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BSI Standards Publication

Fertilizers — Determination of N-(2-nitrophenyl)phosphoric triamide (2-NPT) in urea and fertilizers containing urea — Method using high-performance liquid chromatography (HPLC)

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Fertilizers - Determination of N-(2-nitrophenyl)phosphoric triamide (2-NPT) in urea and fertilizers containing urea - Method using high-performance liquid chromatography (HPLC)

Engrais - Dosage du N-(2-nitrophényl) triamide phosphorique (2-NPT) dans l'urée et les engrais contenant de l'urée - Méthode par chromatographie liquide à haute performance (HPLC)

Düngemittel - Bestimmung von N-(2-Nitrophenyl)Phosphorsäure-Triamid (2-NPT) in Harnstoff und harnstoffhaltigen Düngemitteln - Verfahren mit Hochleistungs-Flüssigchromatographie (HPLC)

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Foreword

This document (EN 16075:2011) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2012, and conflicting national standards shall be withdrawn at the latest by May 2012.

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1 Scope

This European Standard specifies a method for the determination of N-(2-nitrophenyl)phosphoric triamide (2-NPT) in urea or in fertilizers containing urea using high-performance liquid chromatography (HPLC).

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 Principle

The sample of fertilizer is dissolved in water or extracted with water. 2-NPT is determined using reversed phase high-performance liquid chromatography with an UV detector.

5 Reagents

Use only reagents of recognized analytical grade and distilled or demineralised water (conductivity less than 0,5 mS/m, according to EN ISO 3696:1995, grade 3).

5.1 2 N-(2-nitrophenyl)phosphoric triamide, of known purity.

5.2 Standard solution of 2-NPT, ρ = 1 g/l.

Weigh 1 /R g of 2-NPT, where R is the purity of 2-NPT, to the nearest 0,1 mg into a 1 000 ml measuring flask. Dissolve with water, make up to the mark with water and mix well. This solution is stable for about 4 weeks.

5.3 Methanol.

5.4 n-hexane.

6 Apparatus

6.1 Analytical balance, measuring accuracy 0,1 mg or better.

6.2 Ultrasonic bath.

6.3 Syringe filter, for aqueous solutions; pore size 0,45 µm.

6.4 HPLC device.

6.5 Sampling issuing system.

6.6 UV detector.

6.7 Reversed phase HPLC column[1)].

6.8 Laboratory glassware.

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 Preparation of the test solution

Weigh approximately 5 g of the sample (m) into a 100 ml volumetric flask to the nearest 0,1 mg, add approximately 70 ml of water, dissolve in the ultrasonic bath (6.2) and make up to the mark with water. Filtrate this solution straight into a vial through a syringe filter (6.3) to remove any conditioning agents. This solution is used for the determination.

NOTE If paraffin is used as conditioning agent, see 8.2.

8.2 Preparation of the test solution in the presence of paraffin's as conditioning agents

Weigh approximately 5 g of the sample (m') into a 100 ml measuring flask to the nearest 0,1 mg, add approximately 70 ml of water, dissolve in the ultrasonic bath (6.2) and fill up to the mark with water. Add 2 ml of n-hexane (5.4) to this solution, shake thoroughly and fill the solution into a separation funnel. The n-hexane with the dissolved paraffin lies above the aqueous phase with the active substance. Filtrate the aqueous phase straight into a vial through a syringe filter (6.3).

8.3 Preparation of the calibration solutions

To obtain the calibration curve, prepare the following dilution series according to Table 1 from the 2-NPT standard solution (5.2) (each in a 100 ml measuring flask):

1) Synergi Fusion-RP80 4 µm, 250 mm × 4,6 mm or equivalent. This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Table 1 — Preparation of the dilution series

Mass concentration of 2-NPT mg/l	**Volume of standard solution** (5.2) µl
0,5	50
1,0	100
5,0	500
10,0	1 000
20,0	2 000

The calibration solutions shall be prepared daily.

