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Anorganska gnojila - Določevanje oligomerov metilenuree s tekočinsko kromatografijo visoke ločljivosti (HPLC)

Inorganic fertilizers - Determination of methylen-urea oligomers using high-performance liquid chromatography (HPLC)

Anorganische Düngemittel - Bestimmung von Methylen-Harnstoff-Oligomeren mittels Hochleistungsflüssigkeitschromatographie (HPLC)

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Inorganic fertilizers - Determination of methylen-urea oligomers using high-performance liquid chromatography (HPLC)

Anorganische Düngemittel - Bestimmung von Methylen-Harnstoff-Oligomeren mittels Hochleistungsflüssigkeitschromatographie (HPLC)

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European foreword

This document (prEN 15705:2022) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document, together with prEN 17864:2022, will supersede EN 15705:2010.

In comparison with EN 15705:2010, the following technical modifications have been made:

— EN 15705:2010 is split into two parts:

  — Method A of EN 15705:2010 is given in prEN 17864:2022.

  — Method B of EN 15705:2010 is given in this document.

1 Scope

This document specifies a method for the determination of methylen-urea (MU) oligomers in inorganic fertilizers using high-performance liquid chromatography (HPLC).

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

NOTE By the condensation of urea and formaldehyde, several oligomers are formed, such as methylen–diurea (MDU), dimethylen-triurea (DMTU), trimethylen-tetraurea (TMTU) and higher oligomers. The three molecules named here are the most soluble in water, while 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.

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 1482-2, *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*

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 and EN 12944-2 apply.

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

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

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

4 Principle

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

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

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

5 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water, free from carbon dioxide and all nitrogenous compounds (grade 3 according to EN ISO 3696:1995).

5.1 Acetonitrile, HPLC-grade.

5.2 Urea, p.a., 46,6 % of total nitrogen.

5.3 Methylen-diurea (MDU), synthesized and purified by a special laboratory[1], having 42,4 % of total nitrogen.

5.4 Dimethylen-triurea (DMTU), synthesized and purified by a special laboratory[1], having 41,2 % of total nitrogen.

5.5 Trimethylen-tetraurea (TMTU), synthesized and purified by a special laboratory, having 40,6 % of total nitrogen.

6 Apparatus and equipment

Usual laboratory glassware and equipment and, in particular, the following.

6.1 Analytical balance, capable for weighing to of the nearest 0,1 mg.

6.2 Technical balance, capable of weighing to of the nearest 0,01 g.

6.3 HPLC-system, equipped with an UV-detector.

An example of a suitable column and analytical conditions are reported in Annex C.

A guard-column is recommended.

An acceptable chromatogram, as shown as example in Annex B, can be achieved using the column and analytical conditions reported in Annex C.

6.4 Ultrasonic bath.

6.5 Magnetic stirrer.

6.6 Disposable filter, 0,45 µm.

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 Calibration

8.1.1 Stock solution of urea, mass concentration $\rho \approx 1\,000$ mg/kg

Weigh $100/P$ mg of urea (5.2), where P is the purity of urea expressed as a pure number, to the nearest 0,1 mg and put into an empty and dry 100 ml volumetric flask, weighed before to the nearest 0,01 g. Add 50 ml of water and dissolve the urea in an ultrasonic bath (6.4) for about 10 min. Make up approximately to the mark with water and homogenize. Weigh the full flask to the nearest 0,01 g and record the net mass. Store at room temperature, well closed. This stock solution is stable for one week.

[1] 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).

8.1.2 Stock solution of methylen-diurea, $\rho \approx 1\,000$ mg/kg

Weigh $50/P$ mg of MDU (5.3), where P is the purity of MDU expressed as a pure number, to the nearest 0,1 mg and put into an empty and dry 50 ml volumetric flask, weighed before to the nearest 0,01 g. Add 40 ml of water and dissolve the MDU in an ultrasonic bath (6.4) for about 10 min (if necessary gently warm). Make up approximately to the mark with water and homogenize. Weigh the full flask to the nearest 0,01 g and record the net mass. Store at room temperature, well closed. This stock solution is stable for three weeks.

8.1.3 Stock solution of dimethylen-triurea, $\rho \approx 1\,000$ mg/kg

Weigh $50/P$ mg of DMTU (5.4), where P is the purity of DMTU expressed as a pure number, to the nearest 0,1 mg and put into an empty and dry 50 ml volumetric flask, weighed before to the nearest 0,01 g. Add 40 ml of water at 60 °C and dissolve the DMTU in an ultrasonic bath (6.4) for about 10 min. Make up approximately to mark with water and homogenize. Weigh the full flask to the nearest 0,01 g and record the net mass. Store at room temperature, well closed. This stock solution is stable for three weeks.

8.1.4 Stock solution of trimethylen-tetraurea, $\rho \approx 100$ mg/kg

Weigh $10/P$ mg of TMTU (5.5), where P is the purity of TMTU expressed as a pure number, to the nearest 0,1 mg and put into an empty and dry 100 ml volumetric flask, weighed before to the nearest 0,01 g. Add 80 ml of water at 60 °C and dissolve the TMTU in an ultrasonic bath (6.4) for about 10 min. Make up approximately to mark with water at 60 °C and homogenize. Weigh the full flask to the nearest 0,01 g and record the net mass. Store at room temperature, well closed. This stock solution is stable for three weeks.

