SLOVENSKI STANDARD
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Gnojila - Določevanje sredstev za kelatiziranje v gnojilih s kromatografijo - 2. del: Določevanje železovih kelatov z [o,o] EDDHA, [o,o] EDDHMA in HBED ali količine sredstev za kelatiziranje s kromatografijo ionskih parov

Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 2: Determination of Fe chelated by [o,o] EDDHA, [o,o] EDDHMA and HBED, or the amount of chelating agents, by ion pair chromatography

Düngemittel - Bestimmung von Chelatbildnern in Düngemitteln mit Chromatographie - Teil 2: Bestimmung von Fe chelatisiertem [o,o] EDDHA, [o,o] EDDHMA und HBED, oder der Menge der Chelatbildner, mit Ionen-Paarchromatographie

Engrais - Détermination des agents chélatants dans les engrais par chromatographie - Partie 2: Détermination du fer chélaté [o,o] EDDHA, [o,o] EDDHMA et HBED, ou de la quantité d'agents chélatants, par chromatographie d'appariement d'ions

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Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 2: Determination of Fe chelated by [o,o] EDDHA, [o,o] EDDHMA and HBED, or the amount of chelating agents, by ion pair chromatography

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Düngemittel - Bestimmung von Chelatbildnern in Düngemitteln mit Chromatographie - Teil 2: Bestimmung von Fe chelatisiertem [o,o] EDDHA, [o,o] EDDHMA und HBED, oder der Summe der Chelatbildner, mit Ionen-Paarchromatographie

This European Standard was approved by CEN on 11 September 2017.

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

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European foreword

This document (EN 13368-2:2017) 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of an identical text or by endorsement, at the latest by May 2018 and conflicting national standards shall be withdrawn at the latest by May 2018.

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 supersedes EN 13368-2:2012.

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

In comparison with EN 13368-2:2012, the following changes have been made:

a) determination of the chelating agent added to the scope and to the title;

b) a derivatization method for the determination of the chelating agent explained in Clause 4, Principle;

c) a new option added for the preparation of the Fe-[*o,o*] EDDHA solution, starting from a Fe-[*o,o*] EDDHA standard, in 6.6;

d) calculation of the mass fraction of the chelating agents included in Clause 10 including Formulae (3) and (4);

e) Table 2 enlarged by the precision data concerning the 2014 inter-laboratory test;

f) information on the type of standard used for Fe-[*o,o*] EDDHA samples and the possibility to report on the chelating agent contents included in Clause 12;

g) results of the inter-laboratory test performed in 2014, part A and B respectively, added (A.3 and A.4);

h) complete names of chelating agents in Annex C technically revised;

i) editorially revised.

EN 13368, *Fertilizers — Determination of chelating agents in fertilizers by chromatography* consists of the following parts:

— *Part 1: Determination of EDTA, HEEDTA and DTPA by ion chromatography*

— *Part 2: Determination of Fe chelated by [o,o] EDDHA, [o,o] EDDHMA and HBED, or the amount of chelating agents, by ion pair chromatography*

— *Part 3: Determination of [S,S]-EDDS by ion pair chromatography*

According to the CEN-CENELEC Internal Regulations, the national standards organizations 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, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard specifies a method for the chromatographic determination of the iron chelated by each individual *ortho*(hydroxy)-*ortho*(hydroxy) isomer of the chelating agents [*o,o*] EDDHA, [*o,o*] EDDHMA and by HBED in fertilizers containing one or more of these substances, except for [*o,o*] EDDHMA and HBED mixes. The method allows the identification and the determination of the total concentration of water soluble iron chelates of these chelating agents. Also, after derivatization with Fe, the soluble amount of the chelating agents can be determined when other micro-nutrients, beside Fe are present in fertilizers containing [*o,o*] EDDHA, [*o,o*] EDDHMA or HBED.

This method is applicable to EC fertilizers covered by Regulation (EC) No 2003/2003 [4]. It is applicable to a mass fraction of the metal chelated of at least 0,625 %.

