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**Organic and organo-mineral fertilizers — Determination of the biuret content by
high-performance liquid chromatography (HPLC)**

Einführendes Element — Haupt-Element — Ergänzendes Element

Élément introductif — Élément central — Élément complémentaire

ICS:

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European foreword

This document has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

This is a working document

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This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

Introduction

Regulation (EU) 2019/1009 [4] lays down the rules on the making available on the market of EU fertilizing products and the specific safety and quality requirements for the defined product function categories (PFCs). Organic and organo-mineral fertilizers have been classified as PFC 1(A) and PFC 1(B).

This document defines test methods for the determination of the biuret content to be used for organic and organo-mineral fertilizers in order to measure the compliance with the related requirement in Regulation (EU) 2019/1009 [4].

When heating urea near or above its melting point (132 °C) during the manufacturing of urea, ammonia slowly evolves and several different substances, including biuret, are formed. Excessively high concentrations of biuret can have detrimental effects on plants growth.

1 Scope

This document is applicable to fertilizing products, which are classified as PFC 1(A) and PFC 1(B) or the PFC 1(A) and PFC 1(B) component in PFC 7 of Regulation (EU) 2019/1009 [4]. This method is recommended in case the blend would contain organic matter. This method was tested only on one blend during the ILS [*Blend description*].

This document specifies a method for the determination of the biuret content by high-performance liquid chromatography (HPLC) with UV detector. The method is applicable to organic and organo-mineral fertilizers containing urea.

Nowadays, there is a method standardized as EN 15479 that allows the determination of biuret in urea by spectrophotometric detection. Organic and organo-mineral fertilizers contain organic matter and other compounds apart from urea that would interfere in a spectrophotometric method. HPLC allows an accurate determination of biuret by separating it from possible interfering compounds [2] [3].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 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 following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

biuret

imidodicarbonic diamide

member of the class of condensed ureas, formed by the condensation of two molecules of urea.

Note 1 to entry: Condensed ureas are the compounds formed by the condensation of two or more molecules of urea.”

4 Principle

The biuret content of the fertilizer sample is extracted with an aqueous acetonitrile solution and separated by reversed-phase liquid chromatography. Detection of peaks is performed using an ultraviolet (UV) detector. Quantification is performed with calibration curve using external standards.

5 Reagents

All water must have a specific conductivity of not more than 0.2 ms/m at 25°C and must be free of the compounds to be detected. All reagents shall be of recognized analytical grade.

5.1 Acetonitrile, HPLC grade, ≥ 99,9 %.

5.2 Biuret

The purity of these reagents shall be $\geq 97\%$, and the calculation of biuret content within fertilizer should be based on the purity claimed on the label.

5.3 Eluent, used for HPLC (mobile phase), acetonitrile: water 85 : 15 (volume fraction)

For the preparation of the eluent, the following tasks shall be performed:

- a) mix 150 ml water with 850 ml acetonitrile (5.1);
- b) filter the solution by 0,22 μm membrane filter (6.5) as pretreatment;
- c) ultrasonic degas (6.4) for 10 min before used, alternatively an in-line degasser can be used.

5.4 Biuret standard solution (0,5 mg/ml)

For the preparation of the biuret standard solution (0,5 mg/ml), the following tasks shall be performed:

- a) weigh approximately 0,50 g to the nearest 0,1 mg of biuret (5.2) and dissolve it by mobile phase (5.3);
- b) transfer it into a 1 l volumetric flask;
- c) bring to volume with mobile phase (5.3) and mix it thoroughly.

6 Apparatus

6.1 High performance liquid chromatograph, with UV detector.

6.2 Chromatographic column, amino column or amino-propyl column.

6.3 Injection loop.

6.4 Ultrasonic bath.

6.5 Membrane filter, for organic solvents, pore size of 0,22 μm .

6.6 Micro-syringe, with a volume between 5 μl and 50 μl .

The micro-syringe is only necessary in case of using manual injection.

6.7 Syringe-driven filter, syringe with membrane filter for organic solvents (for example Polytetrafluoroethylene, PTFE), pore size of 0,22 μm .

6.8 Centrifuge.

6.9 Sieve, aperture size of 0,50 mm.

7 Procedure

7.1 Sampling

Sampling is not part of this document.

