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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 (CLHP) — Isobutylidène diurée et crotonylidène diurée (méthode A) et oligomères de méthylène-urée (méthode B)*

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# Foreword

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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[4] 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.

# 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 ±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 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 $\rho$(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 $\rho$(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.

#### 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 µm 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, $w_{N(IBDU)}/w_{N(CDU)}$ in percent (g/100 g), according to Formulae (1) and (2):

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

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

where

0,322 is the 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.[2)]

**6.2.5 Dimethylenetriurea (DMTU)**, synthesized and purified by a special laboratory, 41,2 % of total nitrogen.[2)]

**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 ±0,1 mg.

**6.3.3 Technical balance**, capable of weighing to an accuracy of ±0,01 g.

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

**6.3.5 Ultrasonic bath**.

2) 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 | $NH_2$ column, 5 µm, 250 mm × 4,6 mm[3)]<br>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-$NH_2$ 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.

10 min. Make up approximately to the mark with water (6.2.1) at 60 °C 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.

#### 6.4.2.5 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 a clean and dry 100 ml volumetric flask (to the nearest 0,01 g), before transferring in 1 g (6.3.2) (to the nearest 0,1 mg) of each stock solution. Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask and record the net weight.

— Calibration solution 2: Record the weight (6.3.3) of a clean and dry 100 ml volumetric flask (to the nearest 0,01 g) before transferring in 3 g (6.3.2) (to the nearest 0,1 mg) of each stock solution. Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask and record the net weight.

— Calibration solution 3: Record the weight (6.3.3) of an empty and dry 100 ml volumetric flask (to the nearest 0,01 g) before transferring in 5 g (6.3.2) (to the nearest 0,1 mg) of each stock solution. Make up approximately to the mark with water (6.2.1) and mix thoroughly. Weigh (6.3.3) the full flask and record the net weight.

The content (approximate) of the methylene urea oligomers in the three standard solutions is described in Table 3.

**Table 3 — Content (approximate) of the methylene urea oligomers**

| Parameter | Content of urea mg/kg | Content of MDU mg/kg | Content of DMTU mg/kg | Content of TMTU mg/kg |
|---|---|---|---|---|
| Standard 1 | 10 | 10 | 10 | 1 |
| Standard 2 | 30 | 30 | 30 | 3 |
| Standard 3 | 50 | 50 | 50 | 5 |

Gently warm at 60 °C the stock solutions 6.4.2.3 and 6.4.2.4 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.

### 6.4.3 Preparation of the test solution

Weigh 0,5 g (6.3.2) of the ground sample to <0,1 mm to the nearest 0,1 mg and put it into a 2 000 ml clean and dry beaker. Fill the beaker with an amount of approximately 950 ml of water (6.2.1) and add some boiling stones to help the boiling. Boil directly for 30 min.

Weigh (6.3.3) an empty and dry 1 l volumetric flask (to the nearest 0,01 g) before transferring the content into the beaker, without the boiling stones. Wash the beaker well with boiling water. Make up

approximately to mark the flask with boiled water and mixed thoroughly. Weigh (6.3.3) the full flask and record the net weight.

If the injection will not be performed in a short time, keep the flask in a water bath 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.

#### 6.4.4 Measurement

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

#### 6.4.5 Important annotations

The oligomers are analytically separated at a temperature of 60 °C in 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.

### 6.5 Calculations

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 2 and Table 3 regarding the amounts of stock solution, the content of the different methylene urea oligomers in milligrams per kilograms will be calculated by the system. The calculated values are equal to the percentage mass concentration of urea, methylene diurea, dimethylene triurea, and trimethylene tetraurea in the analysed sample of fertilizer.

Calculate the response factor of an oligomer, $RF_O$, according to Formula (3):

$$RF_O = \frac{m_1}{A_1} \tag{3}$$

where

$m_1$ is the mass of that oligomer in 100 g of the standard solution;

$A_1$ is the peak area of that oligomer in that standard solution.

The response factor in percent from the three calibration solutions should be averaged using the Formula (4) ($RF_{MDU}$, for example):

$$RF_{MDU} = \frac{RF_{MDUO} \times A_{MDU}}{m_s} \times 100 \tag{4}$$

where

$RF_{MDUO}$ is the 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 weight), in milligrams.

