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Fertilizers — Determination of complexing agents in fertilizers — Identification of heptagluconic acid by chromatography

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

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Engrais - Détermination des agents complexants dans les engrais - Identification de l'acide heptagluconique par chromatographie

Düngemittel - Bestimmung von Komplexbildnern in Düngemitteln - Identifikation von Heptaglukonsäure mit Chromatographie

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European foreword

This document (EN 16847:2016) 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of an identical text or by endorsement, at the latest by July 2016, and conflicting national standards shall be withdrawn at the latest by July 2016.

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1 Scope

This European Standard specifies a chromatographic method which allows the identification of heptagluconic acid (HGA) in fertilizers containing heptagluconic acid metal complexes.

This method is applicable to EC fertilizers containing complexed micro-nutrients, which are covered by Regulation (EC) No 2003/2003 [1].

NOTE For the complete names of the chelating agents mentioned in this document, see Annex E.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

The method is based on demetalation with phosphoric acid of the micronutrient HGA complex present in an aqueous solution of the sample.

The complexing agent is then identified and determined by high-performance liquid chromatography.

The separation is carried out on an NH_2 phase bonded to silica column and an aqueous solution of phosphoric acid and acetonitrile as eluent.

The detection is based on UV photometry at 210 nm.

5 Interferences

a) High concentrations of phosphate in the sample solution can create a large peak preventing the identification/determination of HGA.

b) High concentrations of chloride, sulfate and nitrate do not interfere in the identification/determination of the complexing agent.

c) The presence of the chelates of EDDHSA, [*o,o*]EDDHA, [*o,o*]EDDHMA, EDTA, DTPA, CDTA, HEEDTA, IDHA as well as the corresponding chelating agents do not interfere since they are separated from HGA.

   These substances can be detected in the chromatogram by the appearance of a peak at larger retention times. Therefore, the presence of these kinds of substances shall be taken into account when successive injections are scheduled.

d) The presence of gluconic acid does interfere in the determination of the complexing agent.

e) The presence of aspartic acid, humic substances and lignosulfonic acid may interfere with the identification/determination of HGA.

6 Apparatus

Usual laboratory equipment, glassware, and the following:

6.1 Magnetic stirrer.

6.2 Chromatograph,

equipped with:

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

b) an injection valve with a 20 µl injection loop or equivalent;

c) a NH_2 column; internal diameter: 4,6 mm; column length: 250 mm; dp = 5 µm [1)];

d) a NH_2 guard-column (recommended);

e) a UV-Vis detector with a 210 nm-filter;

f) an integrator.

6.3 Chromatographic conditions,

according to Table 1.

Table 1 — Chromatographic conditions

Flow rate	Eluent A (7.4) %	Eluent B (7.5) %
1 ml/min	75	25

6.4 Balance,

Balance, with an accuracy of ± 0,1 mg.

6.5 Membrane filters.

Micro membrane filters resistant to aqueous solutions, with porosity of respectively 0,45 µm and 0,2 µm.

7 Reagents

Use only reagents of recognized analytical grade.

[1)] Phenosphere NH_2 80A 5 µm 250×4,6 mm or equivalent. This is an example of suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products can be used if they can be shown to lead to the same results.

7.1 Water,

conforming to EN ISO 3696, degassed by boiling before use.

7.2 Sample preparation solvent.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % and 25 ml of methanol in a 1 l volumetric flask. Dilute to the mark with water and homogenize.

7.3 HGA stock solution,

$c(HGA_{acid})$ = 19 893 mg/l.

This solution shall be freshly prepared daily, because of the formation of the corresponding lactone if it let standing for a long period of time.

Weigh to an accuracy of 0,1 mg about 2 500 mg of the heptagluconic acid, sodium salt dihydrate (CAS # 10094-62-9), c > 99 %, add 50 ml of water in a 100 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize.

7.4 Eluent A: *ortho*-phosphoric acid,

$c(H_3PO_4)$ = 30 mmol/l and methanol.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % (mass concentration) and 25 ml of methanol (HPLC grade) in a 1 l volumetric flask. Dilute to the mark with water and homogenize. Before use, filter the solution through a 0,45 µm membrane filter (6.5).