NOTE 1 The substances EDDHA (ethylenediamine-N,N'-di[(hydroxyphenyl)acetic acid] and EDDHMA (ethylenediamine-*N,N'*-di[(hydroxymethylphenyl)acetic acid] exist as several different isomeric forms. Positional isomers for the hydroxyl or methyl groups (in *ortho, meta,* and *para* positions) as well as stereo isomers (*meso* and dl-racemic forms) are known. Both *meso* and dl-racemic forms of the [*ortho,ortho*] EDDHA and [*ortho,ortho*] EDDHMA are positional isomers for the hydroxyl groups allowed by the Regulation (EC) No 2003/2003. Since *para, meta and ortho* methyl positional isomers of the EDDHMA present quite similar stability, they could be grouped: in the method here described the *para, meta* and *ortho* methyl positional isomers of the [*o,o*] EDDHMA are considered together. HBED (N,N'-bis(2-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid) does not present isomeric forms.

NOTE 2 At present, analytically pure standards only exist for [*ortho,ortho*] EDDHA, [*ortho,ortho*] EDDHMA and HBED. All other substances being unavailable as a standard, the influence of their eventual presence in the samples (with respect to the sensitivity and the selectivity of this method) has not been studied.

NOTE 3 The *meso* and the dl-racemic forms of [*o,o*] EDDHA and [*o,o*] EDDHMA can be determined separately by this method.

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 iron chelates are separated and determined by isocratic ion-pair high-performance liquid chromatography. When an iron chelate (anion) is added to a polar fluid (eluent), containing a large cation, an ion pair is formed. This ion pair is retained by a non-polar solid phase (stationary phase). The strength of the retention depends on the molecular size and its acidity. Then, each iron chelate presents a characteristic retention time depending on the chelating agent, and it is separated from the other substances present in the sample. The separation is carried out on a reverse phase silica column and an aqueous solution of TBA^+ (tetrabutylammonium) and acetonitrile as eluent. The detection is based on photometry at 280 nm.

For metal chelates different from Fe, a derivatization method may be used to form the Fe chelates, and then the chelating agent can be determined by the isocratic ion-pair high-performance liquid chromatography here presented.

NOTE For additional information, see [5], [6] and [7].

5 Interferences

No interferences have been detected. Iron chelates with HBEP, EDDHSA, EDTA, DTPA, CDTA, HEEDTA, [*p,p*] EDDHA, [*o,p*] EDDHA, IDHA as well as the chelating agents do not interfere since they are separated from Fe-[*o,o*] EDDHA, Fe-[*o,o*] EDDHMA or Fe-HBED. Fe-[*o,o*] EDDHA does not interfere with Fe-[*o,o*] EDDHMA or Fe-HBED. In the case that Fe-[*o,o*] EDDHMA and Fe-HBED are present in the same sample, an overlapping of the *meso* isomer of the Fe-[*o,o*] EDDHMA and Fe-HBED peaks may occur with some equipment depending on the column used. The use of a different column of the same type can solve this problem.

NOTE For the complete names of the abbreviations of chelating agents used in this document, see Annex C.

6 Reagents

6.1 General.

All reagents shall be of recognized analytical grade. Water used for the preparation of eluents, standards, and sample solutions shall conform to EN ISO 3696, grade 1 and shall be degassed and free of organic contaminants. If products with a declared purity of less than 99 % are used for the preparation of standard solutions, a correction should be made in order to obtain exactly the required concentration in the solution.

If there is any doubt of the purity of the standard, it is necessary to determine it.

NOTE For this determination, a titrimetric method can be used. See Annex B for a general method using an automatic titrator. Manual titration could also be adequate.

6.2 Sodium hydroxide solution, c(NaOH) = 0,1 mol/l.

Dissolve 4 g of NaOH in pellet form in a 1 l volumetric flask with water (6.1). Dilute to the mark and homogenize.

The incorporation of CO_2 from the atmosphere should be carefully avoided. Otherwise, the dissolution of chelating agents (see 6.6, 6.7 and 6.8) can be incomplete.

