Draft Method proposal: determination of glucoheptonic acid (HGA) in fertilizers.

1 Scope

This document describes a chromatographic method which allows the identification as well as the determination of the content of glucoheptonic acid (HGA) in fertilizers containing glucoheptonic acid, metal complexes.

This method concerns to EC fertilizers containing complexed micronutrients, which are covered by Regulation (EC) 2003/2003.

2 Principle

The method is based on the demetalation with Phosphoric Acid of the micronutrients associated with the complexing agent present in an aqueous extract of the sample.
The complexing agent is then identified and determined by ion-pair high-performance liquid chromatography.
The separation is carried out on an NH2 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.

3 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 and sulfate do not interfere in the identification/determination of the complexing agent.

c) The presence of nitrate and of the chelates of EDDHSA, EDDHA, EDDHMA, EDTA, DTPA, CDTA, HEDTA as well as the corresponding chelating agents do not interfere since they are separated from HGA (1).

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

4 Apparatus

Usual laboratory equipment, glassware, and the following:

4.1. Magnetic stirrer.

4.2. Chromatograph, equipped with:

[1] These substances are separated from HGA and can be detected in the chromatogram by the apparition of a peak at larger retention times. Therefore, the presence of these kinds of substances must be taken into account when successive injections are scheduled.

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 NH2 column; internal diameter: 4.6mm; column length: 150mm; dp = 5µm ([2])

d) a NH2 guard-column (recommended);

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

f) an integrator

4.3. Chromatographic conditions

Time	Flow rate	% Eluent A (5.4.)	%Eluent B (5.5.)
-	1 ml/min	75	25

4.4. Balance, capable of weighing to an accuracy of 0.1mg.

4.5. Membrane filters: Micromembrane filters resistant to aqueous solutions, with porosity of respectively 0.45 µm and 0.2 µm.

5. Reagents

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

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. Dilute to the mark and homogenize.

5.3. Sample preparation solvent

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

5.4. HGA stock solution, c **(HGA $_{acid}$) = 19893 mg/l ([3]).**

Weigh to an accuracy of 0.1 mg about 2500 mg of the glucoheptonic acid, sodium salt dihydrate c >99%, add 50 ml of water in a 100 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize.

([2]) Phenosphere NH_2 80A 5µm 250x4,6mm or equivalent. This is an example of suitable product available commercially.

([3]) This solution should be freshly prepared every time and immediately injected, because of the formation of the corresponding lactone if it lets stand for a long period of time.

5.5. Eluent A: *ortho*-Phosphoric Acid c (H_3PO_4) = 35 mmol/l and Methanol

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85%(wt.in water) and 25 ml of Methanol (HPLC grade) in a 1 litre volumetric flask. Dilute to the mark with water and homogenize. Before use, filter the solution through a 0.45 µm membrane filter (4.4).

5.6. Eluent B: Acetonitrile (HPLC grade)

6. Procedure

6.1. Identification and quantification of HGA in a fertilizer sample

6.1.1. Preparation of the HGA-Metal Complex sample solution

The weight of the sample is dependent of the metal content declared in the product.
Weight into a 150 ml beaker, approximately the amount of sample described in the following table, to an accuracy of 0.1mg:

% Total metal declared (w/w)	weight of sample (mg)
10-15	300
5-10	500
<5	1000

Add 50 ml of (5.3.) and dissolve it with a magnetic stirrer (4.1.) during 5 min. Make up to volume in a 100ml volumetric flask with (5.3.). Let the solution stand overnight in darkness to allow the metal phosphate to form.

6.1. 2. Preparation of the calibration solutions

Pipette a volume (*V* ml) (See Table 1) of the HGA stock solution (5.4.) in six 100ml-volumetric flasks respectively. Make up to volume with the sample preparation solution (5.3.) and homogenize.

Solution	***V ml***	***Concentration of HGA (acid)[4] (mg HGA/l)***
1	1	199
2	2	398
3	6	1194
4	8	1591
5	10	1989
6	16	3183

([4]) The molecular weight of glucoheptonic acid, sodium salt dihydrate corresponds to 284g/mol, whereas the acid form has a molecular weight of 226 g/mol.

6.1.3. Chromatographic analysis

Immediately before injection, all standard and sample solutions shall be filtered through a 0.2 µm membrane filter (4.5.).
After stabilization of the chromatographic conditions (4.3.), inject the standard solutions (6.2.2.) into the chromatographic system (4.2).
The major peak obtained corresponds to glucoheptonic acid [(5)].
Adjust the attenuation on the integrator, in order to obtain a suitable range for the HGA peak from the standard solution. See typical chromatogram in *Annex A*. Measure the retention time.

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

Inject the sample solution 6.1.1. 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: glucoheptonic 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 differs not much of 0.3 min and they could be distinguished by two separated peaks depending on the type of column used.

NOTE: 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 could be observed in e.g. the copper complex or in mixtures of complexes. To avoid this effect, a previous pretreatment of the sample with a resin could be done following the procedure of *Annex C*.

7. Expression of the results

7.1. Glucoheptonic acid content

Calculate the glucoheptonic acid ([6]) content in the fertilizer, expressed as mass fraction in percent, according to the following equation:

$$W_{HGA} = \frac{m_1}{m_2} * 100$$

where,

([5]) When the standard solution is not freshly prepared two defined peaks appears in the chromatogram, one corresponding to the lactone (at lower retention times) and the other corresponding to the glucoheptonic acid. See note 4.

([6]) M_w = 226 g/mol.

