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Fertilizers, soil conditioners and beneficial substances — Simultaneous determination of N-(n-Butyl) thiophosphoric triamide and dicyandiamide by high-performance liquid chromatography

Engrais, Amendements et Substances Bénéfiques — Détermination Simultanée du N-buthylthiophosphore Triamide (NBPT) et du Dicyandiamide (DCD) par Chromatographie Liquide à Haute Performance (HPLC)

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Contents

Page

Foreword

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This document was prepared by Technical Committee ISO/TC 134, *Fertilizers, soil conditioners and beneficial substances*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The stability of urea today is receiving greater attention due to a major increase in no-tillage or minimum-tillage crop production. N-(n-butyl) thiophosphoric triamide (NBPT) can slow urea breakdown by controlling the activity of the urease enzyme. Dicyandiamide (DCD) temporarily inhibits nitrification by deactivating the enzyme of ammonia monooxygenase (AMO) in ammonia-oxidizing microbes. This document provides a method to determine the content of NBPT and DCD in nitrogen fertilizers simultaneously, which will help governmental authorities, fertilizer producers and consumers around the world. Also, it can save time and protect the environment by reducing experimental waste.

Fertilizers, soil conditioners and beneficial substances — Simultaneous determination of N-(n-Butyl) thiophosphoric triamide and dicyandiamide by high-performance liquid chromatography

1 Scope

This document specifies the analytical method for the simultaneous determination of N-(n-butyl) thiophosphoric triamide (NBPT) and dicyandiamide (DCD) in fertilizers by high-performance liquid chromatography (HPLC) method.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8157, *Fertilizers, soil conditioners and beneficial substances — Vocabulary*

ISO 8358, *Solid fertilizers — Preparation of samples for chemical and physical analysis*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8157 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
N-(n-butyl) thiophosphoric triamide
NBPT
white crystalline solid major urease inhibitor of commercial and practical importance in agriculture

Note 1 to entry: CAS Registry Number®[1] 94317-64-3.

3.2
dicyandiamide
DCD
2-cyanoguanidine
nitrification inhibitor and slow-release nitrogen source that has 4 % to 5 % solubility in water

Note 1 to entry: DCD, $C_2H_4N_4$ (CAS 461-58-5).

1 Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4 Principle

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

5 Reagents

During the analysis unless otherwise stated, use only reagents of recognized analytical grade (AR) and only water in accordance with ISO 3696 with electrical resistivity ≥18,2 MΩ·cm.

5.1 Acetonitrile (ACN), HPLC grade.

5.2 N-(n-butyl) thiophosphoric triamide (NBPT), assay, of a mass fraction ≥98 %.

5.3 Dicyandiamide (DCD) assay, of a mass fraction ≥99 %.

5.4 Stock solutions.

5.4.1 NBPT stock solution (1 000 mg/l).

Weigh to the nearest 0,1 mg, approximately 250 mg of NBPT into a 250 ml volumetric flask and dissolve to volume with water.

5.4.2 DCD stock solution (1 000 mg/l).

Weigh to the nearest 0,1 mg, approximately of 250 mg DCD into a 250 ml volumetric flask and dissolve to volume with water.

5.5 Standard solutions.

Prepare standard solutions (including both NBPT and DCD) in volumetric flasks in concentrations ranges listed in Table 1.

The concentration ranges for standard solutions may be adjusted according to the levels expected in the samples.

Table 1 — Preparation of standard solutions

Standard solution	NBPT mg/l	DCD mg/l
Blank	0	0
Standard 1	5,0	1,0
Standard 2	10,0	2,5
Standard 3	25,0	10,0
Standard 4	50,0	25,0
Standard 5	100,0	100,0

6 Apparatus and materials

The usual laboratory apparatus and, in particular, the following shall be used.

6.1 HPLC apparatus with photo-diode array (PDA) detector (dual or multiple wavelengths, recommended) or UV detector.

6.2 Analytical balance, to the nearest of 0,1 mg.

6.3 Ultrasonic bath.

6.4 Membrane filter, 0,45 µm, with the usual filtration equipment.

7 Test procedure

7.1 General

Two replicate experiments shall be done for the determination.

7.2 Preparation of test sample

For solid fertilizers, prepare a test portion by reducing the fertilizer sample to 100 g in accordance with ISO 8358. Grind the sample until it passes through a sieve of aperture size 0,5 mm and mix until homogenous. Place in a clean and dry bottle with a lid. For liquid fertilizers, shake the fertilizer sample up until homogenous and pour out 100 ml. Place in a clean and dry bottle with a lid.

7.3 Preparation of the test solution

Accurately weigh to the nearest 0,1 mg, an appropriate amount (0,1 g to 3 g) of the ground test portion and mix with 100 ml ultra-pure water into a 250 ml volumetric flask and dissolve using an ultrasonic bath for 20 min. Make up the volume to the mark with water. Filter one part of the homogenized sample solution through the membrane filter (6.4).

7.4 HPLC conditions

7.4.1 Column: 250 mm × 4,6 mm C18 reversed-phase column.

7.4.2 Injection volume: 10 µl.

7.4.3 Detector: PDA detector (214 nm for DCD, 205 nm for NBPT, recommended) or UV-detector with absorbance set to 214 nm only.

7.4.4 Eluent: Mixture of acetonitrile (5.1) and water, gradient shown in Table 2.

Table 2 — Gradient elution schedule

Time min	Acetonitrile %	Water %
0	5	95
3	5	95
10	25	75
16	25	75
17	5	95
31	5	95

7.3.5 Flow rate: 1,0 ml/min.

7.5 Determination of standard working solutions and sample test solutions

7.5.1 Determination of standard working solutions

Use NBPT and DCD standard solutions (5.5) to prepare the standard working solutions given in Table 1. Plot the standard curves using the concentration of NBPT and DCD, corresponding to the peak areas obtained in the test.

7.5.2 Determination of sample test solutions

Test a blank solution and the sample test solutions under the same conditions as the standards. Use the retention times to identify NBPT and DCD, and derive the concentrations of NBPT and DCD, in the test solutions from the standard curves, as shown in Annex A.

It is highly recommended that the DCD shall be determined under the wavelength of 214 nm, while the NBPT shall be determined under the wavelength of 205 nm.

Only if the HPLC equipped with a single-wavelength UV detector, consider testing the DCD and NBPT both under the wavelength of 214 nm.

The blank solution is prepared in the same manner as the test solutions, except for adding any test samples.

If the response value (peak area) of any compound in a test solution exceeds the linear calibration range of a standard solution, appropriate dilutions should be prepared.

8 Calculation and expression of results

8.1 General

The mass fraction of NBPT and DCD, w, in the unit of %, is calculated as follows:

$$w = \frac{\rho \times V \times f}{m \times 10\,000}$$

where

ρ is the mass concentration of NBPT and DCD, expressed in milligrams per litre of the test solutions;

V is the volume of the test solutions, expressed in millilitres;

f is the dilution factor of the test solutions;

m is the mass of the test portion, expressed in grams.

The determination result is the arithmetic average of two parallel determination results and shall be rounded off to three significant figures.

8.2 Precision

8.2.1 Ring test

Details of ring test on the precision of the method are summarized in Annex B.

8.2.2 Repeatability, r

For DCD content of all levels, the repeatability limit, r, is $0{,}067w - 0{,}014$, in mass fraction percentage.