Organo-mineral fertilizers — Determination of the N-(n-butyl)thiophosphoric triamide (NBPT) urease inhibitor content

1 Scope

This document specifies a method for the determination of the urease inhibitor N(nbutyl)thiophosphoric triamide (NBPT) and its oxidate form NBPTO in urea based organo-mineral fertilizers, using the liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MSQQQ).

It is applicable to organo-mineral fertilizers,

NOTE: It is possible to apply this method to inorganic fertilizers; in this case a validation is carried out by the laboratory for the procedure and data generated.

2 Principle

This analytical method is based on the principles of liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MSQQQ) for determination of the separated inhibiting compounds NBPT and NBPTO.

3 Reagents

3.1 General

Use only reagents of recognized analytical grade and distilled water or ultrapure water for LC-MS.

3.2 Reagents for liquid chromatography

3.2.1 Methanol, LC-MS grade.

3.2.2 Formic acid, LC-MS grade.

3.2.3 Ammonium formate, p.a.

3.2.4 Water, ultrapure LC-MS grade or distilled water.

3.2.5 N-(n-butyl)thiophosphoric triamide (NBPT), CAS n. 94317-64-3, minimum 98 %.

3.2.6 N-(n-butyl)phosphoric triamide (NBPTO), CAS n. 25316-39-6, minimum 80%.

3.2.7 Urea, p.a.

3.3 Calibration standards

3.3.1 Stock solution, mass concentration ρ_{NBPT} = 0,20 mg/ml

Weigh 50 mg NBPT (3.2.5) into a 250-ml-measuring flask and dissolve to volume with water (5.2.3). Store at +4 °C ± 1 °C for no more than 2 days.

3.3.2 Stock solution, ρ_{NBPTO} = 0,20 mg/ml

Weigh 50 mg NBPTO (3.2.6) into a 250-ml-measuring flask and dissolve to volume with water (5.2.3). Store at +4°C ± 1 °C for no more than 2 days.

3.3.3 Blank solution, water (3.2.4).

4 Equipment and consumables

Disposable equipment is acceptable in the same way as reusable glassware if the specifications are similar. Ordinary laboratory equipment, and particularly the following.

4.1 Analytical scale, capable of weighing to the nearest 0,0001 g.

4.2 Graduated pipettes, for volumes 5 ml, 10 ml, 15 ml, 20 ml, 25 ml.

4.3 One-mark volumetric glass flasks Class A, capacity 100 ml and 250 ml.

4.4 Horizontal shaker agitator

4.5 Qualitative filter paper

4.6 LC-MSQQQ

4.6.1 Operative Conditions

- Column: a non-endcapped silica-based HSS PFP/Fluoro-Phenyl Reversed Phase HPLC column, 3,5 µm 4,6 mm x 150 mm (designed for low pH separations that require alternative selectivity compared to a fully endcapped, high coverage C18 phase, usable for UPLC separations)
- Mobile phase A: Water (3.2.4) with 0,1 % formic acid (3.2.2) and 5 mmol ammonium formate (3.2.3)
- Mobile phase B: Methanol (3.2.1) with 0,1% formic acid (3.2.2)
- Flow rate: 0,6 mL/min
- Column temperature: 30 °C
- Injection volume: 20 µL
- Run time: 15 min
- Expected Retention Time, t_R of NBPT: 8 min.
- Expected Retention Time, t_R of NBPT-O: 6.9 min.

t	A	B
min	%	%
0	90	10
2	90	10
7	5	95
10	5	95
10,1	90	10
15	90	10

- Detector: MS triple quadrupole (MSQQQ)
- Electrospray ionization (ESI)
- Positive polarity
- Gas temperature: 135 °C
- Gas flow: 12 l/min
- VCap: 4 000 V

	Molecular ion	Fragment ion	CollisionEnergy
Qualifier	168	151	15
Quantifier	168	74	15

5 Procedure

5.1 Preparation of the test portion

An amount of 5 g of the test sample shall be weighted to the nearest 0,0001 g, transferred to a 250 ml volumetric flask (4.3), added a volume of 150 ml of water (3.2.4) and dissolved by shaking with agitator (4.4) for 30 min at a rate that keeps the sample in suspension.
It shall be made up to volume with water, homogenized thoroughly and filtered with filter paper (4.5).
An aliquot portion of the extract in water (3.2.4) shall be diluted in one or more steps so that the final concentration of the molecules to be determined falls within the calibration range.
All samples shall be injected in duplicate.

5.2 Calibration

Calibration shall be performed before the analysis.

