

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 15451:2009

<https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009>

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 15451

July 2008

ICS 65.080

Supersedes CEN/TS 15451:2006

English Version

Fertilizers - Determination of chelating agents - Determination of iron chelated by EDDHSA by ion pair chromatography

Engrais - Dosage des agents chélatants - Dosage du fer chélaté par EDDHSA par chromatographie d'appariement d'ions

Düngemittel - Bestimmung von Chelatbildnern - Bestimmung von Eisen-chelatisiertem EDDHSA mit Ionen-Paarchromatographie

This European Standard was approved by CEN on 30 May 2008.

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.

CEN members are the national standards bodies of 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.

[SIST EN 15451:2009](https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009)

<https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009>



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

Management Centre: rue de Stassart, 36 B-1050 Brussels

Contents

| | Page |
|---|------|
| Foreword..... | 3 |
| 1 Scope | 4 |
| 2 Normative references | 4 |
| 3 Principle | 4 |
| 4 Interferences | 4 |
| 5 Reagents | 4 |
| 6 Apparatus | 5 |
| 7 Sampling and sample preparation | 6 |
| 8 Procedure | 7 |
| 9 Expression of the result | 7 |
| 10 Precision for Fe ³⁺ /EDDHSA..... | 8 |
| 11 Test report | 8 |
| Annex A (informative) Chromatogram for Fe ³⁺ /EDDHSA | 10 |
| Annex B (informative) Spectra UV-visible for Fe ³⁺ /EDDHSA | 11 |
| Annex C (informative) Results of the inter-laboratory tests | 12 |
| Annex D (informative) Complete names of chelating agents | 14 |
| Bibliography | 15 |

Foreword

This document (EN 15451:2008) 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 January 2009, and conflicting national standards shall be withdrawn at the latest by January 2009.

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 CEN/TS 15451:2006.

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

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 the United Kingdom.

(standards.iteh.ai)

[SIST EN 15451:2009](https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009)

<https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009>

EN 15451:2008 (E)**1 Scope**

This European Standard specifies a method for the chromatographic determination of the total amount of iron chelated by EDDHSA in commercial products.

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 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Principle

The iron chelates are determined by ion-pair high-performance liquid chromatography. When an iron chelate (anion) is added to a polar fluid (eluent), containing a large cation (ion-pair reagent), 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. Each iron chelate presents a characteristic retention time and a characteristic spectrum 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 480 nm. For Fe³⁺/EDDHSA a gradient method is required.

4 Interferences

No interferences have been detected. Iron chelates with o,o-EDDHA, o,p-EDDHA, o,o-EDDHMA, EDTA, DTPA, CDTA, HEDTA, as well as the chelating agents do not interfere since they are separated from Fe³⁺/EDDHSA.

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 should be water grade1 according to EN ISO 3696:1995.
- c) When reagents 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 the required concentration in the solution.

5.2 Sodium hydroxide solution, c(NaOH) = 0,5 mol/l

Dissolve 20 g of NaOH in pellet form in a 1 litre-volumetric flask with water free of carbon dioxide. Dilute to the mark and homogenize.

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

Dissolve 4 g of NaOH in pellet form in a 1 litre volumetric flask with water free of carbon dioxide. Dilute to the mark and homogenize.

5.4 Hydrochloric acid solution, $c(\text{HCl}) = 1,0 \text{ mol/l}$

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

5.5 Hydrochloric acid solution, $c(\text{HCl}) = 0,1 \text{ mol/l}$

Dilute 50 ml of hydrochloric acid (5.4) to 500 ml with water.

5.6 Iron (III) nitrate solution, $\rho(\text{Fe}) = 1\,050 \text{ mg/l}$

Dissolve 0,7594 g of iron (III) nitrate nonahydrate $[\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}]$ in 100 ml of water. Transfer to a 100 ml-volumetric flask. Dilute to the mark with water and homogenize. Check (for example by AAS) that the Fe concentration of this solution is $(1\,050 \pm 30) \text{ mg/l}$.

As the $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ is deliquescent, for preparation of the Fe^{3+} /EDDHSA solution (5.7) it shall be added in solution of a known concentration.

5.7 Fe^{3+} /EDDHSA solution, $\rho(\text{Fe}) = 100 \text{ mg/l}$

Dissolve $0,233\,0 \text{ g} \times 100/P$, where P is the complexometric purity of the standard in percentage of the acid form H_6EDDHSA obtained by either manual or automatic photometric titration with a $\text{Fe}(\text{III})$ standard at constant $\text{pH} = 6$ (pH stat system), of ethylene diamine di(2-hydroxy-5-sulfophenyl) acetic acid and its condensation products (EDDHSA) in 150 ml of water and 2,7 ml of NaOH (5.2). After complete dissolution, add 25 ml of the Fe solution (5.6) to the chelating agent solution stirring about 5 min. The solution is adjusted to $\text{pH} 7,0$ with NaOH solution (5.3). 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 (250 ml) with water.

