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Fertilizers — Determination of N-(1,2-dicarboxyethyl)-D,L-aspartic acid (Iminodisuccinic acid, IDHA) using high-performance liquid chromatography (HPLC)

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National foreword

This British Standard is the UK implementation of EN 15950:2010.

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Engrais - Dosage de l'acide N-(1,2-dicarboxyéthyl)-D,L aspartique (acide iminodisuccinique, IDHA) par chromatographique liquide haute performance (HPLC)

Düngemittel - Bestimmung von N-(1,2-Dicarboxyethyl)-D,L-asparaginsäure (Iminodibernsteinsäure, IDHA) mit Hochleistungs-Flüssigchromatographie (HPLC)

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Foreword

This document (EN 15950:2010) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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1 Scope

This European Standard specifies a method for the determination of N-(1,2-dicarboxyethyl)-D,L-aspartic acid (Iminodisuccinic acid (IDHA)) in fertilizers.

The method is applicable to all fertilizers containing IDHA as chelating agent for contents > 0,5 % (g/100 g).

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 12944-1:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 1: General terms*

EN 12944-2:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 2: Terms relating to fertilizers*

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1:1999 and EN 12944-2:1999 apply.

4 Principle

IDHA is extracted with water, and measured and detected by reversed phase HPLC using UV detection at 260 nm after total conversion of the chelating agent into its iron-(III)-chelate by adding an excess of a solution of iron-(III)-nitrate (details see Clause 8 and the following). In the HPLC-chromatogram IDHA is represented by two dominant peaks, the first one representing the R,S-isomer (50 %), and the second one representing the R,R- as well as the S,S-isomer (each 25 %).

5 Interferences

Other chelating agents such as DTPA, o,o-EDDHA or o,p-EDDHA do not interfere the determination of IDHA. EDTA may interfere with the determination of IDHA with some equipment, especially with certain columns in the HPLC-equipment (see Table A.2).

6 Reagents

Use only reagents of recognized analytical grade.

6.1 **Water,** distilled or demineralized (grade 1 according to EN ISO 3696:1995).

6.2 **Tetra-n-butylammonium hydrogen sulfate** ($C_{16}H_{37}NO_4S$), for ion pair chromatography.

Also tetra-n-butylammonium bromide or -chloride may be used. In that case the pH needs to be adjusted (see 6.9).

6.3 Tetra-n-butylammonium hydroxide, w ($C_{16}H_{37}NO$) = 40 % in water.

6.4 Iron (III) nitrate nonahydrate, p.a.

6.5 Nitric acid, $w(HNO_3)$ = 65 %, p.a.

6.6 Hydrochloric acid, c(HCl) = 1 mol/l and c(HCl) = 0,1 mol/l.

6.7 Sodium hydroxide, c(NaOH) = 1 mol/l and c(NaOH) = 0,1 mol/l.

6.8 Buffer solution, pH = 8,0.

Adjust 0,1 mol/l boron as boric/borate buffer at pH = 8,0 with hydrochloric acid or sodium hydroxide.

NOTE Commercial buffers may be used. The buffer solution should not contain any phosphate or chelating agents like EDTA or others.

6.9 Eluent solution.

In a 1 l volumetric flask dissolve 2,5 g tetra-n-butylammonium hydrogen sulfate (6.2) (= 7,36 mmol), 1,7 ml tetra-n-butylammonium hydroxide (6.3) and 0,04 ml nitric acid (6.5) in water and make up to volume. The pH of this solution is approximately 2,5.

In the case that tetra-n-butylammonium bromide or -chloride is used instead of tetra-n-butylammonium hydrogen sulfate, weigh 2,37 g or 2,05 g respectively and adjust pH to approximately 2,5 with additional nitric acid (6.5).

6.10 Derivatisation reagent.

In a 100 ml volumetric flask dissolve 4,4 g tetra-n-butylammonium hydrogen sulfate (6.2) (= 12,95 mmol) and 1,5 g iron(III)nitrate nonahydrate (6.4) in water and make up to volume. The pH of this solution is approximately 1,5.