7.5 Eluent B: acetonitrile (HPLC-grade).

8 Procedure

8.1 Preparation of the HGA-metal complex sample solution

The mass of the test portion to be used to prepare the sample solution is dependent on the declared metal content of the product.

NOTE Sample preparation may be conducted according to EN 1482-2, see [6].

Weigh into a 150 ml beaker, approximately the amount of sample specified in Table 2, to an accuracy of 0,1 mg:

Table 2 — Amount of sample

Declared metal content % (mass fraction)	**Mass of test portion** mg
10 to 15	300
5 to 10	500
< 5	1 000

Add 50 ml of sample preparation solvent (7.2) and dissolve it with a magnetic stirrer (6.1) during 5 min. Make up to volume in a 100 ml volumetric flask with sample preparation solvent (7.2). Let the solution stand overnight in darkness to allow the metal phosphate to form.

8.2 Preparation of the calibration solutions

Pipette a volume (V ml) (see Table 3) of the HGA stock solution (7.3) in six 100 ml volumetric flasks respectively. Make up to volume with the sample preparation solution (7.2) and homogenize. Let the solution stand overnight.

Table 3 — Composition of the calibration solutions

Solution	V ml	**Concentration of HGA (acid)** mg HGA/l
1	1	199
2	2	398
3	6	1 194
4	8	1 591
5	10	1 989
6	16	3 183

NOTE The molecular mass of heptagluconic acid, sodium salt dihydrate corresponds to 284 g/mol, whereas the acid form has a molecular mass of 226 g/mol.

8.3 Chromatographic analysis

Immediately before injection, all calibration and sample solutions shall be filtered through a 0,2 µm membrane filter (6.5).

After stabilization of the chromatographic conditions (6.3), inject the calibration solutions (8.2) into the chromatographic system (6.2).

The major peak obtained corresponds to heptagluconic acid.

NOTE 1 Since the calibration solutions are not freshly prepared (see 8.2), two defined peaks may appear in the chromatograms, one tentatively assigned to the lactone and the other corresponding to the heptagluconic acid.

Adjust the attenuation on the integrator, in order to obtain a suitable range for the HGA peak from the standard solution. A typical chromatogram is given in Figure A.1. Measure the retention time.

Draw the calibration curve with the value of the chromatographic peak of the calibration solutions versus the HGA (acid) concentration (mg/l) in the standards.

Inject the sample solution (8.1). Identify the complexing agent by the retention time of the obtained peaks, and if diode array detector is used, confirm it with its UV-visible spectrum (see Annex B).

Measure the area of the peak for the sample solution corresponding to the complexing agent and determine the concentration in (mg/l) using the calibration graph. See Annex A for integration considerations.

NOTE 2 Heptagluconic acid can co-exist in two different isomers: alpha and beta. Both isomers can be found in commercial products. The retention times of both isomers differ in less than 0,3 min and they can be distinguished by two separated peaks depending on the type of column used.

NOTE 3 The first part of some chromatograms could present a set of peaks that can disturb dramatically the measurement of the value of the HGA peak. This effect can be observed in e.g. the copper complex or in mixtures of complexes.

9 Calculation of the heptagluconic acid content and expression of the results

Calculate the heptagluconic acid (*Mw* = 226 g/mol) content, w_{HGA}, in the fertilizer, expressed as mass fraction in percent, according to Formula (1).

$$w_{HGA} = \frac{\rho}{M} \times 10 \quad (1)$$

where

ρ is the concentration of HGA in milligrams per litre determined with the calibration graph;

M is the mass of the sample taken for analysis in milligrams.

NOTE In commercial products, the molar ratio HGA: Metal (HGA measured according this method and the amount of complexed metals determined according to EN 15962, see [2]) is typically above 0,5 (see Annex C for further explanation) [2)].

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test has been carried out in 2014 with nine participating laboratories and three different samples (one Zn-HGA liquid fertilizer, one Fe-HGA solid fertilizer and one micronutrient mix solid fertilizer). The statistical results are summarized in Annex D. Repeatability and reproducibility were calculated according to ISO 5725-2, see [5].