6.3 Hydrochloric acid solution, c(HCl) = 1,0 mol/l.

Dilute 88 ml of hydrochloric acid (mass fraction 35 % HCl) to 1 000 ml with water.

6.4 Hydrochloric acid solution, c(HCl) = 0,1 mol/l.

Dilute 50 ml of hydrochloric acid 1,0 mol/l (6.3) to 500 ml with water.

6.5 Iron-nitrate solution, ρ(Fe) = 1 050 mg/l.

Dissolve 0,759 4 g of ferric nitrate 9-hydrate ($Fe(NO_3)_3 \cdot 9H_2O$) in 100 ml of water. Check (for example by AAS) that the Fe concentration in this solution amounts 1 050 mg/l ± 30 mg/l.

NOTE As the $Fe(NO_3)_3 \cdot 9H_2O$ is deliquescent it will be added in solution of a known concentration.

6.6 Fe-[*o,o*] EDDHA solution, ρ(Fe) = 100 mg/l.

Depending on the availability, either 6.6.1 or 6.6.2 procedures can be used to prepare the standard stock solution.

6.6.1 Preparation from Fe-[*o,o*] EDDHA.

Dissolve 5/P g (where P is the purity of the solid standard in percentage of Fe chelated), in 50 ml of water (6.1) in a 100 ml beaker and make up to 500 ml in a volumetric flask with water. The standard obtained in this way may be stored in darkness for one year.

6.6.2 Preparation from [*o,o*] EDDHA.

Dissolve 0,322 1 g (see 6.1) of ethylenediamine-N,N'-di[(*ortho*-hydroxyphenyl)acetic acid] in 350 ml of water (6.1) and 27 ml of NaOH (6.2) in a 500 ml beaker. Add 50 ml of the Fe solution (6.5) to the chelating agent solution, stirring for about 5 min.

Adjust the solution to pH 7,0 with NaOH solution (6.2). Let the solution stand overnight in darkness to allow excess Fe to precipitate as oxide. Filter quantitatively through a cellulose filter and make up to 500 ml in a volumetric flask with water (6.1). The standard obtained in this way may be stored in darkness for one year.

6.7 Fe-[*o,o*] EDDHMA solution, ρ(Fe) = 100 mg/l.

Dissolve 0,347 1 g (see 6.1) of ethylenediamine-N,N'-di[(*ortho*-hydroxy-*para*-methylphenyl)acetic acid] [the *para*methyl isomer of [*o,o*] EDDHMA, (see Note 1 in Clause 1)] in 350 ml of water (6.1) and 27 ml of NaOH (6.2) in a 500 ml beaker. Add 50 ml of the Fe solution (6.5) to the chelating agent solution, stirring for about 5 min.

Adjust the solution to pH 7,0 with NaOH solution (6.2). Let the solution stand overnight in darkness to allow excess Fe to precipitate as oxide. Filter quantitatively through a cellulose filter and make up to 500 ml in a volumetric flask with water (6.1). The standard obtained in this way may be stored in darkness for one year.

6.8 Fe-HBED solution, ρ(Fe) = 100 mg/l.

Dissolve 0,347 1 g (see 6.1) of N,N'-bis(2-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid (HBED) or 0,379 7 g if HBED·HCI is used in 350 ml of water (6.1) and 27 ml of NaOH (6.2) in a 500 ml beaker. Add 50 ml of the Fe solution (6.5) to the chelating agent solution, stirring for about 5 min.

Adjust the solution to pH 5,0 with HCI solution (6.4) or NaOH solution (6.2). Let the solution stand overnight in darkness to allow excess Fe to precipitate as oxide. Filter quantitatively through a cellulose filter and make up to 500 ml in a volumetric flask with water (6.1). The standard obtained in this way may be stored in darkness for one year.