Following general rules for declaration in regulations to declare the content of the compounds as percentage mass fraction of nitrogen, calculate the contents, $w_{N(UREA)}/w_{N(MDU)}/w_{N(DMTU)}/w_{N(TMTU)}$ in percent (g/100 g), according to Formulae (5), (6), (7), and (8):

$$w_{N\,urea} = w_{urea} \times 0{,}466 \tag{5}$$

$$w_{N\,MDU} = w_{MDU} \times 0{,}424 \tag{6}$$

$$w_{N\,DMTU} = w_{DMTU} \times 0{,}412 \tag{7}$$

$$w_{N\,TMTU} = w_{TMTU} \times 0{,}406 \tag{8}$$

where

0,424 is the conversion factor for the content of MDU in the fertilizer into nitrogen content;

0,412 is the conversion factor for the content of DMTU in the fertilizer into nitrogen content;

0,406 is the conversion factor for the content of TMTU in the fertilizer into nitrogen content;

0,466 is the conversion factor for the content of urea in the fertilizer into nitrogen content.

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.

## 7 Precision method A and method B

### 7.1 Inter-laboratory test

Inter-laboratory tests have been carried out in 2006 (for IBDU and CDU – method A) with 11 participating laboratories and 2008 (for methylene urea oligomers – method B) with 10 participating laboratories and two different samples of fertilizers. Repeatability and reproducibility were calculated according to ISO 5725-1 and ISO 5725-2.

The values derived from these inter-laboratory tests may not be applicable to concentration ranges and matrices other than those given in Annex A.

### 7.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, was not more than 5 % of the cases exceeding the values of *r* given in Table 4 (method A) and Table 5 (method B).

### 7.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, was not more than 5 % of the cases exceeding the values of *R* given in Table 4 (method A) and Table 5 (method B).

**Table 4 — Mean values, repeatability, and reproducibility limits for method A**

| Sample | $\overline{x}$ % | $r$ % | $R$ % |
|---|---|---|---|
| Method A IBDU 1 | 17,838 | 1,312 | 3,222 |
| Method A IBDU 2 | 35,411 | 1,297 | 3,874 |
| Method A CDU | 38,264 | 0,922 | 3,257 |

**Table 5 — Mean values, repeatability, and reproducibility limits for method B**

| Sample, MU-oligomer | $\overline{x}$ % | $r$ % | $R$ % |
|---|---|---|---|
| Method B NPK 1, MDU | 2,50 | 0,23 | 0,83 |
| Method B NPK 1, DMTU | 1,51 | 0,20 | 0,70 |
| Method B NPK 1, TMTU | 0,59 | 0,27 | 0,48 |
| Method B NPK 2, MDU | 2,29 | 0,52 | 0,98 |
| Method B NPK 2, DMTU | 2,14 | 0,38 | 0,79 |
| Method B NPK 2, TMTU | 1,14 | 0,42 | 1,11 |

## 8 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 a reference to this International Standard, i.e. ISO 25705:2016;

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;

g) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents occurred when performing the method, which might have influenced the test result(s).

# Annex A
(informative)

# Results of the inter-laboratory tests

The precision of method A has been determined in the year 2006 in an inter-laboratory trial with 11 laboratories participating and carried out on 3 samples of fertilizer (for IBDU and CDU) and in the year 2008 for Method B with 10 participating laboratories and carried out on two samples of fertilizers (for methylene urea). The statistical results are given in Table A.1, Table A.2, and Table A.3.

