Determination of Mono-, Di-, Tri- and Polysaccharides

A-07.3

Key words

Instrumental HPTLC - quantitative analysis - qualitative identification - densitometry (absorbance) - post chromatographic derivatization - food analysis - process control - carbohydrates

Scope

The analysis of sugars, e.g. in various food stuff, is important for manufacturer, authorities and private investigation laboratories. This analysis can easily and rapidly be performed by planar chromatography.

Depending on the mixture of sugars in a sample 3 different separation systems can be employed.

The extracts are chromatographed on silica gel or amino plates, respectively without prior derivatization. Post chromatographic derivatization with diphenyl amine-aniline-phosphoric acid-reagent follows for all standards and samples simultaneously. Using the amino plate the sugars fluoresce by simply heating the plate. Densitometric evaluation is done by absorbance measurement at 620 nm or by fluorescence measurement at 366/>400 nm. The limit of detection for fluorescent zones is about 10 ng.

Literature

R. Gauch, E. Leuenberger, E. Baumgartner, J. Chromatogr. **174**, 195-200 (1979)

R. Klaus, W. Fischer, H.E. Hauck, Chromatographia **28**, 364-366 (1989).

K. Patsch, S. Netz, W. Funk, J. Planar Chromatography **1**, 39-45 (1988), Part 1.

K. Patsch, S. Netz, W. Funk, J. Planar Chromatography **1**, 177-179 (1988), Part 2.

Advantages of using HPTLC for this analytical task

- Simple sample preparation, no pre-chromatographic derivatization
- Simultaneous quantitative determination of several saccharides in contrast to enzymatic methods
- High sample throughput

Chemicals

Acetone
Acetonitrile
Water dist.
Diphenylamine
Aniline chloride
Methanol
Butanol
Phosphoric acid, 86 %
Boric acid, 0.5 %

Standards:

Raffinose	Sucrose
Melezitose	Galactose
Lactose	Glucose
Maltose	Fructose

Sample preparation

Depending on type of foodstuff dissolve or extract sample with water, and filter if necessary. Co-extracted proteins, which would interfere with chromatographic analysis, can be precipitated by adding twice the amount of acetone (cold).

Dilute the solutions with methanol to a final concentration of about 0.2 mg/mL per saccharide.

Standard solutions

Dissolve 0.5 mg of those saccharides which shall be determined in 10 mL dist. water and dilute 1:4 with water (125 ng/µL).

Chromatogram layers

1) To separate mono-, di- and trisaccharides: HPTLC Silica gel Merck 60 F_{254}, 20x10 cm
2) To separate polysaccharides: HPTLC Silica gel Merck 60 (without F), 20x10 cm
3) To separate glucose from fructose: HPTLC Merck NH_2 F_{254s}, 20x10 cm

Sample application

With CAMAG Linomat as 7 mm bands, track distance 3 mm, distance from left edge 20 mm, distance from lower edge 8 mm; delivery rate 15 s/µL = 17 applications per plate.

Application scheme:

S1	U1	U2	S2	U3	U4	S3	U5	U6	S4	...	
2	2	2	3	2	2	4	2	2	5	...	µL/band

S1- S4 standards in different concentrations; U unknowns

Alternatively the Automatic TLC Sampler can be used.

Chromatography

In CAMAG Twin Trough chamber 20x10 cm

1) To separate mono-, di- and trisaccharides, except glucose from fructose:
   - 10 min conditioning with developing solvent.
   - Develop three times with acetonitrile - water 85:15; migration distance 70 mm each.
   - Dry with hair drier held at a distance of about 40 cm for 20 min (at 40-45°C). After final run, dry in the same way or at 100°C for 2 min in an oven.
2) To separate polysaccharides:
   - 10 min conditioning with developing solvent.
   - Develop with butanol - methanol - water 50:25:20; migration distance 70 mm.
   - Dry plate for 15 min with hair drier.
3) To separate glucose from fructose:
   - Develop with acetonitrile -water - 0.5% aqueous solution of boric acid 76:24:10; migration distance 75 mm.
   - Dry plate for 5 min with hair drier.

Derivatization

- By dipping for 3 s with CAMAG Chromatogram Immersion Device in diphenylamine reagent (2.4 g diphenylamine + 2.4 g aniline chloride in 200 mL methanol, then add 20 mL 86% phosphoric acid*) followed by heating at about 120°C for 10-15 min.
- Using NH_2-plates the sugars fluoresce simply by heating the plate at about 130°C for 5 min. (Some sugars may require a higher temperature and time.)

Note: Dipping the plate into a solution of paraffin/n-hexane 1:2 enhances the fluorescence intensity by a factor 2-3.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by absorbance at 620 nm with tungsten lamp or by fluorescence at 366/>400 nm with mercury lamp, slit dimension 0.2x4 mm, detection limit for fluorescent zones about 10 ng/zone.

* For the NH_2-plate use acetone as solvent; the solution is stable in the dark for several weeks.