5.8 Eluent for the determination

SIST EN 15451:2009

Two solutions are used to make a linear gradient eluent:
<https://standards.iteh.ai/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009>

- Solution A: Add 3,33 ml of TBAOH [40 % (mass fraction) Tetrabutylammonium hydroxide solution in water] to 500 ml of water. Adjust pH to 6,0 with HCl (5.4 and 5.5). Add 350 ml of acetonitrile (HPLC grade) and make up to volume in a 1 litre-volumetric flask with water. Filter through 0,2 μm membrane filter (6.4 b).
- Solution B: Add 3,33 ml of TBAOH [40 % (mass fraction) Tetrabutylammonium hydroxide solution in water] to 150 ml of water. Adjust pH to 6,0 with HCl (5.4 and 5.5). Add 750 ml of acetonitrile (HPLC grade) and make up to volume in a 1 litre-volumetric flask with water. Filter through 0,2 μm -membrane filter (6.4 b).

NOTE 1 TBACl or TBABr may be used, providing that pH is adjusted to 6,0 with NaOH or HCl.

NOTE 2 Tetrabutylammonium bisulfate should be avoided because peak retention times and shape may significantly change.

6 Apparatus**6.1 General**

Usual laboratory equipment, glassware, and:

6.2 Magnetic stirrer

Magnetic stirrer with magnets.

EN 15451:2008 (E)

6.3 Chromatograph

Chromatograph equipped with:

- a) gradient pump delivering the eluent at a flow rate of 1,5 ml/min (see Table 1);
- b) injection valve with a 20 µl injection loop;
- c) C18 column; 150 mm×3,9 mm ID; dp = 5 µm¹⁾;
- d) use of a C18 guard column is recommended;
- e) UV/VIS-detector with a 480 nm-filter or diode array;
- f) integrator.

Table 1 — Gradient table

| Time min | Flow rate ml/min | A % | B % | Curve type |
|-------------|---------------------|--------|--------|------------|
| | 1,5 | 100 | 0 | Linear |
| 5 | 1,5 | 100 | 0 | Linear |
| 6 | 1,5 | 0 | 100 | Linear |
| 11 | 1,5 | 0 | 100 | Linear |
| 12 | 1,5 | 100 | 0 | Linear |
| 20 | 1,5 | 100 | 0 | Linear |

6.4 Membrane filters

- a) Micromembrane filters resistant to aqueous solutions, with porosity of 0,45 µm;
- b) Micromembrane filters resistant to organic solutions, (e.g.: nylon micromembrane filters) with porosity of 0,2 µm.

7 Sampling and sample preparation

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

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

NOTE 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 in a mortar to a particle size less than 1 mm.

1) SYMMETRY™ C18, from WATERS cat.n°: WAT054205, or equivalent are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

8 Procedure

8.1 Preparation of the sample solution of iron chelates

Weigh approximately 125 mg within 0,1 mg, into a 100 ml-beaker (for samples with more than 4 % of chelated iron weigh 80 mg and for samples with less than 2 % of chelated iron weigh 250 mg). Add 80 ml of water. Stir on a magnetic stirrer for 30 min. Dilute to the mark in a 100 ml-volumetric flask with water and homogenize.

8.2 Preparation of the calibration solutions

Pipette a volume (V ml) (see Table 2) of the Fe^{3+} /EDDHSA standard solution (5.7) in six 100 ml-volumetric flasks respectively. Make up to volume with water and homogenize.

Table 2 — Preparation of the calibration curve of Fe^{3+} /EDDHSA

| Solution | V ml | Concentration of Fe in the form of Fe^{3+} /EDDHSA mg Fe/l |
|----------|-----------|--|
| 1 | 1 | 1,0 |
| 2 | 5 | 5,0 |
| 3 | 10 | 10,0 |
| 4 | 25 | 25,0 |
| 5 | 50 | 50,0 |
| 6 | 100 | 100 |

<https://standards.iteh.ai/catalog/standards/sist/6b60bc23-d38b-4f56-9bdd-6466a7188be5/sist-en-15451-2009>

8.3 Chromatographic analysis

Immediately before injection, all solutions should be filtered through a 0.45 μm -membrane filter (6.4 a). Inject the standard solutions (8.2.) into the chromatographic system (6.3). Measure the retention times and the areas of the Fe^{3+} /EDDHSA chromatographic peaks for all solutions. Two chromatographic peaks appear for Fe^{3+} /EDDHSA, corresponding with the different forms of Fe^{3+} /EDDHSA (see Annex A). Draw the calibration graph with the added values of the two chromatographic peaks of the standard solutions of Fe^{3+} /EDDHSA versus the chelated iron concentration (mg Fe/l) in the standards.

Inject the sample solution (8.1). Identify the chelating agent by the retention time of the obtained peaks (see Annex A) and if diode array detector is used, confirm with its UV-visible spectrum (see Annex B). Measure the areas of the peaks for the sample solution corresponding with the iron chelated by EDDHSA as chelating agent. Determine the concentration of the iron chelated (mg Fe/l) using the calibration graph.

NOTE Normally the two chromatographic peaks in Fe^{3+} /EDDHSA appear overlapped. For integration baseline correction as indicated in Annex A, Figure A.1, can be used.

9 Expression of the result

9.1 Fe in Fe-chelates

In case of the iron chelates prepared as in 8.1, the percentage by weight of the Fe in the form of Fe^{3+} /EDDHSA in the fertiliser, w_{Fe} , is equal to: