SLOVENSKI STANDARD

SIST EN 15688:2008

01-oktober-2008

Gnojila - Doloļ evanje inhibitorja ureaze N-(n-butil)tiofosfortriamida (NBPT) s tekoļ insko kromatografijo visoke loļ ljivosti (HPLC)

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

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

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

Ta slovenski standard je istoveten z: EN 15688:2008

ICS:

65.080	Gnojila	Fertilizers

SIST EN 15688:2008 **en,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15688

August 2008

ICS 65.080

English Version

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

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. EN 15688:2008: E

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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of an identical text or by endorsement, at the latest by February 2009, and conflicting national standards shall be withdrawn at the latest by February 2009.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:

$$w_{NBPT} = 100 \frac{A}{R \times V \times m \times 4} \qquad (1)$$

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:

$$R = \frac{R_{C1} + R_{C2} + R_{C3}}{3} = \frac{A_{C1} + A_{C2} + A_{C3}}{(\rho NBPT_{C1} \times V_{C1}) + (\rho NBPT_{C2} \times V_{C2}) + (\rho NBPT_{C3} \times V_{C3})} \qquad (2)$$

where

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

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

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

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

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

V_{C1}, V_{C2}, V_{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.