**Table A.1 — Statistical results of the inter-laboratory tests (method A)**

| Parameter | IBDU 1 | IBDU 2 | CDU |
|---|---|---|---|
| Year of the test | 2006 | 2006 | 2006 |
| Number of participating laboratories | 11 | 11 | 11 |
| Number of laboratories after eliminating outliers | 11 | 9 | 10 |
| Level mean value, (g/100 g) | 17,838 | 35,411 | 38,264 |
| Repeatability standard deviation $s_r$, (g/100 g) | 0,473 | 0,468 | 0,333 |
| Coefficient of variation $CV_r$ (%) | 2,65 | 1,32 | 0,87 |
| Repeatability limit $r$ (2,77 $s_r$) (g/100 g) | 1,312 | 1,297 | 0,922 |
| Reproducibility standard deviation, $s_R$ (g/100 g) | 1,163 | 1,399 | 1,176 |
| Coefficient of variation $CV_R$ (%) | 6,52 | 3,95 | 3,07 |
| Reproducibility limit $R$ (2,77 $s_R$) (g/100 g) | 3,222 | 3,874 | 3,257 |

**Table A.2 — Statistical results of the inter-laboratory tests for sample NPK 1 (method B)**

| Parameter | MDU | DMTU | TMTU |
|---|---|---|---|
| Year of the test | 2008 | 2008 | 2008 |
| Number of participating laboratories | 10 | 10 | 10 |
| Number of laboratories after eliminating outliers | 8 | 9 | 9 |
| Level mean value, (g/100 g) | 2,50 | 1,51 | 0,59 |
| Repeatability standard deviation $s_r$, (g/100 g) | 0,08 | 0,08 | 0,10 |
| Coefficient of variation $CV_r$ (%) | 3,31 | 4,85 | 16,83 |
| Repeatability limit $r$ (2,77 $s_r$) (g/100 g) | 0,23 | 0,20 | 0,27 |
| Reproducibility standard deviation, $s_R$ (g/100 g) | 0,30 | 0,25 | 0,17 |
| Coefficient of variation $CV_R$ (%) | 11,99 | 16,88 | 29,29 |
| Reproducibility limit $R$ (2,77 $s_R$) (g/100 g) | 0,83 | 0,70 | 0,48 |

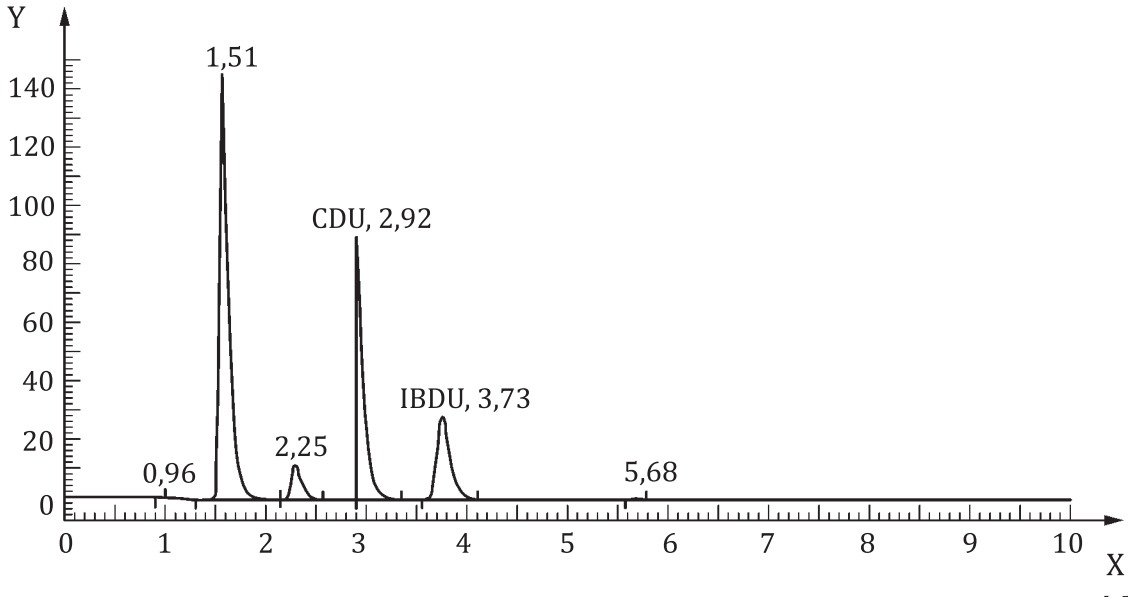
**Table A.3 — Statistical results of the inter-laboratory tests for sample NPK 2 (Method B)**

