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Organsko-mineralna gnojila - Identifikacija kompleksirajočih agensov - 2. del: Metoda s tekočinsko kromatografijo visoke ločljivosti (HPLC)

Organo-mineral fertilizers - Identification of complexing agents - Part 2: Method using high-performance liquid chromatography (HPLC)

Organisch-mineralische Düngemittel - Identifizierung von Komplexbildnern - Teil 2: Verfahren mittels Hochleistungs-Flüssigkeitschromatographie (HPLC)

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Organo-mineral fertilizers - Identification of complexing agents - Part 2: Method using high-performance liquid chromatography (HPLC)

Organisch-mineralische Düngemittel - Identifizierung von Komplexbildnern - Teil 2: Verfahren mittels Hochleistungs-Flüssigkeitschromatographie (HPLC)

This draft Technical Specification is submitted to CEN members for Vote. It has been drawn up by the Technical Committee CEN/TC 260.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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Contents

Page

European foreword

This document (FprCEN/TS 17784-2:2021) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

This document is currently submitted to the Vote on TS.

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

Introduction

Micronutrients are considered to be, in plant nutrition, a number of elements known to be needed in small amounts for proper plant growth and development. The most common are Iron (Fe), Manganese (Mn), Molybdenum (Mo), Copper (Cu), Zinc (Zn) and Boron (B).

If an organo-mineral fertilizer contains a substance, or one of the substances in the mixture, which is intended to enhance the long term availability to plants of micronutrients in the EU fertilizing product, that substance can be either a chelating agent or a complexing agent.

The incorporation of heptagluconic acid as complexing agent in organo-mineral fertilizers is intended to enhance the long term availability to plants of micronutrients in such EU fertilizing products.

WARNING — Users of this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety issues, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this document are carried out by suitably trained staff.

1 Scope

This document specifies a chromatographic method which allows the identification of heptagluconic acid (HGA) in organo-mineral fertilizers containing heptagluconic acid metal complexes.

NOTE For the complete names of the chelating agents mentioned in this document, see Annex D.

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.

EN 12944-1, *Fertilizers and liming materials and soil improvers - Vocabulary - Part 1: General terms*

EN 12944-2, *Fertilizers and liming materials and soil improvers - Vocabulary - Part 2: Terms relating to fertilizers*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1 and EN 12944-2 and the following apply.

ISO and IEC maintain terminological 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
complexing agent
organic substance forming a flat or steric structure with one di- or tri-valent transition metal cation (zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) or cobalt (Co))

4 Principle

The method is based on demetallation with phosphoric acid of the micronutrient HGA complex present in an aqueous solution of the sample.

The complexing agent is then identified and determined by high-performance liquid chromatography.

The separation is carried out on an NH_2 phase bonded to silica column and an aqueous solution of phosphoric acid and acetonitrile as eluent.

The detection is based on UV photometry at 210 nm.

5 Interferences

Current knowledge on eventual interferences from other substances is summarized in the list below:

a) High concentrations of phosphate in the sample solution can create a large peak preventing the identification/determination of HGA.

b) High concentrations of chloride, sulfate and nitrate do not interfere in the identification/determination of the complexing agent.

c) The presence of the chelates of EDDHSA, [o,o] EDDHA, [o,o] EDDHMA, EDTA, DTPA, CDTA, HEEDTA, IDHA as well as the corresponding chelating agent do not interfere since they are separated from HGA.

These substances can be detected in the chromatogram by the appearance of a peak at larger retention times. Therefore, the presence of these kinds of substances shall be taken into account when successive injections are scheduled.

d) The presence of gluconic acid does interfere in the determination of the complexing agent.

e) The presence of aspartic acid, humic substances and lignosulfonic acid can interfere with the identification/determination of HGA.

6 Reagents

All reagents shall be of recognized analytical grade.

6.1 Water

All water used should conform to EN ISO 3696, be degassed and be free of organic contaminants.

6.2 Sample preparation solvent.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % and 25 ml of methanol in a 1 l volumetric flask. Dilute to the mark with water and homogenize.

6.3 HGA stock solution.

The mass concentration (ρ) of this solution is ρ(HGA) = 19 893 mg/l.

This solution shall be freshly prepared daily, because of the formation of the corresponding lactone if it is let standing for a long period of time.

Weigh to the nearest 0,1 mg about 2 500 mg of the heptagluconic acid, sodium salt dihydrate (CAS # 10094-62-9) 99 % (mass concentration), add 50 ml of water in a 100 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize.

