Application note | 000916

Food analysis

Simple screening of natural sugars for detecting adulteration in honey

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Keywords

Honey, sugars, sucrose, glucose, fructose, quality control, Vanquish Core HPLC, Vanquish Refractive Index Detector, Chromeleon CDS, adulteration

Application benefits

- A robust HPLC-RID method for the quantification of glucose, fructose, and sucrose in honey
- Simple and reproducible workflow fulfilling the food regulatory requirements
- Cost-efficient quality test

Goal

To develop a robust HPLC-RID method for the determination of sugar content in honey samples to evaluate their quality and to assess the possibility of adulteration

Introduction

Honey is a truly natural product that is consumed all over the world and is known for its high nutritional value, sweetness, and health benefits. Almost two million tons are produced annually worldwide, which is remarkable considering that each beehive can produce about 20 kg of honey per year. Due to the production limitations as a natural product and high production costs, honey adulteration is a major problem, fueled by readily available, cheaper sweeteners and sugar syrups. According to the Food Fraud Database, honey ranks as the third most frequent target for food adulteration, only surpassed by milk and olive oil.[1] Similarly, the European Union has identified honey to be most at risk for food fraud.[2]

Honey consists essentially of sugars (79%)—predominantly fructose and glucose—water (17%), and other components (4%), such as vitamins and minerals in trace quantities (Figure 1).

Figure 1. Natural honey composition

One approach to assess the quality and address adulteration is to determine sugar content and detect abnormal sugar profiles. The Codex Alimentarius[3], the internationally accepted standards for foods issued by the Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), and the European Directive[4], define the specific sugar amount range for fructose, glucose, and sucrose as parameter to determine honey quality (Table 1).

Table 1. A summary of sugar composition criteria of the EU Directive and the Codex Alimentarius

	Sugar content
Fructose and glucose content (sum of both)	**not less than 60 g/100 g** for honeydew honey, blends of honeydew honey with blossom honey
	not less than 45 g/100 g for any honey not listed above
Sucrose content	**not more than 15 g/100 g** for lavender *(Lavandula spp)*, borage *(Borago officinalis)*
	not more than 10 g/100 g for false acacia *(Robinia pseudoacacia)*, alfalfa *(Medicago sativa)*, Menzies banksia *(Banksia menziesii)*, French honeysuckle *(Hedysarum)*, red gum *(Eucalyptus camadulensis)*, leatherwood *(Eucryphia lucida, Eucryphia milliganii)*, *Citrus* spp.
	not more than 5 g/100 g for any honey not listed above

Multiple techniques can be used for carbohydrates analysis.[5] The Thermo Scientific™ Vanquish™ Refractive Index Detector (RID) is a universal detector for analysis of substances that lack a UV chromophore, so it is ideal for sugar analysis and quantification. Its sensitivity complies with regulatory demand allowing the wide and repeatable measurement range required in routine food control. The Vanquish RID is easily integrated in Vanquish LC systems with optimized fluidic connections and single-point intelligent control through the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). The detector provides excellent reproducibility and sample throughput by supporting fast separations with high data collection rates. Alternatively, pulsed amperometric detection (PAD) is particularly powerful in combination with high-performance anion exchange (HPAE) as it permits direct quantification of nonderivatized carbohydrates at low-picomole levels, usually used for floral origin and adulteration type determination purposes. This application note evaluates a HPLC method featuring the Vanquish RID for the separation and measurement of fructose, glucose, and sucrose (Figure 2) as a routine, cost-efficient solution for the quality assessment and adulteration determination of honey samples.

