APPLICATION NOTE

No. 1158

HPAE-PAD determination of carbohydrates in honey to evaluate samples for quality and adulteration

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Key words
Dionex CarboPac PA210-4 µm column, food adulteration, sugar syrup, honey sugars authenticity

Introduction

Honey is a complex mixture of sugars produced in nature by honeybees. It consists mainly of sugars but also contains small amounts of proteins (enzymes), amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. The sugar composition of honey is mainly dependent on its floral source and differs in various honeys. It is also affected by climate, processing, and storage conditions.[1] Fructose and glucose are the major components and account for 85–95% of the honeybee honey sugars. Their concentrations of fructose and glucose, as well as their ratios are useful parameters for the classification of monofloral honeys.[2] The remaining carbohydrates are a mixture of at least 11 disaccharides, 11 trisaccharides, and several larger oligosaccharides.[3] Minor honey sugars may be useful for the determination of floral origin and may act as a "fingerprint" for a sample's floral source.[4] Besides the reducing sugars (glucose and fructose), the amount of sucrose is a very important indicator for evaluating honey quality. Previous studies

have shown that the amount of sucrose can be used to differentiate the adulteration of honey samples by sugar syrups.[5, 6] High levels of sucrose may indicate a variety of adulterations, such as adding cheap sweeteners, like cane sugar or refined beet sugar, during early harvest. Due to these factors, various regulations require a minimum amount of reducing sugars and a maximum amount of sucrose among other honey quality parameters. The Codex Alimentarius Committee on Sugars (2001) specified a maximum value of 5 g of sucrose in 100 g of floral honey (Codex Standard for Honey, 2001).[7] Therefore, carbohydrate analysis is important as a honey quality parameter and for floral origin determinations.

High Performance Anion Exchange chromatography coupled with Pulsed Amperometric Detection (HPAE-PAD) is one of the most useful techniques for carbohydrate determinations. Many research groups have used HPAE-PAD for the quantification of the carbohydrates in honey.[4, 8–12] Cordella, et al. demonstrated that the HPAE-PAD technique can be used in an automated chemometric approach for honey characterization. They employed chemometric tools such as principal component analysis (PCA), linear discriminant analysis (LDA), and partial least squares (PLS), to process the samples' HPAE-PAD chromatograms to distinguish between honeys of different floral origin. They further elaborated upon this approach for detecting and quantifying the presence of industrial sugar syrup in honey samples with a detection limit of as low as 10% added syrup.[11, 12]

In this application note, we developed a HPAE-PAD method to assay fructose and glucose, and to measure the entire profile of di- and trisaccharides in honey. In this method, separation of individual honey sugars was achieved on the recently introduced Thermo Scientific™ Dionex™ CarboPac™ PA210-4µm column.[13] The Dionex CarboPac PA210-4µm column was developed to provide fast, high-resolution separations for most mono- through tetrasaccharides in a variety of food and beverage samples. These columns are packed with a hydrophobic, polymeric, microporous anion exchange resin stable over the entire pH range of 0–14. Carbohydrate detection was by PAD with a gold working electrode and therefore no sample derivatization was required. In this work, honey sugars of 12 different honey samples have been characterized and quantitated by HPAE-PAD. In addition, HPAE-PAD profiling was demonstrated as a method to study the adulteration of honey samples with commercial sugar syrups.

Goal

To develop an HPAE-PAD method for the determination of carbohydrates in honey samples to evaluate their quality and for the assessment of adulteration

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ system, including:
  - SP Single Pump or DP Dual Pump
  - DC Detector/Chromatography Compartment
  - ED Electrochemical Detector (No cell, P/N 072042)
  - ED Cell (no reference or working electrode; P/N 072044)
  - ED Cell Polypropylene support block for use with disposable electrodes* (P/N 062158)
  - Gold on PTFE Disposable Electrode (P/N 066480)
  - pH-Ag/AgCl Reference Electrode (P/N 061879)
  - EG Vacuum Degas Conversion Kit (P/N 063353)
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option (P/N 074926)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 column (P/N 075550)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software was used for all data acquisition and processing.

** This method can also be executed with a conventional gold working electrode, though all the data presented in this application note were collected with disposable gold working electrodes. This method could be run on a Dionex Integrion system equipped with an electrochemical detector.*

Consumables

- 10 µL PEEK Sample Loop (P/N 042949)
- Thermo Scientific Nalgene Syringe Filters, PES, 0.2 µm (Fisher Scientific, P/N 09-740-61A)
- AirTite All-Plastic Norm-Ject Syringes, 5 mL, sterile (Fisher Scientific, P/N 14-817-28)
- Vial Kit, 10 mL Polypropylene with Caps and Septa (P/N 055058)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane (1000 mL, 0.2 µm pore size, Fisher Scientific P/N 09-740-46)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Trehalose, Fluka (P/N 90208)
- D-Glucose, Sigma-Aldrich (P/N G8270)
- D-Fructose, Sigma-Aldrich (P/N F2543)
- Sucrose, Sigma-Aldrich (P/N S-9378)
- Isomaltose 98%, Sigma-Aldrich (P/N I7253-100MG)
- Melezitose hydrate, Sigma-Aldrich (P/N M5375)
- Kojibiose, Sigma-Aldrich (P/N K476-1MG)
- Raffinose pentahydrate, Sigma-Aldrich (P/N R0250-25G)
- Gentiobiose, Sigma-Aldrich (P/N G3000)
- 1-Kestose, Sigma-Aldrich (P/N 72555)
- Turanose, Sigma-Aldrich (P/N T2754)
- Palatinose, Sigma-Aldrich (P/N P2007)
- Erlose, Sigma-Aldrich (P/N E1895-50MG)
- Maltose monohydrate, Sigma-Aldrich (P/N M5885)

Experimental conditions

System	ICS-5000⁺ HPIC System
Columns	Thermo Scientific™ Dionex™ CarboPac™ PA210 Guard, 4 × 30 mm (P/N 088955) Dionex CarboPac PA210 Analytical, 4 × 150 mm (P/N 088953)
Eluent Source	EGC 500 KOH
Eluent	0–25 min: 30 mM KOH 25–30 min: 100 mM KOH 30–45 min: 30 mM KOH
Flow Rate	0.8 mL/min
Injection Volume	10 µL
Inject Mode	Push full
Loop Overfill Factor	5
Detection	Pulsed amperometry, Gold on PTFE Disposable Gold Working Electrode, Ag/AgCl reference

