BS ISO 19746:2017

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Determination of urea content in urea-based fertilizers by high performance liquid chromatography (HPLC)

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee CII/37, Fertilisers and related chemicals.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Published by BSI Standards Limited 2017

ISBN 978 0 580 87120 7

ICS 65.080

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 March 2017.

Amendments/corrigenda issued since publication

Date	Text affected

BS ISO 19746:2017

INTERNATIONAL STANDARD

ISO 19746

First edition
2017-02

Determination of urea content in urea-based fertilizers by high performance liquid chromatography (HPLC)

Détermination de la teneur en urée dans les engrais à base d'urée par chromatographie liquide à haute performance (CLHP)

Reference number
ISO 19746:2017(E)

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This document was prepared by Technical Committee ISO/TC 134, *Fertilizers and soil conditioners*.

Introduction

Urea is the most widely used source of nitrogen fertilizers worldwide and is used in a variety of forms such as pure urea, in combination with other nutrients, in complex fertilizers, and as reacted or modified ureas[2].

Due to the rapid hydrolysis of urea in the environment, especially when applied at the soil surface[3], efforts have been made to modify urea to slow down this loss process. Slow release nitrogen fertilizers such as methylene urea compounds, controlled release N fertilizers such as sulfur-coated urea (SCU) or polymer-coated urea (PCU), and stabilized N fertilizers containing additives (urease inhibitors and nitrification inhibitors) are examples of products aimed at containing the rapid hydrolyses of urea in the soil.

Accurate determination of urea in urea-based fertilizers is desirable for regulatory and product quality purposes. This is especially true for those fertilizers in which the urea content is physically or chemically modified. Most of these modified fertilizers contain some amounts of free and unreacted urea which is readily available N and therefore could not be accounted as part of the slow or controlled release N component[5].

Determination of urea content in urea-based fertilizers by high performance liquid chromatography (HPLC)

1 Scope

This document specifies the test procedure for determining the urea content in urea-based fertilizers, including urea, urea aldehydes [methylene urea fertilizers, isobutylene diurea (IBDU), crotonylidene diurea (CDU)], urea triazone fertilizers, urea ammonium nitrate (UAN), sulfur- and polymer-coated urea (SCU and PCU), as well as compound fertilizers containing urea. The method is based on High Performance Liquid Chromatography (HPLC).

The proposed method is an extension of the AOAC Official Method 2003.14 which was collaboratively studied for the "Determination of Urea in Water-Soluble Urea-Formaldehyde Fertilizer Products and in Aqueous Urea Solutions" in 2003. The method was published in the Journal of AOAC in 2004[4] and was granted the First Action in 2003 and the Final Action in 2008.

This method also applies to the determination of biuret content in urea containing fertilizer with the results published in the J. AOAC in 2014[5]. This method was adopted by the International Organization for Standardization (ISO) as a Committee Draft (ISO/CD 18643) in 2014, and after review and the Ring Test Analyses[6].

NOTE This HPLC method can also be utilized to analyse Crotonylidene diurea (CDU) and Isobutylidene diurea (IBDU) contents within those above-mentioned fertilizers, in addition to EN 15705[1].

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.

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

3 Terms and definitions

No terms and definitions are listed in this document.

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

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

4 Principles

The urea content in urea-based fertilizer is extracted by aqueous acetonitrile mobile phase and separated from other contents by High Performance liquid chromatography (HPLC) on an aminopropyl column. The urea peak is detected by a UV detector attached to the HPLC.

5 Reagents

WARNING — Acetonitrile is flammable and toxic. The related operations shall be performed in a laboratory fume hood. This document does not point out all possible safety problems, and the user shall bear the responsibility to take proper safety and health measures, and ensure the

operations are compliant with the conditions stipulated by the related laws and regulations of the state.

Use only water conforming to grade 3 of ISO 3696:1995.