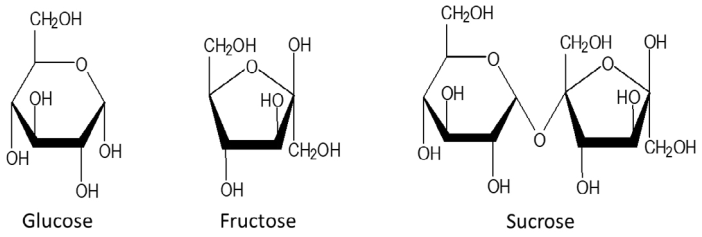

Figure 2. Chemical structures of glucose, fructose, and sucrose

Experimental

Table 2. Chemicals

Chemical name	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Acetonitrile, Optima™ LC/MS grade, Fisher Chemical™	A955-1
D(-)-Fructose, Certified AR for analysis, Fisher Chemical™	F/1952/48
D(+)-Glucose monohydrate, extra pure, SLR, meets analytical specification of Ph. Eur., BP, Fisher Chemical™	G/0400/60
D(+)-Sucrose, 99+%, for analysis, Thermo Scientific™	220902500
D(+)-Maltose monohydrate 90%, Sigma-Aldrich	112569
Quality of Honey Quality Control Material, Fera Science Ltd	FCQH2-HON2QC T2849QC

Table 3. Sample handling

Item name	Part number
LP vortex mixer, Thermo Scientific™	88880017
Fisherbrand™ Analytical balance, Fisher Scientific™	15917500
F1-ClipTip™ variable volume single channel pipettes, Thermo Scientific™	4641210N 4641200N 4641230N
SureSTART™ 9 mm screw caps, Level 2 high-throughput applications, Thermo Scientific™	6ASC9ST1
SureSTART™ 2 mL glass screw top vials, Level 2 high-throughput applications, Thermo Scientific™	6ASV9-1P

Table 4. Instrumentation

Module	Part number
Vanquish Core HPLC system consisting of:	
Vanquish Core System Base	VC-S01-A-02
Vanquish Quaternary Pump C	VC-P20-A-01
Vanquish Split Sampler CT	VC-A12-A-02
Vanquish Column Compartment H Passive pre-heater, SST, 1 µL	VH-C10-A-03 6732.0170
Vanquish Refractive Index Detector	VC-D60-A01

Standards and sample preparation

Standard solutions: Stock solutions of the sugars were prepared by dissolving 500 mg of each carbohydrate in 10 mL deionized water to make an individual 50,000 mg/L stock standard. The stock standards solutions were used to produce mixed standards solutions, with the final concentration levels described in Table 5 by dilution in deionized water.

Table 5. Concentration in mg/L of the sugar calibration standards

	Calibration standard (mg/L)					
Sugar	**Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**Level 6**
Sucrose	5	10	20	50	100	200
Glucose	25	50	100	250	500	1000
Fructose	25	50	100	250	500	1000

Check standards: As best practices in routine quantification assays, internal quality controls were prepared to check the method accuracy in the low, middle, and high concentration ranges, respectively 6, 30, 90 mg/L for sucrose and 30, 150, 950 mg/L for both glucose and fructose.

Honey samples: Seven different commercial honey products were purchased at local stores. Various types from multiple vendors were selected as a representative panel of commercial offerings (i.e., acacia, thyme, flowers). They were labeled from HN1 to HN7. Each honey sample was prepared by dissolving 500 mg in 10 mL of deionized water and diluting by 50 and 100 to achieve a 1:500 and 1:1000 fold dilution, respectively.

Chromatography Data System

Chromeleon 7.3 Chromatography Data System (CDS) was used for data acquisition and analysis. Quality control laboratories need to rely on easy-to-use systems and robust methods so that lab productivity is optimized and results are delivered with high confidence. The Vanquish Core HPLC system combined with the new Vanquish RID is fully integrated into Chromeleon software and offers a unique experience for routine labs thanks to innovative eWorkflow™ procedures (Figure 3), which take the operator from samples to reliable results in as little as two clicks. The operator just selects an instrument, specifies the number of samples and the starting vial position in the autosampler, and begins the analysis. The software then runs the analysis, processes the data, calculates the results, and automatically generates the report.

