BS EN 15688:2008

Fertilizers — Determination of urease inhibitor N-(n-butyl)thiophosphoric triamide (NBPT) using high-performance liquid chromatography (HPLC)

ICS 65.080

BSI British Standards

National foreword

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Engrais - Détermination de l'inhibiteur d'uréase N-(n-butyl) triamine thiophosphorique (NBPT) par chromatographie liquide haute performance (HPLC)

Düngemittel - Bestimmung von Urease-Hemmstoff N-(n-Butyl)-thiophosphortriamid (NBPT) mit Hochleistungs-Flüssigchromatographie (HPLC)

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Foreword

This document (EN 15688:2008) 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of an identical text or by endorsement, at the latest by February 2009, and conflicting national standards shall be withdrawn at the latest by February 2009.

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1 Scope

This document specifies a method for the quantitative determination of the urease inhibitor N-(*n*-butyl)thiophosphoric triamide (NBPT) content in water-soluble matrices, i.e. urea based fertilizers using high performance liquid chromatography (HPLC).

NOTE Pure NBPT reference material is required as a standard for the quantitative calibration.

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, *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

This analytical method is based on the principles of liquid chromatography, with absorption in the ultraviolet region for detection of the separated compounds.

5 Reagents

5.1 General

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

5.2 Reagents for liquid chromatography

5.2.1 Acetonitrile, HPLC-grade or spectroscopic grade.

5.2.2 Water, Milli-Q purified or equivalent quality.

5.2.3 N-(*n*-butyl)thiophosphoric triamide (NBPT), e. g. Sigma, B-3292, min 98 % *n*-Butyl ThioPhosphoric Triamide or TRC, catnr. B694000[1].

5.2.4 Urea, p.a. quality.

5.3 Calibration standards

5.3.1 Stock solution, ρ = 0,20 mg NBPT/ml.

Weigh 50 mg NBPT into a 250-ml-measuring flask and dissolve to volume with water.

5.3.2 Standard solution, 0,01 mg/ml.

Dilute 5,00 ml of the NBPT stock solution (5.3.1) to a volume of 100,00 ml in water.

5.3.3 Standard solution, 0,05 mg/ml.

Dilute 25,00 ml of the NBPT stock solution (5.3.1) to a volume of 100,00 ml in water.

5.3.4 Standard solution, 0,12 mg/ml.

Dilute 60,00 ml of the NBPT stock solution (5.3.1) to a volume of 100,00 ml in water.

5.3.5 Blank solution, water.

6 Apparatus

6.1 HPLC apparatus

6.1.1 Automatic mixing of binary eluent systems

6.1.2 Automatic injector device, capable of injecting 30 µl

6.1.3 UV absorbance detector, operable down to 200 nm in wavelength

6.1.4 Recording peak area integrating device

6.2 HPLC conditions

Two alternative systems may be used, as described in Table 1. Columns equivalent to those mentioned under A and B, may be used.

[1] Sigma B-3292 and TCR, catnr B694000 are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

Table 1 — Alternative HPLC systems A and B

Parameter	A	B
Column	C18 Nucleosil (250 mm×4 mm) 5 µm	LiChroSpher RP-8 (250 mm×4 mm) 5 µm
Mobile phase	MeCN + H_2O 10 + 90	MeCN + H_2O 15 + 85
Flow rate	1,0 ml/min	1,0 ml/min
Injection volume	30 µl	30 µl
Detection	UV absorption at 205 nm	UV absorption at 203 nm
Run time	30 min to 35 min	20 min to 25 min
Expected t_R NBPT	**8 min**	**7 min**

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 [1].

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

8 Procedure

8.1 Preparation of the test portion

Weigh to the nearest 0,001 g an amount of 5 g of the test sample and dissolve by stirring to volume of 250 ml in water.

8.2 Calibration

Use the standard solutions or stock solution to determine the retention time of NBPT in the HPLC system.

