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**Fertilizers — Determination of  
urea condensates using high-  
performance liquid chromatography  
(HPLC) — Isobutylidenediurea and  
crotonylidenediurea (method A) and  
methylen-urea oligomers (method B)**

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*Engrais — Dosage des condensats d'urée par chromatographie  
liquide haute performance (CLHP) — Isobutylidène diurée et  
crotonylidène diurée (méthode A) et oligomères de méthylène-urée  
(méthode B).*

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

ISO 25705 was prepared by CEN/TC 260 (as EN 15705:2010) and was adopted by Technical Committee ISO/TC 134, *Fertilizers and soil conditioners*. The following modifications were made.

- The references to EN 1482-1 and EN 1482-2 were changed to ISO 14820-1 and ISO 14820-2.
- In 5.2.1 and 6.2.1, the general text (not related to the water) was moved directly under 5.2 and 6.2, respectively.
- In Table 1, the word “approximate” was added to the column headers for IBDU and CDU.
- In 5.4.3 and 6.4.3, “the sample grounded” was changed to “the ground sample”.
- In 6.4.3, “pieces of glass” and “pieces” were replaced by “boiling stones”.
- In 6.4.2.1, 6.4.2.2, 6.4.2.3, and 6.4.2.4, “by placing the flask in the” was added before “ultrasonic bath”.
- In 6.4.2.1, 6.4.2.2, 6.4.2.3, 6.4.2.4, 6.4.2.5 and 6.4.3, “homogenize” was changed to “mix thoroughly”.
- In 6.4.2.5, “before transferring into” was changed to “before transferring in” three times.
- In the keys for Figures B.2, B.3, B.4, C.2, C.3, C.4 and C.5, the units for the areas were added.

## Introduction

Fertilizers containing the condensates of urea and specified aldehydes (with crotonaldehyde called crotonyliden diurea or CDU, with isobutyraldehyde called isobutylidene diurea or IBDU, with formaldehyde called urea formaldehyde or methylene urea or MU) are covered by Regulation (EC) 2003/2003, Annex I<sup>[4]</sup> as nitrogenous fertilizers. The methods described in this International Standard enable the quantitative determination of these condensates and the determination of the solubility of the MU-oligomers according to the Regulation.

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# Fertilizers — Determination of urea condensates using high-performance liquid chromatography (HPLC) — Isobutylidenediurea and crotonylidenediurea (method A) and methylen-urea oligomers (method B)

## 1 Scope

This International Standard specifies methods for the determination of isobutylidene diurea (IBDU), Crotonylidene diurea (CDU) (method A) and methylene-urea oligomers (MU) (method B) in fertilizers using high-performance liquid chromatography (HPLC).

The method is applicable to all fertilizers which do not contain interfering organic compounds.

## 2 Normative references

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

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

ISO 14820-2, *Fertilizers and liming materials — Sampling and sample preparation — Part 2: Sample preparation*

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

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

## 3 Terms and definitions

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

## 4 Sampling and sample preparation

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 14820-1.

Sample preparation shall be carried out in accordance with ISO 14820-2.

## 5 Method A: Determination of CDU and IBDU

### 5.1 Principle

The sample is extracted with water and, after appropriate dilution, analysed using a suitable HPLC system.

### 5.2 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

**5.2.1 Water**, distilled or demineralized water (grade 3 according to ISO 3696).

5.2.2 Acetonitrile, HPLC-grade.

5.2.3 Isobutylidene diurea and Crotonylidene diurea, in their pure form.

### 5.3 Apparatus

5.3.1 Laboratory equipment and glassware, for preparation of solutions and dilutions.

5.3.2 Analytical balance, capable of weighing to an accuracy of  $\pm 0,1$  mg.

5.3.3 HPLC-system, with UV-detector.

5.3.4 Ultrasonic bath.

5.3.5 Magnetic stirrer.

5.3.6 Disposable filter, 0,45  $\mu\text{m}$ .

### 5.4 Procedure

#### 5.4.1 System parameters of HPLC

Analytical/separating column: silica column with C18 reverse phase<sup>1)</sup>

Detection wavelength: 200 nm

Eluent: acetonitrile/water: 10/90 (volume fraction)

Flow rate: 1 ml/min

Temperature: ambient temperature

Injection volume: 20  $\mu\text{l}$

#### 5.4.2 Calibration

##### 5.4.2.1 Stock solution IBDU $\rho(\text{IBDU}) = 100 \text{ mg/l}$

Weigh 100/ $R$  mg of IBDU (5.2.3), where  $R$  is the purity of IBDU, into a 1 000 ml flask and add about 900 ml of water (5.2.1). Dissolve in an ultrasonic bath (5.3.4) for about 10 min, followed by stirring on a magnetic stirrer (5.3.5) for about 1 h. Make up to volume. Filtration is not necessary.