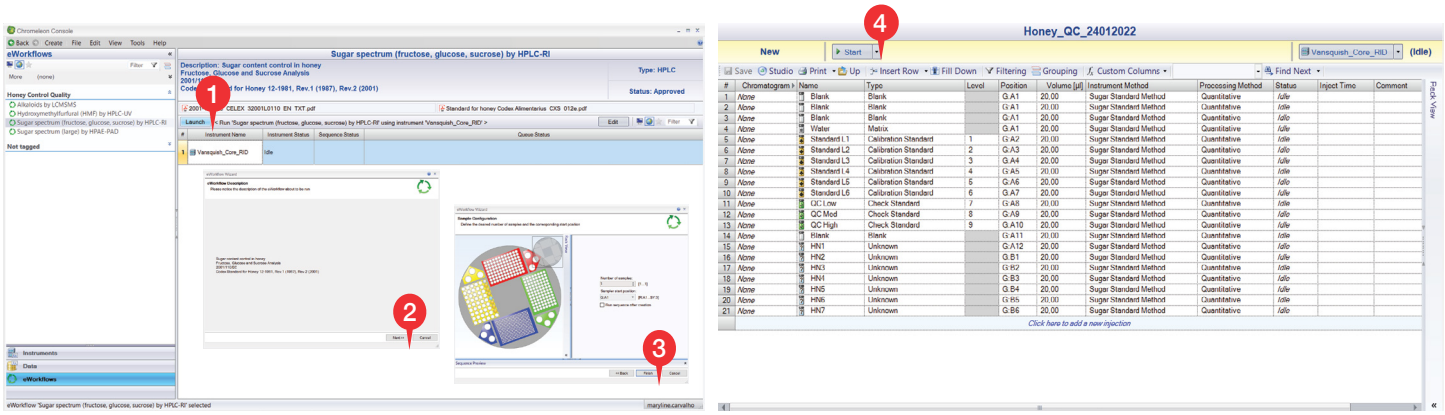

Figure 3. eWorkflow workflow

Table 6. Method conditions

Column	Thermo Scientific™ HyperREZ™ XP Carbohydrate Pb^{2+} (300 mm × 7.7 mm, 8 µm), P/N 69108-307780 with guard cartridge, P/N 69108-903027	Thermo Scientific™ Hypersil GOLD™ Amino (150 mm × 4.6 mm, 3 µm), P/N 25703-154630 with guard cartridge, P/N 25703-014001
Mobile phase	100% Water	Acetonitrile:water (v:v, 80/20)
Run time	25 min	10 min
Flow rate	0.6 mL/min	1.2 mL/min
Column temperature	70 °C (forced air), passive preheater	45 °C (still air), passive preheater
Autosampler temperature	4 °C	4 °C
Autosampler wash solvent	Water:methanol:isopropanol:acetonitrile (v:v:v:v, 45:15:15:15)	Water:methanol:isopropanol:acetonitrile (v:v:v:v, 45:15:15:15)
Injection volume	20 µL	10 µL
Detector settings	Temperature: 35 °C Response time: 1 s Data collection rate: 5 Hz	Temperature: 35 °C Response time: 1 s Data collection rate: 5 Hz

Results and discussion

HyperREZ XP Carbohydrate column

The HyperREZ XP Carbohydrate column is a polymer-based column designed for the determination of carbohydrates, saccharides, organic acids, and alcohols and was selected in this work as it offers the best user experience thanks to its stability and reproducibility. The lead form was preferred over the other cation forms (calcium and sodium) as it provides the best separation of fructose, glucose, and sucrose as well as maltose, which can be a potential natural interference in honey samples and is known to be used as an adulterant sugar (Figure 4).

To investigate reliability and robustness, this study was conducted in two different labs in Germany and France using two different Vanquish Core HPLC systems and two different HyperREZ XP Carbohydrate Pb^{2+} columns, each from different lots. Before starting a series of analyses, an equilibration run of two hours is required to ensure baseline stabilization and achieve minimum noise drift.

