SLOVENSKI STANDARD
SIST EN ISO 10504:2001

01-maj-2001

Škrobni derivati - Določanje sestave glukoznih, fruktoznih in hidrogeniranih glukoznih sirupov - Metoda tekočinske kromatografije visoke ločljivosti (ISO 10504:1998)

Starch derivative - Determination of the composition of glucose syrups, fructose syrups and hydrogenated glucose syrups - Method using high-performance liquid chromatography (ISO 10504:1998)

Stärke und Stärkederivate - Bestimmung der Zusammensetzung von Glucosesirup, Fructosesirup und hydriertem Glucosesirup - Hochleistungsflüssigchromatographisches Verfahren (ISO 10504:1998)

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 a haute performance (ISO 10504:1998)

Ta slovenski standard je istoveten z: **EN ISO 10504:2000**

ICS:

67.180.20	Škrob in izdelki iz njega	Starch and derived products

SIST EN ISO 10504:2001 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 10504

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English version

Starch derivatives - Determination of the composition of glucose syrups, fructose syrups and hydrogenated glucose syrups - Method using high-performance liquid chromatography (ISO 10504:1998)

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This European Standard was approved by CEN on 15 January 2000.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN ISO 10504:2000 E

Foreword

The text of the International Standard from Technical Committee ISO/TC 93 "Starch (including derivatives and by-products)" of the International Organization for Standardization (ISO) has been taken over as an European Standard by CEN/CS.

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 August 2000, and conflicting national standards shall be withdrawn at the latest by August 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 10504:1998 has been approved by CEN as a European Standard without any modification.

NOTE: Normative references to International Standards are listed in annex ZA (normative).

INTERNATIONAL STANDARD

ISO 10504

First edition
1998-10-01

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

Reference number
ISO 10504:1998(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10504 was prepared by Technical Commitee ISO/TC 93, *Starch (including derivatives and by-products).*

Annex A of this International Standard is for information only.

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 standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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.

a) Cation type:

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

— 200 mesh to 400 mesh in the dry form.

b) Anion type:

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

— 200 mesh to 400 mesh in the dry form.

NOTE While resins meeting these specifications are available from more than one supplier, their performance is variable. Experience in several laboratories shows that the resins sold by Bio-Rad [1] (AG50W - X4, AG3 - X4) perform satisfactorily.

5 Apparatus

5.1 Liquid chromatograph, equipped with the following:

— a pump, pulseless, that delivers a constant flow, at the rate required;

— a differential refractometer, thermostatically controlled;

— a 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.

1) This information is given for the convenience of 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.

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.

NOTE There are a few systems available but with varying efficiency. The Bio-Rad [2] guard cartridges 125-0118 have been shown in several laboratories to be the most effective in all respects.

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.

NOTE 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.

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

Instal 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.

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