| Parameter | MDU | DMTU | TMTU |
|---|---|---|---|
| Year of the test | 2008 | 2008 | 2008 |
| Number of participating laboratories | 10 | 10 | 10 |
| Number of laboratories after eliminating outliers | 9 | 9 | 9 |
| Level mean value, (g/100 g) | 2,29 | 2,14 | 1,14 |
| Repeatability standard deviation $s_r$, (g/100 g) | 0,19 | 0,14 | 0,15 |
| Coefficient of variation $CV_r$ (%) | 8,16 | 6,41 | 13,36 |
| Repeatability limit $r$ (2,77 $s_r$) (g/100 g) | 0,52 | 0,38 | 0,42 |
| Reproducibility standard deviation, $s_R$ (g/100 g) | 0,35 | 0,29 | 0,40 |
| Coefficient of variation $CV_R$ (%) | 15,42 | 13,37 | 34,96 |
| Reproducibility limit $R$ (2,77 $s_R$) (g/100 g) | 0,98 | 0,79 | 1,11 |

# Annex B
(informative)

# Chromatogram and calibration curves method A

## B.1 Chromatogram




**Key**

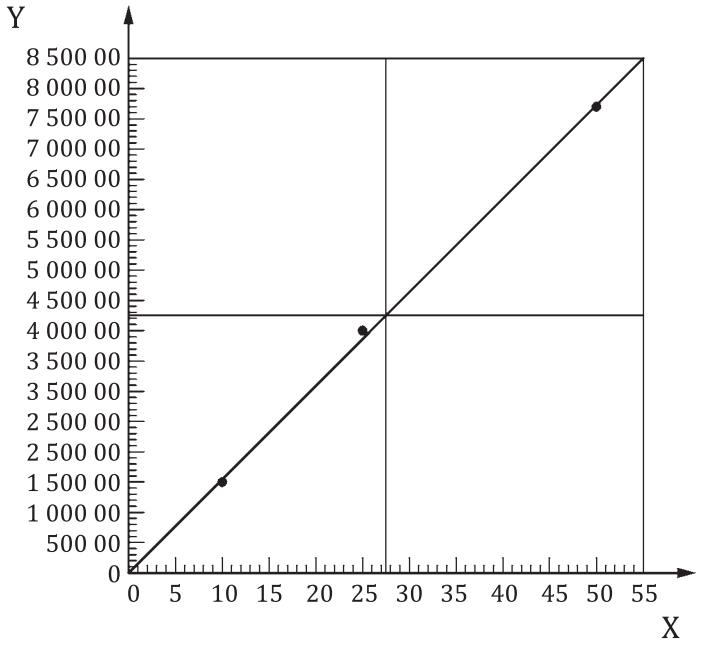
X retention time (min)

Y intensity (mV)

| Peak identification: | RT | Component |
|---|---|---|
| | 0,96 | Deadtime |
| | 1,51 | Inorganic components |
| | 2,25 | Urea |
| | 2,92 | Crotonylidenediurea |
| | 3,73 | Isobutylidenediurea |
| | 5,68 | Not identified peak |

**Figure B.1 — Chromatogram**

## B.2 Calibration curves

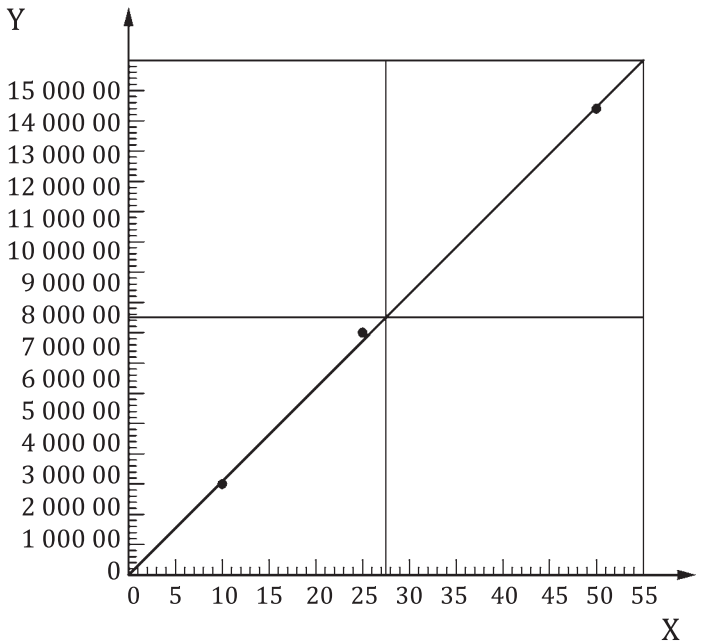



**Key**

X concentration (mg/l)