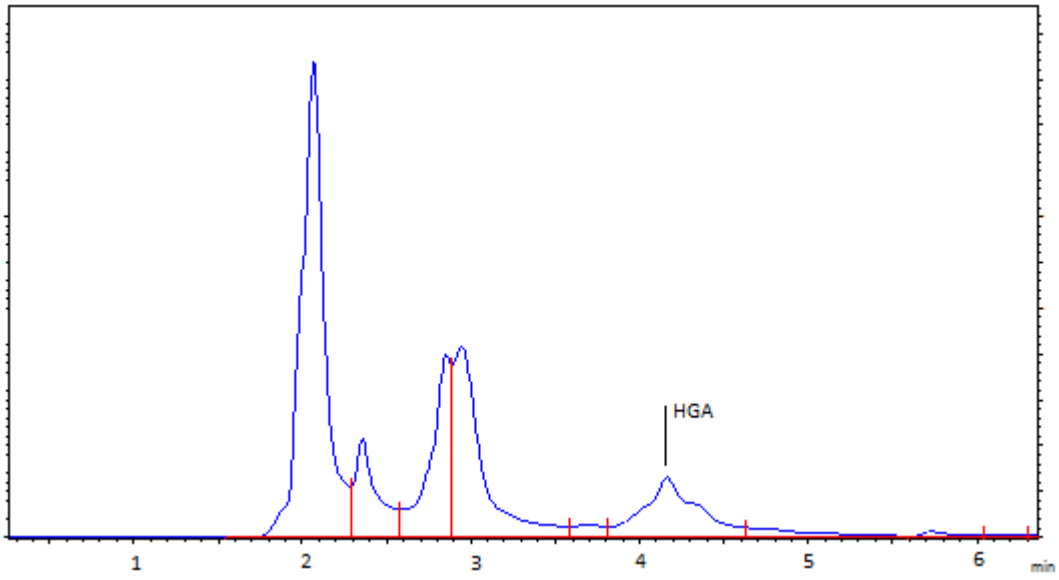
m_1 is the concentration of HGA in milligrams per liter determined by the calibration graph

m_2 is the mass of the sample taken for analysis in milligrams per liter

NOTE: The amount of HGA measured may differ in a 50% of the equimolar expected value, with respect of the complexed metal concentration (Method EN 15962) ([7]).

([7]) This kind of products can form metal complexes of stoichiometry HGA-M (1:1), (1:2) or (2:1); Pecsok L., Juvet R.S., J.Am.Chem.Soc. 77, 1959, 202-206; Escandar, Sala, Can. J. Chem. 1998, 70(7), 2053-5057.

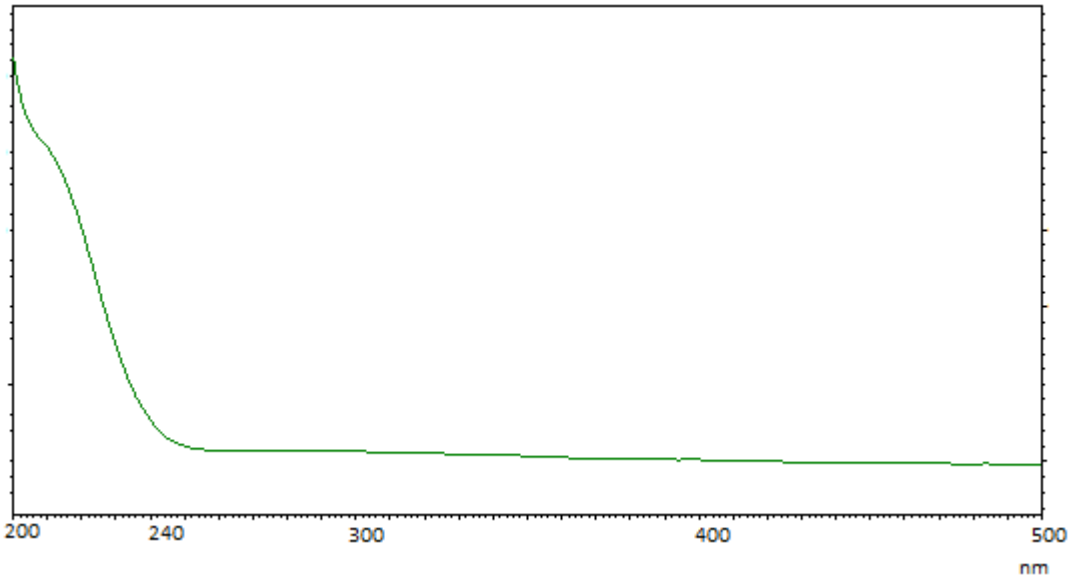
Annex A



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

NOTE: If the chromatogram clearly shows a peak tail, the HGA peak could be tangential integrated or the resin procedure of *Annex C* should be applied to this sample.

Annex B



Annex C

Peaks overlapping and baseline shifts may be corrected using a previous treatment of the sample with a chelating resin. Also demetalation is completely attained using this procedure.

The resin [8] characteristics should be:

Matrix: Macroporous styrene divinylbenzene

Functional groups: Iminodiacetic acid

Ionic form: Na+

Harmonic mean size: 0.50 - 0.65 mm

Uniformity coefficient < 1.7

Weight Cation Capacity (mol_c/kg) > 4.45

An alliquot of 10 ml of the Heptagluconate complex solution (6.1.1.) is allowed to interact with an amount of resin that provides ten times more cation capacity that the amount of metal declared in the Heptagluconate complex, in a plastic bottle (25 ml) or test tube (25 ml) with stopper, in a through and fro shaker at 120 rpm for 20 h. After that time, the sample is conveniently filtered with a cellulose filter for quantitative analysis.
Immediately before injection, the sample solution shall be filtered through a 0.2 µm membrane filter (4.5.).
Inject the solution into the chromatographic system (4.2).

[8] (Amberlite® IRC 7481 with a Weight Cation Capacity (eq/kg) > 4.45 or equivalent is an example