Waveform

Time(s)	Potential (V) Integration
0.00	+0.1
0.20	+0.1 Begin
0.40	+0.1 End
0.41	–2.0
0.42	–2.0
0.43	+0.6
0.44	–0.1
0.50	–0.1

Backpressure	~ 3700 psi
Background	40–50 nC
Noise	~30 pC /min peak-to-peak
Run Time	45 min

Preparation of solutions and reagents

Standard Solutions

Honey sugar standard: Dissolve 0.1 g of sugar standard in a 100 mL DI water to make a 1000 mg/L stock standard. Maintain the stock solution at -20 °C until needed. Using this stock standard, prepare working standards fresh weekly. Store working standard at 4 °C.

Working standards

See Table 1.

Sample preparation

Preparation of honey samples: Dissolve 0.1 g of honey sample in 100 mL of DI water to achieve a 1:1000 fold dilution. Store the honey sample at -20 °C. Filter through a 0.2 µm filter before analysis.

Preparation of adulterated honey samples: For adulteration experiments, six commercial honey samples and five commercial sugar syrup samples are used.

Dilute the honey samples and sugar samples (1:3000 fold) by dissolving 0.1 g sample in 300 mL DI water. Then mix the sugar syrup sample and honey sample in a 20:80 ratio. For honey sample HS6 mix the sugar syrup sample and honey sample in a 10:90 ratio. Filter through a 0.2 µm filter before analysis.

Table 1. Working standards.

No.	Sugar	Stock solution (mg/L)	Standard 1 (mg/L)	Standard 2 (mg/L)	Standard 3 (mg/L)	Standard 4 (mg/L)	Standard 5 (mg/L)
1	Trehalose	100	0.40	0.80	2.00	5.00	10.0
2	Glucose	1000	20.0	40.0	75.0	100	150
3	Fructose	1000	40.0	75.0	100	150	200
4	Sucrose	100	0.40	2.00	4.00	8.00	20.0
5	Isomaltose	100	1.00	2.50	5.00	10.0	20.0
6	Melezitose	100	0.50	1.00	2.00	4.00	10.0
7	Raffinose	100	0.20	0.50	1.00	2.50	5.00
8	Gentiobiose	100	0.20	0.40	0.80	2.00	4.00
9	1-Kestose	100	0.50	1.00	2.00	4.00	10.0
10	Turanose	1000	2.00	5.00	10.0	25.0	50.0
11	Palatinose	100	0.20	0.40	0.80	2.00	4.00
12	Maltose	1000	2.00	5.00	10.0	25.0	50.0
13	Erlose	100	1.00	2.50	5.00	10.0	20.0

Precautions

1. Each run must have a 5 min wash step and a 15 min equilibration step to ensure retention time reproducibility.

2. When running sugar syrup samples, an additional longer column wash of 10 h using 100 mM KOH is needed after about 15–20 injections (before starting wash, remove ED cell from the flow path and also reverse the column order).

3. The working electrode shows some loss of peak area response (~10–12%) over 3–4 weeks of continuous sample runs, and thus calibration standards should be run daily for the best results.

Results and discussion

Separation

Honey sugars were separated using a Dionex CarboPac PA210-Fast-4µm column (150 × 4 mm) in series with a Dionex CarboPac PA210 guard column (50 × 4 mm). A solution of honey sugar standards was prepared and an aliquot (10 µL) of the solution was injected onto the column and eluted at 0.8 mL/min with 30 mM hydroxide produced by the system's eluent generator. Figure 1 displays the chromatogram of the honey sugars standard showing the separation of 15 sugar standards in a single run. Of the 15 sugars, two are monosaccharides (glucose and fructose), nine are disaccharides (trehalose, sucrose, kojibiose, gentiobiose, turanose, palatinose, nigerose, isomaltose and maltose) and four are trisaccharides

