BRITISH STANDARD

BS EN 15452:2008

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

ICS 65.080

British Standards

National foreword

This British Standard is the UK implementation of EN 15452:2008. It supersedes DD CEN/TS 15452:2006 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CII/37, Fertilisers and related chemicals.

A list of organizations represented on this committee can be obtained on request to its secretary.

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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

Foreword

This document (EN 15452:2008) 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of an identical text or by endorsement, at the latest by January 2009, and conflicting national standards shall be withdrawn at the latest by January 2009.

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1 Scope

This European Standard 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 European 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 Currently, 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.

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

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 3696:1995, grade 1.

c) When products with a declared purity (P) 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, ρ(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/ ± 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) mg ×100/P, where P is the complexometric purity of the standard in percentage of the acid form o,p-H_4-EDDHA obtained by either manual or automatic photometric titration with a Fe(III)standard at constant pH = 6 (pH stat system), 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

6.1 General

Usual laboratory equipment, glassware, and:

6.2 Magnetic stirrer

Magnetic stirrer with magnets.

6.3 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 µm1);

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

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

f) integrator.

6.4 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 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.

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:

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

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

where:

V is the amount of standard solution used in millilitre;

$m^{\mathrm{c}}{}_{\mathrm{o,p}}$ 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 ml	o,p-EDDHA	
		d/l isomer-1	d/l isomer-2
		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 (6.4). Inject the standard solutions (8.2) into the chromatographic system (6.3). 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.

9 Expression of results

The mass fraction, w, in percent of Fe chelated by o,p-EDDHA in the fertilizer is equal to:

$$w_{\mathrm{o,p-Fe}} = \frac{\rho_{\mathrm{o,p-d/l-1}} + \rho_{\mathrm{o,p-d/l-2}}}{m_{\mathrm{s}}} \times 25 \qquad (3)$$

where

$\rho_{\mathrm{o,p\text{-}d/l\text{-}1}}$	is the mass concentration of Fe chelated by the first set of stereoisomers (d/l-1) of o,p-EDDHA, in milligram per litre
$\rho_{\mathrm{o,p\text{-}d/l\text{-}2}}$	is the mass concentration of Fe chelated by the second set of stereoisomers (d/l-2) of o,p-EDDHA, in milligram per litre
m_{s}	is the mass of the sample taken for analysis, in milligrams.

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test was carried out in 2005 with 12 participating laboratories and 4 different commercial samples. The results of this inter-laboratory test are summarized in Annex B. Repeatability and reproducibility were calculated according to ISO 5725-2 [2].

10.2 Repeatability

The absolute difference between two independent test results obtained with the same method on identical test material, in the same laboratory, by the same operator, using the same equipment within a shot interval of time, will in not more than 5 % of the cases exceed the values of r given in Table 2.

10.3 Reproducibility

The absolute difference between two single test results obtained with the same method on identical test material, in different laboratories, by different operators, using different equipment, will in not more than 5 % of the cases exceed the values of R given in Table 2.

Table 2 — Mean values, repeatability and reproducibility limits

Sample	o,p-Fe %	p	n	r	R
Sample 1	2,17	10	2	0,037	0,25
Sample 2	1,33	10	2	0,051	0,29
Sample 3	0,47	9	2	0,047	0,16
Sample 4	0,15	8	2	0,0070	0,25

o,p-Fe: overall mean concentration in percent of Fe chelated by o,p-EDDHA
p: number of participating laboratories
n: number of replicates
r: repeatability limit, in g/100 g of o,p-Fe
R: reproducibility limit, in g/100 g of o,p-Fe

NOTE The reproducibility limits obtained for the lower concentrations of Fe chelated by o,p-EDDHA are higher than the tolerances recognized by Regulation (EC) 2003/2003 [3] for micronutrient content.

