

BS EN 13368-1:2014



BSI Standards Publication

# Fertilizers — Determination of chelating agents in fertilizers by chromatography

Part 1: Determination of EDTA, HEEDTA and DTPA by ion chromatography

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**National foreword**

This British Standard is the UK implementation of EN 13368-1:2014. It supersedes BS EN 13368-1:2001 which is withdrawn.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 in fertilizers by chromatography - Part 1: Determination of EDTA, HEEDTA and DTPA by ion chromatography

Engrais - Détermination des agents chélatants dans les engrais par chromatographie - Partie 1: Détermination du EDTA, HEEDTA et DTPA par chromatographie ionique

Düngemittel - Bestimmung von Chelatbildnern in Düngemitteln mit Chromatographie - Teil 1: Bestimmung von EDTA, HEEDTA und DTPA mit Ionenchromatographie

This European Standard was approved by CEN on 16 November 2013.

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## Foreword

This document (EN 13368-1:2014) 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of an identical text or by endorsement, at the latest by July 2014, and conflicting national standards shall be withdrawn at the latest by July 2014.

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This document supersedes EN 13368-1:2001.

The following modifications have been made:

- a) the title of the European Standard revised;
- b) referenced European Standards on vocabulary added to Clause 2;
- c) Clause 3 Terms and definitions added;
- d) the sampling method is not part of the standard, informative reference to EN 1482-1 added;
- e) Annex A: complete names of chelating agents technical revised;
- f) Bibliography revised;
- g) editorially revised.

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## 1 Scope

This European Standard specifies a method for the chromatographic determination of the total amount of each of the individual chelating agents EDTA, HEEDTA, and DTPA in fertilizers containing one or more of these substances. The method allows the identification and the determination of the total water soluble fraction of each of these chelating agents. It does not allow to distinguish between the free form and the metal bound form of the chelating agents.

NOTE EDTA, HEEDTA and DTPA are abbreviations used in this European Standard for the sake of simplicity. For complete names see Annex A.

This method applies to fertilizers containing chelates of one or more of the following micro-nutrients: cobalt, copper, iron, manganese, zinc and with a mass fraction of at least 0,1 %.

## 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 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, *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 micro-nutrients associated with the chelating agents present in an aqueous extract of the sample are replaced by iron(III). The iron chelates are separated and determined by ion chromatography. The separation is based on anion exchange, by elution with a nitrate acetate solution. The detection is based on UV photometry at 330 nm, after post-column reaction with diluted perchloric acid.

## 5 Interferences

Several substances can interfere, to a degree largely dependent on the type of column used. With the column described in 7.2, the following phenomena have been observed.

- a) Injection of solutions having high concentrations of salts can cause shifts in the retention times, mostly decreasing the retention when compared to the standard solutions. In these cases, the identity of the peaks can be confirmed by standard addition.

- b) Solutions having high concentrations of salts can also create a large signal at the void volume, poorly resolved from the HEEDTA peak.
- c) High concentrations of nitrate, carbonate, sulfate, and phosphate do not interfere. On the other hand, large amounts of chloride create a negative fronting peak poorly resolved from the DTPA peak, and altering its peak shape.
- d) Compounds, related to the group of polyamino polycarboxylic acids can interfere. While signals for [o,o] EDDHA, [o,o] EDDHMA, and EDDHSA are not detected, relatively weak signals are observed for NTA and CDTA. Under some conditions NTA may coelute with HEEDTA or EDTA.

NOTE EDDHA, EDDHMA, EDDHSA, NTA and CDTA are abbreviations used in this European Standard for the sake of simplicity. For complete names see Annex A.

- e) No signals have been detected for the following complexing agents: citrate, oxalate, tartrate, phthalate, and 20 naturally occurring amino acids.

## 6 Reagents

**6.1 Reagents of recognized analytical grade and water** conforming to EN ISO 3696, degassed by boiling before use.

**6.2 Nitric acid**,  $c(\text{HNO}_3) = 7,2 \text{ mol/l}$ .

Nitric acid, diluted 1 + 1 with water.

**6.3 Sodium hydroxide solution**,  $c(\text{NaOH}) = 0,5 \text{ mol/l}$ .

Dissolve 20 g of NaOH in pellet form in a 1 l volumetric flask with water. Dilute to the mark and homogenize.

**6.4 EDTA stock solution**,  $c(\text{EDTA}) = 2 \text{ mmol/l}$ .

Dissolve 372 mg of the disodium dihydrogen salt of ethylene diamine tetraacetic acid dihydrate in 400 ml of water in a 500 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize. Store the solution in a plastic bottle.

**6.5 HEEDTA stock solution**,  $c(\text{HEEDTA}) = 2 \text{ mmol/l}$ .

Dissolve 380 mg of the trisodium salt of hydroxyethyl ethylene diamine triacetic acid dihydrate in 400 ml of water in a 500 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize. Store the solution in a plastic bottle.