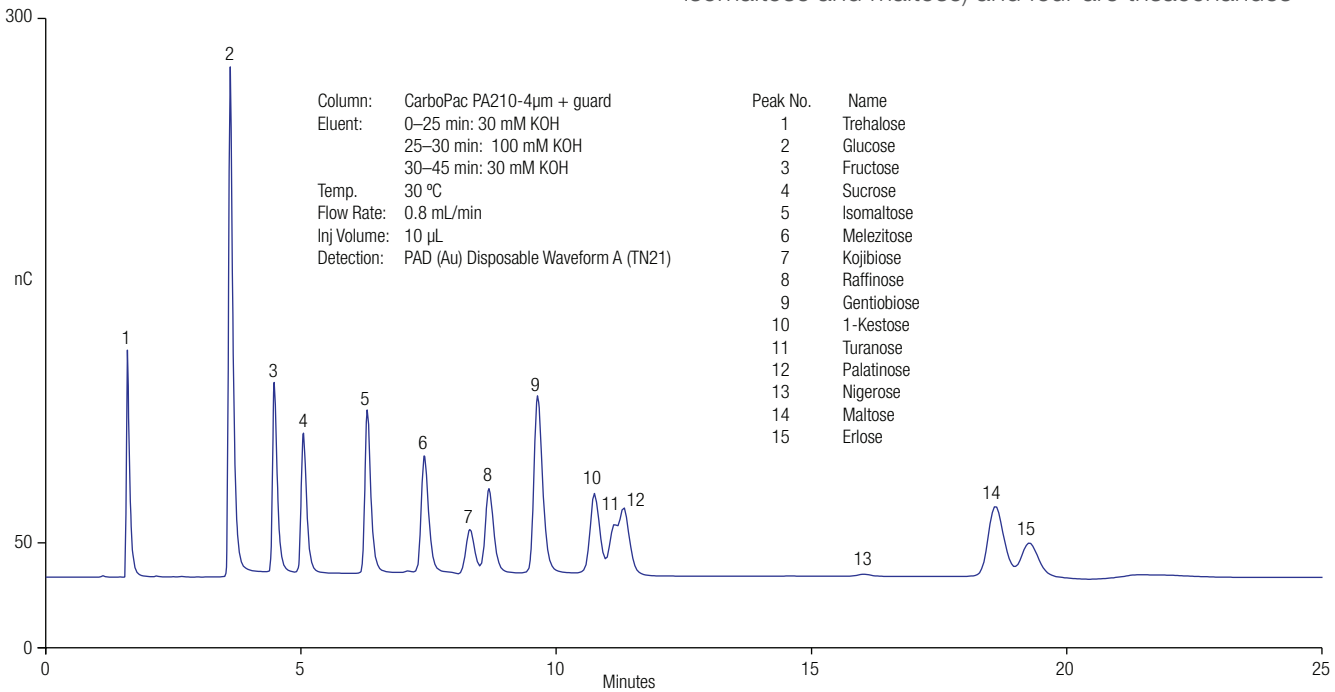

Figure 1. Chromatogram of the 15 honey sugar standards mix

(melezitose, raffinose, 1-kestose, and erlose). All 15 honey sugars were separated within 25 min with good resolution, except turanose/palatinose, which are poorly resolved under these conditions. It is noteworthy that standards containing turanose (Figure 2) showed a slight increase of the baseline (~ 0.5 nC) around 4 min. This is due to partial on-column hydrolysis of turanose to fructose and glucose, resulting in the baseline rise at the retention times where they elute. This effect was observed earlier and with additional experiments it was demonstrated that the potential impact on the determination of turanose, glucose, and fructose was negligible.[14]

Honey sugar analysis

For this study, we purchased 12 commercial honey samples (Table 2) and analyzed them using HPAE-PAD. Figures 3–6 show the chromatograms of the 12 honey samples. For all 12 investigated honey samples, reducing sugars, fructose and glucose, were found to be the major constituents, and their amounts were within the limits established by The Codex Alimentarius Committee on Sugars (2001).[7]

Table 2. List of commercial honey samples.

Honey sample	Floral source
HS1	Clover
HS2	Clover, sunflower and alfalfa
HS3*	Wildflowers
HS4	Manuka tree
HS5	Clover
HS6	Mixed
HS7	Manuka tree
HS8	Mixed
HS9	Clover
HS10*	Wildflower
HS11*	Blackberry blossoms
HS12*	Mixed

**Local beekeeper honey*

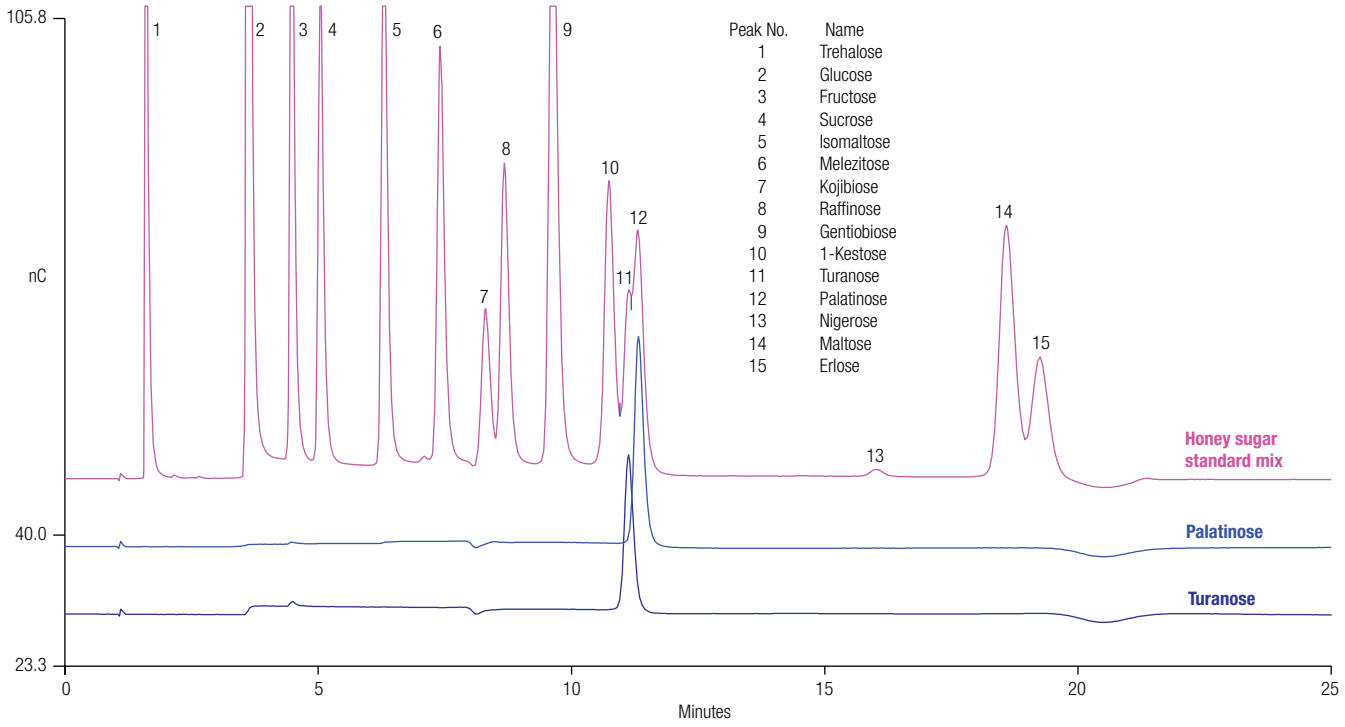

Figure 2. Chromatogram of 5 mg/L turanose, 5 mg/L palatinose and the 15 honey sugar mix standards.

