but not violent. Stop heating and allow to stand for at least 3 hours at ambient temperature. Transfer the liquid quantitatively to a 250 ml graduated flask, ignoring undissolved iron. Make up to the mark with water and mix carefully. Transfer by pipette a portion containing a maximum of 100 mg nitrogen into a 300 ml Kjeldahl flask. Add 15 ml concentrated sulfuric acid (4.9), 0.4 g copper oxide or 1.25 g copper sulfate (4.24) and a few glass beads.

Heat moderately at first in order to initiate the reaction, then more strongly until the liquid becomes colourless or slightly greenish and white fumes appear. After cooling, transfer the solution quantitatively to the distillation flask, dilute to about 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 Method 8a, 7.1.1.2.

(7.2.3) *Blank test*

Carry out a blank test under the same conditions (omitting only the sample), and use this value in the calculation of the final result.

(7.2.4) *Expression of result*

$\%N(total)=(aA)\times 0.28M$

where:

a = ml of standard solution of sodium or potassium hydroxide (0.2 M) used for the blank, carried out under the same conditions as the analysis.

A = ml of standard solution of sodium or potassium hydroxide (0.2 M) used for the analysis.

M = mass of the sample, in grams, present in the aliquot part taken for analysis.

Total nitrogen excluding nitric nitrogen

7.3 7.3.1 Transfer by pipette into a 300 ml Kjeldahl flask an aliquot portion of the filtrate (7.1) containing not more than 50 mg of nitrogen. Dilute to 100 ml with water, add 5 g ferrous sulfate (4.13), 20 ml concentrated sulfuric acid (4.9) and a few glass beads to control boiling (425). Heat moderately at first then more strongly until white fumes appear. Continue the reaction for 15 minutes. Stop heating, introduce 0.4 g copper oxide or 1.25 g copper sulfate (4.24) as catalyst, resume heating and maintain production of white fumes for 10 – 15 minutes. After cooling, transfer the contents of the Kjeldahl flask quantitatively to the distillation flask (5.1). Dilute to about 500 ml with water and add a few granules of pumice stone (4.25). Connect the flask to the distillation apparatus and continue the determinations as in Method 8a, 7.1.1.2.

(7.3.2) *Blank test*

See 7.2.3.

(7.3.3) *Expression of result*

$\%N(total)=(aA)\times 0.28M$

where:

a = ml of standard solution of sodium or potassium hydroxide (0.2 M) used for the blank.

A = ml of standard solution of sodium or potassium hydroxide (0.2 M) used for the analysis.

M = mass of the sample, in grams, present in the aliquot part taken for analysis.

Nitric nitrogen **is obtained: by 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)

Ammoniacal nitrogen

7.5.—(7.5.1) *In the presence of ureic nitrogen*

Transfer by pipette into the dry flask of the apparatus (5.2) an aliquot portion of the filtrate (7.1) containing a maximum of 20 mg ammoniacal nitrogen. Connect up the apparatus. Place in the 300 ml Erlenmeyer flask 50.0 ml standard 0.05 M sulfuric acid solution (4.14) and an amount of distilled water such that the level of the liquid is about 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 about 50 ml and mix. To avoid foaming during aeration add several drops of octan-1-ol (4.15). Add 50 ml saturated potassium carbonate solution (4.16) and immediately begin to expel the ammonia thus released from the cold suspension. A strong current of air is necessary (flow rate of about 3 litres per minute) and should be purified beforehand by passing it through washing flasks containing dilute sulfuric acid and dilute sodium hydroxide. Instead of using air under pressure, a vacuum may be used (water pump) provided that the connections between the apparatus are air tight. The liberation of ammonia is generally complete after three hours. However, it is desirable to make certain of this by changing the Erlenmeyer flask. When the process is finished, disconnect the Erlenmeyer flask from the apparatus, rinse the end of the intake tube and the walls of the Erlenmeyer flask with a little distilled water and titrate the excess acid against standard 0.1 M sodium hydroxide solution (4.17).

