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SIST EN 13368-2:2007

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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 and o,o-EDDHMA by ion pair chromatography

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

Düngemittel - Bestimmung von Chelatbildnern in Düngemitteln mit Chromatographie - Teil 2: Bestimmung von Fe chelatisiertem o,o-EDDHA und o,o-EDDHMA mit Ionen-Paarchromatographie

This European Standard was approved by CEN on 24 February 2007.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

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 Management Centre has the same status as the official versions.

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## Foreword

This document (EN 13368-2:2007) 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of an identical text or by endorsement, at the latest by October 2007, and conflicting national standards shall be withdrawn at the latest by October 2007.

This document supersedes EN 13368-2:2001.

EN 13368:2001 consisted of two parts:

- Fertilizers — Determination of chelating agents in fertilizers by ion chromatography — Part 1: EDTA, HEDTA and DTPA
- Fertilizers — Determination of chelating agents in fertilizers by ion chromatography — Part 2: EDDHA and EDDHMA

For technical reasons, the second element in the title of this document has been modified (the word "ion" has been deleted). Consequently, the title of Part 1 of EN 13368 should be revised in the same way. There is agreement that the replacement of Part 1 by a new edition only for harmonization of the title should be avoided. Therefore, it is proposed to harmonize the title within the next revision procedure.

Following the revised title of Part 2, the title of EN 13368-1 should be revised as follows:

- Fertilizers — Determination of chelating agents in fertilizers by chromatography — Part 1: Determination of EDTA, HEDTA and DTPA

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, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

## 1 Scope

This document 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 and o,o-EDDHMA in fertilizers containing one or both of these substances. 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.

NOTE 1 The substances EDDHA (ethylene diamine di(hydroxy phenyl) acetic acid) and EDDHMA (ethylene diamine di(hydroxy methyl phenyl)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.

NOTE 2 At present, analytically pure standards only exist for ortho,ortho-EDDHA and ortho,ortho-EDDHMA. 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.

This procedure concerns EC fertilizers covered by Regulation (EC) No 2003/2003 [5]. It is applicable to a mass fraction of the metal chelated of at least 0,625 %.

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## 2 Normative references

The following referenced documents are indispensable for the application 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 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 3696:1987)*

## 3 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<sup>+</sup> (tetrabutylammonium) and acetonitrile as eluent. The detection is based on photometry at 280 nm.

NOTE For additional information see [3] and [4].

## 4 Interferences

No interferences have been detected. Iron chelates with HBED, HBEP, EDDHSA, EDTA, DTPA, CDTA, HEDTA, p,p-EDDHA, o,p-EDDHA, as well as the chelating agents do not interfere since they are separated from Fe-o,o-EDDHA and Fe-o,o-EDDHMA.

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

## 5 Reagents

### 5.1 General

- a) All reagents shall be of recognized analytical grade;
- b) all water used for the preparation of eluent, standards, and sample solutions shall conform to EN ISO 3696, grade 1 and shall be degassed, and free of organic contaminants;
- c) if for the preparation of standard solutions products with a declared purity of less than 99 % are used, 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 A for a general method using an automatic titrator. Manual titration could be also adequate.

### 5.2 Sodium hydroxide solution, [SIST EN 13368-2:2007](https://standards.iteh.ai/catalog/standards/sist/c5d99651-e6f0-404b-bc74-74a626531517/sist-en-13368-2-2007)

$c(\text{NaOH}) = 0,1 \text{ mol/l}$ .

Dissolve 4 g of NaOH in pellet form in a 1 litre volumetric flask with water (see 5.1 b)). Dilute to the mark and homogenize.

NOTE The incorporation of  $\text{CO}_2$  from the atmosphere should be carefully avoided. Otherwise dissolution of chelating agents (see 5.5) can be incomplete.

### 5.3 Hydrochloric acid solution,

$c(\text{HCl}) = 1,0 \text{ mol/l}$ .

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

### 5.4 Hydrochloric acid solution,

$c(\text{HCl}) = 0,1 \text{ mol/l}$ .

Dilute 50 ml of hydrochloric acid 1,0 mol/l (5.3) to 500 ml with water.

### 5.5 Fe-o,o-EDDHA solution,

$\rho(\text{Fe}) = 100 \text{ mg/l}$ .

Dissolve 0,759 4 g of ferric nitrate 9-hydrate ( $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) in 100 ml of water. Check (for example by AAS) that the Fe concentration in this solution is  $1\,050 \text{ mg} \pm 30 \text{ mg Fe/l}$ .

NOTE As the  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  is deliquescent it will be added in solution of a known concentration.

