BRITISH STANDARD

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Fertilizers — Spectrophotometric determination of biuret in urea

ICS 65.080

British Standards

National foreword

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Foreword

This document (EN 15479:2009) has been prepared by Technical Committee CEN/TC 260 "Fertilizers and liming materials", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2009, and conflicting national standards shall be withdrawn at the latest by July 2009.

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1 Scope

This European Standard specifies a method for the determination of biuret in urea. The method is applicable to urea and urea-based fertilizers.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 1482-2, *Fertilizers and liming materials — Sampling and sample preparation — Part 2: Sample preparation*

EN 12944-1:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 1: General terms*

EN 12944-2:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 2: Terms relating to fertilizers*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1:1999 and EN 12944-2:1999 apply.

4 Principle

In an alkaline medium in the presence of potassium sodium tartrate, biuret and bivalent copper form a violet cupric compound. The absorbance of the solution is measured at a wavelength of about 546 nm.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and distilled or demineralized water, free from carbon dioxide and ammonia (grade 3 according to EN ISO 3696:1995). The quality of the water is particularly important in this determination.

5.2 Methanol

5.3 Sulfuric acid solution, c = approximately 0,05 mol/l.

5.4 Sodium hydroxide solution, c = approximately 0,1 mol/l.

5.5 Alkaline solution of potassium sodium tartrate

In a graduated one-litre flask, dissolve 40 g of sodium hydroxide in 500 ml of water and leave to cool. Add 50 g of potassium sodium tartrate ($NaKC_4H_4O_6$ $4H_2O$). Make up to the mark and leave standing for 24 h before use.

5.6 Solution of copper sulfate

In a graduated one-litre flask dissolve 15 g of copper sulfate ($CuSO_4$ $5H_2O$) in 500 ml of water. Make up to the mark.

5.7 Freshly prepared biuret standard solution

In a 250 ml graduated flask, dissolve 0,250 g of pure biuret[1] in water. Make up to 250 ml. 1 ml of this solution contains 0,001 g of biuret.

5.8 Indicator solution

In a graduated 100 ml flask, dissolve 0,1 g of methyl red in 50 ml of 95 % ethanol, make up to 100 ml with water. Filter if any insoluble remain. This indicator is red in acid solution and yellow in alkaline solution.

6 Apparatus

6.1 Spectrometer or photometer

With filters with a sensitivity and precision to permit measures of less than 0,5 % factor of transmission to be produced. The factor of transmission, T, is given by the following equation:

$$T = \frac{J}{Jo} \tag{1}$$

The opacity, O, is given by the following equation:

$$O = \frac{Jo}{J} \tag{2}$$

The absorbance, E, is given by the following equation:

$$E = \log O \tag{3}$$

The absorbance per unit of optical run, k, is given by the following equation:

$$k = \frac{E}{s} \tag{4}$$

The coefficient of specific absorbance, K, is given by the following equation:

$$K = \frac{E}{\rho \times s} \tag{5}$$

where

Jo is the intensity of the beam of monochromatic rays (of a determinate wavelength) before it passes through a transparent body;

J is the intensity of this beam after the passage;

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[1] Biuret can be purified beforehand by washing with an ammoniacal solution (10 %), then with acetone and dried in a vacuum.

s is the thickness of the layer in centimetres;

ρ is the mass concentration in milligrams per litre;

k is the specific factor for each substance in the Lambert-Beer law.

6.2 Graduated flasks, capacity of 100 ml, 250 ml and 1 000 ml.

6.3 Graduated pipettes, capacity of 2 ml, 5 ml, 10 ml, 20 ml, 25 ml and 50 ml,

or a **25 ml burette**, graduated to 0,05 ml.

6.4 Beaker, capacity 250 ml.

6.5 Glass column for cation exchange resin

Height 20 cm, diameter 25 mm, with a bottom plate of nylon gauze with a mesh width of 0,1 mm and ending in a narrow outlet with tap.

The column is filled with cation exchange resin (strong cation: e. g. Duolite C20) to a height of about 120 mm. Before using the column, the resin has first to be regenerated with 100 ml hydrochloric acid (hydrochloric acid, approximately 4 mol/l solution) and subsequently rinsed with water until the eluent is free from acid. After each determination the resin has to be regenerated. The usable capacity of the cation resin has to be great enough to be sure all the ammonia is caught. The total volume of the resin is about 60 cm^3; the usable capacity is about 60 milli-equivalents.

7 Sampling and sample preparation

Sampling is not part of the method specified in this document. A recommended sampling method is given in EN 1482-1.

Sample preparation shall be carried out in accordance with EN 1482-2.

8 Procedure

8.1 Preparation of the calibration curve

Transfer 0 ml, 2 ml, 5 ml, 10 ml, 20 ml, 25 ml and 50 ml aliquots of biuret standard solution (5.7) into a series of seven graduated 100 ml flasks. Make up the volumes to about 50 ml with water, add one drop of indicator (5.8) and neutralize, if necessary, with sulfuric acid 0,05 mol/l (5.3). Stir in 20 ml of the alkaline tartrate solution (5.5) then 20 ml of the copper sulfate solution (5.6).