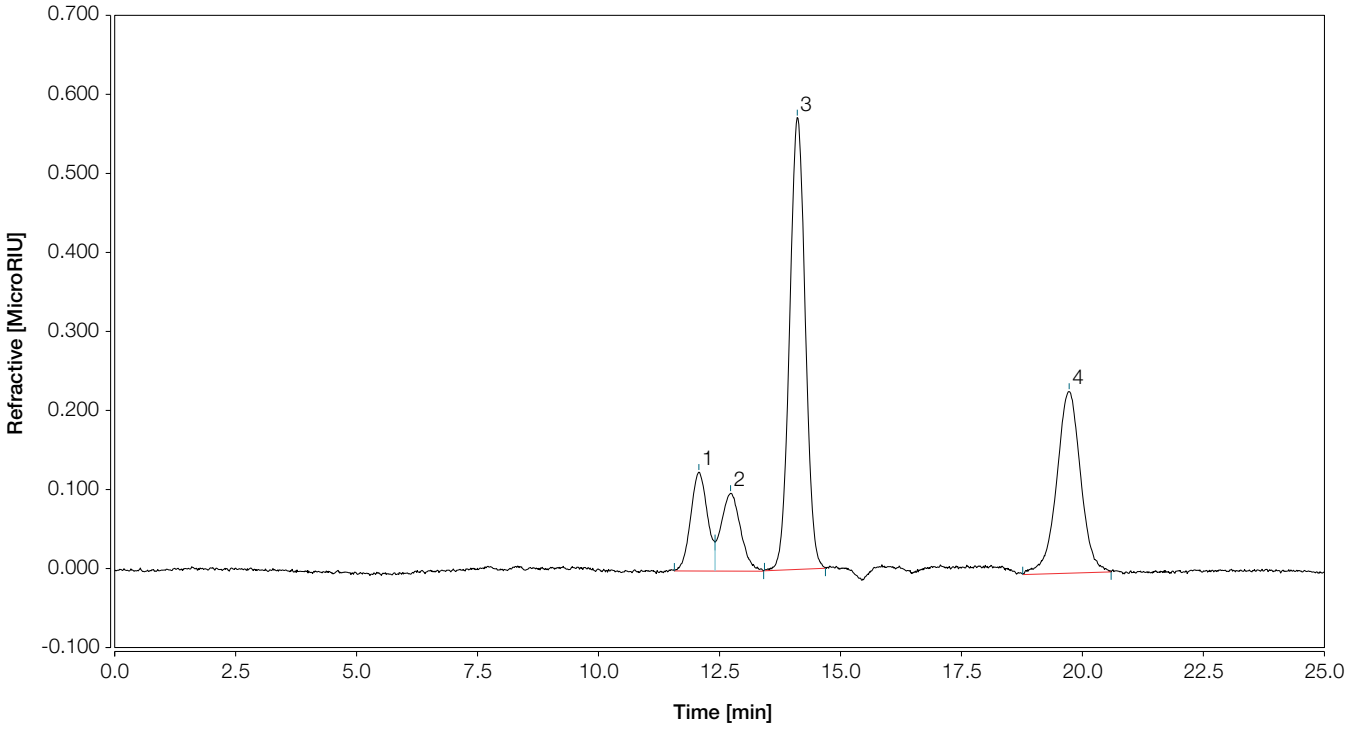

Figure 4. Chromatogram profile corresponding to a mixed standards solution. The separation of sucrose (1), maltose (2), glucose (3), and fructose (4) was obtained with a HyperREZ XP Carbohydrate Pb^{2+} column in isocratic mode. Mixed standard concentrations are respectively 10 mg/L for sucrose and maltose, and 50 mg/L for glucose and fructose.

Inter-system relative standard deviation for retention time values was less than 1% for all compounds of interest (Table 7). This is particularly important because processing methods do not have to be adapted from one system to another when a method is transferred.

Table 7. Relative standard deviation of retention time and relative peak area for each sugar

Sugar	Retention time relative standard deviation (%) (N=30)	Relative peak area relative standard deviation (%) (N=30)
Sucrose	0.68	2.16
Glucose	0.55	0.65
Fructose	0.30	1.36

Method linearity and performance

Calibration curves were obtained using averaged peak areas from two replicate injections of standard mixtures. The concentration range was set from 5 to 200 mg/L for sucrose and from 25 to 1,000 mg/L for both fructose and glucose. All components showed excellent method linearity and curve fits, with the coefficient of determination R^2 >0.9999 (Table 8). Relative standard deviations for all check standards were below 5%. In addition, the method accuracy was verified by analyzing a honey quality control material from Fera Science Ltd., which included the individual sugars fructose, glucose, and sucrose, as well as hydroxymethylfurfural (HMF) and diastase. Three samples were prepared from the honey quality control following the procedure described for commercial honey specimens. The amount deviation for sucrose was 9%, which was still within the acceptable concentration range published in Fapas QC Material Data Sheet, while for both fructose and glucose it was below 2%. The external quality control results demonstrate confidence for monitoring sugar content in honey samples.

