APPLICATION NOTE

Quantification of carbohydrates in various matrices

A-136.1
PRO

Keywords

Monosaccharides, polysaccharides, oligosaccharides, honey, fermentation, sugars, food, HPTLC PRO

Introduction

The quantification of carbohydrates can be challenging due to their high polarity, low volatility, their lack of a chromophore and their common occurrence in complex matrices [1-5]. HPTLC separates mono- and oligosaccharides after minimal sample preparation and sensitively detects and quantifies these compounds after post-chromatographic derivatization. With the two methods developed for the HPTLC PRO System described herein, many samples can be rapidly analyzed at low running costs [5]. The HPTLC PRO System allows to autonomously process up to five HPTLC plates without manual intervention. Run time for each plate is reduced compared to conventional HPTLC due to the optimized chamber geometry, the reduced equilibration time, and parallel processing of plates within the System (while the second plate is applied the first is developed). Two different methods (A and B) are suitable for different analytical tasks and can be applied for various sugar-containing sample matrices, *e.g.* fermentation broth, after selection of a suitable sample preparation [4].

Scope

The two methods (A and B) are suitable for the analysis and quantification of carbohydrates in various matrices like syrups, honeys, and molasses. The selectivity of the two methods is different and the elution strength of method B is higher. Method A is better suited for the separation of small carbohydrates (like fructose and glucose), and with method B also large carbohydrates and sugar alcohols are migrating. The content of each carbohydrate can be determined by multi-level calibration. For quantification, a single-wavelength scan at 370 nm after derivatization with aniline-diphenylamine-phosphoric acid (ADPA) reagent is shown.

NOTE: The presented results are to be regarded as examples only!

Recommended devices

HPTLC PRO Module PLATE STORAGE, HPTLC PRO Module APPLICATION, HPTLC PRO Module DEVELOPMENT, Derivatizer, TLC Visualizer 2, TLC Scanner 4, *visionCATS* 3.1 (or higher)

Samples

10.0 mg of each sample (*e.g.* honey) are mixed with 10.0 mL of 50% aqueous acetonitrile and sonicated for 10 min. If not completely dissolved, samples are centrifuged and the supernatants are used as test solutions. Fermentation broth samples can be directly applied or after dilution with 50% aqueous acetonitrile. Sample preparation for other matrices (chocolate, biscuits, propolis, malt drink, milk) is described in [4].

References

System suitability test (SST): Universal HPTLC Mix (UHM) from [6]

Identification: carbohydrates are individually dissolved in 50% aqueous acetonitrile at a concentration of 1.0 mg/mL

Quantification: dilution of the corresponding reference(s) in 50% aqueous acetonitrile to different concentration levels (linear working range for fructose, sucrose, maltose, and glucose from LOQ_{370} nm to 125 ng/zone, see [5])

Chromatography

Stationary phase	HPTLC glass plates Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	20 tracks, band length 6.0 mm, track distance 8.5 mm, distance from left edge 18.0 mm, distance from lower edge 8.0 mm Identification and SST: apply 1.0 µL of each reference solution, 2.0 µL of the UHM and 3.0 µL of test solutions Quantification: apply 1.0 µL of reference solutions and 1.0 µL of non-diluted and/or diluted test solutions (*e.g.* 1:20 diluted for the quantification of fructose and glucose in honey)
Developing solvent	*Method (A):* *n*-butanol – isopropanol – aqueous boric acid (5.0 mg/mL) 3:5:1 (*V/V*) *Method (B):* ethyl acetate – methanol – aqueous boric acid (5.0 mg/mL) – acetic acid 50:40:10:2 (*V/V*)
Development	*Method (A):* plates are developed in the HPTLC PRO Module DEVELOPMENT after activation at 0% relative humidity (molecular sieve) for 10 min, followed by 90 s of pre-conditioning with developing solvent at 30% pump power. *Method (B):* Plates are developed in the HPTLC PRO Module DEVELOPMENT after activation at 0% relative humidity (molecular sieve) for 10 min.

