SLOVENSKI STANDARD
SIST EN 13368-2:2013

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Nadomešča:
SIST EN 13368-2:2007

Gnojila - Določevanje kelatov v gnojilih s kromatografijo - 2. del: Določevanje železovih kelatov z o,o-EDDHA, o,o-EDDHMA in HBED 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 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 mit Ionen-Paarchromatographie

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Engrais - Détermination des agents chélatants dans les engrais par chromatographie - Partie 2: Détermination du fer chélaté o,o-EDDHA, o,o-EDDHMA et HBED par chromatographie d’appariement d’ions

Ta slovenski standard je istoveten z: **EN 13368-2:2012**

ICS:

65.080	Gnojila	Fertilizers

SIST EN 13368-2:2013 **en,fr,de**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 13368-2

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English Version

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

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This European Standard was approved by CEN on 6 July 2012.

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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.

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Foreword

This document (EN 13368-2:2012) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and lliming 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of an identical text or by endorsement, at the latest by February 2013, and conflicting national standards shall be withdrawn at the latest by February 2013.

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 13368-2:2007.

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

The following has been added to the former edition (EN 13368-2:2007) of this European Standard:

a) new substance Fe-HBED added to the scope and to the title;

b) requirements concerning the use of different columns in order to avoid overlapping of the *meso* isomer of Fe-[*o*,*o*] EDDHMA and Fe-HBED peaks added in Clause 5, Interferences;

c) iron-nitrate solution and Fe-HBED solution added in Clause 6, Reagents;

d) the sampling method is not part of the standard; informative reference to EN 1482-1 added;

e) Table 1 enlarged by a column for total chelate;

f) typical chromatogram of a commercial Fe-HBED sample added (Figure 4);

g) Formula (2) on calculation of Fe-HBED added;

h) Table 2 enlarged by the precision data concerning determination of Fe-HBED;

i) A.2: Results of the inter-laboratory test performed in 2010 added;

j) Annex C: Complete names of chelating agents technical revised;

k) Bibliography revised.

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This European Standard "*Fertilizers — Determination of chelating agents in fertilizers by ion 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 by ion pair chromatography

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.

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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. It does not determine the free form of the chelating agents.

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.

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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.

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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 1482-2, *Fertilizers and liming materials — Sampling and sample preparation — Part 2: Sample preparation*

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 an apolar 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.

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

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5 Interferences

No interferences have been detected. Iron chelates with HBEP, EDDHSA, EDTA, DTPA, CDTA, HEEDTA, p,p-EDDHA, o,p-EDDHA, 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 recognised analytical grade. All 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.

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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 homogenise.

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NOTE 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 is 1 050 mg ± 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.

Dissolve 0,322 1 g (see 6.1) of ethylenediamine-N,N′-di[(*ortho*-hydroxyphenyl)acetic acid] in 350 ml of water 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.

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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 volume (500 ml) in a volumetric flask with water. The standard obtained in this way can 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 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 volume (500 ml) in a volumetric flask with water. The standard obtained in this way can 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·HCl is used in 350 ml of water 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 HCl 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 volume (500 ml) in a volumetric flask with water. The standard obtained in this way can be stored in darkness for one year.

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6.9 **Eluent for the determination**

Add 20 ml of TBAOH (mass fraction 40 % Tetrabutylammonium hydroxide solution in water) to 600 ml of water. 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.

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NOTE TBACl or TBABr can 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];

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[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.

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

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

f) an integrator.

7.3 **Balance,** capable of weighing to 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.

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.

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9 Procedure

9.1 Preparation of the sample solution

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

For samples declaring more than 6 % 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 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 and homogenise.

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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 shown in Table 1.