6.4 Eluent A: *ortho*-phosphoric acid.

The molar concentration (c) of this solution is $c(H_3PO_4)$ = 30 mmol/l and methanol.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % (mass concentration) and 25 ml of methanol high-performance liquid chromatography (HPLC) grade in a 1 l volumetric flask. Dilute to the mark with water and homogenize. Before use, filter the solution through a 0,45 µm membrane filter (7.5).

6.5 Eluent B: acetonitrile (HPLC-grade).

7 Apparatus

Usual laboratory equipment, glassware, and the following:

7.1 Magnetic stirrer.

7.2 Chromatograph.

Equipped with:

a) an isocratic pump delivering the eluent at a flow rate of 1 ml/min;

b) an injection valve with a 20 µl injection loop or equivalent;

c) a NH_2 column[1]; internal diameter: 4,6 mm; column length: 250 mm; dp = 5 µm;

d) a NH_2 guard-column (recommended);

e) a UV-VIS detector with a 210 nm-filter;

f) an integrator.

7.3 Chromatographic conditions.

According to Table 1.

Table 1 — Chromatographic conditions

Flow rate	Eluent A (6.4)	Eluent B (6.5)
	%	%
1 ml/min	75	25

7.4 Balance.

Balance, with a maximum permissible error of ±0,1 mg.

7.5 Membrane filters.

Micro membrane filters resistant to aqueous solutions, with porosity of respectively 0,45 µm and 0,2 µm.

8 Sampling and sample preparation

Sampling and sample preparation are not part of the method specified in this document.

Recommended sampling methods are given in EN 1482-1 and, for sample preparation, in EN 1482-2.

9 Procedure

9.1 Preparation of the HGA-metal complex sample solution.

The mass of the test portion to be used to prepare the sample solution is dependent on the declared metal content of the product.

Weigh into a 150 ml beaker, approximately the amount of sample specified in Table 2, to the nearest 0,1 mg:

Table 2 — Amount of sample

Declared metal content	Mass of test portion
% (mass fraction)	mg
10 ≤ to 15	300
5 ≤to < 10	500
< 5	1 000

[1] PhenoSphere™ NH_2 80A 5 µm 250 mm x 4,6 mm or equivalent is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Add 50 ml of sample preparation solvent (6.2) and dissolve it with a magnetic stirrer (7.1) during 5 min. Make up to volume in a 100 ml volumetric flask with sample preparation solvent (6.2). Let the solution stand overnight in darkness to allow the metal phosphate to form.

9.2 Preparation of the calibration solutions.

Pipette a volume (*V* in ml) (see Table 3) of the HGA stock solution (6.3) in six 100 ml volumetric flasks respectively. Make up to volume with the sample preparation solution (6.2) and homogenize. Let the solution stand overnight.

Table 3 — Composition of the calibration solutions

Solution	*V* ml	Concentration of HGA mg HGA/l
1	1	199
2	2	398
3	6	1 194
4	8	1 591
5	10	1 989
6	16	3 183

NOTE The molecular mass of heptagluconic acid, sodium salt dihydrate corresponds to 284 g/mol, whereas HGA has a molecular mass of 226 g/mol.

9.3 Chromatographic analysis.

Immediately before injection, all calibration and sample solutions shall be filtered through a 0,2 µm membrane filter (7.5).

After stabilization of the chromatographic conditions (7.3), inject the calibration solutions (9.2) into the chromatographic system (7.2).

The major peak obtained corresponds to heptagluconic acid.

NOTE 1 Since the calibration solutions are not freshly prepared (see 9.2), two defined peaks can appear in the chromatograms, one tentatively assigned to the lactone and the other corresponding to the heptagluconic acid.

Adjust the attenuation on the integrator, in order to obtain a suitable range for the HGA peak from the standard solution. A typical chromatogram is given in Figure A.1. Measure the retention time.

Draw the calibration curve with the value of the chromatographic peak of the calibration solutions versus the HGA concentration (mg/l) in the standards.

Inject the sample solution (9.1). A typical chromatogram is given in Figure A.2. Identify the complexing agent by the retention time of the obtained peaks, and if diode array detector is used, confirm it with its UV-visible spectrum (see Figure B.1).

Measure the area of the peak for the sample solution corresponding to the complexing agent and determine the concentration in (mg/l) using the calibration graph. See Annex A for integration considerations.

NOTE 2 Heptagluconic acid can co-exist in two different isomers: alpha and beta. Both isomers can be found in commercial products as shown in Figure A.3. The reaction times of both isomers differ in less than 0,3 min and they can be distinguished by two separated peaks depending on the type of column used.