
Gnojila - Določevanje N-(n-butil)-triamida tiofosforne kisline (NBPT) in N-(n-propil)-triamida tiofosforne kisline (NPPT) - Metoda tekočinske kromatografije visoke ločljivosti (HPLC)

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)

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

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

Ta slovenski standard je istoveten z: EN 16651:2015

ICS:

65.080

Gnojila

Fertilizers

SIST EN 16651:2015

en,fr,de

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[SIST EN 16651:2015](#)

<https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-b989b37acf17/sist-en-16651-2015>

EUROPEAN STANDARD

EN 16651

NORME EUROPÉENNE

EUROPÄISCHE NORM

May 2015

ICS 65.080

English Version

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)

This European Standard was approved by CEN on 16 April 2015.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

[SIST EN 16651:2015](https://standards.iteh.ai/standards/sis/16651-2015)

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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

Contents	Page
Foreword.....	3
1 Scope	4
2 Normative references	4
3 Terms and definitions	4
4 Principle.....	4
5 Reagents.....	4
6 Apparatus	5
7 Sampling.....	6
8 Procedure	6
8.1 Preparation of the test solution.....	6
8.2 Calibration	6
8.3 Blank test.....	6
8.4 Calculation and expression of the results	6
8.4.1 Concentration of NBPT	6
8.4.2 Concentration of NPPT	7
8.4.3 External response factors.....	7
8.4.4 Calculation of the total content	8
8.5 Environmental aspects	8
9 Precision.....	8
9.1 Inter-laboratory test.....	8
9.2 Repeatability.....	8
9.3 Reproducibility.....	8
10 Test report	9
Annex A (informative) Results of the inter-laboratory test	10
Bibliography	11

iTech STANDARD PREVIEW
(standards.iteh.ai)

[SIST EN 16651:2015](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-4686-9e14-b989b37ac117/sist-en-16651-2015)

[https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-4686-9e14-](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-4686-9e14-b989b37ac117/sist-en-16651-2015)

[b989b37ac117/sist-en-16651-2015](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-4686-9e14-b989b37ac117/sist-en-16651-2015)

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST EN 16651:2015](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-b989b37acfl7/sist-en-16651-2015)

<https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-b989b37acfl7/sist-en-16651-2015>

EN 16651:2015 (E)**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.

STANDARD PREVIEW
(standards.iteh.ai)

4 Principle

SIST EN 16651:2015

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, $\rho(\text{NBPT}) = 0,20 \text{ mg/ml}$ and $\rho(\text{NPPT}) = 0,12 \text{ mg/ml}$.

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, $\rho(\text{NBPT}) = 0,01 \text{ mg/ml}$ and $\rho(\text{NPPT}) = 0,006 \text{ mg/ml}$.

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, $\rho(\text{NBPT}) = 0,05 \text{ mg/ml}$ and $\rho(\text{NPPT}) = 0,030 \text{ mg/ml}$.

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, $\rho(\text{NBPT}) = 0,12 \text{ mg/ml}$ and $\rho(\text{NPPT}) = 0,072 \text{ mg/ml}$.

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 \times 4 mm) 5 μm or equivalent.

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[SIST EN 16651:2015](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-b989b37acfl7/sist-en-16651-2015)

<https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-b989b37acfl7/sist-en-16651-2015>

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

[SIST EN 16651:2015](https://standards.iteh.ai/catalog/standards/sist/929b73d9-aba3-468b-9ef4-99392a11f5e4/en-16651-2015)

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):

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

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

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):

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

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):

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

where

- $R_{\text{C1}}, R_{\text{C2}}, R_{\text{C3}}$ are the response factors of the calibration standard;
- $A_{\text{C1}}, A_{\text{C2}}, A_{\text{C3}}$ are the peak areas of the calibration standard;
- $\rho_{\text{NBPTC1}}, \rho_{\text{NBPTC2}}, \rho_{\text{NBPTC3}}$ are the mass concentrations of NBPT of the calibration standard C1, C2 and C3, in milligrams per millilitre;
- $V_{\text{C1}}, V_{\text{C2}}, V_{\text{C3}}$ 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):

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