6.9 Eluent for the determination.

Add 20 ml of TBAOH (mass fraction 40 % Tetrabutylammonium hydroxide solution in water) to 600 ml of water (6.1). Adjust to pH 6,0 with hydrochloric acid solution (6.3 and 6.4). Add 300 ml of acetonitrile (HPLC grade) and make up to volume in a 1 l volumetric flask with water. Filter through a 0,2 µm membrane filter (7.4 b) and degas.

TBACl or TBABr may be used, providing that pH is adjusted to 6,0 with NaOH or HCl.

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 1,5 ml/min;

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

c) a C-18 column; internal diameter:3,9 mm; column length: 150 mm; dp = 5 µm[1)];

d) a C-18 guard column (recommended);

e) a UV/VIS-detector with a 280 nm-filter;

f) an integrator.

7.3 Balance, with an accuracy of ± 0,1 mg.

7.4 Membrane filters, including:

a) micro membrane filters resistant to aqueous solutions, with porosity of 0,45 µm;

b) micro membrane filters resistant to organic solutions (e.g. polyamide 66 micro membrane filters), with porosity of 0,2 µm.

8 Sampling and sample preparation

Sampling is not part of the method specified in this document. A recommended sampling method is given in EN 1482-1 [1].

Sample preparation shall be carried out in accordance with EN 1482-2.

For the size reduction of samples with a high amount of chelating agents, it is not recommended to use a high speed laboratory mill. It is more convenient to grind the sample to a particle size less than 1 mm.

1) SYMMETRY™ C18, from WATERS, LiChroCART® Purospher® RP-18, from MERCK or equivalent are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

9 Procedure

9.1 Preparation of the sample solution for iron chelates

Weigh, to the nearest 0,1 mg, 200 mg of the iron chelate into a 250 ml beaker. Add 200 ml of water (6.1). Stir using a magnetic stirrer for 1 h. Transfer quantitatively into a 250 ml volumetric flask. Dilute to the mark with water and homogenize.

For samples declaring more than 5 % of chelated Fe, use a 500 ml volumetric flask.

For liquid samples, weigh an amount equivalent to 200 mg of solid.

9.2 Preparation of the sample solution for other micronutrient chelates

Weigh, to the nearest 0,1 mg, 1 g of the chelate into a 250 ml beaker. Add 200 ml of water (6.1). Stir using a magnetic stirrer for 1 h. Transfer quantitatively into a 250 ml volumetric flask. Dilute to the mark with water (6.1) and homogenize. Filter through a paper filter. Pipette 20 ml of the solution into a 50 ml beaker. Add 10 ml of the iron(III) solution (6.5), homogenize, and allow to stand for 15 min. Adjust the pH to 9,0 ± 0,1 with sodium hydroxide solution (6.2) and allow to stand for another 15 min. Transfer quantitatively into a 100 ml volumetric flask. Dilute to the mark with water (6.1) and homogenize.

For samples declaring more than 5 % of chelated metals, use in the first step a 500 ml volumetric flask.

For liquid samples, weigh an amount equivalent to 200 mg of solid.

9.3 Preparation of the calibration solutions

Pipette *V* ml (see Table 1) of the Fe-[*o,o*] EDDHA (6.6) or Fe-[*o,o*] EDDHMA (6.7) or Fe-HBED (6.8) standard solution into six 100 ml volumetric flasks. Make up to volume with water (6.1) and homogenize.

Table 1 — Composition of the calibration solutions

Solution	*V* ml	Fe concentration in the ***rac*** chelate mg/l	Fe concentration in the *meso* chelate mg/l	Total Fe concentration in chelate mg/l
1	5	2,5	2,5	5,0
2	10	5,0	5,0	10,0
3	20	10,0	10,0	20,0
4	30	15,0	15,0	30,0
5	40	20,0	20,0	40,0
6	50	25,0	25,0	50,0

NOTE 1 As the standard chelates solutions should be 50 % *meso*- and 50 % dl-racemic optical isomers, the concentration of the total iron chelate is the sum of the concentrations given in Table 1.

NOTE 2 Since HBED does not present optical isomers the total concentrations can be used.