In the case that tetra-n-butylammonium bromide or -chloride is used instead of tetra-n-butylammonium hydrogen sulfate, weigh 4,17 g or 3,60 g respectively and adjust pH to approximately 1,5 with additional nitric acid (6.5).

7 Apparatus

Laboratory equipment and glassware for preparation of solutions and dilutions.

7.1 Analytical balance, capable of weighing to an accuracy of ± 0,1 mg.

7.2 pH-meter with electrode.

7.3 HPLC-system, with Diode Array Detector (DAD) or UV-detector.

7.4 Membrane filters, micro membrane filters resistant to aqueous solutions, with porosity of 0,45 µm.

8 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.

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 with a mortar. Special care shall be taken with NPK samples due to their high hygroscopicity.

9 Procedure

9.1 System parameters of HPLC

— Analytical/separating column:	silica column with C18 or C8 reverse phase, 5 µm, 250 mm × 4,6 mm [1)] The use of a guard column is recommended.
— Detection wavelength:	260 nm
— Eluent:	according to 6.9
— Flow rate:	0,5 ml/min
— Temperature:	Constant between 20 °C and 40 °C
— Run time:	20 min
— Injection volume:	20 µl

9.2 Calibration

9.2.1 Stock IDHA solution: ρ(IDHA) = 1 000 mg/l

Weigh 338,3 mg × 100/R, where R is the purity of the tetra sodium salt of IDHA in percent, equivalent to 250,0 mg of free acid, into a 250 ml beaker, add about 150 ml of water (6.1) and dissolve either by using an ultrasonic bath or stirring on a magnetic stirrer for about 15 min. When using an ultrasonic bath, the solution should be cooled down to room temperature before the next step.

Measure the pH of the solution. By the use of hydrochloric acid (6.6) or sodium hydroxide (6.7) and the pH-meter (7.2) adjust the pH to 8,0 ± 0,1. Then add 20 ml of buffer solution (6.8). Transfer into a 250 ml volumetric flask, make up to volume and homogenize. This solution shall be used on the day of its preparation.

9.2.2 Calibration solution

Into six beakers (e.g. 25 ml) take volumes from the stock solution (9.2.1) according to Table 1, make up to 10 ml with water (6.1) and add 3 ml of the derivatisation reagent (6.10).

Homogenize and filtrate the solution by using the micro membrane filters (7.4) into the auto sampler vial.

Inject the standard solutions into the chromatographic system. The evaluation of calibration is carried out manually or by means of a suitable PC-aided (computerized) calculation method. Measure the retention times

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1) LiChrosorb RP-18 or RP-8 5 µm 250/4,6 mm or equivalent is an example of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute any endorsement by CEN of these products.

and the areas of the two IDHA isomer peaks for all solutions. Draw a calibration line with the sum of the peak areas of the standard solutions versus the IDHA concentration (mg/l), according to Table 1.

Table 1 — Preparation of calibration solutions

Parameter	ml of stock solution	ml of water	Content of IDHA mg/l
Standard 1	1,00	9,00	100
Standard 2	2,00	8,00	200
Standard 3	4,00	6,00	400
Standard 4	6,00	4,00	600
Standard 5	8,00	2,00	800
Standard 6	10,00	0,00	1 000

9.3 Preparation of the test solution

Weigh an amount of the sample grounded to < 0,25 mm according to Table 2 to the nearest 0,1 mg and flush into a 250 ml beaker, add about 150 ml of water (6.1) and solve either by using an ultrasonic bath or by stirring on a magnetic stirrer for about 15 min. When using an ultrasonic bath, the solution should be cooled down to room temperature before the next step.

Table 2 — Preparation of sample solutions

Sum of chelated micro-nutrients in the sample % (e.g. Fe, Cu, Mn, Zn, Co)	Weight of the sample portion g
> 5	0,25
> 1 to ≤ 5	1,0
≤ 1	5,0

Using hydrochloric acid (6.6) or sodium hydroxide (6.7) and the pH-meter (7.2) adjust the pH to 8,0 ± 0,1. Then add 20 ml of buffer solution (6.8). Transfer into a 250 ml volumetric flask, make up to volume with water (6.1) and homogenize. Filtrate the solution by using the micro membrane filters (7.4) (about 7 ml to 10 ml). This solution shall be used on the day of its preparation.