Dissolve 0,322 1 g (see 5.1.) of ethylene diamine di(o-hydroxy phenyl)acetic acid in 350 ml of water and 27 ml of NaOH (5.2) in a 500 ml beaker. Add 50 ml of the Fe solution to the chelating agent solution stirring for about 5 min.

Adjust the solution to pH 7,0 with NaOH solution (5.2). 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.

### 5.6 Fe-o,o-EDDHMA solution,

$\rho(\text{Fe}) = 100 \text{ mg/l}$ .

Dissolve 0,347 1 g (see 5.1) of ethylene diamine di(o-hydroxy-p-methyl phenyl)acetic acid (o,o-EDDHMA) in 350 ml of water and 27 ml of NaOH (5.2) in a 500 ml beaker. Add 50 ml of the Fe solution to the chelating agent solution stirring for about 5 min.

Adjust the solution to pH 7,0 with NaOH solution (5.2). 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.

### 5.7 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 HCl (5.3 and 5.4). 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  $\mu\text{m}$  membrane filter (6.4 b)) and degas.

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

## 6 Apparatus

Usual laboratory equipment, glassware and the following:

### 6.1 Magnetic stirrer

### 6.2 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  $\mu\text{l}$  injection loop;



- c) a C-18 column; internal diameter :3,9 mm; column length: 150 mm; dp = 5 µm<sup>1</sup>;
- d) the use of a C-18 guard column is recommended;
- e) a UV/VIS-detector with a 280 nm-filter;
- f) an integrator.

### 6.3 Balance,

capable of weighing to an accuracy of 0,1 mg.

### 6.4 Membrane filters

- a) Micro membrane filters resistant to aqueous solutions, with porosity of 0,45 µm.
- b) Micro membrane filters resistant to organic solutions(e.g. nylon micro membrane filters), with porosity of 0,2 µm.

## 7 Preparation of the sample

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.

## 8 Procedure

[SIST EN 13368-2:2007](https://standards.iteh.ai/catalog/standards/sist/c5d99651-e6f0-404b-bc74-74a626521517/sist-en-13368-2-2007)

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### 8.1 Preparation of the sample solution

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 homogenize.

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

### 8.2 Preparation of the calibration solutions

Pipette V ml (see Table 1) of the Fe-o,o-EDDHA (5.5) or Fe-o,o-EDDHMA (5.6) standard solution into six 100 ml volumetric flasks. Make up to volume with water and homogenize.

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<sup>1</sup> SYMMETRY™ C18, from WATERS cat.nº: WAT054205, or equivalent is an example of a suitable product 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.

**Table 1 — Composition of the calibration solutions**

| Solution | V (ml) | Concentration of rac chelate | Concentration of meso chelate |
|----------|--------|------------------------------|-------------------------------|
|          |        | (mg Fe/l)                    | (mg Fe/l)                     |
| 1        | 5      | 2,5                          | 2,5                           |
| 2        | 10     | 5,0                          | 5,0                           |
| 3        | 20     | 10,0                         | 10,0                          |
| 4        | 30     | 15,0                         | 15,0                          |
| 5        | 40     | 20,0                         | 20,0                          |
| 6        | 50     | 25,0                         | 25,0                          |

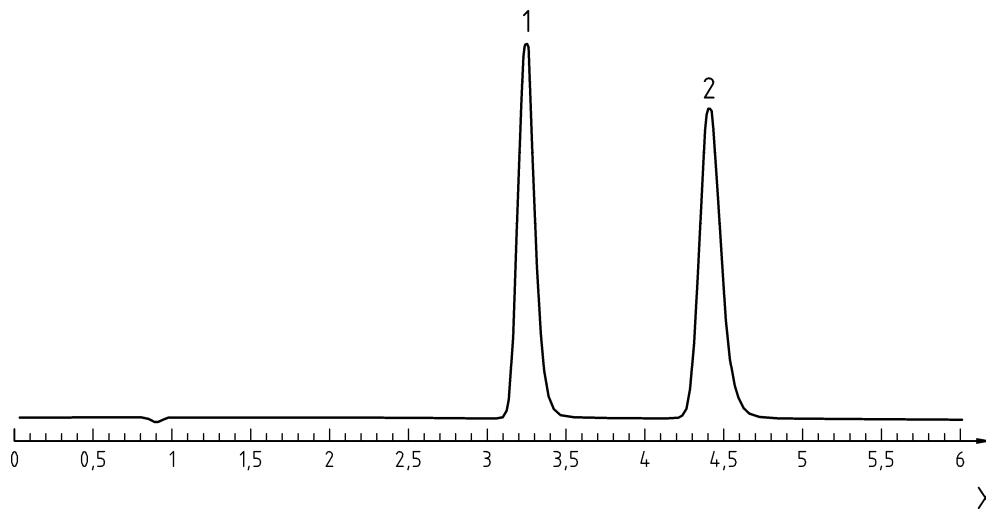
NOTE As the standard chelates solutions should be 50 % meso- and 50 % dl-racemic optical isomers, the concentration of the total iron chelate is the sum of the concentrations shown in Table 1.