The values derived from this inter-laboratory test might not be applicable to concentration ranges and matrices other than those given in Annex D. Moreover, slight modifications (mainly waiting time in the preparation of the calibration solution) were introduced in the method here presented after the ring test.

10.2 Repeatability

The absolute difference between two independent test results obtained using 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 no more than 5 % of the cases exceed the values of *r* given in Table 4.

10.3 Reproducibility

The absolute difference between two single test results obtained using the same method on identical test material, in different laboratories, by different operators using different equipment, will in no more than 5 % of the cases exceed the values of *R* given in Table 4.

Table 4 — Mean values, repeatability and reproducibility limits

Sample	$\bar{x}$	*r*	*R*
	%	%	%
HGA content (%)			
Sample 1 Zn-HGA	17,1	2,7	7,1
Sample 2 Fe-HGA	44,7	1,4	18,3
Sample 3 Mix HGA	33,4	2,5	17,5

[2)] This kind of products can form metal complexes of stoichiometry HGA-M (1:1), (1:2) or (2:1); see Bibliography [3] and [4].

11 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 European Standard;

c) test results obtained expressed as percentage of HGA in the fertilizer;

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

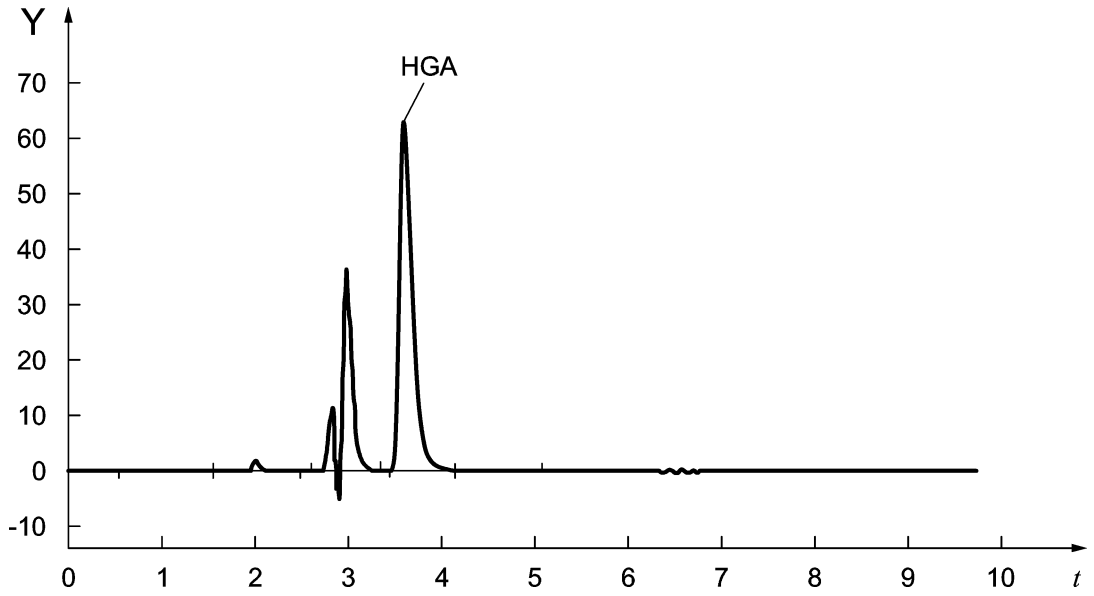
e) date when the analysis was finished;

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)