5.1 Acetonitrile, LC grade.

5.2 Mobile phase, 150 ml water + 850 ml acetonitrile.

Filter the mobile phase solution using a 0,22 micron membrane and degas with nitrogen gas for 10 min as a pre-treatment before use.

5.3 Urea standard solutions, (0,5 mg/ml = 500 ppm).

Weigh 0,5 g of high purity urea, dissolve in mobile phase (**5.2**) and transfer into a 1 l volumetric flask. Dilute the solution to volume with mobile phase (**5.2**) and mix thoroughly.

6 Apparatus

Ordinary laboratory apparatus and the following equipment/instruments:

6.1 Ultrasonic bath.

6.2 High performance liquid chromatograph, with UV detector.

6.3 Microsyringe, 5 µl~50 µl.

6.4 Syringe-driven Filter, with organic filter membrane of 0,22 µm pores.

6.5 Injection loop, volume of 10 µl.

6.6 Sieve, with the aperture size of 0,50 mm.

6.7 2,00 micron filter paper.

7 Procedures

7.1 Preparation of test sample

7.1.1 For dry urea fertilizers, simply take 500 g of a divided sample as the test sample. For compound fertilizers, take a reduced lab sample of 100 g, grinding until it passes through a sieve of aperture size 0,5 mm, and mix thoroughly for reasons of homogeneity. Place the sample in a clean and dry bottle with lid.

7.1.2 For liquid urea fertilizers, take 100 ml of a homogeneous solution. Filter any particulate through 2,0 micron filter paper.

For all of these samples, use ultrasonic mixing for 10 min to assure complete dissolution. Filter to remove any undissolved portion through a 2,0 micron filter paper.

7.2 Preparation of the test solution

A minimum of two replicate experiments shall be done for the analysis of the urea content in fertilizer samples.

Weigh 0,1 g~0,5 g of the dry or the liquid test sample (accurately to 0,000 2 g, with urea content of 1 mg~2 mg ca.) into a 25 ml beaker, add 10 ml of the mobile phase (5.2) and dissolve using an ultrasonic bath for 10 min. Transfer the sample to a 25 ml volumetric flask, dilute to volume with the mobile phase (5.2), mix thoroughly and leave standing. Filter with a syringe filter to obtain the test solution.

7.3 Preparation of urea working standard solution

According to Table 1, pipette 0,00 ml, 0,50 ml, 1,00 ml, 3,00 ml, 5,00 ml and 10,00 ml of the urea standard solution (5.3) into six separate 25 ml volumetric flasks respectively. Dilute with the mobile phase (5.2) and make up to the mark, then mix thoroughly. Finally, filter each solution through 0,22 micron organic filter membranes.

Table 1 — Preparation of urea working standard solution

Volume of urea standard solution ml	Mass of urea mg
0,00[a]	0,00
0,50	0,25
1,00	0,50
3,00	1,50
5,00	2,50
10,00	5,00

[a] Blank solution.

7.4 HPLC conditions

Recommended operating conditions for the HPLC method are listed in Table 2. Other HPLC conditions that will achieve the same separation efficiency may be used.

Table 2 — Recommended operating conditions for the HPLC method

Chromatographic column[a]	Aminopropyl column	RP-8[c,d]
Flow rate[b]	1,0 ml/min	1,0 ml/min
Inject volume	10 µl	20 µl
Column temperature	35 °C	35 °C
Detector wavelength	195 nm	200 nm

[a] Examples of Aminopropyl columns are: Phenomenex Spherex-NH2column, 260 mm × 4,6 mm, Part No.00G-0017-E0 and ThermoFisher APS-2 HYPERSIL 250 mm × 4,6 mm, with 5 um particle size (Part No.30705-254630).

[b] Flow rate is a convenient parameter to optimize for specific products, i.e. 1,0 ml/min or 1,3 ml/min. See Annex A for retention times utilizing these two different flow rates (1,0 ml vs. 1,3 ml/min for triazone solutions).