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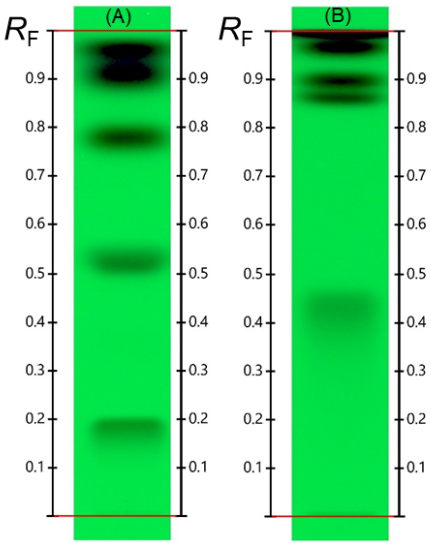
Developing distance	70 mm (from the lower edge)
Plate drying	*Method (A):* Drying for 15 min in the HPTLC PRO Module DEVELOPMENT *Method (B):* Drying for 5 min in the HPTLC PRO Module DEVELOPMENT
Derivatization reagent	ADPA reagent Preparation: 2.0 g of diphenylamine and 2.0 mL of aniline are dissolved in 80.0 mL of methanol, and 10.0 mL of *o*-phosphoric acid (85%) are added. The mixture is shaken until any precipitate is dissolved, and then another 10.0 mL of methanol are added. Use: Derivatize (Derivatizer: 3 mL, yellow nozzle, spraying level 6), heat the plate at 110°C for 10 min.
Documentation	With the TLC Visualizer 2: Underivatized at UV 254 nm (required for the detection of the UHM) and derivatized at white light RT
Densitometry	Densitometric analyses for quantification are performed in absorbance mode with the TLC Scanner 4 at 370 nm, slit dimension 5.0 x 0.3 mm, scanning speed 20 mm/s, data resolution 25 µm/step; spectra recording from 350 to 800 nm.

Results

SST: Zones must be detected at UV 254 nm prior to derivatization

(A) R_F 0.14, R_F 0.53, R_F 0.78 ± 0.025
(B) R_F 0.43, R_F 0.86, R_F 0.90 ± 0.025



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Identity:

List of carbohydrates and their corresponding R_F values

Carbohydrate	R_F (A)	R_F (B)	Carbohydrate	R_F (A)	R_F (B)
Galacturonic acid	0.06	0.13	Ribose	0.36	0.59
Glucuronic acid	0.09	0.17	Sucrose	0.40	0.56
Raffinose	0.18	0.32	Glucose	0.44	0.64
Maltotriose	0.21	0.37	Arabinose	0.44	0.64
Lactose	0.24	0.41	Mannose	0.48	0.63
Fructose	0.24	0.48	Xylose	0.48	0.71
Trehalose	0.32	0.48	Fucose	0.52	0.71
Maltose	0.33	0.52	Rhamnose	0.64	0.78
Galactose	0.36	0.55	Deoxyribose	0.68	0.81

