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Starch derivatives — Determination of the composition of glucose syrups, fructose syrups and hydrogenated glucose syrups — Method using high-performance liquid chromatography

Produits dérivés de l'amidon — Détermination de la composition des sirops de glucose, des sirops de fructose et des sirops de glucose hydrogénés — Méthode par chromatographie en phase liquide à haute performance

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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The committee responsible for this document is ISO/TC 93, *Starch (including derivatives and by-products)*.

This second edition cancels and replaces ISO 10504:1998, of which it constitutes a minor revision.

Starch derivatives — Determination of the composition of glucose syrups, fructose syrups and hydrogenated glucose syrups — Method using high-performance liquid chromatography

1 Scope

This International Standard describes a high-performance liquid chromatographic (HPLC) method for measuring the composition of dextrose solutions, glucose syrups, fructose-containing syrups, hydrogenated glucose syrups, sorbitol, mannitol and maltitol. The constituents are mainly glucose, maltose, maltotriose, fructose, sorbitol, mannitol, maltitol and malto-oligosaccharides.

The use of a column packed with cation-exchange resin is essential.

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.

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

ISO 5381:1983, *Starch hydrolysis products — Determination of water content — Modified Karl Fischer method*

3 Principle

Saccharide components are separated using high-performance liquid chromatography. Separation is achieved using a cation-exchange column with water as the eluent. The eluted components are detected by means of a differential refractometer, and quantified using an electronic integrator.

4 Reagents

All reagents used shall be of recognized analytical reagent grade.

4.1 Special distilled water.

The water used may be double-distilled of quality grade 1 in accordance with ISO 3696. The most suitable is demineralized water, which prevents contamination of the ion-exchange resin.

The water should be filtered by passage through a 0,22 µm filter. Also, it should be degassed by treatment under vacuum, or by use of an in-line degassing unit. The water should be maintained under an inert atmosphere, and preferably at 70 °C to inhibit microbial growth.

NOTE Some commercial water-purification devices produce water which is both filtered and degassed.

4.2 Primary standard solutions.

Prepare solutions (see Annex A) containing 10 % (or less) dry matter, according to the sensitivity of the refractometer, with compositions as close as possible to that of the samples to be analysed.

NOTE Suitable reference materials for the constituents listed in Clause 1 can be obtained from established chemical companies.

4.3 Ion-exchange resins, for off-line demineralization of samples.

Salts present in the sample will co-elute from the column, and will be detected by the refractometer, causing errors in the determination. These salts shall first be removed by ion-exchange resins. The most convenient way is to have an in-line guard column cartridge system (5.5), but this may also be carried out off-line using the following resins[1)]:

a) Cation type:

   1) strong cation exchanger, 4 % cross-linked polystyrene divinylbenzene, in the H^+ form;

   2) 200 mesh to 400 mesh in the dry form;

b) Anion type:

   1) weak anion exchanger, 4 % cross-linked polystyrene divinylbenzene support containing tertiary amine groups, in the free base form;

   2) 200 mesh to 400 mesh in the dry form.

5 Apparatus

5.1 Liquid chromatograph; equipped with the following.

5.1.1 Pump, pulseless, that delivers a constant flow, at the rate required.

5.1.2 Differential refractometer, thermostatically controlled.

5.1.3 Thermostatically controlled column oven, capable of maintaining the column at temperatures up to 95 °C, to within ± 0,5 °C.

5.2 Sample injector, comprising a loop injector (manual or part of autosampler) with a capacity of 20 µl or less.

5.3 Integrator, comprising an electronic integrator with calculating and recording capabilities, compatible with the voltage output of the detector.

5.4 Separation column, comprising a pre-packed cation-exchange column in the form best suited for the analysis. The recommended resin is 6 % to 8 % cross-linked sulfonated polystyrene divinylbenzene with a bead diameter of 9 µm to 25 µm.

NOTE Acceptable columns are available from several major column suppliers.

5.5 Guard columns, custom-prepared dual-cartridge system, inserted unheated in-line, to demineralize the sample.[2)]

5.6 Sample filtration system, comprising a syringe to which suitable membrane disc filters can be attached. These should be of 0,45 µm pore size.

Commercially available syrups are usually highly refined, and a 0,45 µm filter is suitable. However, if blockage of the chromatograph is too frequent, a 0,22 µm filter should be used.

1) While resins meeting these specifications are available from more than one supplier, their performance is variable. Experience in several laboratories has shown that the resins AG® 50W-X4 and AG® 3-X4 perform satisfactorily. (AG® 50W-X4 and AG® 3-X4 are trade names of products supplied by Bio-Rad. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.)

2) There are a few systems available but with varying efficiency. The Bio-Rad guard cartridges 125–0118 have been shown in several laboratories to be the most effective in all respects. (This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.)

6 Procedure

6.1 Choice of column

For general applications, a cation-exchange resin in the calcium form should be used, in particular for fructose syrups and hydrogenated glucose syrups. However, the separation of maltose at a high content from maltotriose is difficult when the maltotriose content is about 6 % or more. In such instances better resolution is achieved with a cation-exchange resin in either the potassium or sodium form.