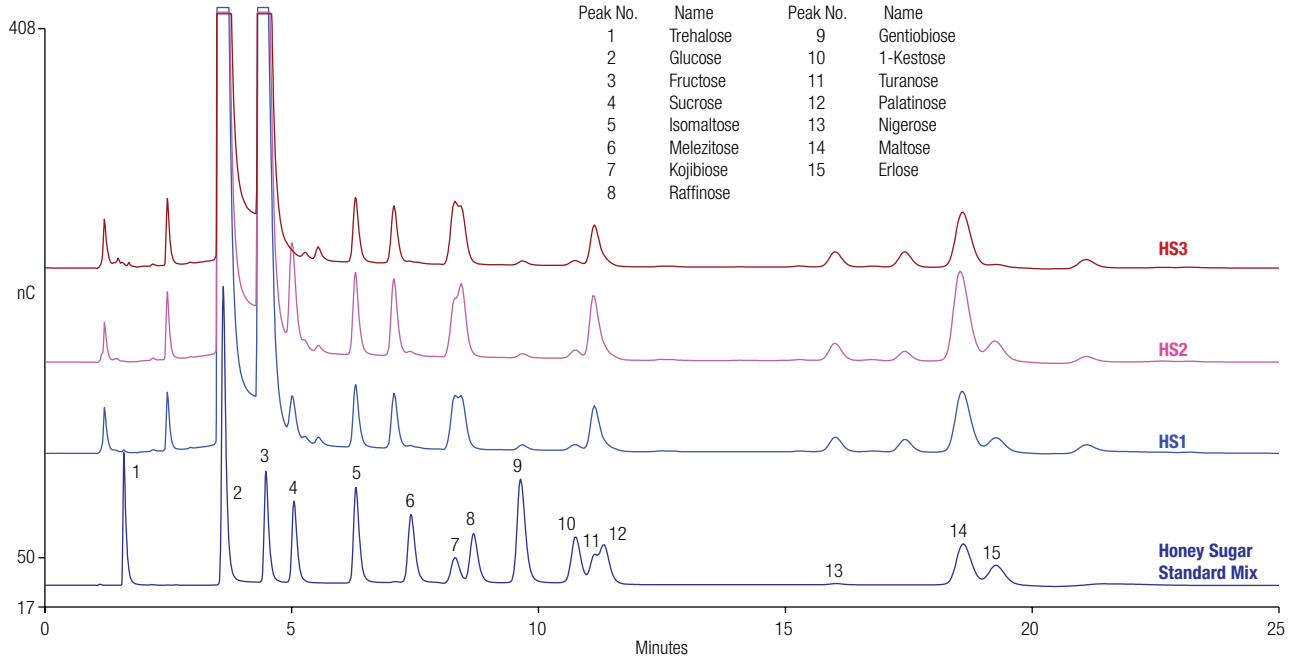

Figure 3. Chromatogram of honey samples (HS1- HS3) along with the 15 sugar standard mix.

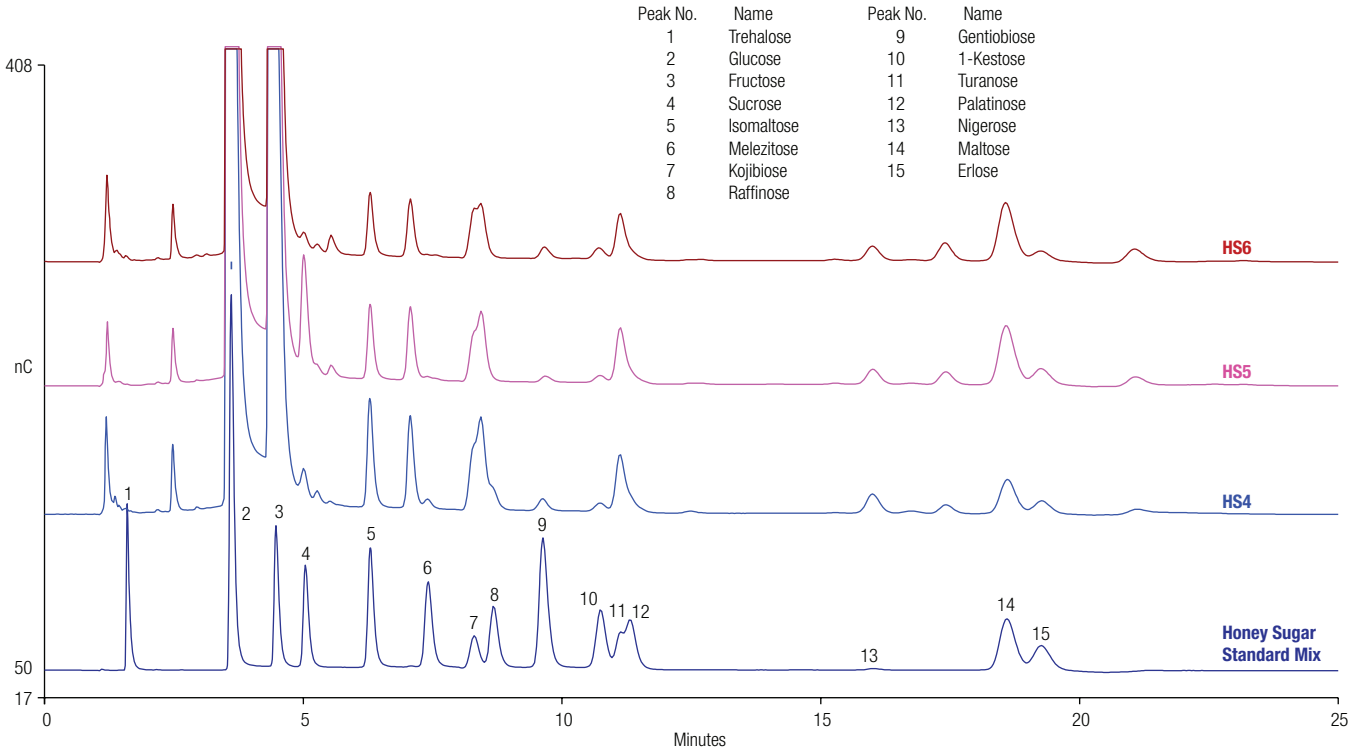

Figure 4. Chromatogram of honey samples (HS4- HS6) along with the 15 sugar standard mix.