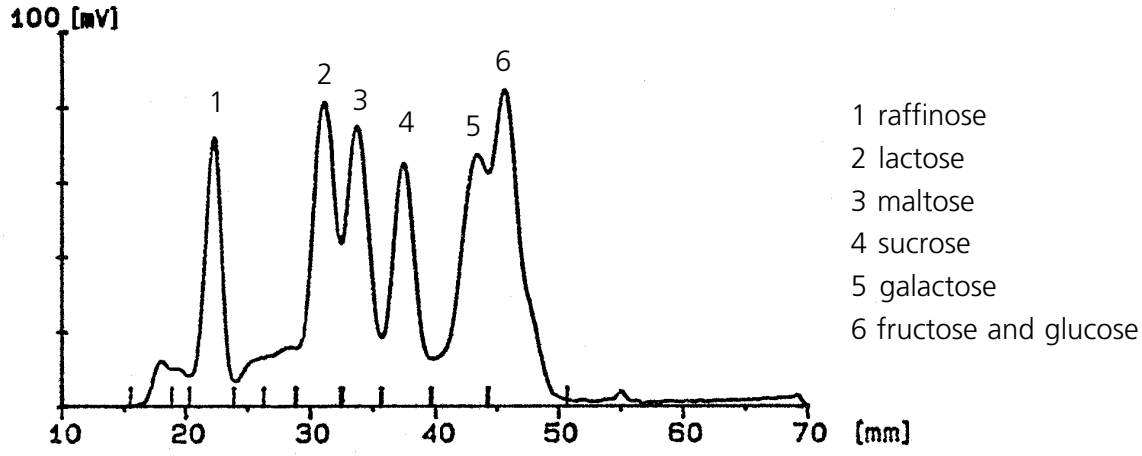

Fig. 1: Densitogram by 620 nm of 7 different saccharides (plate/chromatography see 1.)

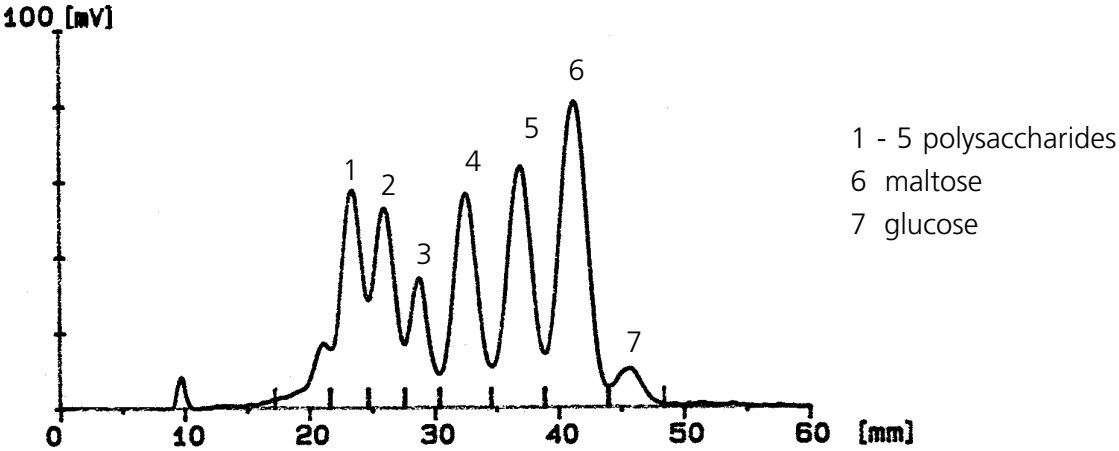

Fig. 2: Densitogram by 620 nm of an unknown sample of polysaccharides; maltose and glucose (plate/chromatography see 2.)

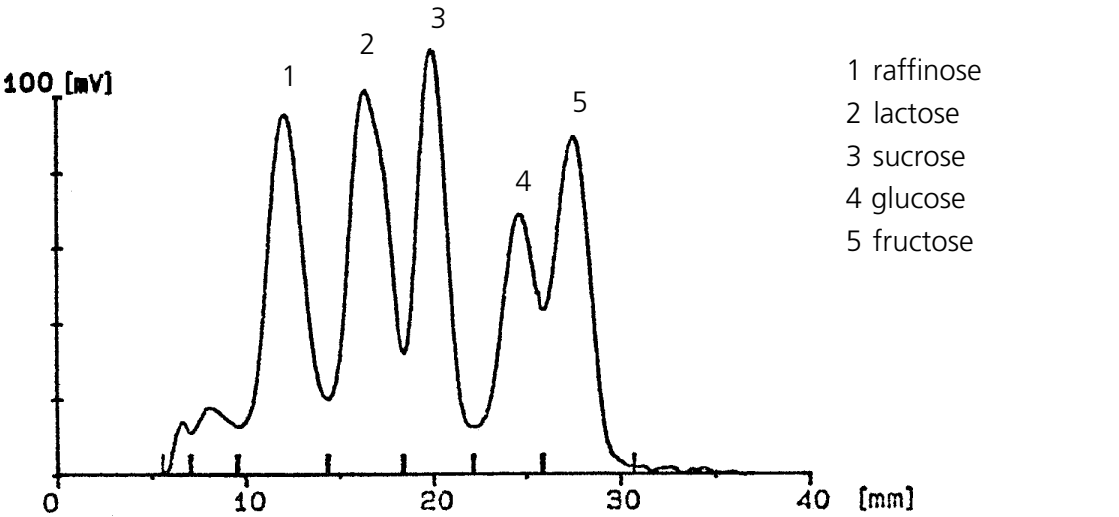

Fig. 3: Densitogram by 620 nm of 5 different saccharides (plate/chromatography see 3.)