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Fertilizers — Determination of N-(n-Butyl)thiophosphoric acid triamide (NBPT) and N-(n-Propyl)thiophosphoric acid triamide (NPPT) — Method using high-performance liquid chromatography (HPLC)

National foreword

This British Standard is the UK implementation of EN 16651:2015.

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Fertilizers - Determination of N-(n-Butyl)thiophosphoric acid triamide (NBPT) and N-(n-Propyl)thiophosphoric acid triamide (NPPT) - Method using high-performance liquid chromatography (HPLC)

Engrais - Dosage du N-(n-butyl)triamide de l'acide thiophosphorique (NBPT) et du N-(n-propyl)triamide de l'acide thiophosphorique (NPPT) - Méthode par chromatographie liquide à haute performance (HPLC)

Düngemittel - Bestimmung von N-(n-Butyl)thiophosphorsäuretriamid (NBPT) und N-(n-Propyl)thiophosphorsäuretriamid (NPPT) - Verfahren mit Hochleistungs-Flüssigchromatographie (HPLC)

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CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 16651:2015) 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of an identical text or by endorsement, at the latest by November 2015, and conflicting national standards shall be withdrawn at the latest by November 2015.

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1 Scope

This European Standard specifies a method for the quantitative determination of the urease inhibitors N-(n-Butyl)thiophosphoric acid triamide (NBPT, CAS-No. 94317-64-3) and N-(n-Propyl)thiophosphoric acid triamide (NPPT, CAS-No. 916809-14-8) content in urea based fertilizers using high-performance liquid chromatography (HPLC).

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.

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)*

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 fertilizer sample is diluted in water, grade 1. The concentration of N-(n-Butyl)thiophosphoric acid triamide (NBPT) and N-(n-Propyl)thiophosphoric acid triamide (NPPT) is determined in diluted aqueous solution by high-performance liquid chromatography (HPLC) using an UV detector at 205 nm.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water, grade 1 according to EN ISO 3696.

5.2 Acetonitrile, HPLC grade.

5.3 Urea, analytical pure.

5.4 NBPT, N-(n-Butyl)thiophosphoric acid triamide, CAS-No. 94317-64-3, analytical pure.

5.5 NPPT, N-(n-Propyl)thiophosphoric acid triamide, CAS-No. 916809-14-8, analytical pure.

5.6 Eluent.

Mix in a 1 000 ml volumetric flask (6.2), 750 ml of water (5.1) and 250 ml acetonitrile (5.2) and filter through a micro filter (6.3).

5.7 Stock solution, ρ(NBPT) = 0,20 mg/ml and ρ(NPPT) = 0,12 mg/ml.

Weigh in a 250 ml graduated flask (6.2), an amount of 50 mg NBPT and 30 mg NPPT. Add approximately 200 ml of water (5.1) and shake until NBPT and NPPT are diluted. Fill up to the mark with water (5.1) and shake. This stock solution may be used within 24 h.

5.8 Calibration solution C1, ρ(NBPT) = 0,01 mg/ml and ρ(NPPT) = 0,006 mg/ml.

Fill into a 100 ml graduated flask (6.2), an amount of 5,00 ml stock solution (5.7). Fill up to the mark with water (5.1). This calibration solution may be used within 24 h.

5.9 Calibration solution C2, ρ(NBPT) = 0,05 mg/ml and ρ(NPPT) = 0,030 mg/ml.

Fill into a 100 ml graduated flask an amount of 25,00 ml stock solution (5.7). Fill up to the mark with water (5.1). This calibration solution may be used within 24 h.

5.10 Calibration solution C3, ρ(NBPT) = 0,12 mg/ml and ρ(NPPT) = 0,072 mg/ml.

Fill into a 100 ml graduated flask an amount of 60,00 ml stock solution (5.7). Fill up to the mark with water (5.1). This calibration solution may be used within 24 h.

6 Apparatus

6.1 Analytical balance, capable of weighing to an accuracy of 0,001 g.

6.2 Volumetric flasks, capacity 100 ml, 250 ml and 1 000 ml.

6.3 Micro filter, pore size 0,45 µm.

6.4 One way shot, capacity 5 ml.

6.5 Vials, capacity 2 ml.

6.6 Appropriate shaking equipment.

6.7 HPLC apparatus, consisting of the following parts:

6.7.1 HPLC instrument with UV-Detector, and optional an auto sampler and column oven.

6.7.2 HPLC separation column, e.g. LiChroSpher 100 RP-8 (250 mm × 4 mm) 5 µm or equivalent.

The HPLC conditions based on this column are given in Table 1.