Chromatograms of the standard and a typical sample solution

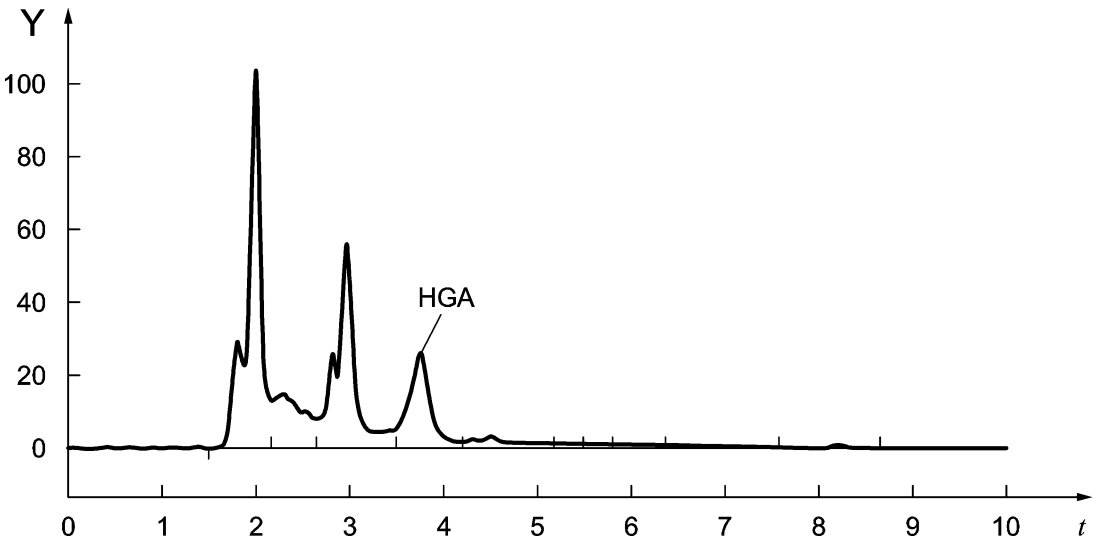


Key

t time in minutes

Y absorbance units

Figure A.1 — Standard

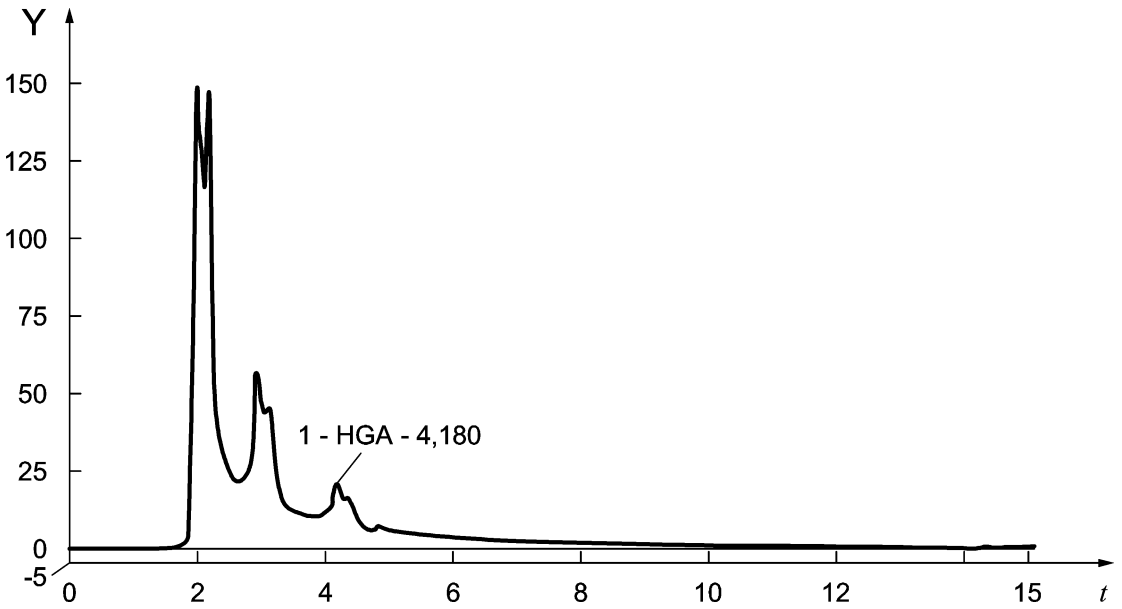


Key

t time in minutes

Y absorbance units

Figure A.2 — Sample solution



Key

t time in minutes

Y absorbance units

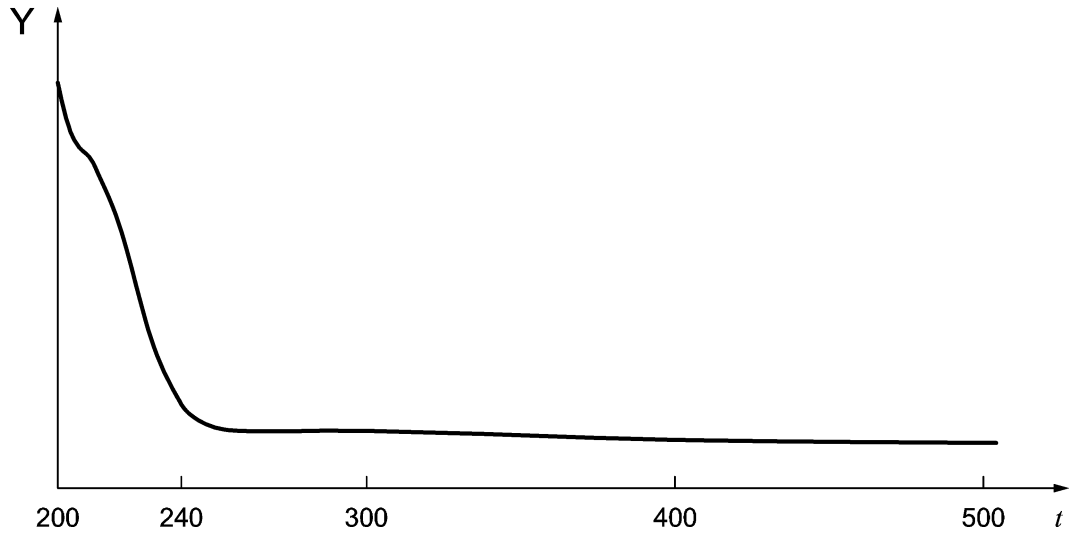
Figure A.3 — Sample solution with isomer separation

The form and the number of the first peaks of the chromatogram can differ depending on the metal complex. Those peaks are not assigned.

Baseline integration should be done.

Annex B
(informative)

Absorption spectra of the HGA



Key

t time in minutes

Y absorbance units

Figure B.1 — Absorption spectra of the HGA

Annex C
(informative)

Calculation of the molar ratio HGA:Metal

The molar ratio HGA:Metal (*MR*) is relevant for the HGA identification in micro-nutrient complex fertilizers. It is defined as the number of moles of HGA divided by the sum of the number of moles of all complexed micro-nutrient metals according to EN 15962, see [2]. An example for a Fe, Mn, Zn and Cu fertilizer is given in Formula (C.1).

$$MR = \frac{w_{HGA} / 226{,}0}{w_{Fe} / 55{,}85 + w_{Mn} / 54{,}94 + w_{Zn} / 65{,}38 + w_{Cu} / 63{,}55} \quad \text{(C.1)}$$

Also as example in the inter-laboratory test done to study the precision of the method (see Clause 10 and Annex D), the HGA:metal ratios obtained, using the nominal metal concentrations were calculated as explained in Table C.1.