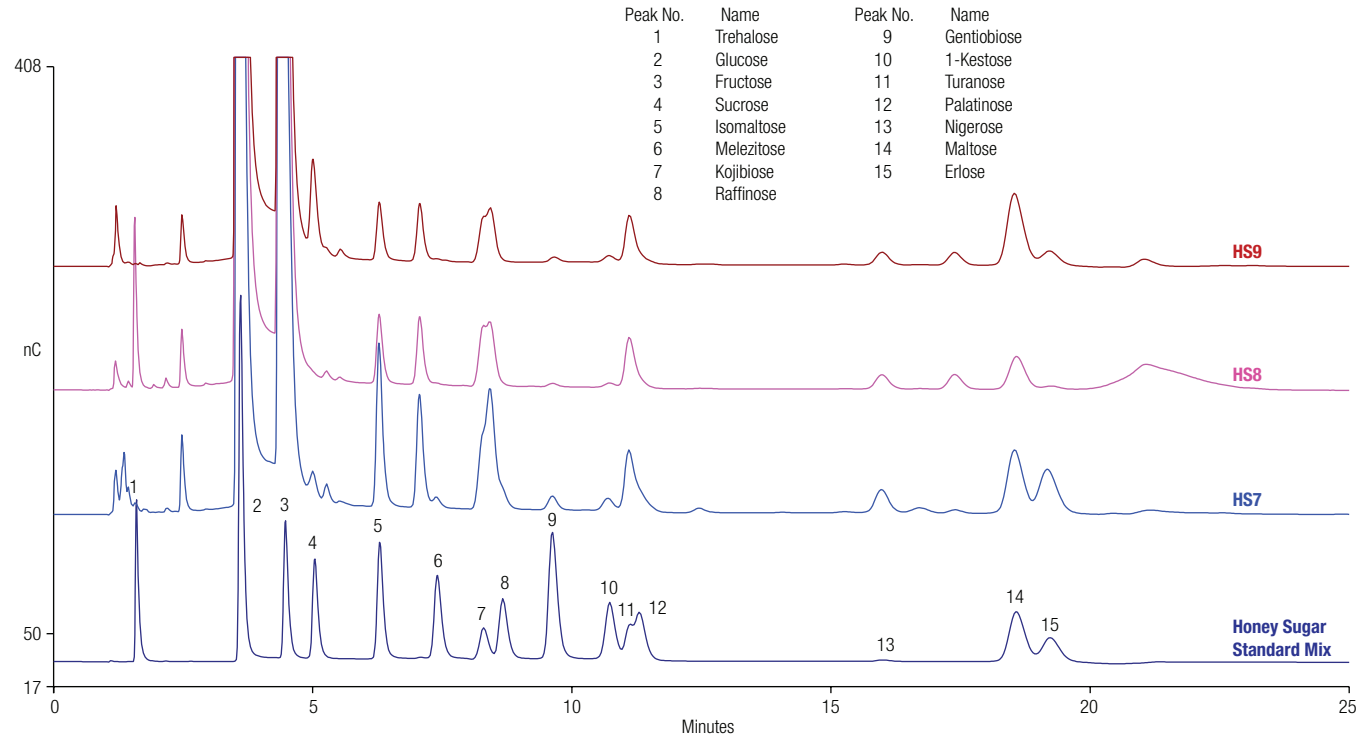

Figure 5. Chromatogram of honey samples (HS7- HS9) along with the 15 sugar standard mix.

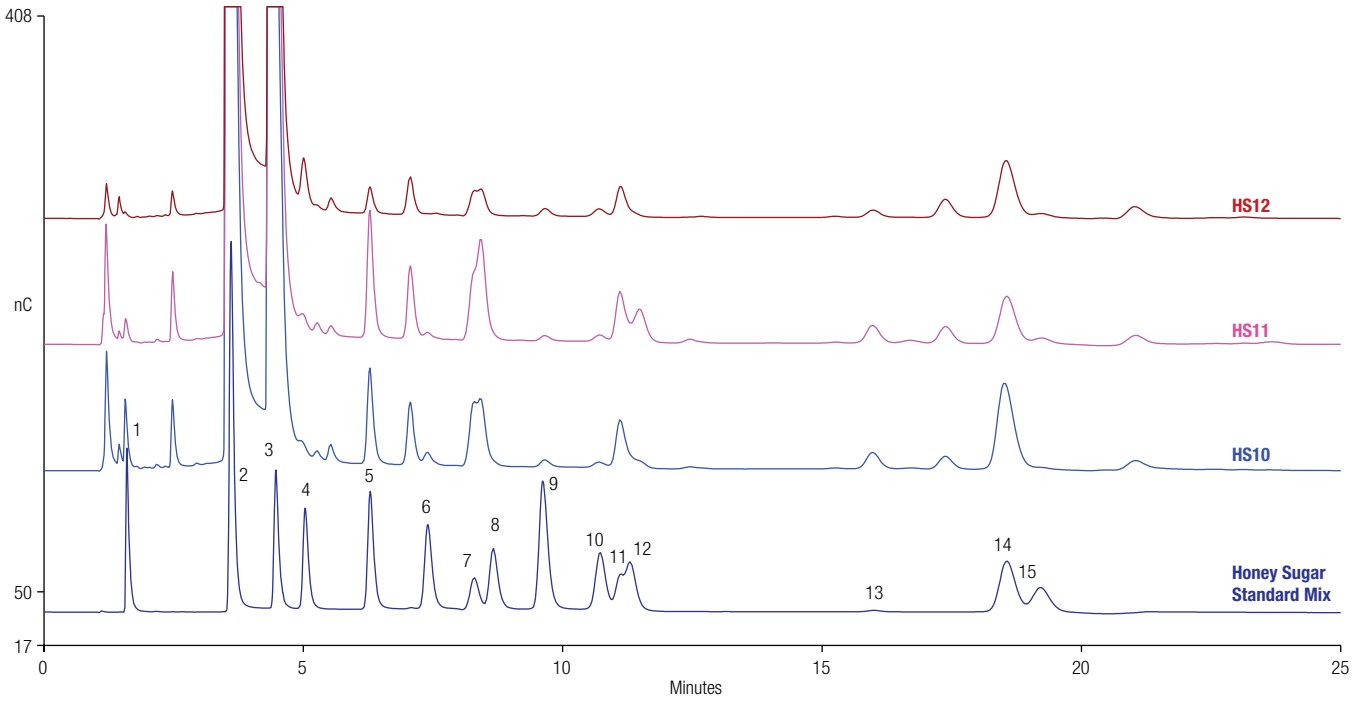

Figure 6. Chromatogram of honey samples (HS10- HS12) along with the 15 sugar standard mix.