**6.6 DTPA stock solution**,  $c(\text{DTPA}) = 2 \text{ mmol/l}$ .

Dissolve 393 mg of diethylene triamine pentaacetic acid in 10 ml of NaOH (6.3) in a 50 ml beaker. After dissolution, transfer quantitatively into a 500 ml volumetric flask, dilute to the mark with water and homogenize. Store the solution in a plastic bottle.

**6.7 EDTA/HEEDTA/DTPA standard mixtures.**

Into a set of three volumetric flasks of 100 ml, pipette respectively 2,5 ml, 5 ml and 10 ml of each stock solution of 2 mmol/l (6.4, 6.5 and 6.6). Dilute to the mark with water and homogenize. These solutions contain a mixture of EDTA, HEEDTA and DTPA, in concentrations of respectively 0,05 mmol/l, 0,1 mmol/l and 0,2 mmol/l. They should be used within two days.

### 6.8 Eluent nitrate (50 mmol/l) and acetate (50 mmol/l).

Dissolve 4,10 g of anhydrous sodium acetate ( $\text{NaCH}_3\text{COO}$ ) in a mixture of 800 ml of water and 6,95 ml of nitric acid (6.2) in a 1 l volumetric flask. Dilute to the mark with water and homogenize. Adjust the pH of the eluent to  $2,75 \pm 0,20$ . Before use, filter the solution through a  $0,45 \mu\text{m}$  membrane filter (7.3).

NOTE The concentration of the eluent influences the speed and the efficiency of the separation, which can be carried out with eluent concentrations varying between 25 mmol/l and 75 mmol/l of nitrate and acetate. At low concentrations, an improved separation between the void signal, the HEEDTA and the EDTA peaks can be obtained, while at high concentrations a better DTPA peak shape is observed.

### 6.9 Iron(III) nitrate solution.

Dissolve 5 g of ferric nitrate nonahydrate ( $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) in a mixture of 800 ml of water and 21 ml of nitric acid (6.2) in a 1 l volumetric flask. Dilute to the mark with water and homogenize. Store the solution in a plastic bottle.

NOTE Nitric acid is added in order to stabilize the solution and to allow the complete replacement of other chelated micro-nutrients by iron(III).

### 6.10 Perchloric acid solution, 2 %.

Dilute 29 ml of perchloric acid (70 %  $\text{HClO}_4$ ,  $\rho = 1,67 \text{ g/ml}$ ) to 1 l with water.

## 7 Apparatus

Usual laboratory equipment, glassware, and the following:

7.1 **Rotary shaker**, capable of operating at a rotational speed of about  $35 \text{ min}^{-1}$  to  $45 \text{ min}^{-1}$ .

7.2 **Chromatograph**, equipped with:

- an isocratic pump delivering the eluent (6.8) at a flow rate of 0,5 ml/min;
- an injection valve with an injection loop of about 50  $\mu\text{l}$ ;
- an anion exchange separator column 10  $\mu\text{m}$  particles – 2 % substrate x – linking 100 mmol<sub>c</sub> capacity (per column) – alkyl quaternary ammonium – medium high (hydrophobic)<sup>1)</sup>;

NOTE 1 The column mentioned in c) is not exclusive. Any anion exchange column with comparable capacity, hydrophobicity, and selectivity can probably be used.

NOTE 2 Columns can, in their native state, exhibit some sensitivity towards various anions (e.g. nitrate, sulfate, phosphate) which can disappear after treatment with excess iron. It can be necessary to precondition the column prior to its use by repeated injections, at least 10, of the iron(III) solution (6.9), followed by equilibration under eluent flow for at least 6 h.

After many injections, especially of products having complex matrices, the column may lose some of the capacity and the separating efficiency. Substances like phenolic compounds (e.g. [o,o] EDDHA, humic acids) can be strongly adsorbed on the column particles. Suspended and colloidal matter can block the column entrance and disturb the eluent flow. The column manufacturer cleaning instructions are consulted for the suitable cleaning procedure.

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1) A combination of IonPac AS 7 separator and Ion Pac AG 7 guard column from Dionex Co, Sunnyvale, CA USA, or equivalent are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.



The injection of un-dissolved matter severely decreases the lifetime of the column. Solutions should always be allowed to equilibrate, and then filter through a 0,2 µm membrane filter before injection.

- d) a post-column reagent delivery module, delivering the reagent (6.10) at a flow rate between 0,5 ml/min and 0,6 ml/min;

NOTE 3 The post-column reagent stabilizes the eluting ferric chelates and suppresses the signal of some possibly interfering substances. For the analysis of samples with simple matrices, the post column reaction can be considered as superfluous.

- e) an UV/VIS detector with a 330 nm filter, output range set at an absorbance of 0,1;

NOTE 4 Ferric chelates of EDTA, HEEDTA, and DTPA have a useful spectral absorbance between 250 nm and 350 nm. The absorbance at 254 nm offers a better sensitivity, but also produces a noisier background, more sensitive to interferences.

- f) an integrator.

**7.3 Membrane filters**, micro membrane filters, resistant to aqueous solutions, with porosity of respectively 0,45 µm and 0,2 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 in a mortar to a particle size less than 1 mm.

