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Gnojila - Določevanje kondenzatov sečnine s tekočinsko kromatografijo visoke ločljivosti (HPLC) - Izobutilidendiurea in krotonilidendiurea (metoda A) in oligomeri metilenuree (metoda B)

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

Düngemittel - Bestimmung von Harnstoffkondensaten mit Hochleistungs-Flüssigchromatographie (HPLC) - Isobutylidendiharnstoff und Crotonylidendiharnstoff (Verfahren A) und Methylenharnstoff-Oligomere (Verfahren B)

Engrais - Détermination de condensates d'urea par chromatographie liquide à haute performance (CLHP) - Isobutylidenediurea et crotonylidenediurea (méthode A) et methyleneurea-oligomeres (méthode B)

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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)

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

Düngemittel - Bestimmung von Harnstoffkondensaten mit Hochleistungs-Flüssigchromatographie (HPLC) - Isobutylidendiharnstoff und Crotonylidendiharnstoff (Verfahren A) und Methylenharnstoff-Oligomere (Verfahren B)

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Foreword

This document (CEN/TS 15705:2009) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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1 Scope

This document specifies methods for the determination of isobutylidenediurea (IBDU), crotonylidenediurea (CDU) (method A) and methylene-urea oligomers (MU) (method B) in fertilizers using high-performance liquid chromatography (HPLC).

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

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 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 [1].

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

5 Method A: Determination of CDU and IBDU

5.1 Principle

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

5.2 Reagents

5.2.1 General

Use only reagents of recognized analytical grade and distilled or demineralized water (grade 3 according to EN ISO 3696:1995).

5.2.2 Acetonitrile, p.a., HPLC-grade;

5.2.3 Isobutylidenediurea and crotonylidenediurea, 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 for weighing to an accuracy of ± 0,1 mg;

5.3.3 HPLC-system, with UV-detector;

5.3.4 Ultrasonic bath;

5.3.5 Magnetic stirrer;

5.3.6 Disposible filter, 0,45 µm.

5.4 Procedure

5.4.1 System parameters of HPLC

Analytical/separating column: silica column with C18 reverse phase [1]

Detection wavelength: 200 nm

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

Flow rate: 1 ml/min

Temperature: ambient temperature

Injection volume: 20 µl

5.4.2 Calibration

5.4.2.1 Stock solution IBDU ρ(IBDU) = 100 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 ρ(CDU) = 100 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.

[1] E.g. LiChrosorb RP-18 7µm 250/4 mm or equivalent.

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).

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	Content of CDU mg/l
Standard1	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 sample grounded to < 0,2 mm to the nearest 0,1 mg and flush 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, $w_{N(IBDU)}/w_{N(CDU)}$ in percent (g/100 g), according to the following equations:

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

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

where

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

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

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

NOTE By the condensation of urea and formaldehyde developed no only one compound, as it is by the reaction of urea and crotonaldehyde or isobutylaldehyde, but oligomers like methylen–diurea (MDU), dimethylen-triurea (DMTU), trimethylen-tetraurea (TMTU) and higher oligomers. 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 analyzed using a suitable HPLC system.

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

In the HPLC-diagram methylen-urea oligomers are represented by different peaks: urea, methylen-diurea, dimethylen-triurea; trimethylen-tetraurea are, in the mean time, the most soluble and important.

6.2 Reagents

6.2.1 General

Use only reagents of recognized analytical grade and distilled or demineralized water (grade 3 according to EN ISO 3696:1995).

6.2.2 Acetonitrile, p.a., HPLC-grade;

6.2.3 Urea, p.a., 46,6 % of total nitrogen;

6.2.4 Methylen-diurea (MDU), synthesized and purified by a special laboratory, 42,4 % of total nitrogen;

6.2.5 Dimethylen-triurea (DMTU), synthesized and purified by a special laboratory, 41,2 % of total nitrogen;

6.2.6 Trimethylen-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 for weighing to an accuracy of ± 0,1 mg;

6.3.3 Technical balance, capable for weighing to an accuracy of ± 0,01 g;

6.3.4 HPLC-system, equipped with an UV-detector;

6.3.5 Ultrasonic bath;

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_2 column, 5 µm, 250 mm × 4,6 mm [2)]
	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

2) e.g. Supelcosil LC-NH_2 or equivalent.

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 an empty 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 in an ultrasonic bath (6.3.5) for about 10 min. Make up approximately to the mark with water (6.2.1) and homogenize. 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 methylen-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 in an 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 homogenize. 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 dimethylen-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 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) at 60 °C and dissolve the DMTU in an ultrasonic bath (6.3.5) for about 10 min. Make up approximately to mark with water (6.2.1) and homogenize. 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 trimethylen-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 an empty 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 in an ultrasonic bath (6.3.5) for about 10 min. Make up approximately to mark with water (6.2.1) at 60 °C and homogenize. 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.

6.4.2.5 Calibration solutions

For calibration, prepare three solutions according to Table 2.

Table 2 — Preparation of calibration solutions

Parameter	urea stock solution g	MDU stock solution g	DMTU stock solution g	TMTU stock solution g
Standard 1	1	1	1	1
Standard 2	3	3	3	3
Standard 3	5	5	5	5

— Calibration solution 1: record the weight (6.3.3) of an empty and dry 100 ml volumetric flask (to the nearest 0,01 g), before transferring into 1 g (6.3.2) (to the nearest 0,1 mg) of each stock solution. Make up approximately to mark with water (6.2.1) and homogenize. Weigh (6.3.3) the full flask and record the net weight.