8.1.5 Calibration solutions

For calibration, prepare three solutions according to Table 1.

Table 1 — Preparation of calibration solutions

Calibration solution	Urea stock solution	MDU stock solution	DMTU stock solution	TMTU stock solution
	g	g	g	g
1	1	1	1	1
2	3	3	3	3
3	5	5	5	5

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

Calibration solution 2: record the mass of an empty and dry 100 ml volumetric flask (to the nearest 0,01 g) before transferring into 3 g (to the nearest 0,1 mg) of each stock solution. Make up approximately to mark with water and homogenize. Weigh the full flask and record the net mass.

Calibration solution 3: record the mass of an empty and dry 100 ml volumetric flask (to the nearest 0,01 g) before transferring into 5 g (to the nearest 0,1 mg) of each stock solution. Make up approximately to mark with water and homogenize. Weigh the full flask and record the net mass.

The content (approximate) of the methylen-urea oligomers in the three calibration solutions is described in Table 2.

Table 2 — Content (approximate) of the methylen-urea oligomers

Calibration solution	Urea stock solution mg/kg	MDU stock solution mg/kg	DMTU stock solution mg/kg	TMTU stock solution mg/kg
1	10	10	10	1
2	30	30	30	3
3	50	50	50	5

Gently warm at 60 °C the stock solutions 8.1.3 and 8.1.4 before transferring, to ensure the complete solubility of the DMTU and TMTU respectively.

All the calibration solutions shall be prepared fresh daily.

All the calibration solutions for the HPLC set up shall be brought at 60 °C before injection.

8.2 Preparation of the test solution

Weigh 0,5 g of the sample grounded to < 0,1 mm to the nearest 0,1 mg and put it into a 2 000 ml (“dry” is unnecessary) beaker. Fill the beaker with an amount of approximately 950 ml of water and some pieces of glass to help the boiling. Boil directly for 30 min.

Weigh an empty and dry 1 l volumetric flask (to the nearest 0,01 g) before transferring into the content of the beaker, without the pieces of glass. Wash well the beaker with boiling water and make up approximately to mark the flask with boiling water and homogenize. Weigh the full flask and record the net mass.

The oligomers are analytically separated at a temperature of 60 °C of the column oven. In order to reach a quicker temperature alignment between column oven and eluents, it is recommended to adjust the eluents to 60 °C as well.

If the injection will not be performed in a short time, keep the flask in a bath water at 70 °C to 80 °C.

Filter 1 ml into the HPLC injection vial and inject.

In the case where no auto sampler is available, manually inject 20 µl of this solution.

8.3 Measurement

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

9 Calculations and expression of the result

The calculation is 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 and Table 2 regarding the amounts of stock solution, the content of the different methylen-urea oligomers in milligrams per kilograms are calculated by the system. The calculated values are equal to the percentage mass concentration of urea, methylen-diurea, dimethylen-triurea, trimethylen-tetraurea in the analysed sample of fertilizer.

Calculate the average response factor of an oligomer, RF_O, according to the following Formula:

$$RF_O = (m_1/A_1 + m_2/A_2 + m_3/A_3)/3 \quad (1)$$

where

m_1 is the mass of that oligomer in 100 g of the calibration solution 1;

A_1 is the peak area of that oligomer in the calibration solution 1;

m_2 is the mass of that oligomer in 100 g of the calibration solution 2;

A_2 is the peak area of that oligomer in the calibration solution 2;

m_3 is the mass of that oligomer in 100 g of the calibration solution 3;

A_3 is the peak area of that oligomer in the calibration solution 3.

The concentration in percent on the sample of MDU, for example, is calculated using the following Formula:

$$c_{MDU} = RF_{MDU} \times A_{MDU} / m_s \times 100 \quad (2)$$

where

RF_{MDU} is the average response factor of the MDU-oligomer;

A_{MDU} is the peak area of the MDU in the sample;

m_s is the mass of the test portion (sample mass), in mg.

In order to declare the content of the compounds as percentage mass fraction of nitrogen, calculate the contents, $w_{N(urea)}$, $w_{N(MDU)}$, $w_{N(DMTU)}$, $w_{N(TMTU)}$ in percent (g/100 g), according to the following Formulae:

$$w_{N_{urea}} = w_{urea} \times F_{urea} \quad (3)$$

$$w_{N_{MDU}} = w_{MDU} \times F_{MDU} \quad (4)$$

$$w_{N_{DMTU}} = w_{DMTU} \times F_{DMTU} \quad (5)$$

$$w_{N_{TMTU}} = w_{TMTU} \times F_{TMTU} \quad (6)$$

where

F_{urea} is the conversion factor for the content of urea in the fertilizer into nitrogen content, i.e. 0,466;

F_{MDU} is the conversion factor for the content of MDU in the fertilizer into nitrogen content, i.e. 0,424;

F_{DMTU} is the conversion factor for the content of DMTU in the fertilizer into nitrogen content, i.e. 0,412;

F_{TMTU} is the conversion factor for the content of TMTU in the fertilizer into nitrogen content, i.e. 0,406.

To convert the mass fraction in mg/kg to the mass concentration in mg/l, consider the density of the water at 60 °C, which is 0,983 24 g/ml.