6.2 System start-up

Install the column in the oven, and connect the guard columns (5.5) (if used) to the inlet. It is not necessary to heat the guard columns. Connect the injector to the inlet of the column (or guard columns, if used), and connect the outlet of the column to the detector inlet. Arrange that the detector effluent goes to waste.

Start the pump at a rate of 0,1 ml/min, and pass the solvent through the column. Set the correct temperature for the column according to the supplier's recommendations. Enter the control parameters into the integrator. When the column temperature is stable, increase the solvent flow rate to 0,5 ml/min and purge the reference cell. Refer to the refractometer instruction manual to set the detector for correct measurement of the signal from the sample cell. Set the required attenuation.

6.3 Calibration of column

6.3.1 In accordance with the method specified in ISO 5381, determine the water content of every separate substance to be used for preparing the mixed primary standard solutions (see Annex A).

For higher polyols (tri-itol and above), no commercial standards are available.

6.3.2 Prepare a standard solution of each separate substance (see 4.2) and, using the same conditions as those to be used for the analysis, inject an aliquot portion several times into the column. At least three results, based on integrator response, should show a variation of ± 0,1 % or less for the major constituent. Calculate an average result for all components.

NOTE For the single primary substances, an assumption is made that each sugar has the same relative response, and that the normalized area percentage figures reflect the true analysis. To obtain the required level of higher molecular weight species, a dextrin, or a fraction especially prepared from a starch hydrolysate, can be used.

6.3.3 Prepare mixtures of the single substances to give compositions as close as possible to those of the samples to be analysed. These should be prepared at the chosen concentration (see 4.2).

NOTE An example is given in Annex A.

6.3.4 Inject the chosen aliquot portion twice into the chromatograph. The quantity injected shall be large enough to give measurable peaks of minor constituents, while the major component is within the detector range for linear response.

6.3.5 Check the area of the peaks on the chromatogram. There should be a maximum deviation of ± 0,2 % for the major peak areas on at least two chromatograms.

The response factor, r_x, for component x, is calculated as follows:

$$r_x = \frac{m_x}{a_x}$$

where

m_x is the actual percentage of component x present in the standard solution;

a_x is the area percentage of the normalized chromatogram attributed to component x.

The response factors are usually equivalent to, or close to unity. If a deviation of more than 2 % is observed, then the chromatographic system should be checked, especially the integration parameters.

6.4 Sample preparation

6.4.1 When in-line demineralization is used, dilute the sample to the chosen concentration (see 4.2), and filter through a 0,45 µm filter.

If such a system is not used, then the sample shall first be treated off-line (see 6.4.2 and 6.4.3).

6.4.2 Mix the ion-exchange resins in a proportion such that equal exchange capacity is obtained. Wash the mixed resins well with water (4.1), then remove any excess, ensuring that the resin remains moist. The resin may be stored like this for several months.

6.4.3 To 15 ml to 20 ml of the liquid sample containing 25 % to 30 % dry matter, add 1,0 g to 1,5 g of the mixed resin. Stir gently for 15 min, then filter to remove the resin. Dilute to the chosen concentration and filter through a 0,45 µm filter.

See the note in 5.6.

6.5 Sample analysis

Inject the chosen aliquot portion of the sample into the chromatograph and perform the analysis as described in 6.3.4. Duplicate analyses should be performed. Record area percentage figures of the total detector response.

7 Calculation

Calculate the content of component x, according to the following equation:

$$c_x = r_x \cdot a_x$$

where

c_x is the calculated percentage of component x in the test sample;

r_x is the previously calculated response factor (see 6.3.5);

a_x is the normalized area percentage of the chromatogram for the component x.

Round the result to one decimal place.

When the system is working at its optimum, area percentage values for the composite standard solution correlate so closely with the known composition of the standard, that a response factor of unity can be applied to all components. When this is the situation, then the percentage of the component equals the area percentage of the component.

8 Precision

8.1 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 cases be greater than the repeatability limit (g/100 g) listed in Table 1 for that type of syrup.

Table 1 — Repeatability limits

Syrup type	Approximate syrup composition	Analyte	Repeatability limit, *r*
	g/100 g		g/100 g
α-D-glucose (Dextrose)	Dextrose 95	Dextrose Maltose	0,90 0,17
α-D-glucose (Dextrose)	Dextrose 45	Dextrose Maltose	1,4 0,70
Maltose	Maltose 80	Maltose	0,36
Maltose	Maltose 48	Maltose	0,39
Fructose	Fructose 42	Fructose Dextrose	0,43 0,23
Fructose	Fructose 9	Fructose Dextrose	0,22 0,55
Sorbitol	Sorbitol 98	Sorbitol	0,81
Maltitol	Maltitol 75	Maltitol Maltotriitol	0,19 0,12

8.2 Reproducibility

The absolute difference between two single results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the reproducibility limit (g/100 g) listed in Table 2 for that type of syrup.