BRITISH STANDARD

BS EN
15451:2008

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

ICS 65.080

British Standards

National foreword

This British Standard is the UK implementation of EN 15451:2008. It supersedes DD CEN/TS 15451:2006 which is withdrawn.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.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was
published under the authority
of the Standards Policy and
Strategy Committee
on 29 August 2008

ISBN 978 0 580 60411 9

Amendments/corrigenda issued since publication

Date	Comments

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.

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

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

Ref. No. EN 15451:2008: E

Contents

Page

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.

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 Fe^{3+}/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 Fe^{3+}/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(HCl) = 1,0 mol/l

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

5.5 Hydrochloric acid solution, c(HCl) = 0,1 mol/l

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

5.6 Iron (III) nitrate solution, ρ(Fe) = 1 050 mg/l

Dissolve 0,7594 g of iron (III) nitrate nonahydrate [$Fe(NO_3)_3 \cdot 9H_2O$] 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 ± 30) mg/l.

As the $Fe(NO_3)_3 \cdot 9H_2O$ 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, ρ(Fe) = 100 mg/l

Dissolve 0,233 0 g×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 Fe(III)standard at constant 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 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

Two solutions are used to make a linear gradient eluent:

a) 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).

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.

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.nº: 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

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:

$$w_{\mathrm{Fe}} = \frac{\rho}{m} \times 0{,}01$$

where

ρ is the mass concentration of Fe in milligrams per litre of the Fe chelated by EDDHSA determined with the calibration graph;

m is the mass of the sample taken for analysis in grams.

10 Precision for Fe^{3+}/EDDHSA

10.1 Inter-laboratory test

Two inter-laboratory tests have been carried out in 2004 and 2005 with 13 and 9 participating laboratories respectively. Three different samples were analyzed. The inter-laboratory tests yielded the data summarized in Annex C. Repeatability and reproducibility were calculated according to ISO 5725-2 [2].

The values derived from the inter-laboratory test in 2005 are presented as the repeatability and reproducibility data of the method presented in 10.2 and 10.3. These results may not be applicable to concentration ranges and matrices other than those given in Annex C.

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 short interval of time, will, in not more than 5 % of the cases, exceed the values of r given in Table 3.

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 3.

Table 3 — Mean values, repeatability and reproducibility limits

Sample	$\bar{x}$	r	R
Fe-EDDHSA-1	3,54	0,08	1,00
Fe-EDDHSA-2	2,82	0,04	0,51
Fe-EDDHSA-3	1,71	0,07	0,44

NOTE Reproducibility limits obtained for iron chelated by EDDHSA are higher than the tolerances recognized by Regulation (EC) 2003/2003 [3] (see Bibliography) for micro nutrients 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 15451;

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

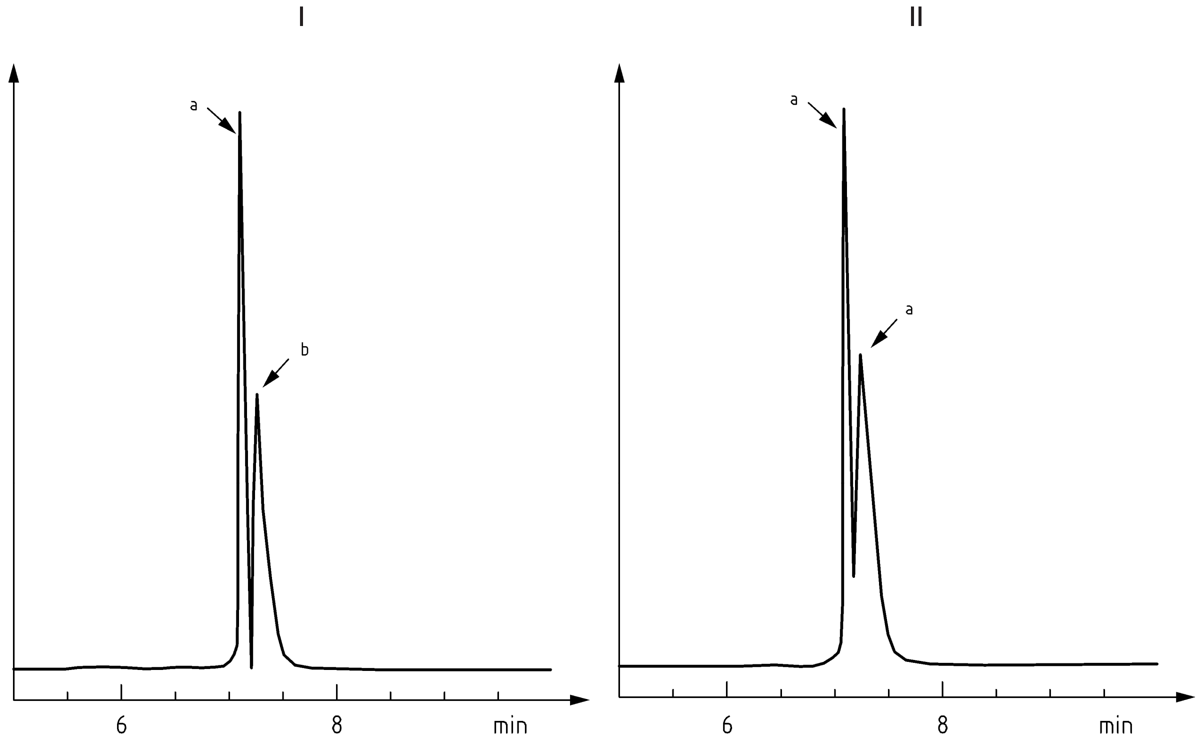
d) date the test was finished;