Y area (mV·min)

**Figure B.2 — Calibration curve CDU**




**Key**

X concentration (mg/l)

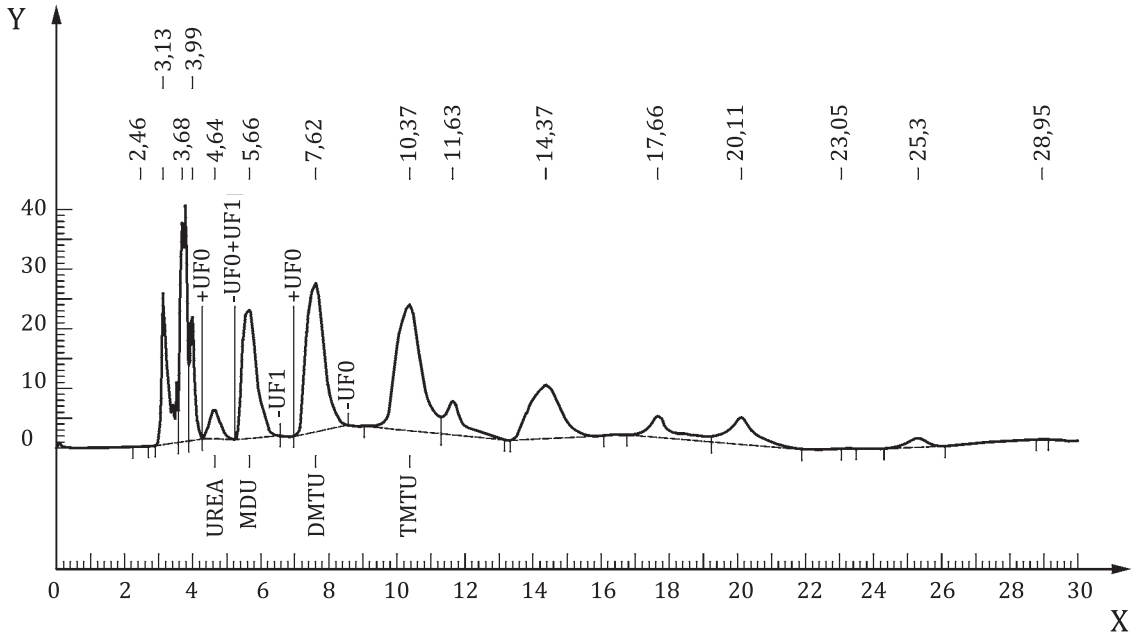
Y area (mV·min)

**Figure B.3 — Calibration curve IBDU**

# Annex C
(informative)

# Chromatogram and calibration curves method B

## C.1 Chromatogram




**Key**

X retention time (min)

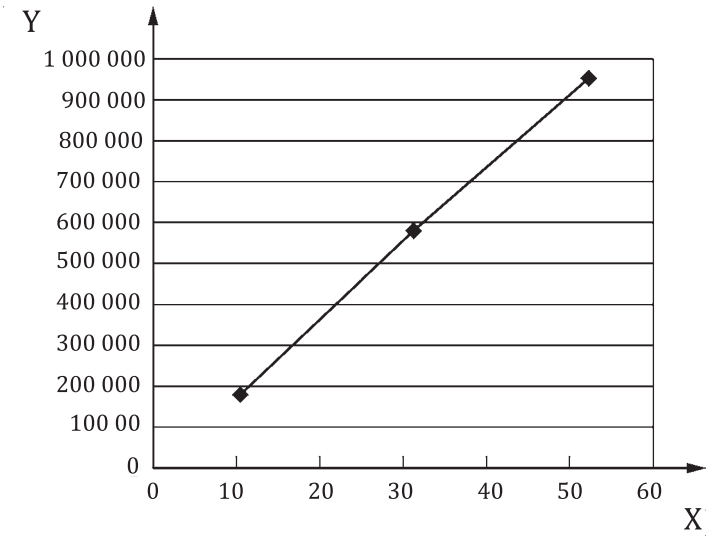
Y intensity (AU)