[c] For the complex triazone liquids, the aminopropyl system has to be used to avoid interferences.

[d] Column used was a LiChroSpher RP-8, 250 mm × 4 mm, 5 um.

The best separate conditions can be determined according to different equipment and climate conditions.

7.5 Preparation of standard curve

Referring to the apparatus operation condition (Table 2), adjust the HPLC apparatus to the best operating conditions. Inject 10 µl of the working standard solution (5.3) and analyse to determine the concentration of the urea in the standard. Each working standard solution shall be determined in a

minimum of two replicates. Draw the standard curve or calculate the linear regression equation using the average peak areas of the urea chromatographic signal and their corresponding mass.

7.6 Determination of the urea content in the test solution

Determine the urea content of each test solution (5.3) using the same method used for the determination of the concentrations of the working standard solutions, measure the peak area, and calculate the urea mass in each test solution according to the standard curve or the linear regression equation. After completing the determination, first wash the chromatographic system with mobile phase (5.2) for 30 min, followed by absolute acetonitrile (5.1) for 30 min. Finally, turn off the apparatus according to the operating procedures.

7.7 Calculation and expression of results

The mass fraction of urea (%), w, is calculated as follows:

$$w = \frac{m_1 \times 10^{-3}}{m} \times 100 \quad (1)$$

where

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

m is the mass of the test portion, in g.

The arithmetic average of the results shall be calculated and reported.

7.8 Precision

7.8.1 Repeatability, *r*

7.8.2 Reproducibility, *R*

NOTE The precision and other statistical data are left blank at this time. These statistical data have been reported for the AOAC collaborative studies for the determination of urea[5] and for the ISO biuret ring test studies[6].

7.9 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the test samples;

b) test method used with reference to this document, i.e. ISO 19746;

c) test results obtained;

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.

All operating details which are not specified in this document or are regarded as optional, together with details of any incidents occurred when performing the method and which might have influenced the test results should be included in the report.

Annex A
(informative)

Examples of the retention times of triuret, biuret, urea and some other ureaforms

Table A.1 — Retention times examples[a]

Flow rates at 1 ml/min and (1,3 ml/min)	
Triuret	3,659 (3,359)
Biuret	4,244 (3,769)
Urea	4,439 (3,875)
Monomethylolurea (MMU)	5,923 (4,552)
Methylenediurea (MDU)	6,823 (5,453)
Dimethylolurea (DMU)	7,497 (5,879)
Hexamethylenetetramine (HMT)	9,577 (6,530)
Triazone	10,0 (7,98)
Crotonylidene diurea (CDU)	6,411

[a] Retention times may vary from column to column slightly (standards should be run first to confirm the retention times).