Table 8. Results of external linear calibration based on standards of Table 5. Calibration equation: (Peak area) = b*(Amount) + a

Sugar	Retention time relative standard deviation (%)	Calibration line parameters		
		a	b	R^2
Sucrose	0.07	0.0015	0.0048	0.99996
Glucose	0.03	-0.0025	0.0045	0.99999
Fructose	0.03	0.0012	0.0022	0.99993

Honey samples results

For all seven investigated honey samples, fructose and glucose were found to be the major constituents, with fructose always in greatest proportion. Their amounts complied to the limits established by the Codex Alimentarius and EU Directive. Sucrose was detected as a minor constituent, and its amount was within the limits established by the Codex Alimentarius and EU Directive. As expected, maltose was detected in all honey samples. Refer to Figures 5 and 6.

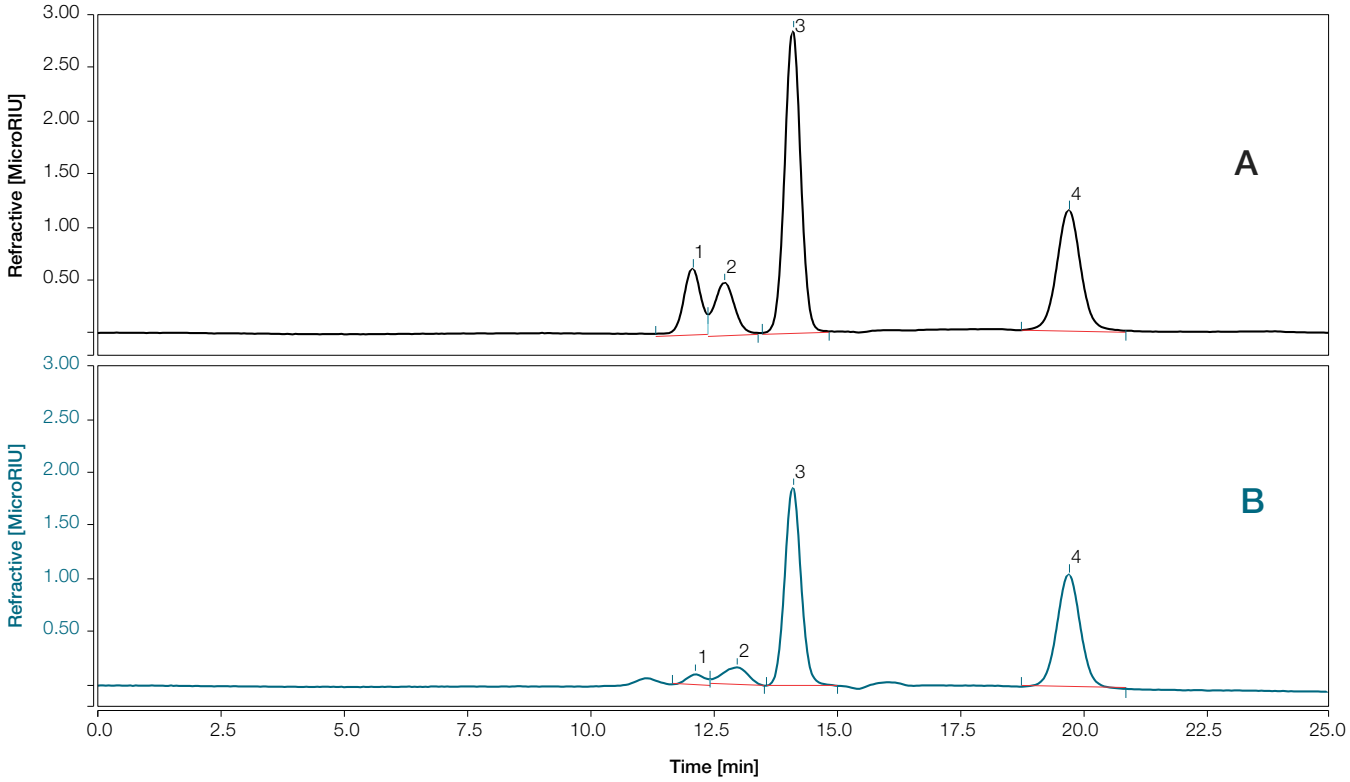

Figure 5. Chromatogram of mixed standard (A) and honey sample HN1 (B). Mixed standard concentrations are respectively 50 mg/L for sucrose (1) and maltose (2), and 250 mg/L for glucose (3) and fructose (4).