##### 5.4.2.2 Stock solution CDU $\rho(\text{CDU}) = 100 \text{ mg/l}$

Weigh 100/ $R$  mg of CDU (5.2.3), where  $R$  is the purity of CDU, into a 1 000 ml flask and add about 900 ml of water (5.2.1). Dissolve in an ultrasonic bath (5.3.4) for about 10 min, followed by stirring on a magnetic stirrer (5.3.5) for about 1 h. Make up to volume. Filtration is not necessary.

##### 5.4.2.3 Calibration solution

For calibration, prepare three solutions according to Table 1 using one-mark (bulb) pipettes and dilute to the mark with water (5.2.1).

1) LiChrosorb RP-18 7  $\mu\text{m}$  250/4 mm is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.



For the determination of the retention time, dilute 10 ml of the stock solution (5.4.2.1) or respectively (5.4.2.2) into two 100 ml flasks and make up to volume with water (5.2.1).

The evaluation of calibration is carried out manually or by means of a suitable PC-aided (computerized) calculation method.

**Table 1 — Preparation of calibration solutions**

Parameter	Amount of stock solution IBDU/CDU ml (to be added to the 100 ml flask)	Content of IBDU mg/l (approximate)	Content of CDU mg/l (approximate)
Standard 1	10	10,0	10,0
Standard 2	25	25,0	25,0
Standard 3	50	50,0	50,0

### 5.4.3 Preparation of the test portion

Weigh 1 g of the ground sample to <0,2 mm to the nearest 0,1 mg and quantitatively transfer into a 1 000 ml volumetric flask with water (5.2.1). Fill the flask to an amount of approximately 900 ml and treat it for 10 min in the ultrasonic bath (5.3.4). Then make up to the mark and stir for 1 h at room temperature on a magnetic stirrer (5.3.5). Dilute 10 ml of the solution in a 100 ml volumetric flask and filter into the HPLC injection vial through a disposable filter (5.3.6).

### 5.4.4 Measurement

Measurement is performed manually or by means of an automatic sample loading system (autosampler).

### 5.4.5 Important annotations

IBDU is able to form urea in aqueous solution. Therefore, the measurement of the calibration and sample solutions shall be completed within one working day.

The concentrations of CDU and IBDU in the sample solutions shall be kept within the calibration limits (5.4.2) to ensure sufficient reproducibility.

## 5.5 Calculation

The calculation can be performed manually or by means of a PC using the calibration parameters in respect to the amount used.

In the case of PC-aided (computerized) calculation and application of Table 1 regarding the amounts of stock solution, the content of IBDU/CDU in milligrams per litre will be calculated by the system. The calculated values are equal to the percentage mass concentration of IBDU/CDU in the analysed sample of fertilizer.

Following general rules for declaration in regulations to declare the content of the compounds as percentage mass fraction of nitrogen, calculate the contents,  $w_{N(\text{IBDU})}/w_{N(\text{CDU})}$  in percent (g/100 g), according to Formulae (1) and (2):

$$w_{N(\text{IBDU})} = w_{\text{IBDU}} \times 0,322 \quad (1)$$

$$w_{N(\text{CDU})} = w_{\text{CDU}} \times 0,326 \quad (2)$$

where

0,322 is the conversion factor for the content of IBDU in the fertilizer into nitrogen content;

0,326 is the conversion factor for the content of CDU in the fertilizer into nitrogen content.

## 6 Method B: Determination of methylene-urea oligomers (MU)

**NOTE** By the condensation of urea and formaldehyde several oligomers like methylene-diurea (MDU), dimethylenetriurea (DMTU), trimethylene-tetraurea (TMTU) and higher oligomers are formed. These three molecules are the most soluble in water, the higher compounds are insoluble in hot water, but their nitrogen is available for plants by microbiological decomposition. Also, urea is always a companion of MU – oligomers.

### 6.1 Principle

The sample is extracted with boiling water and analysed using a suitable HPLC system.

The methylene-urea soluble oligomers are measured and detected by the HPLC-method.

In the HPLC-diagram, methylene-urea oligomers are represented by different peaks: urea, methylene-diurea, dimethylenetriurea; and trimethylene tetraurea are, in the meantime, the most soluble and important.