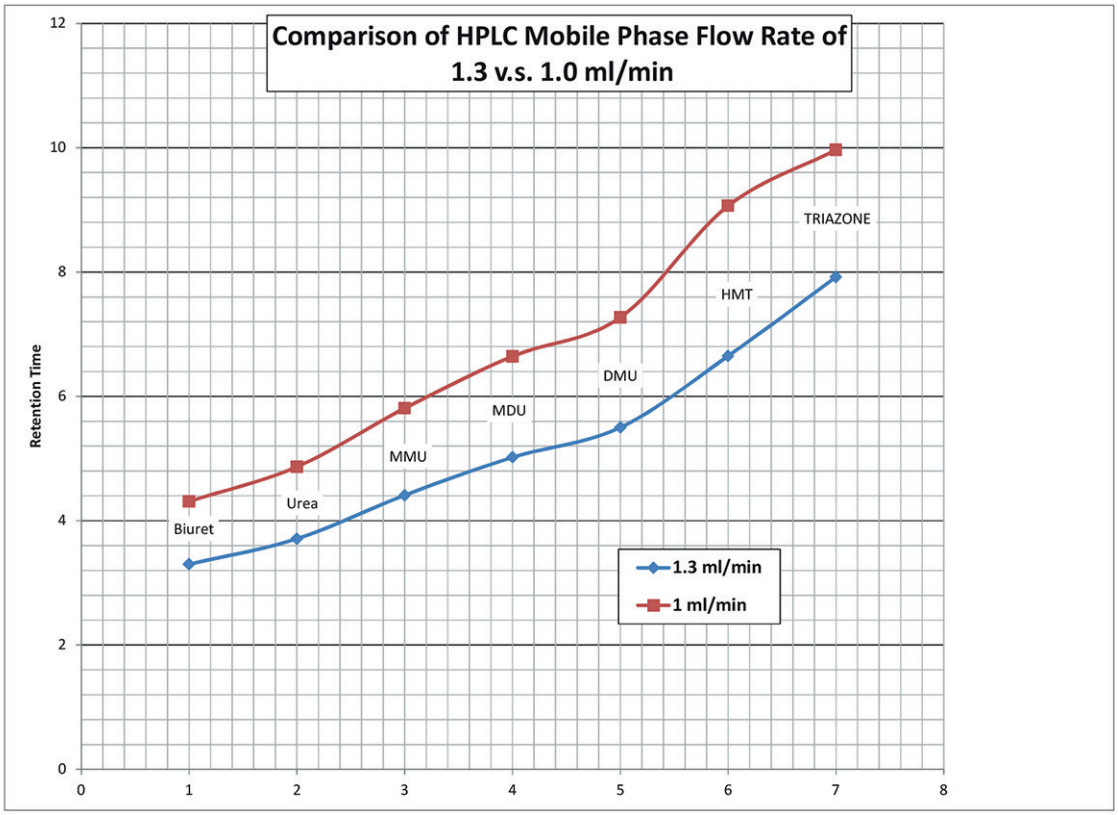

Figure A.1 — Examples of retention times with different flow rates (graphical depiction of retention times)

Examples of chromatograms:

a) urea;

b) Triazone;

c) ureaform chromatogram;

d) sulfur-coated urea chromatogram.

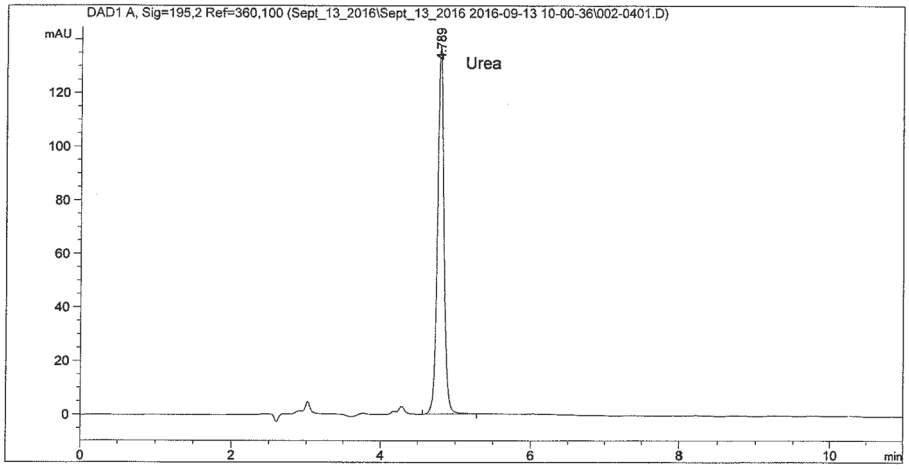

Figure A.2 — Urea chromatogram (urea peak at 4,789 min)