The relative % of monosaccharides, disaccharides, and trisaccharides in the 12 honey samples is calculated based on the relative peak area percentage of the disaccharides and trisaccharides identified in our method and are reported in Table 3. As discussed earlier, the concentrations of fructose and glucose, as well as their ratio, are useful indicators for the classification of monofloral honeys. In almost all types of honey, fructose is the carbohydrate in greatest proportion, and the ratio of fructose to glucose (F/G) is greater than 1 (Table 4).

Table 3. Relative % of mono, di, and trisaccharides in honey samples (HS1-HS12)

Sample	Total Monosaccharides (%)	Total Disaccharides (%)	Total Trisaccharides (%)
HS1	91.4	4.91	0.36
HS2	87.9	7.58	0.38
HS3	91.8	4.32	0.41
HS4	89.9	5.08	0.74
HS5	89.3	6.18	0.38
HS6	90.9	4.36	0.52
HS7	86.2	7.22	0.73
HS8	92.2	4.46	0.36
HS9	90.9	5.76	0.36
HS10	90.0	6.22	0.53
HS11	89.5	4.63	0.45
HS12	93.3	3.74	0.47
Range	86.2–93.3	3.74–7.58	0.36–0.74

Calibration and quantification

Quantification was performed with 13 sugar standards; two monosaccharides (glucose and fructose), seven disaccharides (trehalose, isomaltose, sucrose, gentiobiose, turanose, palatinose, and maltose), and four trisaccharides (melezitose, raffinose, 1-kestose, and erlose). The calibration curves for these 13 sugars are shown in Figure 7. The coefficient of determination (r^2) is greater than 0.999 for all sugars except glucose (r^2 = 0.996) and fructose (r^2 = 0.998). This is due to the larger calibration range and thus high concentrations of glucose and fructose which saturate the detector response above 100–150 mg/L. Over the course of the analysis, the relative standard deviations of the peak area of all 13 peaks (n = 6) ranged from 0.15–0.50%.

The results of the monosaccharide analysis of the 12 honey samples (Table 4) showed that the fructose content varied between 36.0 and 41.9 g/100 g. The glucose content of the samples was within a range of 26.8 to 39.5 g/100 g. The higher concentration of fructose relative to glucose is one way in which honey differs from commercial invert sugar syrup and in honey of good quality the fructose content should exceed that of glucose.[15] The fructose/glucose ratio was within the range of 1.01 to 1.37. The HS4 (manuka honey) sample showed the lowest glucose content among all the honey samples and thus has the highest fructose/glucose ratio. The fructose/glucose and glucose/water ratios are parameters that help predict the tendency of honey to crystallize. Honey with a low fructose to glucose ratio crystallizes more rapidly, whereas honey with a higher fructose to glucose ratio (containing less than 30% glucose) crystallizes quite slowly and can stay liquid for a long time without special treatment. The sum of fructose and glucose (fructose + glucose) contents ranged between 63.7 and 81.4 g/100 g. Although there are no regulatory limits on individual values of fructose and glucose their sum has been fixed at a value of $\geq$ 60 g/100 g as one of the requirements of the international standard for honey established by Codex Alimentarius Commission.[7] The sum of fructose and glucose for the honey samples used in this study all exceeded limit required by the Codex; i.e., 60g/100 g.

Generally, the disaccharide and trisaccharide profile of honey depends upon the sugars and the enzymes present in the bee and nectar.[15] Among disaccharides (Table 5), maltose was the main component in the majority of the honey samples followed by turanose, sucrose, and isomaltose. The sucrose contents of the honey samples ranged from 0.03 to 1.82 g/100 g. For two of the honey samples in our study; HS3 (a local bee keeper honey) and HS8, the sucrose content was very low or not detected. Lower content of sucrose in honey might result from activities of enzymes introduced by bees. The international norm established by the Codex Alimentarius Commission requires that a good quality honey should not contain more than 5 g/100 g sucrose.[7] The values obtained for sucrose contents of the honey samples in this study were all within the limits of international standards. Trehalose, gentiobiose, and palatinose were the other disaccharides found in minor quantities in these 12 honey samples. For trisaccharides (Table 6), four sugars were analyzed and quantitated i.e. melezitose, raffinose, 1- kestose, and erlose. Out of the 12 honey samples, HS4, HS7, and HS10 had higher percentages of trisaccharides. Among trisaccharides, erlose was the main component in majority of the honey samples, ranging from 0.19 to 2.26 g/100g. The melezitose content of the samples ranged from 0.00 to 0.17 g/100 g.