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)

Chromatogram for Fe^{3+}/EDDHSA



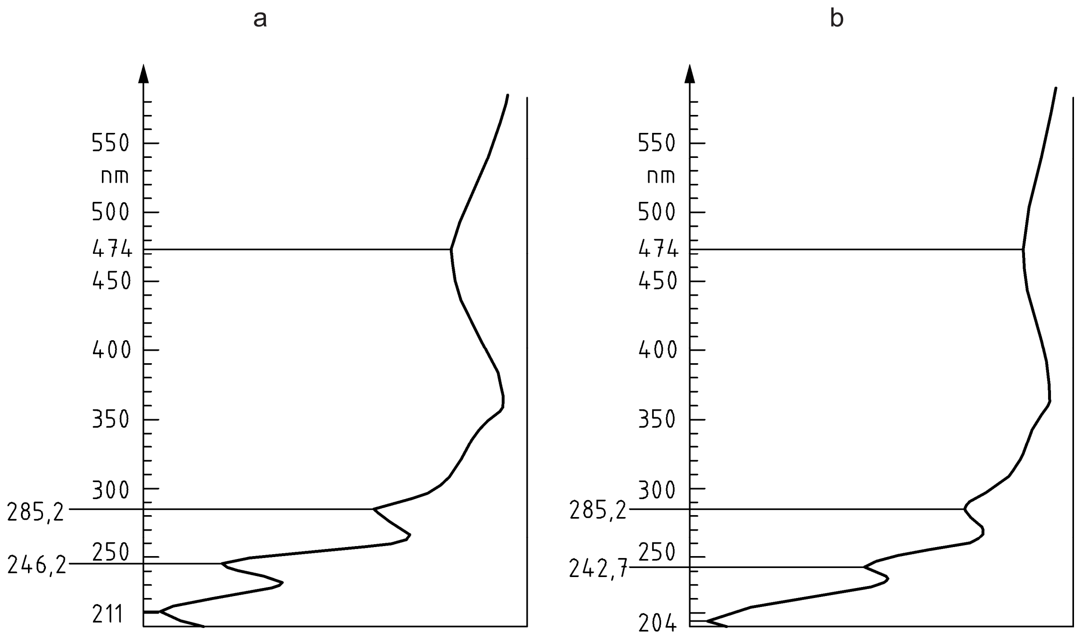
Key

I Commercial sample Fe^{3+}/EDDHSA
II Fe^{3+}/EDDHSA standard (25 $mg \cdot l^{-1}$ Fe)
a and b Different forms of Fe^{3+}/EDDHSA

Figure A.1 — Chromatograms

Annex B
(informative)

Spectra UV-visible for Fe^{3+}/EDDHSA



Key

a and b Different forms of Fe^{3+}/EDDHSA

Figure B.1 — Spectra UV-visible for Fe^{3+}/EDDHSA

Annex C
(informative)

Results of the inter-laboratory tests

C.1 Test samples

Three different commercial Fe chelates containing Fe^{3+}/EDDHSA (one liquid and two solids) were provided to all the participants. Also a standard with EDDHSA as chelating agent (purity of 74,5 %) was provided.