Volumes of 0 ml, 5,0 ml, 10,0 ml, 15,0 ml, 20,0 ml and 25,0 ml of the stock solution (3.3.1 and 3.3.2) shall be introduced into a 100 ml volumetric flask (4.3) and made up to volume with water (3.2.4). The mass concentration of each analyte in the calibration standard solutions is 0 mg/l, 10,0 mg/l, 20,0 mg/l, 30,0 mg/l, 40,0 mg/l and 50,0 mg/l, respectively. The solutions shall be prepared at the time of each batch of analytical determination.

The stock solutions (3.3.1 and 3.3.2) shall be used to determine the retention time of NBPT and NBPTO in the LC system.

The response factor of NBPT and NBPTO shall be calculated by analysis of the calibration standards in the LC system. All standards shall be injected in duplicate.

5.3 Blank test

For each series of determinations, a blank test shall be introduced in each analytical batch using urea sample test (3.2.7) free from NBPT and NBPTO, prepared according to the procedure in 8.2.

6 Calculation and expression of the result

For the preparatory steps to the determination of NBPT and NBPTO, clauses 4, 5, 6, 7 and 8 shall be applied.
The concentration of NBPT and NBPTO in the sample solution is determined according to the principle of external standard.
The mass fraction of NBPT w_{NBPT}, and NBPTO w_{NBPTO} in percent shall be calculated according to formulae (1) and (2):

$$w_{NBPT} = 100 \times \frac{A \times D}{RxVxmx4} \quad (1)$$

$$w_{NBPTO} = 100 \times \frac{A \times D}{RxVxmx4} \quad (2)$$

where:

- A is the peak area for NBPT or NBPTO;
- R is the response factor (see formula 4 and 5) (peak area/µg NBPT or NBPTO);
- V is the injection volume in µl (20 µl);
- m is the mass of the test portion weighed into the sample solution (250 ml), in g.
- D is the dilution factor calculated according to the formula (3):

$$D = \frac{V_1}{V_{p1}} \times \frac{V_2}{V_{p2}} \times \ldots \frac{V_n}{V_{pn}} \quad (3)$$

where:

- $V_{p1,2...n}$ withdrawal volumes of the solutions for each dilution step in ml;

$V_{1,2,...n}$ volumes of the flasks in which dilution is carried out in ml.

The result shall be given as the average of two replicated determinations done on two different test portions from the same sample, with an accuracy of two figures, e.g. 12 %, 1,2 %, 0,12 %.

The external standard response factor, R, is calculated from the average of the peak areas and mass concentrations of NBPT and NBPTO of the 5 calibration standards according to formulae (4) and (5):

$$R_{NBPT} = \frac{R_{C1} + R_{C2} + R_{C3} + R_{C4} + R_{C5}}{5} \quad (4)$$

$$R_{NBPT} = \frac{A_{C1} + A_{C2} + A_{C3} + A_{C4} + A_{C5}}{(\rho NBPT_{C1} \times V_{C1}) + (\rho NBPT_{C2} \times V_{C2}) + (\rho NBPT_{C3} \times V_{C3}) + (\rho NBPT_{C4} \times V_{C4}) + (\rho NBPT_{C5} \times V_{C5})}$$

$$R_{NBPTO} = \frac{R_{C1} + R_{C2} + R_{C3} + R_{C4} + R_{C5}}{5} \quad (5)$$

$$R_{NBPTO} = \frac{A_{C1} + A_{C2} + A_{C3} + A_{C4} + A_{C5}}{(\rho NBPTO_{C1} \times V_{C1}) + (\rho NBPTO_{C2} \times V_{C2}) + (\rho NBPTO_{C3} \times V_{C3}) + (\rho NBPTO_{C4} \times V_{C4}) + (\rho NBPTO_{C5} \times V_{C5})}$$

where:

- R_{C1}, R_{C2}, R_{C3} R_{C4}, R_{C5} response factor of calibration standards;
- A_{C1}, A_{C2}, A_{C3} A_{C4}, A_{C5} peak area of calibration standards;
- $\rho NBPT_{CI}, \rho NBPT_{C2}, \rho NBPT_{C3}, \rho NBPT_{C4}, \rho NBPT_{C5}$ mass concentration of NBPT in each calibration standard C1, C2, C3, C4, C5 in mg/ml;
- $\rho NBPTO_{CI}, \rho NBPTO_{C2}, \rho NBPTO_{C3}, \rho NBPTO_{C4}, \rho NBPTO_{C5}$ mass concentration of NBPTO, in each calibration standard C1, C2, C3, C4, C5 in mg/ml;
- V_{C1}, V_{C2}, V_{C3}, V_{C4}, V_{C5} injection volume of calibration standards in µl.