Table C.1 — HGA:metal (molar ratios, *MR*) calculated for samples used in the inter-laboratory test (see Annex D)

Values	Sample		
	Sample 1 Zn-HGA	Sample 2 Fe-HGA	Sample 3 Mix HGA
w_{HGA} Mean value %	17,1	44,7	33,4
HGA, mol 100 g^{-1}	0,076	0,198	0,148
Metal nominal values %			
w_{Fe}		11,8	8,55
w_{Mn}			4,50
w_{Zn}	5,90		0,63
w_{Cu}			0,27
Metal nominal values mol 100 g^{-1}			
w_{Fe}		0,211	0,153
w_{Mn}			0,081 9
w_{Zn}	0,090 2		0,009 6
w_{Cu}			0,004 2
Sum	0,090 2	0,211	0,249
HGA:Metal (molar ratio, *MR*)	0,84	0,94	0,59

Annex D
(informative)

Statistical results of the inter-laboratory test

D.1 Inter-laboratory test

The precision of the method has been determined in the year 2014 in an inter-laboratory trial with nine laboratories participating and carried out on three samples of fertilizer. Laboratories were initially requested to send the results using tangential linear skim integration. After presenting the results and noting that the results presented large differences with the nominal values of the samples, laboratories were requested to present again the results but providing the baseline to baseline integration. The statistical results for this set of data are given in Table D.1. Slight modifications (mainly waiting time in the calibration curve) were introduced in the method here presented.

