SLOVENSKI STANDARD

SIST-TS CEN/TS 15452:2008

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Gnojila - Določevanje sredstev za kelatiziranje - Določevanje železovih kelatov z o,p-EDDHA s tekočinsko kromatografijo visoke ločljivosti (HPLC)

Fertilizers - Determination of chelating agents - Determination of iron chelated by o,p-EDDHA by reversed phase HPLC

Düngemittel - Bestimmung von Chelatbildnern - Bestimmung von Eisen-chelatisiertem o,p-EDDHA mit Umkehrphasen HPLC

Engrais - Dosage des agents chélatants - Dosage du fer chélaté par o,p-EDDHA par chromatographie liquide a haute performance a polarité de phase inversée

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TECHNICAL SPECIFICATION

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ICS 65.080

English Version

Fertilizers - Determination of chelating agents - Determination of iron chelated by o,p-EDDHA by reversed phase HPLC

Engrais - Dosage des agents chélatants - Dosage du fer chélaté par o,p-EDDHA par chromatographie liquide à haute performance à polarité de phase inversée

Düngemittel - Bestimmung von Chelatbildnern - Bestimmung von Eisen-chelatisiertem o,p-EDDHA mit Umkehrphasen HPLC

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Contents

Page

Foreword

This document (CEN/TS 15452:2006) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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 announce this Technical Specification: Austria, Belgium, 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.

1 Scope

This Technical Specification specifies a method for the chromatographic determination of the amount of iron chelated by each of the individual isomers of the chelating agent ortho-para EDDHA (o,p-EDDHA) in fertilizers. The method allows the identification of this chelating agent and the determination of the water soluble fraction of iron chelated by this chelating agent. The method is not applicable for the determination of the amount of free chelating agent.

NOTE 1 This method has been shown to be also suitable for the determination of the amount of iron chelated by each of the individual isomers of the chelating agent ortho-ortho EDDHA (o,o-EDDHA) in fertilizers.

NOTE 2 o,o-EDDHA and o,p-EDDHA are abbreviations used in this standard for the sake of simplicity. For complete names see Annex C.

NOTE 3 The substances o,o-EDDHA and o,p-EDDHA both exist as different stereoisomers. For o,o-EDDHA a meso form and a d/l pair (the racemic isomers) exist, for o,p-EDDHA two different d/l pairs exist. All four stereoisomers are observed separately in this method.

NOTE 4 Presently, an analytically pure standard only exists for o,o-EDDHA. The method for o,p-EDDHA has been developed with an o,p-EDDHA standard containing an uncertain concentration of o,p-EDDHA.

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.

prEN 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 technique used is reversed phase HPLC with UV detection at 277 nm. The sample is separated on a silica-based reversed phase column using sodium formate, c = 0,015 mol/l, pH = 3,0, and acetonitrile as mobile phase.

For both o,p-EDDHA and o,o-EDDHA, two stereoisomer peaks are observed.

The concentration of iron chelated by o,p-EDDHA (o,p-Fe) is determined according to the external standard method.

4 Interferences

No interferences have been detected. Iron chelates with EDTA, HEDTA, DTPA and EDDHMA do not interfere.

5 Reagents

5.1 General

a) all reagents shall be of recognized analytical grade.

b) all water should conform to EN ISO 3696.

c) when 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 the required concentration in the solution.

5.2 Sodium hydroxide solution

$c(NaOH)$ = 0,1 mol/l

Dissolve 4 g of NaOH in pellet form in water in a 1 000 ml-volumetric flask. Dilute to the mark and homogenize.

5.3 Hydrochloric acid solution

$c(HCl)$ = 4 mol/l

Dilute 395 g of concentrated hydrochloric acid (37 %) to 1 000 ml with water.

5.4 Hydrochloric acid solution

$c(HCl)$ = 0,1 mol/l

Dilute 25 ml of hydrochloric acid (5.3) to 1 000 ml with water.

5.5 Iron (III) nitrate solution

$\rho(Fe)$ = 4 200 mg/l

Dissolve 3,04 g of iron (III) nitrate nonahydrate [$Fe(NO_3)_3 \cdot 9H_2O$] in 80 ml of water. Add 1 ml of hydrochloric acid (5.3). Transfer to a 100 ml-volumetric flask. Dilute to the mark with water and homogenize. Check that the Fe concentration of this solution is 4 200 mg/l ± 100 mg/l for example by AAS or iodometric titration. This solution is stable for approximately one week.

