SLOVENSKI STANDARD
SIST EN 13368-3:2018

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Gnojila - Določevanje sredstev za kelatiziranje v gnojilih s kromatografijo - 3. del: Določevanje [S,S]-EDDS s kromatografijo ionskih parov

Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 3: Determination of [S,S]-EDDS by ion pair chromatography

Düngemittel - Bestimmung von Chelatbildnern in Düngemitteln mit Chromatographie - Teil 3: Bestimmung von [S,S]-EDDS mit Ionen-Paarchromatographie

Engrais - Détermination des agents chélatants dans les engrais par chromatographie - Partie 3: Détermination du [S,S]-EDDS par chromatographie d'appariement d'ions

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Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 3: Determination of [S,S]-EDDS by ion pair chromatography

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This European Standard was approved by CEN on 25 September 2017.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 13368-3:2017) 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of an identical text or by endorsement, at the latest by June 2018, and conflicting national standards shall be withdrawn at the latest by June 2018.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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

The European Standard 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 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, 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 chelating agent [*S,S*]-EDDS in fertilizers. The method allows the identification and the determination of the total concentration of the water-soluble fraction of this chelating agent. It does not allow distinguishing between the free form and the metal bound form of the chelating agent.

This method is applicable to EC fertilizers containing chelates of one or more of the following micro-nutrients: cobalt, copper, iron, manganese and zinc, covered by Regulation (EC) No 2003/2003 [4]. It is applicable to a mass fraction of the metal chelated of at least 0,35 %.

NOTE The substance EDDS (ethylenediamine-*N,N'*-disuccinic acid) exists as several different stereo isomeric forms. [*S,S*] (with CAS Number 20846–91–7), [*R,R*] and [*R,S*] optical isomers are possible. [*S,S*] and [*R,R*] are mirror images with equal chemical characteristics. However only the [*S,S*] isomer is biodegradable. When both are present, they form the racemic mixture. The [*R,S*] isomer (the meso isomer) is only slowly biodegradable.

Only the [*S,S*]-EDDS isomer is allowed by the Regulation (EC) No 2003/2003. Since the Cu chelate of the [*R,R*]-EDDS isomer presents the same stability as the [S,S]-EDDS, both should coelute. The absence of [R,S]-EDDS indicates that only a pure isomer ([*S,S*] or [*R,R*]) exists. An additional test based on polarimetry can be used to ascertain the isomeric characteristic of the sample, or the standard.

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 micronutrients associated with the [*S,S*]-EDDS present in an aqueous extract of the sample are replaced by Cu(II). The Cu(II) chelates are separated and determined by isocratic ion-pair high-performance liquid chromatography. When a copper 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 copper 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 copper acetate and methanol as eluent. The detection is based on photometry at 254 nm.

A derivatization method should be used to form the Cu chelates, so the chelating agent is determined by

the isocratic ion-pair high-performance liquid chromatography here specified.

5 Interferences

No interferences have been detected. Metal chelates with [*o,o*] EDDHA, [*o,o*] EDDHMA, HBED, EDDHSA, EDTA, DTPA, HEEDTA, IDHA, [*o,p*] EDDHA, lignosulfonates and heptagluconate as well as the chelating agents do not interfere since after the Cu derivatization they are separated from Cu-[*S,S*]-EDDS. Since retention times depend on the column type, any interference should be checked if a mixture with other chelating or complexing agents is suspected.

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

6 Reagents

6.1 General.

All reagents shall be of recognized 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 on the purity of the standard substances, it is necessary to determine it.

For the determination of its concentration, a titrimetric method can be used. See Annex B for a photometric method using an automatic titrator. Manual titration can also be adequate.

The isomeric purity can be checked using the method indicated in Annex C.

6.2 Sodium hydroxide solution, c(NaOH) = 0,15 mol/l.

Dissolve 3,0 g of NaOH in pellet form in a 500 ml 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 the chelating agent (see 6.6) can be incomplete.

6.3 Phosphoric acid solution, $c(H_3PO_4)$ = 1,0 mol/l.

Dilute 68 ml of phosphoric acid (mass fraction 85 % H_3PO_4) to 1 000 ml with water.

6.4 Phosphoric acid solution, $c(H_3PO_4)$ = 0,1 mol/l.

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

6.5 Cu acetate solution (for derivatization), c(Cu) approximately 0,02 mol/l.

Dissolve 4,00 g of copper acetate monohydrate ($Cu(CH_3\text{-}COO)_2 \cdot H_2O$) in 1 l of water.

6.6 [*S,S*]-EDDS solution, c(EDDS) = 8,95 mmol/l.

If the EDDS acid form is used (CAS# 20846-91-7), dissolve 26,16/P g (where P is the purity of the solid standard in percentage of EDDS acid form), in 50 ml of water and 20 ml of NaOH (6.2) in a 100 ml beaker and make up to 100 ml in a volumetric flask with water. The standard obtained in this way may be stored in darkness in a fridge (at about 5 °C) for 6 months. If a significant yellowing appears (due to photodecomposition) a fresh standard should be prepared.

If EDDS trisodium salt solution is used instead of the acid form then dissolve 32,07/*P* g (where *P* is the purity of the standard in percentage of EDDS trisodium salt) in 50 ml of water in a 100 ml beaker and make up to 100 ml in a volumetric flask with water.

NOTE This concentration is equivalent to ρ(Fe) = 500 mg/l. Since most commercial samples are Fe chelates, equivalent Fe concentration is given to simplify final calculations.

6.7 Eluent for the determination.

Dissolve 1,00 g of copper acetate monohydrate ($Cu(CH_3\text{-}COO)_2 \cdot H_2O$) (5,00 mmol) in 900 ml of water. Add 2 ml of TBAOH (mass fraction 40 % Tetrabutylammonium hydroxide solution in water). Adjust to pH 2,8 with phosphoric acid solution (6.3 and 6.4). Add 40 ml of methanol (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.

NOTE TBACl or TBABr can be used, providing that pH is adjusted to 2,8 with H_3PO_4.

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 1,0 ml/min;

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

c) a C-18 column[1)], dp = 5 µm, > 99 % end capped; recommended internal diameter: 3,9 mm and column length: 150 mm;

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

e) a UV/VIS-detector with a 254 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.

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.

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.

9 Procedure

9.1 Preparation of the sample solution

Weigh an amount of sample, depending on the declared content of chelated micro-nutrients, to the nearest 0,1 mg, into a beaker of 100 ml or 250 ml, according to Table 1:

Table 1 — Sample mass/volume ratios

Micro-nutrient content %	**Mass of sample** *m* g	**Volume of extract** *V* ml
≤ 4	1,0	100
4 to 10	1,0	250
10 to 16	0,50	250

Add about 80 ml of water for a 100 ml beaker, or about 200 ml of water for a 250 ml beaker. Stir using a magnetic stirrer for 1 h. Transfer quantitatively into a 100 ml or 250 ml volumetric flask. Dilute to the mark with water and homogenize.

Pipette 10 ml of the solution into a 50 ml beaker. Add about 15 ml of water and adjust the pH to 11 with sodium hydroxide solution (6.2). Then add 5,0 ml of copper acetate solution (6.5) and homogenize. After 1 h check the pH and rise, if necessary, above 9,5. Allow to stand for 24 h. Adjust the pH to 7,0 ± 0,5 with phosphoric acid solution (6.3 and 6.4) and allow to stand for another 15 min. Transfer quantitatively into a 50 ml volumetric flask. Dilute to the mark with water and homogenize.

The amount of copper added should be enough to displace all the micro-nutrients. Otherwise, the peak in the chromatogram could appear split.

9.2 Preparation of the calibration solutions

Pipette *V* ml (see Table 2) of the [*S*,*S*]-EDDS solution (6.6) into six 25 ml volumetric flasks. Add 15 ml of water and 2,50 ml of copper acetate solution (6.5). Make up to volume with water and homogenize.

Table 2 — Composition of the calibration solutions

Solution	***V*** ml	**[*S*,*S*]-EDDS concentration** mmol/l	**Equivalent theoretical Fe concentration** mg/l
1	0,500	0,179	10,0
2	1,000	0,358	20,0
3	2,00	0,716	40,0
4	3,00	1,074	60,0
5	4,00	1,432	80,0
6	5,00	1,790	100,0

[*S*,*S*]-EDDS standards should not contain Fe. Since most commercial samples are Fe chelates, equivalent Fe concentrations are given in Table 2 to simplify final calculations.