Transfer 5,00 ml into a beaker (e.g. 25 ml) and add 1,5 ml of the derivatisation reagent (6.10) and make homogeneous. Filtrate the solution by using the micro membrane filters (7.4) into the auto sampler vial.

In the case that no auto sampler is available, manually inject 20 µl of the filtrated solution.

9.4 Measurement

Run the chromatographic analysis and identify the IDHA isomers by the retention time of the obtained peaks (see Figure B.2). Measure the sum of the areas of both peaks. Determine the concentration of IDHA using the calibration graph (see Figure B.3).

The concentration of IDHA in the sample solutions shall be kept within the calibration limits to ensure sufficient reproducibility.

10 Calculation

10.1 Calculation of the content of IDHA in g/100 g of sample

The calculation may be performed manually or by means of a PC using the calibration parameters in respect to the amount used.

In the case of PC-aided (computerized) calculation and application of Table 1 regarding the amounts of stock solution, the concentration of IDHA in milligrams per litre will be calculated by the system.

Calculate the mass fraction of the sum of R,S- and R,R- and S,S-isomers of IDHA (free acid), R_{IDHA}, in g/100 g according to Equation (1).

$$R_{\mathrm{IDHA}} = \frac{A}{40 \times E} \qquad (1)$$

where

A is the IDHA concentration, in milligrams per litre, calculated by the system from the calibration graph;

E is the mass of the test portion, in grams.

10.2 Calculation of the content of IDHA in mmol/100 g of sample

Calculate the content of IDHA, w_{IDHA}, in mmol/100 g according to Equation (2).

$$w_{\mathrm{IDHA}} = \frac{R \times 1000}{249{,}1} \qquad (2)$$

where

R is the mass fraction of IDHA in grams per 100 g of the sample;

249,1 is the mole weight of the free acid of IDHA.

11 Precision for IDHA

11.1 Inter-laboratory test

Two inter-laboratory tests have been carried out in 2008 with eleven and ten participating laboratories and nine different samples of fertilizers. Repeatability and reproducibility were calculated according to ISO 5725-2.

The values derived from this inter-laboratory test may not be applicable to concentration ranges and matrices other than those given in Annex A.

11.2 Repeatability

The absolute difference between two independent single 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.

11.3 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 values of R given in Table 3.

Table 3 — Mean values, repeatability and reproducibility limits

Sample	$\bar{x}$ %	r %	R %
NPK 15-10-15	1,77	0,13	1,11
NPK 10-5-15	2,32	0,27	1,15
NPK 15-15-15	2,03	0,38	1,56
NPK 15-10-20	2,13	0,12	1,93
Cu(IDHA) solid	40,59	1,91	9,22
Fe(IDHA) solid	37,68	2,79	16,83
Fe(IDHA) solution	11,71	0,86	2,81
Mn(IDHA) solid	41,80	0,96	5,42
Zn(IDHA) solid	40,80	1,51	11,91

12 Test report

The test report shall contain at least the following information:

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

b) test method used with reference to this document;

c) test results obtained;

d) date of sampling and sampling procedure (if known);

e) date when the analysis was finished;

f) whether the requirement of the repeatability limit has been fulfilled;

g) all operating details not specified in this document, or regarded as optional, together with details of any incidents occurred when performing the method, which might have influenced the test result(s).

Annex A
(informative)

Results of the inter-laboratory test

The precision of the method has been determined in the year 2008 in two inter-laboratory trials with ten and eleven laboratories participating and carried out on nine samples of fertilizer. The statistical results are given in Table A.1.