UV/VIS spectra (after derivatization) of fructose, maltose, sucrose, and glucose

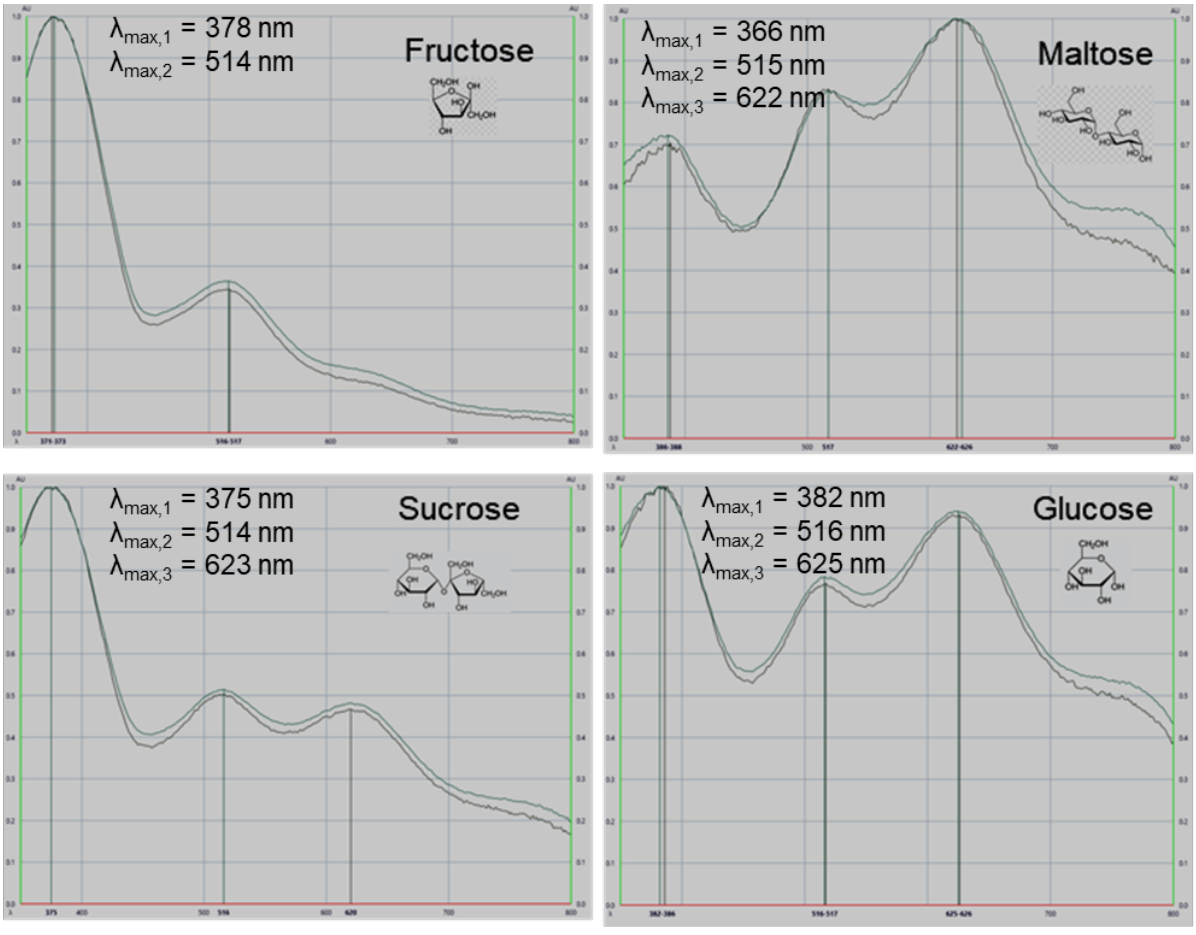

Figure 1: UV/VIS spectra recorded with the TLC Scanner 4 at 350-800 nm after derivatization with ADPA reagent; Note: UV/VIS spectra of other carbohydrates are available in the substance database of the HPTLC Association [7]