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). In Figure 1 and Figure 2 a typical chromatograms of a [*S*,*S*]-EDDS standard and a commercial sample containing Fe-[*S*,*S*]-EDDS, after Cu derivatization, are presented.

a) Identification

The chromatogram should present a chromatographic peak as in the standards, and previous peaks, corresponding to the [*R*,*S*] isomer, should not be present. In Figure 3 the chromatogram and spectra of a EDDS mixture containing all the isomers is presented. If the [*R*,*S*] isomer is detected the synthesis does not produce pure [*S*,*S*]-EDDS. Then also [*R*,*R*]-EDDS isomer is contributing to the [*S*,*S*] peak. In case of doubt, the purity of the isomeric forms could be checked by polarimetry as presented in Annex C.

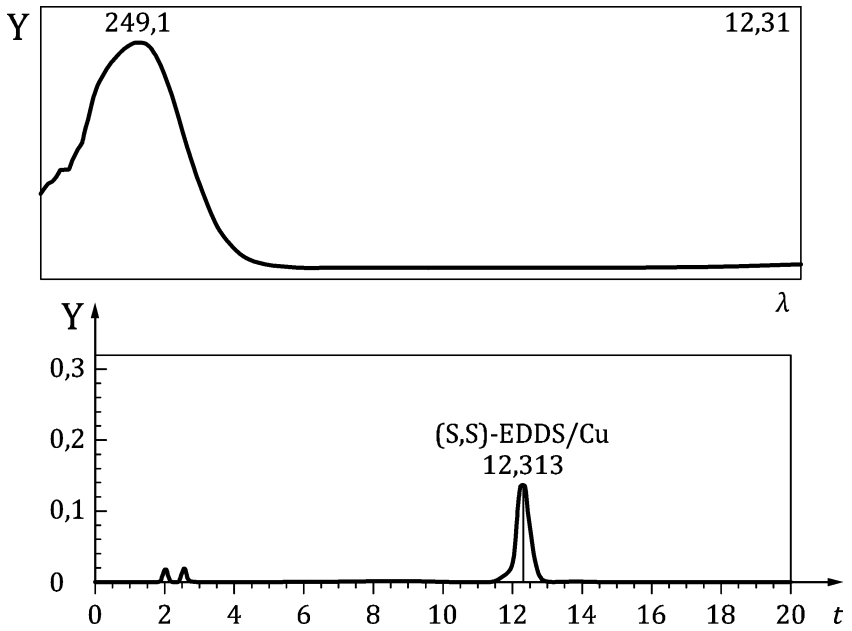
NOTE [*S*,*S*]-EDDS and [*R*,*R*]-EDDS coelute using this method. The [*S*,*S*] and [*R*,*R*] isomers are present together in samples where chemical synthesis lead to a complete isomeric mixture: meso isomer ([*R*,*S*]) accounting approximately to 50 % and the racemic mixture ([*R*,*R*] and [*S*,*S*] isomers) other 50 %. The absence of [*R*,*S*] isomer is a requisite to stablish that only the isomer [*S*,*S*]-EDDS is present.

b) Quantification

Measure the retention times and the areas of the [*S*,*S*]-EDDS isomer for all solutions. Draw the calibration graph with the values of the peak areas of the standard solutions versus the [*S*,*S*]-EDDS or the equivalent iron concentration (mg/l).

[*S*,*S*]-EDDS standards should not contain Fe. Since most commercial samples are Fe chelates, equivalent Fe concentrations are given in Table 2 to simplify final calculations.

Inject the sample solution (see 9.1). Identify the chelating agent by the retention time of the obtained peaks (see Figure 2). Measure the area of the peak of the chelating agent. Determine the concentration of the [*S*,*S*]-EDDS (c) or the equivalent iron concentration (c_{Fe}) chelated by [*S*,*S*]-EDDS (mg Fe/l) using the corresponding calibration graph.



Key

Y absorbance units

t time in minutes

λ wavelength

Figure 1 — Typical chromatogram (down) and spectra in the 200 nm to 600 nm range (up) obtained for a [*S,S*]-EDDS standard solution with an [*S,S*]-EDDS concentration of 0,716 mmol/l (equivalent to a Fe concentration of 40 mg/l)