Table A.1 — Statistical results of the inter-laboratory test

Parameter	**NPK 15-15-15**	**NPK 15-10-20**	**NPK 15-10-15**	**NPK 10-5-15**	**Cu(IDHA) solid**	**Fe(IDHA) solid**	**Fe(IDHA) solution**	**Mn(IDHA) solid**	**Zn(IDHA) solid**
Year of the test	2008	2008	2008	2008	2008	2008	2008	2008	2008
Number of participating laboratories	10	10	10	10	10	11	11	10	10
Number of laboratories after eliminating outliers	10	8	9	9	10	11	11	8	10
Level mean value (g/100g)	2,03	2,13	1,77	2,32	40,59	37,68	11,71	41,80	40,80
Repeatability standard deviation s_r (g/100g)	0,14	0,04	0,05	0,10	0,69	1,01	0,31	0,35	0,54
Coefficient of variation CV_r (%)	6,85	1,98	2,67	4,20	1,70	2,67	2,64	0,83	1,33
Repeatability limit r (2,77 × s_r) (g/100g)	0,38	0,12	0,13	0,27	1,91	2,79	0,86	0,96	1,51
Reproducibility standard deviation s_R (g/100g)	0,56	0,70	0,40	0,41	3,33	6,08	1,01	1,96	4,30
Coefficient of variation CV_R (%)	27,81	32,80	22,71	17,84	8,21	16,12	8,66	4,69	10,54
Reproducibility limit R (2,77 × s_R) (g/100g)	1,56	1,93	1,11	1,15	9,22	16,83	2,81	5,42	11,91

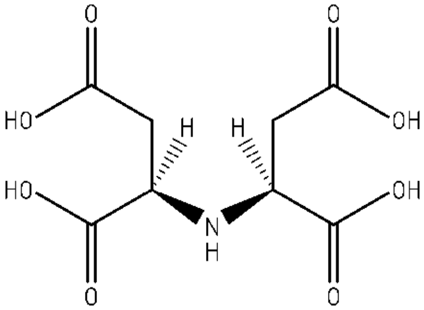
Table A.2 — Retention times of IDHA-isomers and EDTA

Laboratory	**Retention times** min	
	R,S-Isomer	**EDTA**
01	7,23	10,69
02	7,47	8,18
03	13,50	16,55
04	12,1	14,3
05	7,12	11,32
06	10,0	11,1
07	7,57	8,85
09	11,4	14,3
10	10,33	12,05

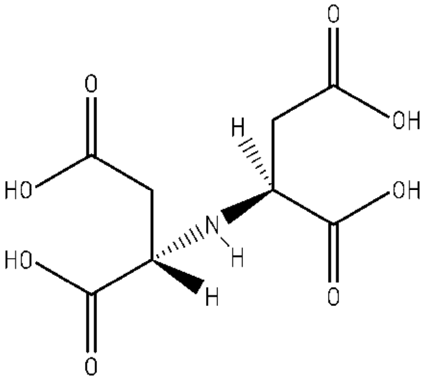
Annex B
(informative)