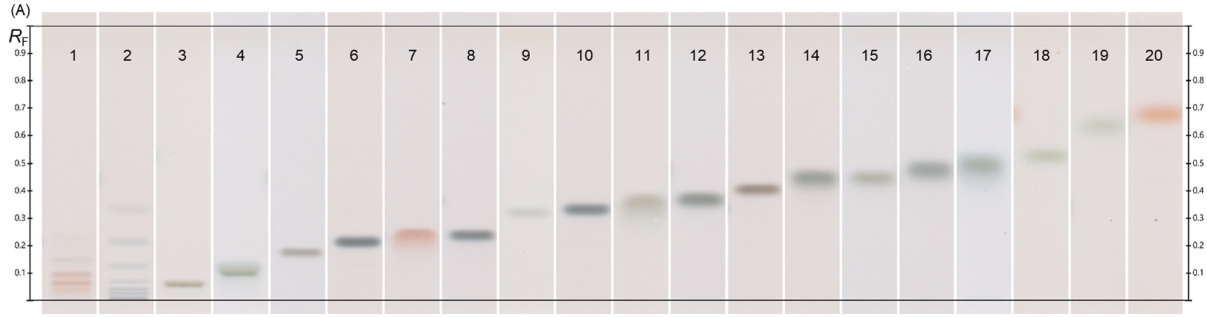

Figure 2: HPTLC chromatograms of carbohydrates obtained with method (A) after derivatization with ADPA reagent at white light RT

Track assignment table for Figure 2

Track	Reference	Track	Reference
1	Fructo-oligosaccharides	11	Ribose
2	Maltodextrin	12	Galactose
3	Galacturonic acid	13	Sucrose
4	Glucuronic acid	14	Glucose
5	Raffinose	15	Arabinose
6	Maltotriose	16	Mannose
7	Fructose	17	Xylose
8	Lactose	18	Fucose
9	Trehalose	19	Rhamnose
10	Maltose	20	Deoxyribose

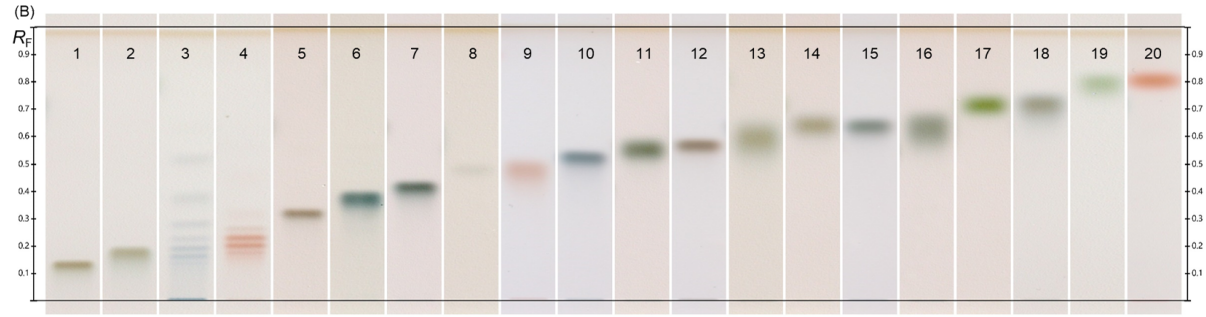

Figure 3: HPTLC chromatograms of carbohydrates obtained with method (B) after derivatization with ADPA reagent at white light RT

Track assignment table for Figure 3

Track	Reference	Track	Reference
1	Galacturonic acid	11	Galactose
2	Glucuronic acid	12	Sucrose
3	Maltodextrin	13	Ribose
4	Fructo-oligosaccharides	14	Arabinose
5	Raffinose	15	Glucose
6	Maltotriose	16	Mannose
7	Lactose	17	Fucose
8	Trehalose	18	Xylose
9	Fructose	19	Rhamnose
10	Maltose	20	Deoxyribose

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Different samples analyzed with method (A)