Table 1 — Example of HPLC conditions [a]

Parameter	Conditions
Column	LiChroSpher 100 RP-8 (250 mm × 4 mm) 5 µm
Mobile phase	Eluent (5.6)
Flow rate	1,0 ml/min
Injection volume	20 µl
UV detection at wavelength	205 nm
Column temperature	Room temperature
Expected retention time NBPT	approximately 7 min
Expected retention time NPPT	approximately 5 min

[a] The use of an equivalent column could lead to different parameters.

7 Sampling

Sampling from fertilizers, which are stored as bulk in storage buildings or warehouse or in packaged form 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 to the nearest 0,001 g an amount of 7,500 g of the test sample into a 250 ml volumetric flask and dissolve in 200 ml water (5.1), shake the flask with shaking equipment (6.6) until the sample is completely dissolved. Fill up to the mark with water (5.1) and mix thoroughly. Fill the solution in a one way shot (6.4), filter through the micro filter (6.3) in a vial (6.5) and then inject the solution in HPLC apparatus (6.7). Solutions should be analysed within 24 h.

8.2 Calibration

Determine the retention times of NBPT and NPPT by using the calibration solutions (5.8, 5.9 and 5.10). Activate the measure with the three different calibration solutions C1, C2 and C3 (manual handling or optional use of an auto sampler).

8.3 Blank test

For each series of determination carry out a blank test using an urea solution containing 30 g urea in 1 000 ml of water. Place the sample in the auto sampler and activate the measure.

8.4 Calculation and expression of the results1)

8.4.1 Concentration of NBPT

Calculate the concentration of NBPT in the sample solution by the external calibration. Calculate the mass fraction of NBPT, w_{NBPT}, in percent of the sample by dividing by the fertilizer content (mass concentration) of the sample solution according to Formula (1):

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1) Calibration curve e.g. calculated by the software of the HPLC apparatus can be used optionally.

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

where

A	is the peak area for NBPT;
R	is the response factor (see Formula (3), peak area/µg NBPT);
V	is the injection volume in microlitres;
m	is the mass of the test portion weighed into the test solution (250 ml), in grams.

8.4.2 Concentration of NPPT

Calculate the concentration of NPPT in the sample solution by the external calibration. Calculate the mass fraction of NPPT, w_{NPPT}, in percent of the sample by dividing by the fertilizer content (mass concentration) of the sample solution according to Formula (2):

$$w_{\text{NPPT}} = 100 \frac{A}{R \times V \times m \times 4} \tag{2}$$

where

A	is the peak area for NPPT;
R	is the response factor (see Formula (4), peak area/µg NPPT);
V	is the injection volume in microlitres;
m	is the mass of the test portion weighed into the test solution (250 ml), in grams.

8.4.3 External response factors

8.4.3.1 Calculate the external standard response factor, R_{NBPT}, from the average of the peak areas and mass concentrations of NBPT of the three calibration standards according to Formula (3):

$$R_{\text{NBPT}} = \frac{R_{\text{C1}} + R_{\text{C2}} + R_{\text{C3}}}{3} = \frac{A_{\text{C1}} + A_{\text{C2}} + A_{\text{C3}}}{(\rho_{\text{NBPTC1}} \times V_{\text{C1}}) + (\rho_{\text{NBPTC2}} \times V_{\text{C2}}) + (\rho_{\text{NBPTC3}} \times V_{\text{C3}})} \tag{3}$$

where

R_{C1}, R_{C2}, R_{C3}	are the response factors of the calibration standard;
A_{C1}, A_{C2}, A_{C3}	are the peak areas of the calibration standard;
ρ_{NBPTC1}, ρ_{NBPTC2}, ρ_{NBPTC3}	are the mass concentrations of NBPT of the calibration standard C1, C2 and C3, in milligrams per millilitre;
V_{C1}, V_{C2}, V_{C3}	are the injection volumes of the calibration solution in microlitres.

8.4.3.2 Calculate the external standard response factor, R_{NPPT}, from the average of the peak areas and mass concentrations of NPPT of the three calibration standards according to Formula (4):

$$R_{\text{NPPT}} = \frac{R_{\text{C1}} + R_{\text{C2}} + R_{\text{C3}}}{3} = \frac{A_{\text{C1}} + A_{\text{C2}} + A_{\text{C3}}}{(\rho_{\text{NPPTC1}} \times V_{\text{C1}}) + (\rho_{\text{NPPTC2}} \times V_{\text{C2}}) + (\rho_{\text{NPPTC3}} \times V_{\text{C3}})} \tag{4}$$

where

R_{C1}, R_{C2}, R_{C3}	are the response factors of the calibration standard;
A_{C1}, A_{C2}, A_{C3}	are the peak areas of the calibration standard;
ρ_{NPPTC1}, ρ_{NPPTC2}, ρ_{NPPTC3}	are the mass concentrations of NPPT of the calibration standard C1, C2 and C3, in milligrams per millilitre;
V_{C1}, V_{C2}, V_{C3}	are the injection volumes of the calibration solution in microlitres.