### 6.2 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

**6.2.1 Water**, distilled or demineralized water (grade 3 according to ISO 3696).

**6.2.2 Acetonitrile**, HPLC-grade p.a. should not be used here. It is not common.

**6.2.3 Urea**, reagent grade (or analytical grade), 46,6 % of total nitrogen.

**6.2.4 Methylene diurea (MDU)**, synthesized and purified by a special laboratory, 42,4 % of total nitrogen.<sup>2)</sup>

**6.2.5 Dimethylenetriurea (DMTU)**, synthesized and purified by a special laboratory, 41,2 % of total nitrogen.<sup>2)</sup>

**6.2.6 Trimethylene tetraurea (TMTU)**, synthesized and purified by a special laboratory, 40,6 % of total nitrogen.

### 6.3 Apparatus

**6.3.1 Laboratory equipment and glassware**, for preparation of solutions and dilutions.

**6.3.2 Analytical balance**, capable of weighing to an accuracy of  $\pm 0,1$  mg.

**6.3.3 Technical balance**, capable of weighing to an accuracy of  $\pm 0,01$  g.

**6.3.4 HPLC-system**, equipped with an UV-detector.

**6.3.5 Ultrasonic bath.**

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<sup>2)</sup> The standard substances MDU and DMTU can be prepared according to the method given in Official Methods of Analysis of AOAC International, AOAC Official Method 983.01, JAOAC 66, 769 (1983).

**6.3.6 Magnetic stirrer.****6.3.7 Disposable filter, 0,45 µm.****6.4 Procedure****6.4.1 System parameters of HPLC**

Analytical/separating column	NH <sub>2</sub> column, 5 µm, 250 mm × 4,6 mm <sup>3)</sup>
	A guard-column is recommended.
Detection wavelength	195 nm (Diode Array detector)
Eluent	acetonitrile/water 85/15 (volume fraction)
Flow rate	1 ml/min
Temperature	60 °C
Run time	30 min
Injection volume	20 µl

**6.4.2 Calibration****6.4.2.1 Stock solution of urea,  $\rho \approx 1\,000$  mg/kg**

Weigh (6.3.2) 100/*R* mg of urea (6.2.3), where *R* is the purity of urea, to the nearest 0,1 mg and put into a clean and dry 100 ml volumetric flask, weighed (6.3.3) before to the nearest 0,01 g. Add 50 ml of water (6.2.1) and dissolve the urea by placing the flask in the ultrasonic bath (6.3.5) for about 10 min. Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask to the nearest 0,01 g and record the net weight. Store at room temperature, well closed. This stock solution is stable for one week.

**6.4.2.2 Stock solution of methylene diurea,  $\rho \approx 1\,000$  mg/kg**

Weigh (6.3.2) 50/*R* mg of MDU (6.2.4), where *R* is the purity of MDU, to the nearest 0,1 mg and put into an empty and dry 50 ml volumetric flask, weighed (6.3.3) before to the nearest 0,01 g. Add 40 ml of water (6.2.1) and dissolve the MDU by placing the flask in the ultrasonic bath (6.3.5) for about 10 min (if necessary, gently warm). Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask to the nearest 0,01 g and record the net weight. Store at room temperature, well closed. This stock solution is stable for three weeks.

**6.4.2.3 Stock solution of dimethylene triurea,  $\rho \approx 1\,000$  mg/kg**

Weigh (6.3.2) 50/*R* mg of DMTU (6.2.5), where *R* is the purity of DMTU, to the nearest 0,1 mg and put into a clean and dry 50 ml volumetric flask, weighed (6.3.3) before to the nearest 0,01 g. Add 40 ml of water (6.2.1) at 60 °C and dissolve the DMTU by placing the flask in the ultrasonic bath (6.3.5) for about 10 min. Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask to the nearest 0,01 g and record the net weight. Store at room temperature, well closed. This stock solution is stable for three weeks.

**6.4.2.4 Stock solution of trimethylene tetraurea,  $\rho \approx 100$  mg/kg**

Weigh (6.3.2) 10/*R* mg of TMTU (6.2.6), where *R* is the purity of TMTU, to the nearest 0,1 mg and put into a clean and dry 100 ml volumetric flask, weighed (6.3.3) before to the nearest 0,01 g. Add 80 ml of water (6.2.1) at 60 °C and dissolve the TMTU by placing the flask in the ultrasonic bath (6.3.5) for about

3) Supelcosil LC-NH<sub>2</sub> is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.