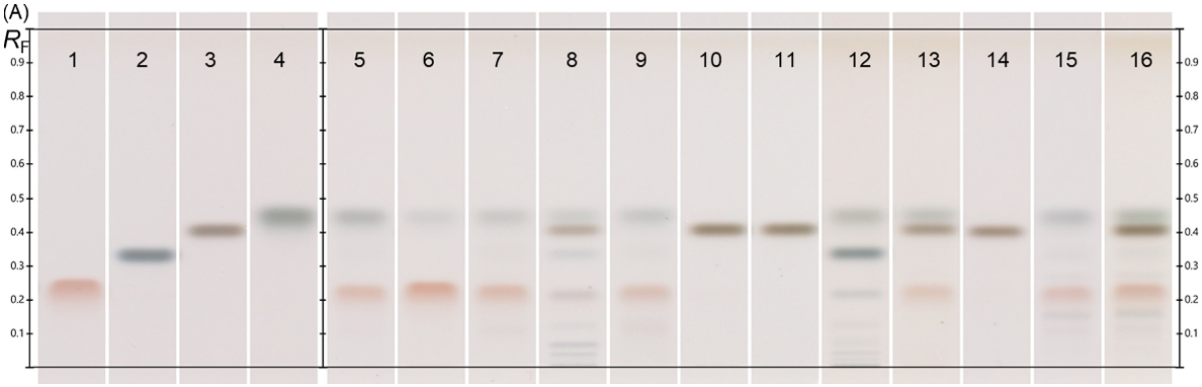

Figure 4: HPTLC chromatograms of selected references and samples obtained with method (A) after derivatization with ADPA reagent at white light RT; Track 1: fructose, track 2: maltose, track 3: sucrose, track 4: glucose, track 5: blossom honey, track 6: agave syrup, track 7: linden blossom honey, track 8: molasses, track 9: honeydew, track 10: sugar from coconut-flowers, track 11: sugar cane, track 12: rice syrup, track 13: syrup from coconut-flowers, track 14: maple syrup, track 15: wild bee honey, track 16: wild bee honey adulterated with maple syrup

Quantification

In many cases the samples have to be diluted for a quantification in the linear working range, *e.g.* for a quantification of fructose and glucose in honey, the samples are diluted 1:20.

Example: quantification of fructose, maltose, sucrose, and glucose in a wild bee honey, in maple syrup, and a wild bee honey adulterated with maple syrup

	Concentration (g/100 g)			
	Fructose	**Glucose**	**Maltose**	**Sucrose**
Maple syrup	*n.d.*	*n.d.*	*n.d.*	67.67
Maple syrup + wild bee honey	31.97	22.42	6.13	68.59
Wild bee honey	31.88	24.86	6.96	*n.d.*

n.d.: not detected

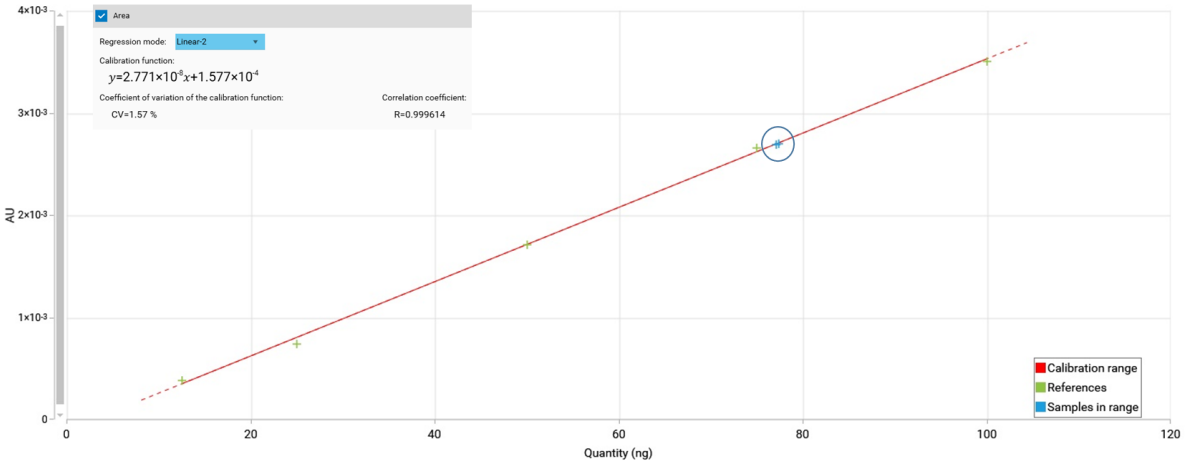

Figure 5: Calibration curve of sucrose (method A); blue circle shows the amount detected in the samples maple syrup and wild bee honey mixed with maple syrup.

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Literature

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