| Peak identification: | RT | Component |
|---|---|---|
| | 4,64 | Urea |
| | 5,66 | MDU |
| | 7,62 | DMTU |
| | 10,37 | TMTU |

NOTE The other not identified peaks are representing components of the NPK-fertilizer.

**Figure C.1 — Chromatogram of the solution of a NPK – fertilizer**

## C.2 Calibration curves

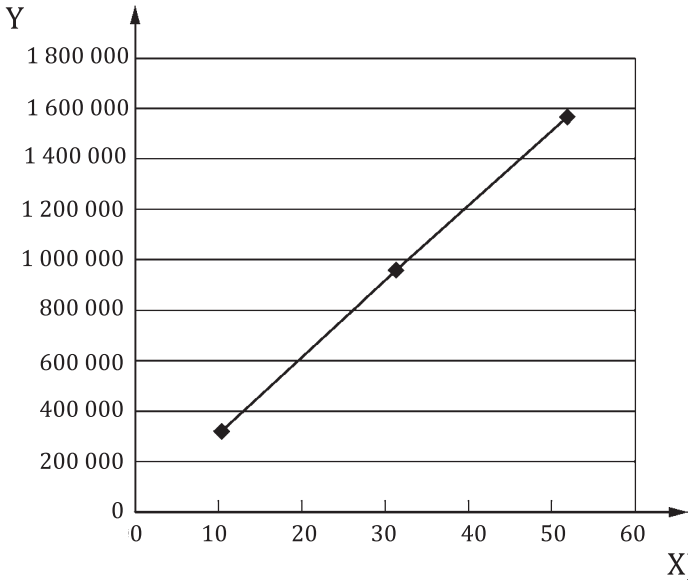



**Key**

X concentration (mg/kg)

Y area (AU·min)

**Figure C.2 — Calibration curve MDU**

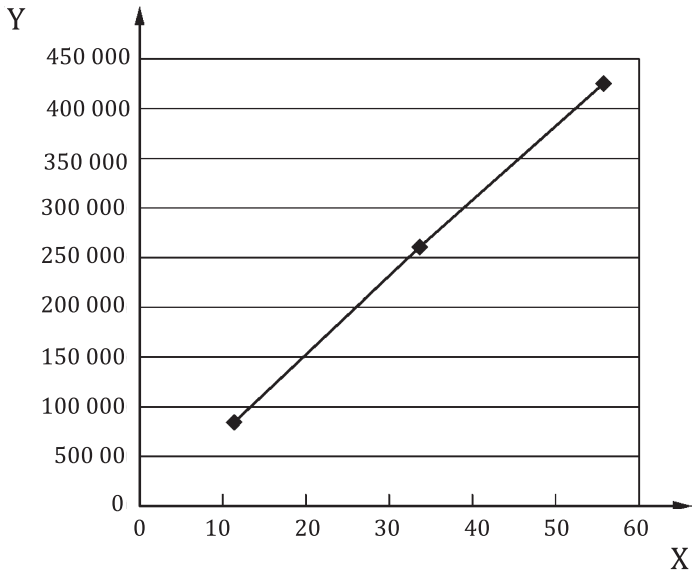



**Key**

X concentration (mg/kg)

Y area (AU·min)

**Figure C.3 — Calibration curve DMTU**




**Key**

X concentration (mg/kg)

Y area (AU·min)

**Figure C.4 — Calibration curve TMTU**

# Bibliography

[1] ISO 14820-1, *Fertilizers and liming materials — Sampling and sample preparation — Part 1: Sampling*

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] *Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers*, Official Journal L 304, 21/11/2003 P. 0001-0194

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