Calculate the response factor of NBPT, R, by analysis of the calibration standards in the HPLC system. All standards should be injected in duplicate.

For calculations, see Clause 9.

8.3 Blank test

For each series of determinations, carry out a blank test using an urea sample according to 5.2.4, which is free from NBPT.

8.4 Control test

The control test depends on validation – linearity and precision of standard injections should be used as system suitability test.

9 Calculation and expression of the result

Determine the concentration of NBPT in the sample solution by the external standard principle. Calculate the mass fraction of NBPT, w_{NBPT}, in percent of the dry sample by dividing by the fertilizer content (mass/volume) of the sample solution according to the following equation:

$$w_{\mathrm{NBPT}} = 100\,\frac{A}{R \times V \times m \times 4} \tag{1}$$

where

A is the peak area for NBPT;

R is the response factor (see formula (2)) (peak area/µg NBPT);

V is the injection volume in micro litre;

m is the mass of the test portion weighed into the sample solution (250 ml), in grams.

The external standard response factor, R, is calculated from the average of the peak areas and mass concentrations of NBPT of the 3 calibration standards according to the following equation:

$$R = \frac{R_{\mathrm{C1}} + R_{\mathrm{C2}} + R_{\mathrm{C3}}}{3} = \frac{A_{\mathrm{C1}} + A_{\mathrm{C2}} + A_{\mathrm{C3}}}{(\rho\mathrm{NBPT}_{\mathrm{C1}} \times V_{\mathrm{C1}}) + (\rho\mathrm{NBPT}_{\mathrm{C2}} \times V_{\mathrm{C2}}) + (\rho\mathrm{NBPT}_{\mathrm{C3}} \times V_{\mathrm{C3}})} \tag{2}$$

where

$R_{\mathrm{C1}}, R_{\mathrm{C2}}, R_{\mathrm{C3}}$ are the response factors of the calibration standards;

$A_{\mathrm{C1}}, A_{\mathrm{C2}}, A_{\mathrm{C3}}$ are the peak areas of the calibration standards;

$\rho\mathrm{NBPT}_{\mathrm{C1}}$ is the mass concentration of NBPT of the calibration standard C1, in milligrams per millilitre;

$\rho\mathrm{NBPT}_{\mathrm{C2}}$ is the mass concentration of NBPT of the calibration standard C2, in milligrams per millilitre;

$\rho\mathrm{NBPT}_{\mathrm{C3}}$ is the mass concentration of NBPT of the calibration standard C3, in milligrams per millilitre;

$V_{\mathrm{C1}}, V_{\mathrm{C2}}, V_{\mathrm{C3}}$ are the injection volumes of the calibration solutions in micro litre.

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test has been carried out in 2006 with 7 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 [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	$\bar{x}$	r	R
Urea based sample 1	0,0255	0,0026	0,0074
Urea based sample 2	0,0553	0,0028	0,0119
Urea based sample 3	0,1119	0,0051	0,0254

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 might have influenced the test result(s).

Annex A
(informative)

Results of the inter-laboratory test

The precision of the method has been determined in the year 2006 in an inter-laboratory trial with 7 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 trial

Parameter	Sample 1	Sample 2	Sample 3
Year of the test	2006	2006	2006
Number of participating laboratories	7	7	7
Number of laboratories after eliminating outliers	7	7	7
mean value, $\bar{x}$ (%)	0,0255	0,0553	0,1119
Repeatability standard deviation s_r, (%)	0,0009	0,0010	0,0018
RSD_r (%)	4	1,8	1,6
Repeatability limit r (2,83 s_r) (%)	0,0026	0,0028	0,0051
Reproducibility standard deviation, s_R (%)	0,0026	0,0043	0,0091
RSD_R (%)	10	7,7	8,1
Reproducibility limit R (2,83 s_R) (%)	0,074	0,0119	0,0254

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] *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, Pp. 1-194

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