These solutions shall be measured with two precision burettes or better still with pipettes.

Make up to 100 ml with water, mix and leave standing for 15 min at (30 ± 2) °C.

With the “0” biuret standard solution as a reference, measure the absorbance of each solution at a wavelength of about 546 nm using cells of a suitable path length.

Plot the calibration curve using the absorbance rate as the ordinate and the corresponding quantities of biuret, in milligrams, as the abscissa.

8.2 Preparation of the solution to be analysed

Weigh, to the nearest 0,001 g, 10 g of the prepared sample; dissolve in about 150 ml of water in a 250 ml graduated flask, and make up to the mark. Filter if necessary.

Weigh to the nearest 0,001 g about 25 g of the ammoniumsulfate containing sample. Transfer into a beaker glass of 250 ml with about 100 ml of water at about 70 °C and dissolve. Filter the solution over a Büchner funnel with a glass fibre filter and wash the filter with approximately 50 ml of water. Transfer the filtrate in a volumetric flask of 250 ml.

Bring the temperature to 20 °C, dilute to the mark with water and mix. Pipette 100 ml of the solution and pass it through the cation resin with a speed of approximately 150 ml/h. Collect the eluate in a 250 ml volumetric flask. Wash the resin with water until a total volume of approximately 220 ml is obtained in the volumetric flask. Dilute to the mark with water and mix. Then proceed as indicated in 8.3.

Elimination of the opalescence: if any colloid substance is present, difficulties might arise during filtering. The solution intended for analysis is in that case prepared as follows:

- dissolve the sample for analysis in 150 ml of water, add 2 ml of 1 mol/l hydrochloric acid and filter the solution through two flat very fine filters into a graduated 250 ml flask. Wash the filters with water and make up to volume. Continue the process according to the method as described in 8.3.

8.3 Determination

According to the presumed biuret content, transfer 25 ml or 50 ml from the solution mentioned in 8.2 with a pipette, place this quantity in a 100 ml graduated flask and neutralize if necessary with sulfuric acid solution or sodium hydroxide solution (5.3 or 5.4) as required, using methyl red as an indicator and add, with the same accuracy as that used when drawing up a calibration curve, 20 ml of the alkaline solution of potassium sodium tartrate (5.5) and 20 ml of the copper solution (5.6). Make up to volume, mix thoroughly and leave standing for 15 min at (30 ± 2) °C.

Then carry out the photometric measurements and calculate the quantity of biuret present in the urea.

9 Calculation and expression of the result

Calculate the mass concentration, ρ_B, of biuret in percent of the sample according to the following equation:

$$\rho_B = \frac{m \times 2,5}{V} \qquad (6)$$

where

m is the mass, in milligrams, of biuret, read from the calibration graph;

V is the volume of the aliquot in millilitre.

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test was carried out in 2004 with 13 participating laboratories and two different samples. This test yielded the data given in Annex A. Repeatability and reproducibility were calculated according to ISO 5725-1.

The values derived from this inter-laboratory test might not be applicable to concentration ranges and matrices other than those given in Annex A.

10.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of the cases exceed the values of r given in Table 1.

10.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of the cases exceed the values of R given in Table 1.

Table 1 — Mean values, repeatability and reproducibility limits

Sample	$\bar{x}$ %	r %	R %
Urea	0,81	0,04	0,20
Urea + S	0,76	0,05	0,38

11 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) test method used with reference to this document;

c) test results obtained expressed as the percentage mass fraction of biuret in the fertilizer;

d) date of sampling and sampling procedure (if known);

e) date when the analysis was finished;

f) whether the requirement of the repeatability limit has been fulfilled;

g) all operating details not specified in this document, or regarded as optional, together with details of any incidents that occurred when performing the method which might have influenced the test result(s).

Annex A
(informative)

Results of the inter-laboratory tests

The precision of the method was established in 2004 by Working Group 7 “Chemical analysis” of CEN/TC 260 “Fertilizers and liming materials” in an inter-laboratory test evaluated in accordance with ISO 5725-1. The statistical results are given in Table A.1.

Table A.1 — Statistical results of the inter-laboratory test

Parameter	**Sample**	
	Urea	Urea + S
Number of participating laboratories	13	9
Number of laboratories after elimination of outliers (accepted test results)	11	6
Mean value $\bar{x}$ (%)	0,81	0,76
Repeatability standard deviation s_r (%)	0,01	0,02
RSD_r (%)	1,7	2,0
Repeatability limit r (%)	0,04	0,05
Reproducibility standard deviation s_R (%)	0,07	0,14
RSD_R (%)	8,9	18,0
Reproducibility limit R (%)	0,20	0,38

Bibliography

[1] EN 1482-1, Fertilizers and liming materials — Sampling and sample preparation — Part 1: Sampling

[2] ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions

[3] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers, Official Journal L 304, 21/11/2003 P. 0001-0194, Annex IV, method 2.5

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