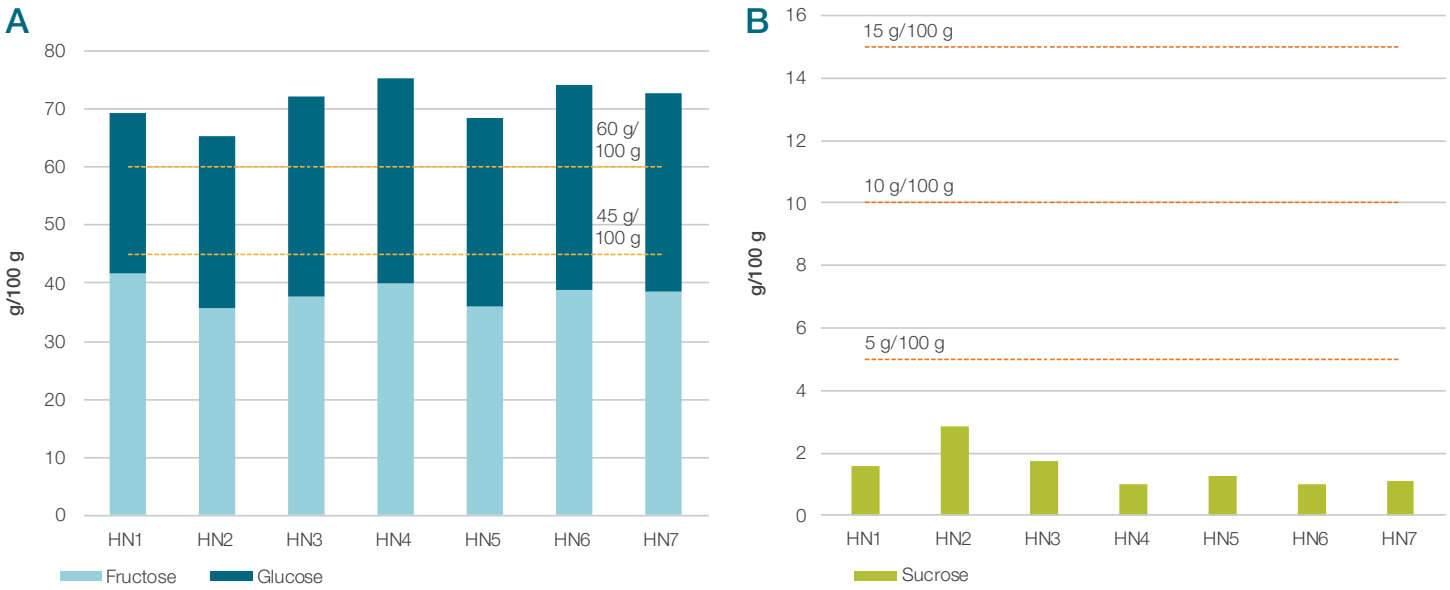

Figure 6. Sugar content results for seven commercial honey samples tested. The limit amounts required by The Codex Alimentarius Committee on Sugars (2001) is reported as an indicator. (A) Results for fructose and glucose, (B) results for sucrose.

Hypersil GOLD Amino column

The Hypersil GOLD Amino column was evaluated for boosting lab productivity by using a silica stationary phase with smaller particle size. A faster method of 10 minutes run time was achieved using a 150 mm long column with 4.6 mm inner diameter and 3 µm particles size. An isocratic method based on a mix of acetonitrile and water (80/20, v/v) as mobile phase composition and 1.2 mL/min as flow rate were defined as the best conditions for resolution of targeted sugars (Figure 7). Although the HyperREZ XP Carbohydrate column is usually preferred as a traditional column for sugar analysis with a simple ACN-free mobile phase, the Hypersil GOLD Amino column can be considered as an alternative for sugars quantitation determination in honey.