D.2 Test Samples

Three different samples have been provided to all the participants. All of them were commercial metal complexes containing HGA (one liquid product and two solid samples).

— Sample 1: Zn-HGA (liquid product); %Zn: 5,9

— Sample 2: Fe-HGA (solid sample); %Fe: 11,8

— Sample 3: Mix-HGA (solid sample); %Fe: 8,55, %Mn: 4,50, %Zn: 0,63, %Cu: 0,27

D.3 Inter-laboratory test procedure

The participant laboratories were requested to perform three replicates of each sample according to the method. The parameter measured was the amount of HGA in the samples expressed as g of HGA 100 g^{-1} of product. One decimal place was specified for each determination.

Samples and protocol were sent to 13 laboratories from six countries and nine laboratories presented initially the results. Afterwards, the laboratories were requested to present the results using the baseline to baseline integration. Eight laboratories presented the results.

Test results, observations and remarks were reported.

D.4 Results and statistical interpretation

Statistical calculations were run on all the results, according to ISO 5725-2, see [5].

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

Table D.1 shows the statistical results of the inter-laboratory test. Reproducibility limit was considered too high for a quantitative method, but acceptable for the identification of HGA in fertilizers, since the complexed metal quantification is done according to EN 15962, see [2].

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

Parameter	HGA content w_{HGA} %		
	Sample		
	Sample 1 Zn-HGA	Sample 2 Fe-HGA	Sample 3 Mix HGA
Number of laboratories	8	8	8
Number of outliers	0	1	1
Number of laboratories after elimination of outliers	8	7	7
Mean value, %	17,1	44,7	33,4
Repeatability standard deviation, (s_r), %	0,96	0,49	0,89
Repeatability limit, (r), %	2,68	1,38	2,49
RSD_r, %	5,60	1,10	2,66
Reproducibility standard deviation (s_R), %	2,54	6,55	6,25
Reproducibility limit, (R), %	7,11	18,33	17,50
RSD_R, %	14,8	14,6	18,7

Annex E
(informative)

Complete names of chelating agents

EDTA	ethylenediaminetetraacetic acid $C_{10}H_{16}O_8N_2$ CAS-No. 60–00–4
HEEDTA	2-hydroxyethylethylenediaminetriacetic acid $C_{10}H_{18}O_7N_2$ CAS-No. 150–39–0
DTPA	diethylenetriaminepentaacetic acid $C_{14}H_{23}O_{10}N_3$ CAS-No. 67–43–6
[*o,o*] EDDHA	ethylenediamine-N,N'-di[(*ortho*-hydroxyphenyl)acetic acid] $C_{18}H_{20}O_6N_2$ CAS-No. 1170–02–1
[*o,o*] EDDHMA	ethylenediamine-N,N'-di[(*ortho*-hydroxymethylphenyl)acetic acid] $C_{20}H_{24}O_6N_2$ CAS-No. 641632–90–8
EDDHSA	ethylenediamine-N,N'-di-[(2-hydroxy-5-sulfophenyl)acetic acid] and its condensation products $C_{18}H_{20}O_{12}N_2S_2$ + n*($C_{12}H_{14}O_8N_2S$) CAS-No. 57368–07–7 and 642045–40–7
NTA	nitrilotriacetic acid $C_6H_9O_6N$ CAS-No. 139–13–9
CDTA	1,2-cyclohexylenediaminetetraacetic acid $C_{14}H_{22}O_8N_2$ CAS-No. 482–54–2
HBED	N,N´-bis(2-hydroxybenzyl)-ethylenediamine-N,N´-diacetic acid $C_{20}H_{24}O_6N_2$ CAS-No. 35998–29–9

Bibliography

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

[2] EN 15962, *Fertilizers - Determination of the complexed micro-nutrient content and of the complexed fraction of micro-nutrients*

[3] PECSOK L., JUVET R.S. *J. Am. Chem. Soc.* 1959, **77** pp. 202–206

[4] ESCANDAR S. *Can. J. Chem.* 1998, **70** (7) pp. 2053–5057

[5] 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*

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

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