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

9.3 Chromatographic analysis

Immediately before injection, all solutions shall be filtered through a 0,45 µm membrane filter (7.4 a). Inject the standard solutions (see 9.2) into the chromatographic system (7.2). Measure the retention times and the areas of the two Fe-[*o,o*] EDDHA or Fe-[*o,o*] EDDHMA isomers (*meso* and dl-racemic) or Fe-HBED peaks for all solutions. For each appropriate iron chelate, draw two calibration graphs with the values of the peak areas of the standard solutions versus the iron concentration (mg/l) in the form of the corresponding chelating agent isomer. For Fe-HBED, only one graph is needed. See Figures 1 to 4.

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NOTE 1 See NOTE 1 in 9.2.

NOTE 2 A statistically significant negative intercept can be caused by the contamination of the column with Fe oxide precipitates. In that case, the column should be replaced or cleaned.

Inject the sample solution (see 9.1). Identify the chelating agent by the retention time of the obtained peaks (see Figures 2, 3 and 4). Measure the areas of the isomer peaks for each chelating agent. Determine the concentration of the iron chelated by each optical isomer (mg Fe/l) using the corresponding calibration graph.

In Figure 3 the typical chromatogram of a commercial product containing Fe-[*o,o*] EDDHMA is shown. Besides meso and dl-racemic isomers of para-methyl ortho-hydroxyl Fe-[*o,o*] EDDHMA two other isomers, assigned to other methyl positional isomers, are found (peaks 3 and 4). Since they have similar stability to the para-methyl isomers (see NOTE 1 in Clause 1), their area should be added to the nearest (1 or 2) peak area.

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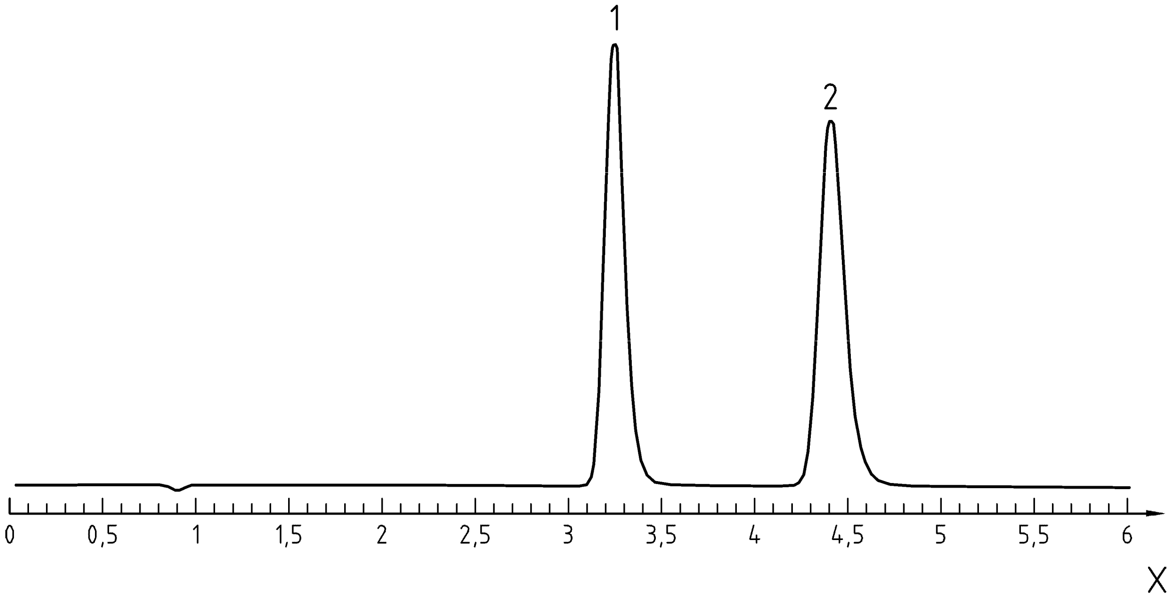

Key

1 dl-racemic Fe-[*o*,*o*] EDDHA

2 meso Fe-[*o*,*o*] EDDHA

X time in minutes

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Figure 1 — Typical chromatogram of a Fe-[*o*,*o*] EDDHA standard solution with an Fe content of 25 mg/l /l

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