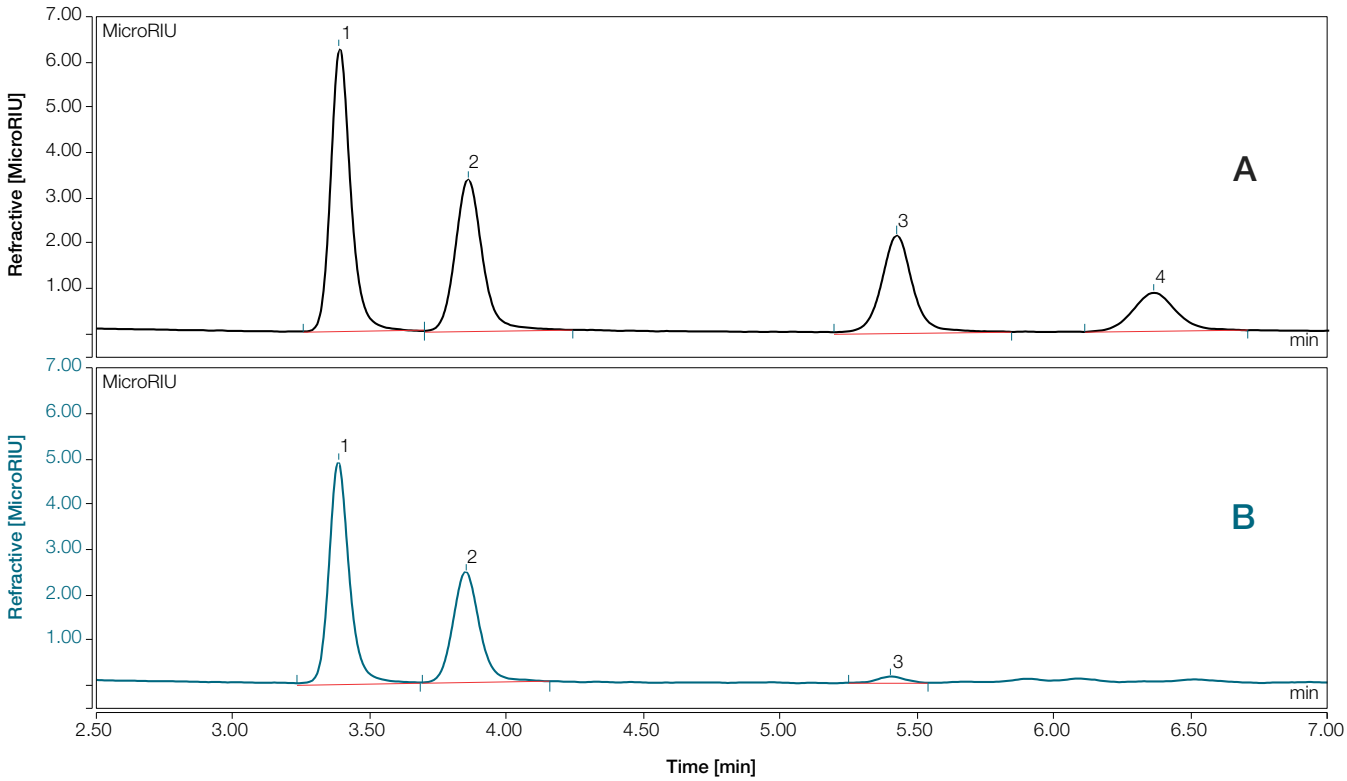

Figure 7. Chromatogram of mixed standard (A) and honey Fapas quality control test material (B). The separation of fructose (1), glucose (2), sucrose (3), and maltose (4) was obtained with a Hypersil GOLD Amino column in isocratic mode. Mixed standard concentrations were 500 mg/L for fructose and glucose and 250 mg/L for sucrose and maltose.

Conclusion

The Vanquish Core HPLC system with Vanquish Refractive Index detector offers a cost-efficient solution to control the content of regulated sugars in honey and determine potential adulteration. It provides robust and sensitive determination of low sucrose concentrations while at the same time detecting high concentrations of the major components, glucose and fructose.

The Hypersil GOLD Amino column achieves a fast separation increasing lab productivity, while the HyperREZ XP Carbohydrate Pb^{2+} column offers the greener chromatography method with a mobile phase 100% aqueous, moving towards conditions more sustainable and eco-friendly.

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