5.6 o,p-Fe-EDDHA standard solution

ρ(o,p-Fe) = 100 mg/l

Weigh to the nearest 0,1 mg (64,5 ± 1)*100/purity (%) mg of o,p-H_4-EDDHA ($m^c_{o,p}$, see 5.1c) in a 100 ml beaker. Add 5 ml of NaOH (5.2) and carefully dissolve the sample by stirring. Add, after complete dissolution, 35 ml of water and 2,5 ml of iron (III) nitrate solution (5.5). Adjust the pH of the solution to 3,0 with NaOH (5.2) or HCl (5.4). Transfer the solution quantitatively to a 100 ml-volumetric flask. Dilute to the mark with water and homogenize.

5.7 Eluent solution

Dissolve 1,0 g of sodium formate in 800 ml of water. Adjust the pH to 3,0 with HCl (5.3 or 5.4). Transfer the solution to a 1 000 ml-volumetric flask. Dilute to the mark with water and homogenize. Filter the solution through a 0,45 µm membrane filter.

Mix 915 ml of the sodium formate solution, pH 3,0, with 85 ml of acetonitrile. Homogenize and degas the solution.

NOTE The quality and the status of the column may have an influence on the retention times. To adjust the retention times, it may be necessary to slightly change the ratio of sodium formate solution and acetonitrile.

6 Apparatus

Usual laboratory equipment, glassware, and:

6.1 Magnetic stirrer

Magnetic stirrer with magnets.

6.2 Chromatograph

equipped with:

a) isocratic pump delivering the eluent at a flow rate of 1,0 ml/min;

b) injection valve with a 20 µl injection loop;

c) C18 modified silica column (ODS-2); 250×4,6 mm ID; d_p = 5 µm[1)];

d) use of a C18 guard column (ODS-2) is recommended;

e) UV/VIS-detector with a 277-nm-filter;

f) integrator.

6.3 Membrane filters

Micromembrane filters resistant to aqueous solutions with porosity of 0,45 µm.

7 Sampling and sample preparation

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

Sample preparation shall be carried out in accordance with prEN 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.

8 Procedure

8.1 Preparation of the sample solution

Weigh to the nearest 0,1 mg approximately (250 ± 10) mg of sample (m_s) in a 250 ml-beaker. Add 200 ml of water and dissolve the sample by stirring for 30 min. Transfer the solution quantitatively to a 250 ml-volumetric flask. Dilute to the mark with water and homogenize.

NOTE If the o,p-Fe concentration is lower than 0,5 % or higher than 5 % the concentration of these iron chelates will be outside the calibration range. For a more accurate determination the intake should be accordingly either increased or decreased.

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1) Spherisorb ODS-2 from Waters or Allsphere ODS-2 from Alltech 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.

8.2 Preparation of the standard solutions

Pipette a volume (V ml) (see Table 1) of the o,p-Fe-EDDHA standard solution (5.6) in six 50 ml-volumetric flasks. Dilute to the mark with water and homogenize. These solutions contain iron chelated by o,p-EDDHA in the approximated concentrations given in Table 1.

The exact mass concentration of iron chelated by o,p-EDDHA in milligrams per litre is given by:

$$\rho_{o,p-Fe} = \rho_{d/l-1} + \rho_{d/l-2}$$

where:

$$\rho_{d/l-1} = \rho_{d/l-2} = 0{,}5 \times \frac{V}{50} \times \frac{m^{c}_{o,p}}{0{,}1} \times \frac{P}{100} \times \frac{55{,}847}{360{,}35}$$

V is the amount of standard solution used in millilitre

$m^{c}_{o,p}$ is the amount of o,p-H_4-EDDHA used for the preparation of the standard solution in milligrams

P is the purity of the calibration standard in percent

NOTE It is assumed that the o,p-EDDHA acid standard contains equal amounts of the two stereoisomers.

Table 1 — Preparation of the standard solutions

Solution	V	o,p-EDDHA	
		d/l isomer-1	d/l isomer-2
	ml	Fe mg/l	Fe mg/l
1	2,5	2,5	2,5
2	5	5,0	5,0
3	10	10,0	10,0
4	15	15,0	15,0
5	20	20,0	20,0
6	25	25,0	25,0

8.3 Chromatographic analysis

Immediately before injection, all solutions should be filtered through a 0,45 µm-membrane filter (6.3). Inject the standard solutions (8.2) into the chromatographic system (6.2). Measure the retention times and the areas of the two o,p-Fe-EDDHA isomers (d/l-1 and d/l-2) for all solutions. Draw a different calibration line for each of the two isomers with the value of the peak areas of the standard solutions versus the exact concentration of Fe (mg/l) chelated by each chelating agent isomer.

Inject the sample solution (8.1). Identify the different isomers by the retention time of the obtained peaks (see Figure A.1). Measure the area of the peak for each isomer. Determine the concentration of the iron chelated (mg/l) for each isomer using the corresponding calibration line.