## 9 Procedure

### 9.1 Preparation of the test solution

Weigh 5 g of the sample ( $m$ ), to an accuracy of 1 mg, into a 250 ml volumetric flask ( $V_0$ ). Add 200 ml of water and put the flask on the rotary shaker (7.1) for 1 h. Make up to the mark with water, homogenize, and filter through a paper filter. If necessary, dilute the filtrate with water, in order to obtain a concentration of chelating agent between 0,02 mmol/l and 0,2 mmol/l. Let  $D$  be the dilution factor. Pipette 20 ml of the solution into a 100 ml beaker. Add 5 ml of the iron(III) solution (6.9), homogenize, and allow to stand for 15 min.

The addition of iron may cause precipitation, especially if phosphate is present in the sample solution. For this reason, the precipitate should be allowed to settle and the solution to equilibrate for 15 min.

### 9.2 Preparation of the standard solutions

Pipette 20 ml of each of the EDTA/HEEDTA/DTPA standard mixtures of respectively 0,05 mmol/l, 0,1 mmol/l and 0,2 mmol/l (6.7) into a set of 100 ml beakers. Add 5 ml of the iron(III) solution (6.9), homogenize, and allow to stand for 15 min.

### 9.3 Chromatographic analysis

Immediately before injection, all solutions shall be filtered through a 0,2 µm membrane filter (7.3). Inject the standard solutions (9.2) into the chromatographic system (7.2). The retention times of the iron chelates are in

the order HEEDTA < EDTA < DTPA. Adjust the attenuation on the integrator, in order to obtain about 75 % of the output range for the EDTA peak from the most concentrated standard solution. See typical chromatogram in Annex B. Measure the retention times and the peak areas for the three chelating agents. Inject the sample solution (9.1). After elution, identify the chelating agent by the retention time, and measure the corresponding peak area. For each appropriate chelating agent, draw a calibration graph with the values of the peak area of the standard solutions versus the corresponding chelating agent concentration ( $C_s$ ) (mmol/l). Determine the concentration of the chelating agent in the sample solution (mmol/l), using the corresponding calibration graph.

## 10 Expression of results

Calculate the mass fraction of the chelating agent (EDTA, HEEDTA or DTPA),  $w_{ca}$ , in percent, expressed as free acid, in the fertilizer according to Formula (1).

$$w_{ca} = \frac{C_s \cdot D \cdot M_w \cdot V_0}{10^4 \cdot m} \quad (1)$$

where

$C_s$  is the concentration of the chelating agent in the test solution in millimole per litre, determined with the calibration graph (9.3);

$D$  is the dilution factor (9.1);

$M_w$  is the molar mass, in grams per mole, of the chelating agent in the acid form, being for EDTA: 292, HEEDTA: 278, DTPA: 393;

$V_0$  is the total volume of the extract (9.1), in millilitres;

$m$  is the mass of the sample taken for extraction, in grams.

## 11 Precision

Results are based on three different ring tests involving between nine and ten laboratories with only five having completed the full range of testing (see [2]).

When only identification of the product is considered repeatability and reproducibility data are not necessary since no interferences have been noted.

Should however this standard be used to measure the quantity of a chelating agent, then, at the 95 % confidence level, repeatability limit and reproducibility limit are:

Repeatability limit  $r = 6 \%$ ,

and

Reproducibility limit  $R = 25 \%$ .

## 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 (EN 13368-1:2014);
- c) test results obtained;
- d) date of the 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)

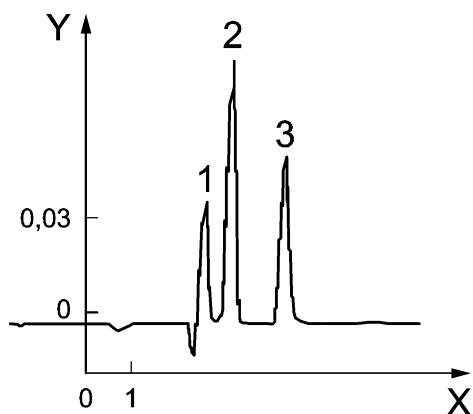
### Complete names of chelating agents

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

## Annex B (informative)

### Chromatogram

A chromatogram obtained after injection of standard mixture containing 0,1 mmol/l of EDTA, HEEDTA and DTPA (9.2) is given in Figure B.1.



#### Key

- Y absorbance
- 1 HEEDTA
- 2 EDTA
- 3 DTPA
- X time (in minutes)

Figure B.1

## 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 Pages 0001-0194
- [2] CEN/TC 260 N 268 Technical Report — Mandate M/051: Study and standardization remit assigned to CEN concerning the analytical method for chelated trace element content in fertilizers
- [3] EN 1482-1, *Fertilizers and liming materials - Sampling and sample preparation - Part 1: Sampling*
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- [5] I. Vande Gucht, *Determination of chelating agents by ion chromatography*, J. Chrom., Vol. 671, June 1994



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