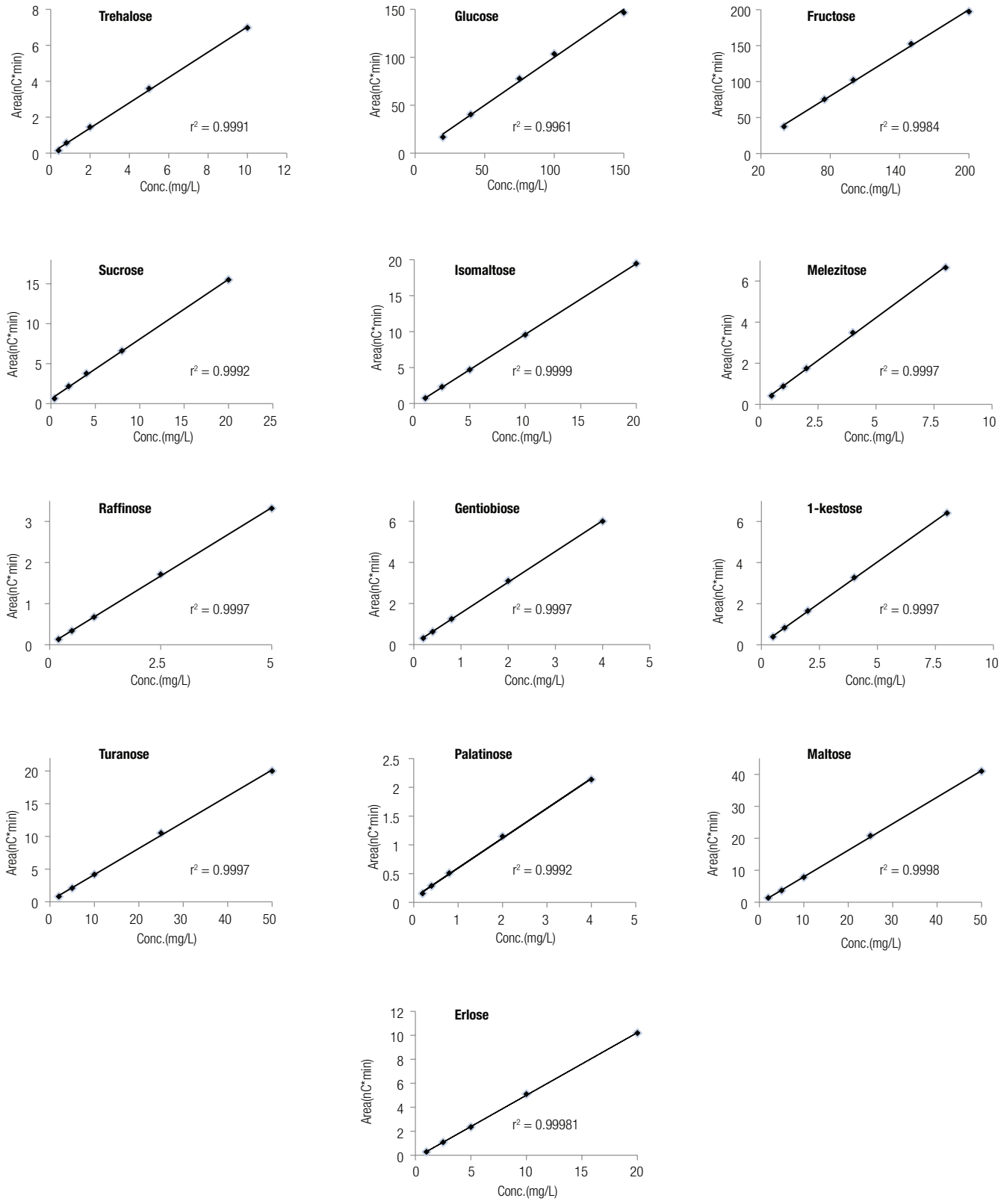

Figure 7. Calibration curves of 13 honey sugar standards.

Table 4. Amount of monosaccharides in honey samples (HS1-HS12).

Sample	Glucose (g/100g)	Fructose (g/100g)	F+G (g/100g)	F/G ratio
HS1	39.5	41.9	81.4	1.06
HS2	36.8	41.1	77.9	1.12
HS3	37.9	39.9	77.8	1.05
HS4	34.3	38.0	72.3	1.11
HS5	35.7	37.9	73.6	1.06
HS6	35.5	37.8	73.2	1.07
HS7	26.8	36.9	63.7	1.37
HS8	37.0	38.1	75.1	1.03
HS9	35.6	38.1	73.7	1.07
HS10	34.9	39.3	74.2	1.13
HS11	34.7	38.3	73.0	1.10
HS12	35.7	36.0	71.8	1.01
Range	(26.8–39.5) g/100g	(36.0–41.9) g/100g	(63.7–81.4) g/100g	(1.01–1.37)

Table 5. Amount of disaccharides in honey samples (HS1-HS12).

Sample	Trehalose (g/100g)	Sucrose (g/100g)	Isomaltose (g/100g)	Gentiobiose (g/100g)	Turanose (g/100g)	Palatinose (g/100g)	Maltose (g/100g)
HS1	0.016	0.668	0.623	0.040	1.48	0.186	1.99
HS2	0.011	1.82	0.817	0.032	2.45	0.260	3.01
HS3	0.027	0.032	0.639	0.033	1.46	0.120	1.77
HS4	0.017	0.351	0.980	0.097	1.97	0.268	1.10
HS5	0.013	1.71	0.680	0.057	1.79	0.260	1.77
HS6	0.031	0.118	0.575	0.093	1.37	0.186	1.79
HS7	0	0.489	1.52	0.125	2.12	0.433	1.99
HS8	0.899	0.025	0.639	0.021	1.50	0.191	1.00
HS9	0.014	1.33	0.541	0.035	1.49	0.170	2.24
HS10	0.317	0.852	0.874	0.052	1.38	0.224	2.29
HS11	0.090	0.219	1.16	0.037	1.67	0.210	1.37
HS12	0.025	0.609	0.282	0.060	0.878	0.075	1.67

Table 6. Amount of trisaccharides in honey samples (HS1-HS12)

Sample	Melezitose (g/100g)	Raffinose (g/100g)	1-Kestose (g/100g)	Erlose (g/100g)
HS1	0.014	0	0.125	0.852
HS2	0.070	0	0.138	1.172
HS3	0.005	0	0.091	0.266
HS4	0.119	0.476	0.143	0.642
HS5	0.011	0	0.135	0.867
HS6	0.003	0	0.203	0.585
HS7	0.142	0.410	0.230	2.261
HS8	0.015	0	0.082	0.186
HS9	0.009	0	0.128	0.795
HS10	0.173	0.155	0.098	0.205
HS11	0.081	0	0.101	0.382
HS12	0	0	0.147	0.284

Raffinose, a trisaccharide composed of galactose, fructose, and glucose, was only found in three honey samples, HS4, HS7, and HS10. It has been reported that honeydew honey contains higher amounts of trisaccharides such as melezitose and raffinose and oligosaccharides compared to blossom honey.[16] In addition to these four trisaccharides, the other commonly found trisaccharides in honey are panose, maltotriose, and theanderose. All the honey samples studied here (HS1–HS12) exhibit a peak at ~ 21.5 min. Generally, panose is the second most common honey trisaccharide after erlose. From the previous published work, we speculate that the peak at ~ 21.5 min is panose.[10, 17–18]

