SLOVENSKI STANDARD
SIST EN 15950:2010

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Gnojila - Določevanje N-(1,2-dikarboksietil) D,L asparaginske kisline (iminodijantarna kislina, IDHA) s tekočinsko kromatografijo visoke ločljivosti (HPLC)

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

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

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

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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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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.

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of an identical text or by endorsement, at the latest by April 2011, and conflicting national standards shall be withdrawn at the latest by April 2011.

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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)*

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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.

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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.

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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.

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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).

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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.

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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

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9.2 Calibration

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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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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.

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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

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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.