C.2 Inter-laboratory test procedure

The inter-laboratory test was done in 2004 and with modifications to the method repeated in 2005. The test samples were sent in 2004 to 14 laboratories from 6 countries, private ones as well as official ones, but only 13 provided results. In 2005 samples were sent to the 12 laboratories from 4 countries that accepted to participate and 9 laboratories presented results.

The participating laboratories were requested to perform two replicates of each sample. The results of the two replicates of each sample must be done with two decimals.

Test results, observations and remarks have been reported on the appropriate sheets.

C.3 Results and statistical interpretation

Statistical calculations have been run on all the results, according to ISO 5725-2 .[2]

Parameters of repeatability and reproducibility have been evaluated for each sample (mean value, standard deviation of repeatability, standard deviation of reproducibility, repeatability, reproducibility, relative standard deviation of repeatability and relative standard deviation of reproducibility).

Table C.1 shows the statistical results of the ring test on repeatability and reproducibility.

Table C.1 — Statistical results

Parameter	Ring test 2004			Ring test 2005		
	Sample			Sample		
	Fe-EDDHSA-1	Fe-EDDHSA-2	Fe-EDDHSA-3	Fe-EDDHSA-1	Fe-EDDHSA-2	Fe-EDDHSA-3
Number of laboratories	13	13	13	9	9	9
Number of outliers	0	1	2	1	1	0
Remaining laboratories	13	12	11	8	8	9
Mean value, gFe/100 g	3,43	2,77	1,66	3,54	2,82	1,71
Repeatability standard deviation (s_r), gFe/100 g	0,05	0,03	0,01	0,03	0,01	0,03
Repeatability limit (r), gFe/100 g	0,15	0,08	0,04	0,08	0,04	0,07
RSD_r, %	1,6	0,98	0,89	0,85	0,44	1,5
Reproducibility standard deviation (s_R), g/100 g	0,49	0,17	0,27	0,36	0,18	0,16
Reproducibility limit (R), gFe/100 g	1,36	0,47	0,76	1,00	0,51	0,44
RSD_R, %	14,2	6,1	16,4	10,1	6,5	9,2
Horwitz Value R	3,3	3,4	3,7	3,3	3,4	3,7
Horrat R index	4, 3	1,8	4,4	3,0	1,9	2,5

Annex D
(informative)

Complete names of chelating agents

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-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
o,o-EDDHMA	ethylenediamine-di-(o-hydroxy-p-methyphenyl)acetic acid	
	$C_{20}H_{24}N_2O_6$	CAS-No. 109172-81-8
EDDHSA	ethylenediamine-di-(o-hydroxysulfophenyl)acetic acid and its condensation products	
	$C_{18}H_{20}N_2O_{12}S_2$	CAS-No. 57368-07-7 and 642045-40-7
CDTA	cyclohexylenediaminetetraacetic acid	
	$C_{14}H_{22}N_2O_8$	CAS-No. 482-54-2

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] GARCIA-MARCO, S.; CREMONINII, M. A.; ESTEBAN, P.; YUNTA, F.; HERNANDEZ-APAOLAZA, L.; PLACUCCI,, G. and LUCENA, J. J., *Gradient ion-pair chromatographic method for the determination of iron N,N'-ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate) by HPLC/APIES-MS.*, *Journal of Chromatography A*, 2005, n° 1064, pp. 67-74.

British Standards Institution (BSI)

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover.
Tel: +44 (0)20 8996 9000 Fax: +44 (0)20 8996 7400

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services.
Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001
Email: orders@bsigroup.com

You may also buy directly using a debit/credit card from the BSI Shop on the Website http://www.bsigroup.com/shop.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre.
Tel: +44 (0)20 8996 7111 Fax: +44 (0)20 8996 7048
Email: info@bsigroup.com

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration.
Tel: +44 (0)20 8996 7002 Fax: +44 (0)20 8996 7001
Email: membership@bsigroup.com

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsigroup.com/BSOL.

Further information about BSI is available on the BSI website at http://www.bsigroup.com.

Copyright

Details and advice can be obtained from the Copyright & Licensing Manager.
Tel: +44 (0)20 8996 7070 Email: copyright@bsigroup.com

BSI Group Headquarters
389 Chiswick High Road,
London W4 4AL, UK
Tel +44 (0)20 8996 9001
Fax +44 (0)20 8996 7001
www.bsigroup.com/standards