8.4 HPLC conditions

Column temperature: room temperature

Elution agent: water/methanol mixture 50+50 (volume fraction)

Flow rate: 0,5 ml/min

Injection volume: 20 µl

Wavelength : 214 nm

The elution agent shall be degassed, e.g. in the ultrasonic bath (6.2).

8.5 HPLC determination

To determine the calibration curve, inject an amount of 20 µl of each calibration solution (see 8.3) three times. The calibration curve can be used to for the content determination if the correlation coefficient ≥ 0,99.

NOTE The correlation coefficient is calculated in accordance with the method of smallest squares.

Inject 20 µl of the test solution two times in succession. The results of these injections shall be within the calibration curve. Otherwise the test solution (8.1 or 8.2) has to be diluted.

9 Calculation and expression of the results

9.1 Calculation

Carry out the evaluation on the basis of the calibration curve over the peak areas.

Calculate the mass fraction of 2-NPT, $w_{2\text{-NPT}}$, in percent according to Equation (1):

$$w_{2-\text{NPT}} = \frac{(A_{\text{pk}} - b) \times V \times 100}{a \times m} \quad (1)$$

where

A_{pk} is the peak area;

b is the ordinate section of the calibration curve;

a is the slope of the calibration curve;

V is the injected volume, in litre;

m is the mass of the test portion, in milligrams, prepared according to 8.1 or 8.2.

After sample preparation according to 8.1 or 8.2 and in the case that the calculation of the content of 2-NPT in milligrams per litre (following the calibration solutions given in Table 1) will be performed automatically by the software of the chromatographic system, calculate the 2-NPT content, $w_{2\text{-NPT}}$, in percent according to Equation (2).

$$w_{2-\text{NPT}} = \frac{A}{500} \tag{2}$$

where

A is the content of 2-NPT in milligrams per litre;

500 is the factor for the conversion of the mass concentration milligrams per litre in percent 2-NPT.

9.2 Expression of results

Calculate the arithmetic mean from both values obtained. Indicate the result to the nearest 0,001 %.

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test has been carried out in 2009 with 11 participating laboratories and 3 different samples of fertilizers. This test yielded the data given in Annex A. Repeatability and reproducibility were calculated according to ISO 5725-1 and ISO 5725-2.

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

10.2 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, will in not more than 5 % of the cases exceed the values of r given in Table 2.

10.3 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, will in not more than 5 % of the cases exceed the values of R given in Table 2.

Table 2 — Mean values, repeatability and reproducibility limits

Sample	$\bar{x}$ mg/kg	r mg/kg	R mg/kg
Urea 1	238,62	21,63	40,10
Urea 2	462,59	39,99	65,01
Urea 3	695,38	38,11	89,91

11 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the test method used with reference to this document;

c) the 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 document, or regarded as optional, together with details of any incidents occurred when performing the method, which may have influenced the test result(s).

Annex A
(informative)

Statistical results of the inter-laboratory test

The precision of the method has been determined in the year 2009 in an inter-laboratory test with 11 laboratories participating and carried out on 3 samples of fertilizer. The statistical results are given in Table A.1.

Table A.1 — Statistical results of the inter-laboratory test

Parameter	Urea 1	Urea 2	Urea 3
Year of the test	2009	2009	2009
Number of participating laboratories	11	11	11
Number of laboratories after eliminating outliers	11	11	11
Mean value, $\bar{x}$, (mg/kg)	238,62	462,59	695,38
Repeatability standard deviation s_r, (mg/kg)	7,81	14,44	13,76
Relative standard deviation of repeatability, RSD_r (%)	3,27	3,12	1,98
Repeatability limit r (2,77 s_r) (mg/kg)	21,63	39,99	38,11
Reproducibility standard deviation, s_R (mg/kg)	14,48	23,47	32,46
Relative standard deviation of reproducibility, RSD_R, (%)	6,07	5,07	4,67
Reproducibility limit R (2,77 s_R) (mg/kg)	40,10	65,01	89,91

Bibliography

[1] EN 1482-1, *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. 1-194

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