11 Test report

The test report shall include at least the following information:

a) All information necessary for complete identification of the sample;

b) Test method used, making reference to this document, i.e. EN 15452;

c) Test results together with the units used to express them;

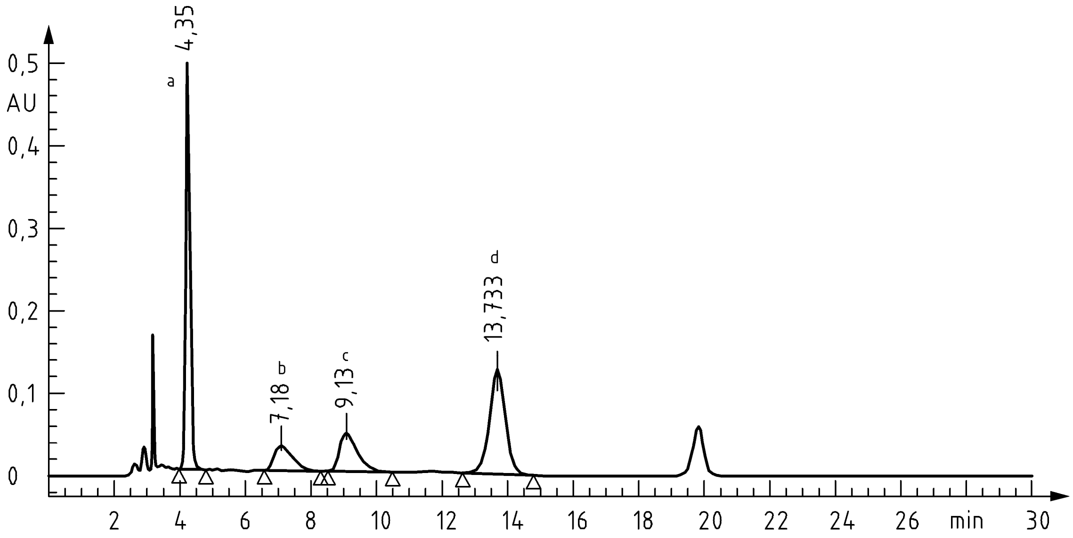
d) Date on which the test was finished;

e) Statement as to whether the requirement for the repeatability limit has been fulfilled;

f) All the procedural steps not specified in this document or carried out optionally, as well as details of any circumstances that occurred while carrying out the method that may have influenced the result(s).

Annex A
(informative)

Typical chromatogram



Key

a racemic o,o-Fe-EDDHA

b d/l-1 o,p-Fe-EDDHA

c d/l-2 o,p-Fe-EDDHA

d meso o,o-Fe-EDDHA

Figure A.1 — Typical chromatogram of a commercial Fe-EDDHA product

Annex B
(informative)

Statistical results of the inter-laboratory test

The statistical results of the inter-laboratory test are given in Table B.1.

Table B.1 — Statistical results

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Number of laboratories	11	11	11	9
Number of outliers	1	1	2	1
Remaining laboratories	10	10	9	8
Mean value, g/100 g	2,17	1,33	0,47	0,15
Repeatability standard deviation (s_r), g/100 g	0,013	0,018	0,017	0,0025
Repeatability limit (r), g/100 g	0,037	0,051	0,047	0,0070
RSD_r, %	0,61	1,4	3,6	1,7
Reproducibility standard deviation (s_R), g/100 g	0,089	0,10	0,057	0,090
Reproducibility limit (R), g/100 g	0,25	0,29	0,16	0,25
RSD_R, %	4,1	7,8	12,3	61,2
Horwitz RSD_H, %	3,6	3,8	4,5	5,3
Horrat index	1,15	2,04	2,75	11,5

Annex C
(informative)

Complete names of chelating agents

o,o-EDDHA	ethylenediamine-di-(o-hydroxyphenyl)acetic acid	
	$C_{18}H_{20}N_2O_6$	CAS-No. 1170-02-1
o,p-EDDHA	ethylenediamine-N-[(ortho-hydroxyphenyl)acetic acid]-N’-[(para-hydroxyphenyl)acetic acid]	
	$C_{18}H_{20}N_2O_6$	CAS-No. 1170-02-1
EDTA	ethylenediaminetetraacetic acid	
	$C_{10}H_{16}N_2O_8$	CAS-No. 60-00-4
HEDTA	hydroxyethylethylenediamine triacetic acid	
	$C_{10}H_{18}N_2O_7$	CAS-No. 150-39-0
DTPA	diethylenetriaminepentaacetic acid	
	$C_{14}H_{23}N_3O_{10}$	CAS-No. 67-43-6
o,o-EDDHMA	ethylenediamine-di-(o-hydroxy-p-methyphenyl)acetic acid	
	$C_{20}H_{24}N_2O_6$	CAS-No. 109172-81-8

Bibliography

[1] EN 1482-1, *Fertilizers and liming materials — Sampling and sample preparation — Part 1: Sampling*

[2] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[3] *Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers,* Official Journal L 304, 21/11/2003 Pp. 1-194

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

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