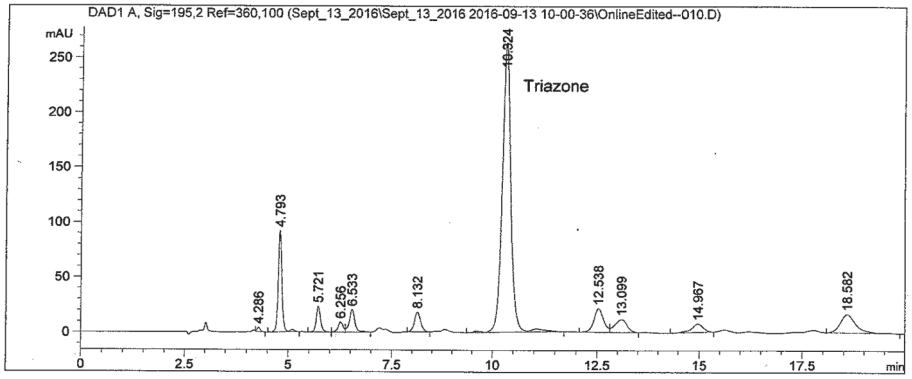

Figure A.3 — Triazone chromatogram (Triazone peak at 10,324 min)

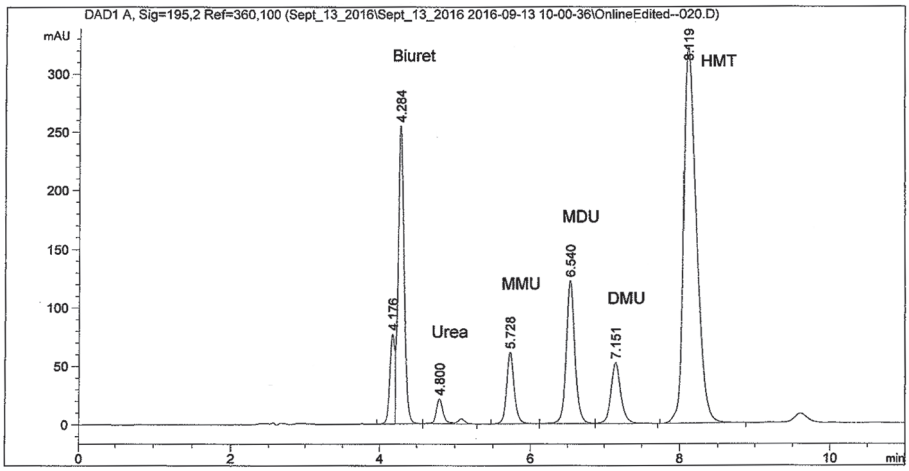

Figure A.4 — Urea-formaldehyde (ureaform) chromatogram

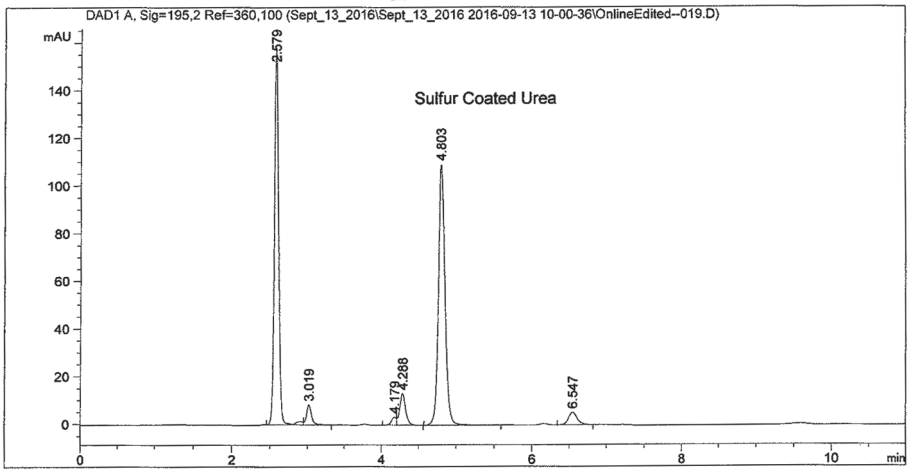

Figure A.5 — Sulfur-coated urea chromatogram (urea peak at 4,803 min)

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