7.2 Calibration solutions

For the calibration solution, as given in Table 1, the operator shall take 0,00 ml, 0,50 ml, 1,00 ml, 3,00 ml, 5,00 ml and 10,00 ml biuret standard solution (5.4) into six separate 25 ml volumetric flasks, respectively, and bring to volume with the mobile phase (5.3). These solutions shall be filtered with a syringe driven filter (6.7).

Table 1 — Calibration solutions

	Volume of biuret standard solution ml	Mass of biuret mg	Concentration of biuret mg/l
Standard 1	0,00	0,00	0
Standard 2	0,50	0,25	10
Standard 3	1,00	0,50	20
Standard 4	3,00	1,50	60
Standard 5	5,00	2,50	100
Standard 6	10,00	5,00	200

A calibration curve shall be prepared by injecting the six calibration solutions (see Table 1) according to the operation procedure of the available instrument (6.1) every day at the beginning of the analysis.

The calibration curve is described by Formula (1).

$$y = m_x + b \quad (1)$$

where

m is the slope of the calibration curve;

b is the y-axis intercept.

7.3 Preparation of the sample

The operator shall grind the sample until it passes through a sieve (6.9).

7.4 Extraction of the sample and preparation of the test solution

For the extraction of the sample and preparation of the test solution, the following tasks shall be performed:

- a) weigh approximately 0,5 g to the nearest 1 mg test sample into a 25 ml beaker;
- b) add 10 ml mobile phase and dissolve using an ultrasonic bath for 10 min;
- c) transfer to a 25 ml volumetric flask and dilute to volume with mobile phase;
- d) mix thoroughly and leave standing;
- e) filter with a syringe-driven filter (6.7) to obtain the test solution.

In some solid fertilizers, the organic matter could saturate the filter. In this case, centrifuge the solution before filtering at 2 000 *g* for 15 min.

7.5 HPLC conditions

Recommended operating conditions of HPLC are listed in Table 2. Other HPLC conditions that can achieve the same separation effects may be used.

Table 2 — HPLC conditions

Parameter	Conditions ^a
Chromatographic column (6.2)	Amino column or Aminopropyl column ^b , 4,6 × 250 mm, with 5 µm packing
Flow rate	1,0 ml/min
Injection volume	10 µl
Column temperature	35 °C
Detector wavelength	195 nm
^a The best separation conditions can be determined according to different equipment. ^b If the column is new or has not been in service for more than a week, the column should be conditioned for 4 h at room temperature with LC-grade <i>iso</i> -propanol at a flow rate that will maintain at least 200 bars column back pressure. This is typically 1 ml/min. After that, the column shall be washed again for 4 h. with acetonitrile (5.1) at a flow rate of 1 ml/min, followed by washing with the mobile phase at a flow rate of 1 ml/min until a stable base line is achieved.	

7.6 Preparation of the standard curve

Referring to the apparatus operation condition, the operator shall adjust the HPLC apparatus to the best condition. As an example, the operator may inject 10 µl of the calibration solutions (7.2) and analyse the series of biuret calibration solutions. Each calibration solution shall be determined twice. The standard curve or linear regression equation will be obtained by the average peak areas of the biuret and the corresponding mass.

7.7 Determination of biuret in the test solutions

The operator shall determine the biuret concentration in the test solution (7.4) with the same method used for the analysis of the calibration solutions, measuring the peak area, and calculating the biuret mass in each test solution according to the standard curve or linear regression equation. After completing the determination, the operator shall wash the system with mobile phase (5.3) for 30 min, and then with degassed absolute acetonitrile (5.1) for another 30 min.

8 Calculations

For calculating the concentration of biuret in the fertilizer, in g per kg, Formula (2) shall be used.

$$w = \frac{m_1}{m} \quad (2)$$

where

m_1 is the mass of biuret in mg, of the test solution, calculated according to the standard curve or linear regression equation corresponding to the peak areas;

m is the mass in g of the test portion.

9 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, CEN/TS 17765:2022;
- c) test results obtained expressed as mg/kg 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 occurred when performing the method, which might have influenced the test result(s).

Bibliography

- [1] EN 15479, *Fertilizers — Spectrophotometric determination of biuret in urea*
- [2] ISO 18643, *Fertilizers and soil conditioners — Determination of biuret content of urea-based fertilizers — HPLC method*
- [3] Fertilizer Research 26: 311-318, 1990. © 1990 Kluwer Academic Publishers
- [4] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003