**8.3 Chromatographic analysis**

Immediately before injection, all solutions shall be filtered through a 0,45 µm membrane filter (6.4 a)). Inject the standard solutions (see 8.2) into the chromatographic system (6.2). Measure the retention times and the areas of the two Fe-o,o-EDDHA or Fe-o,o-EDDHMA isomers (meso and dl-racemic) 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 Fe/l) in the form of the corresponding chelating agent isomer. See Figures 1 and 3.

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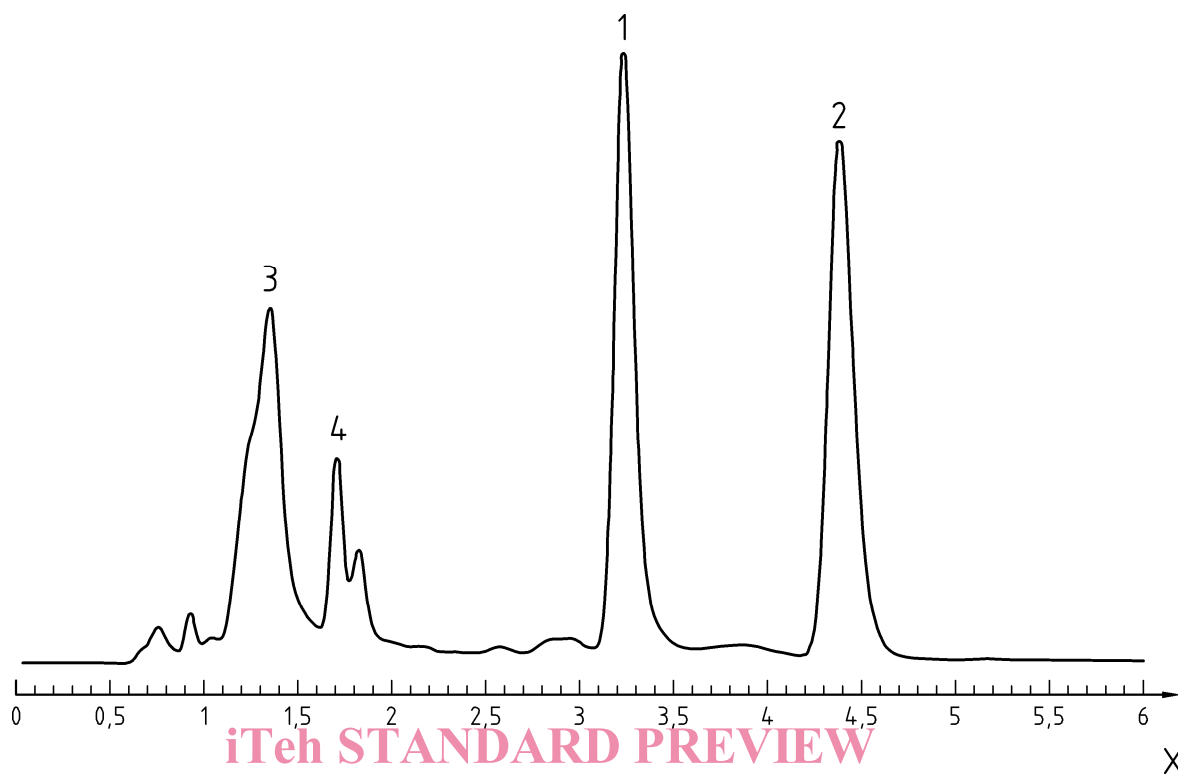
NOTE 1 See NOTE in 8.2.



**Key**

- 1 dl-racemic Fe-o,o-EDDHA
- 2 meso Fe-o,o-EDDHA
- X time in minutes

**Figure 1 — Typical chromatogram of a 25 mg Fe/l of Fe-o,o-EDDHA standard solution**

**Key**

- 1 dl-racemic Fe-o,o-EDDHA [SIST EN 13368-2:2007](https://standards.iteh.ai/catalog/standards/sist/en-13368-2-2007)
- 2 meso Fe-o,o-EDDHA <https://standards.iteh.ai/catalog/standards/sist/c5d99651-e6f0-404b-bc74-74a626531517/sist-en-13368-2-2007>
- 3 ortho-hydroxy para-hydroxy isomer
- 4 para-hydroxy para-hydroxy isomer by-product
- X time in minutes

**Figure 2 — Typical chromatogram of a commercial Fe-o,o-EDDHA product**