Stereo isomers of IDHA, chromatogram and calibration curve

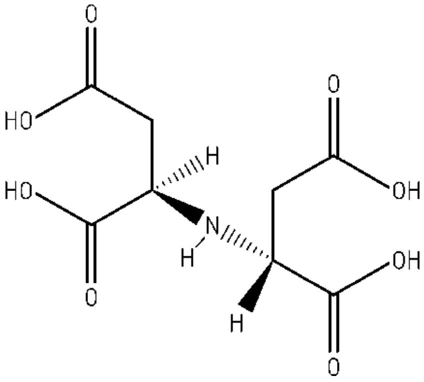
B.1 Stereo isomers of IDHA



a) R,S-isomer



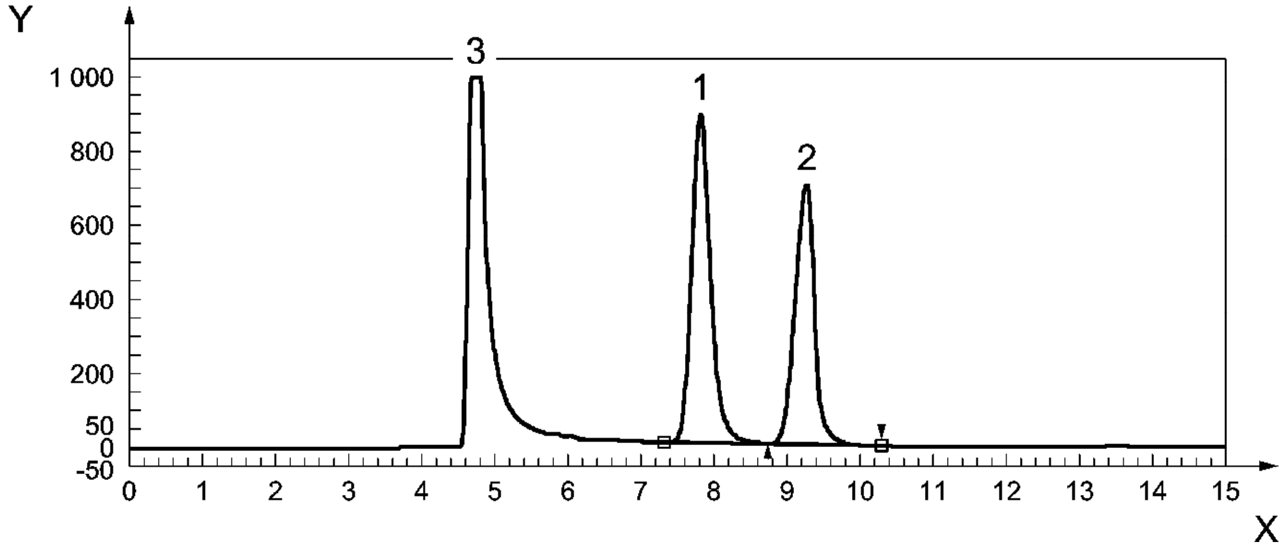
b) S,S-isomer



c) R,R-isomer

Figure B.1 — Scheme of the stereo isomers of IDHA

B.2 Chromatogram



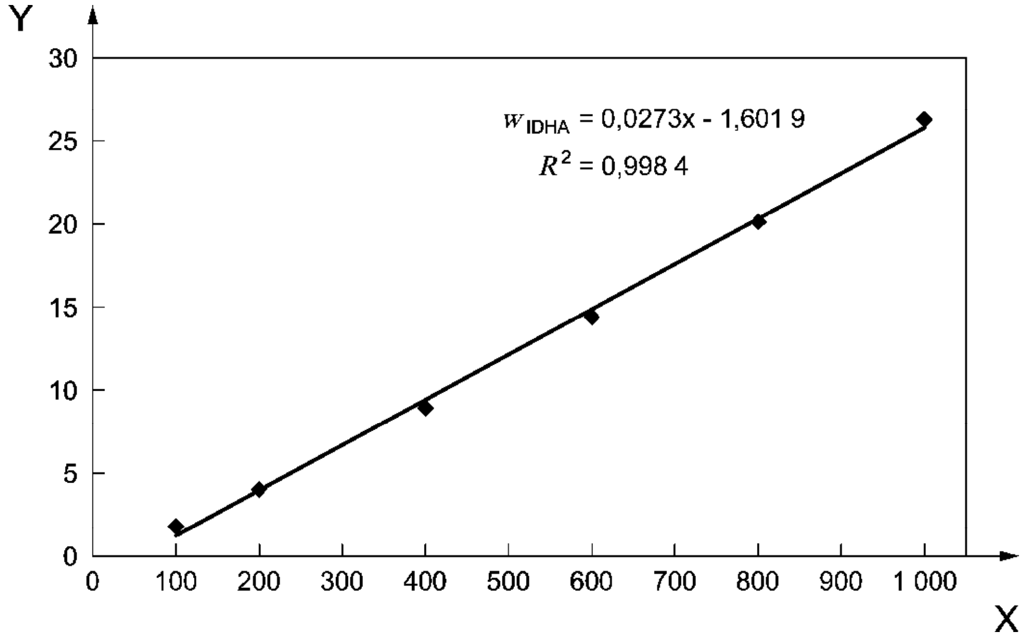
Key

X	Retention time (minutes)
1	R,S-isomer
2	R,R-S,S-isomers
3	Derivatization reagent
Y	Intensity (millivolts)

Figure B.2 — Example of chromatogram

The retention times may change with the used equipment, especially the column. It may be determined by using the standard solution (9.2.2).

B.3 Calibration curve



Key

X Concentration (milligrams per litre)
Y Sum of peak areas × 10^6

Figure B.3 — Calibration curve

Bibliography

[1] 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 P. 0001-0194 and amendments

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

[3] 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*

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