8.4.4 Calculation of the total content

Calculate the total active-ingredient content, w_T by addition of the calculated contents of NBPT and NPPT according to Formula (5).

$$w_T = w_{NBPT} + w_{NPPT} \tag{5}$$

8.5 Environmental aspects

The substances, solutions, eluents and packaging materials should be disposed under consideration of safety data sheets and in accordance with local, state and federal regulations.

9 Precision

9.1 Inter-laboratory test

An inter-laboratory test has been carried out in 2013 with 11 participating laboratories and four different samples of fertilizers. This test yielded the data given in Annex A.

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

9.2 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 no more than 5 % of the cases exceed the values of r given in Table 2 (NPPT) and Table 3 (NBPT).

9.3 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 no more than 5 % of the cases exceed the values of R given in Table 2 (NPPT) and Table 3 (NBPT).

Table 2 — NPPT: Mean values, repeatability and reproducibility limits

Sample	$\overline{x}$	r	R
LIMUS 1 [a]	0,004 6	0,000 5	0,001 0
LIMUS 2 [a]	0,010 7	0,000 8	0,001 7
LIMUS 3	0,023 7	0,001 7	0,003 4
LIMUS 4	0,036 3	0,002 5	0,004 2

[a] A more diluted calibration solution could be added if sample solutions are wanted to be between the calibration points.

Table 3 — NBPT: Mean values, repeatability and reproducibility limits

Sample	$\overline{x}$	r	R
LIMUS 1 [a]	0,015 1	0,001 2	0,002 5
LIMUS 2	0,034 5	0,002 4	0,004 9
LIMUS 3	0,076 9	0,003 9	0,009 2
LIMUS 4	0,116 9	0,009 1	0,014 1

[a] A more diluted calibration solution could be added if sample solutions are wanted to be between the calibration points.

10 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 (EN 16651);

c) the test results obtained;

d) date when the analysis was finished;

e) whether the requirement of the repeatability limit has been fulfilled;

f) 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 2013 in an inter-laboratory trial with 11 laboratories participating and carried out on four samples of fertilizer. Repeatability and reproducibility were calculated according to ISO 5725-2. The statistical results are given in Table A.1 and Table A.2.

Table A.1 — Statistical results of the inter-laboratory trial for NPPT

Parameter	Sample LIMUS 1	Sample LIMUS 2	Sample LIMUS 3	Sample LIMUS 4
Year of the test	2013	2013	2013	2013
Number of participating laboratories	11	11	11	11
Number of laboratories after eliminating outliers	10	9	10	10
mean value, $\overline{x}$ (%)	0,004 6	0,010 7	0,023 7	0,036 3
Repeatability standard deviation s_r, (%)	0,000 2	0,000 3	0,000 6	0,000 9
RSD_r (%)	4,24	2,75	2,57	2,46
Repeatability limit r (2,83 s_r) (%)	0,000 5	0,000 8	0,001 7	0,002 5
Reproducibility standard deviation, s_R (%)	0,000 4	0,000 6	0,001 2	0,001 5
RSD_R (%)	7,76	5,70	5,11	4,14
Reproducibility limit R (2,83 s_R) (%)	0,001 0	0,001 7	0,003 4	0,004 2

Table A.2 — Statistical results of the inter-laboratory trial for NBPT

Parameter	Sample LIMUS 1	Sample LIMUS 2	Sample LIMUS 3	Sample LIMUS 4
Year of the test	2013	2013	2013	2013
Number of participating laboratories	11	11	11	11
Number of laboratories after eliminating outliers	10	11	11	11
mean value, $\overline{x}$ (%)	0,015 1	0,034 5	0,076 9	0,116 9
Repeatability standard deviation s_r, (%)	0,000 4	0,000 9	0,001 4	0,003 2
RSD_r (%)	2,77	2,53	1,81	2,77
Repeatability limit r (2,83 s_r) (%)	0,001 2	0,002 4	0,003 9	0,009 1
Reproducibility standard deviation, s_R (%)	0,000 9	0,001 7	0,003 3	0,005 0
RSD_R (%)	5,84	5,03	4,29	4,31
Reproducibility limit R (2,83 s_R) (%)	0,002 5	0,004 9	0,009 2	0,014 1

Bibliography

[1] EN 1482-1, *Fertilizers and liming materials - Sampling and sample preparation - Part 1: Sampling*

[2] 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*

[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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