(7.5.2) *Blank test*

See 7.2.3.

(7.5.3) *Expression of result*

$$\%N(\text{ammoniacal}) = (aA) \times 0.14M$$

where:

a = ml of standard solution of sodium or potassium hydroxide (0.1 M) (4.17) used for the blank.

A = ml of standard solution of sodium or potassium hydroxide (0.1 M) (4.17) used for the analysis.

M = mass of the sample, in grams, present in the aliquot part taken for analysis.

Ureic nitrogen

7.6.—(7.6.1) *Urease method*

Transfer by pipette into a 500 ml graduated flask, an aliquot portion of the filtrate (7.1) containing not more than 250 mg of ureic nitrogen. To remove 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) with 10% sodium carbonate solution (4.19). Allow to settle and check whether precipitation is complete. Make up to the mark, mix and filter through a fluted filter paper. Transfer by pipette 50 ml of filtrate into the 300 ml Erlenmeyer flask of the apparatus (5.3). Acidify with 2 M hydrochloric acid (4.20) to pH 3.0, measured by means of the pH meter (5.5). Raise the pH to 5.4 by the addition of 0.1 M sodium hydroxide (4.17). To avoid ammonia losses when hydrolysis by urease occurs, close the Erlenmeyer flask by means of a stopper provided with a dropping funnel and a small bubble trap containing

exactly 2 ml standard 0.1 M hydrochloric acid solution (4.21). Introduce through the separating funnel, 20 ml urease solution (4.22). Allow to stand for one hour at 20 – 25 C. Place 25.0 ml of the standard 0.1 M hydrochloric acid solution (4.20) in the dropping funnel, allow to run into the solution, then rinse with a little water. Transfer quantitatively the contents of the bubble trap to the solution contained in the Erlenmeyer flask. Titrate the excess acid using standard 0.1 M sodium hydroxide solution (4.17), until a pH of 5.4 is obtained, measured on the pH meter.

Remarks

1.	After precipitation by barium hydroxide and sodium carbonate solutions, make up to the mark, filter and neutralise as quickly as possible.
2.	The titration may also be carried out using an 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 result*

%N(ureic)=(aA)×0.14M

where:

a = ml of standard solution of sodium or potassium hydroxide (0.1 M) (4.17) used for the blank, carried out in exactly the same conditions as the analysis.

A = ml of standard solution of sodium or potassium hydroxide (0.1 M) (4.17) used for the analysis.

M = mass of the sample, in grams, present in the aliquot part taken for analysis.

(7.6.4) *Gravimetric method using xanthydrol*

Transfer by pipette into a 100 ml beaker an aliquot portion of the filtrate (7.1) containing not more than 20 mg urea. Add 40 ml 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 ml acetic acid (4.11). Add 10 ml xanthydrol solution (4.23) to the filtrate drop by drop, stirring continuously with a glass rod. Allow to stand until the precipitate appears, then stir again for one or two minutes. Allow to stand for one and a half hours. Filter, using a slight reduction in pressure, through a sintered glass crucible (5.6) which has been previously dried and weighed. Wash three times with 5 ml ethanol (4.28), without trying to remove all the acetic acid. Place in an oven at a temperature of 130°C for one hour (do not exceed 145 C). Allow to cool in a desiccator and weigh.

(7.6.5) *Expression of result*

%N(ureic)=6.67×mM

where:

m = mass of the precipitate in grams.

M = mass of the sample, in grams, present in the aliquot part taken for analysis.

Correct for the blank.

Note: Although biuret will also be precipitated by xanthydrol, this should not give rise to a significant error in the determination since its level is generally low in absolute value in compound fertilisers.

(7.6.6) *Difference method*

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

Case	*Nitric N*	*Ammoniacal N*	*Ureic N*
1	Absent	Present	(7.2.4) – (7.5.3)
2	Present	Present	(7.3.3) – (7.5.3)

8 VERIFICATION OF RESULTS

8

8.1 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 solutions of potassium nitrate (4.3), ammonium sulfate (4.4) and urea (4.5).