This method offers several benefits over previous methods. First, the method uses a Dionex CarboPac PA210-Fast-4µm column. Its smaller resin particles (4 µm) compared to the 6–13 µm resins in the earlier Dionex CarboPac columns provide fast, high-resolution separations. The column was developed to provide fast, high-resolution separations for most mono- through tetra-saccharides in a variety of applications including food and beverage analyses. These columns are packed with a hydrophobic, polymeric, microporous anion exchange resin stable over entire pH range of 0–14. The unique pH-stability of this packing allows eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes. The increased resolution is demonstrated by the improved separation of turanose/palatinose, which eluted as a single peak in the previous studies. Another advantage is that this method does not require a sodium acetate eluent for honey analysis and therefore it can use eluent generation. This eliminates eluent preparation errors and the need to handle sodium hydroxide and sodium acetate as required for the preparation of eluents for previous HPAE-PAD honey applications. Eluent generation allows chromatographers to run a full range of gradient and isocratic separations more reliably than manually prepared eluents.

Sample recovery

Method accuracy was evaluated by measuring recoveries of 10 sugar standards spiked into honey samples. For spiking experiments four honey samples were used (HS7–HS10) and spiked with a 10 sugar standard mix at two concentration levels. Figure 8 shows the chromatogram of unspiked and spiked honey sample HS7. The recovery percentages were calculated using the formula shown below:

$$Recovery\ \% = (C_{spiked\ sample} - C_{unspiked\ sample}) / (C_{analyte\ added}) \times 100$$

Tables 7–10 list the percentage recovery results for honey samples HS7 through HS10. For all of the four honey samples spiked, recoveries were in the range of 78.2–113%.

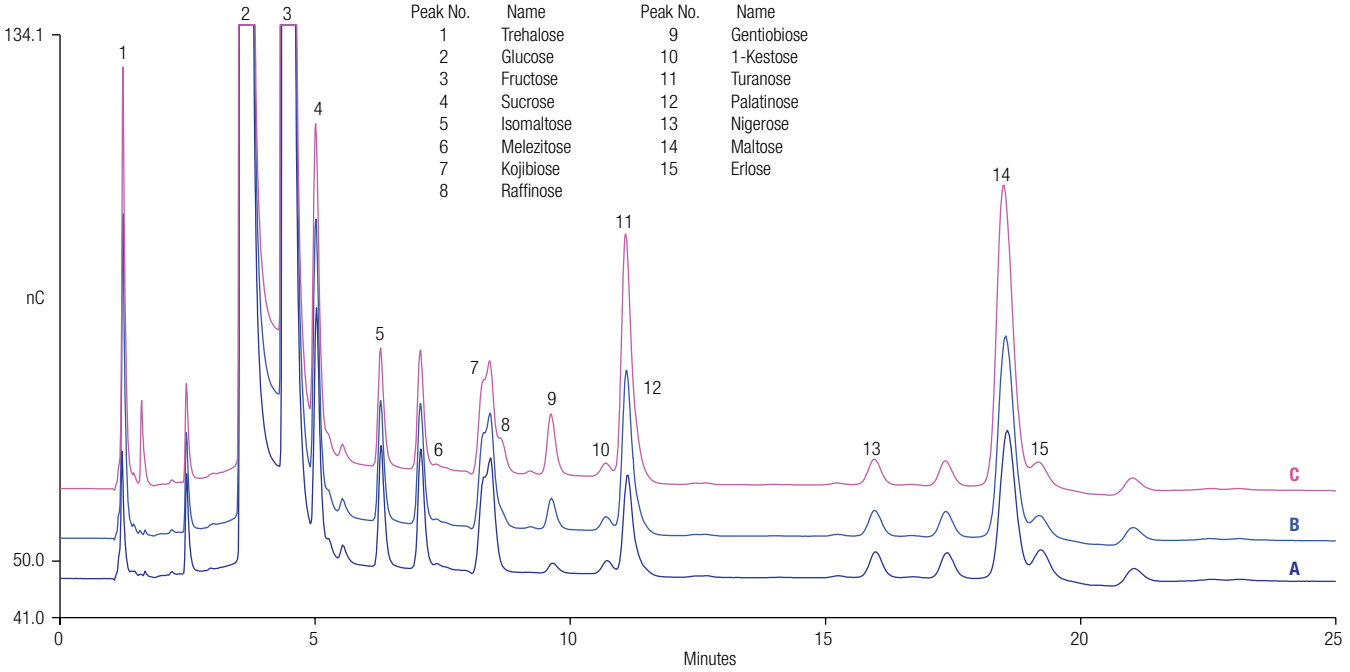

Figure 8. Chromatogram of unspiked HS7 (A), spiked HS7 at spike level 1 (B), and spiked HS7 at spike level 2.

Table 7. Spike recovery results for honey sample HS7.

HS7		Spike Level 1		Spike Level 2	
Carbohydrate	Found (mg/L)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Trehalose	0.18	1.01	94.3	2.6	95.2
Glucose	125	40.3	81.8	54.1	79.5
Fructose	181	50.6	81	75.4	80.9
Sucrose	2.2	2.06	113	3.86	94.2
Raffinose	2.15	0.48	95.2	1.23	98.5
Gentiobiose	0.62	0.38	102	1	96.3
Turanose	10.6	5.02	93.9	12.6	94.6
Palatinose	2.15	0.37	103	1.02	78.9
Maltose	9.85	4.76	85.5	12.6	102

Table 8. Spike recovery results for honey sample HS8.

HS8		Spike Level 1		Spike Level 2	
Carbohydrate	Found (mg/L)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Trehalose	4.5	1.01	95.9	2.6	96.4
Glucose	185	40.3	86.9	54.1	84.1
Fructose	191	50.6	88.9	75.4	89.3
Sucrose	0.2	2.06	112	3.86	98.3
Raffinose	0	0.48	104	1.23	110
Gentiobiose	0.11	0.38	106	1	103
Turanose	7.49	5.02	98.1	12.6	101
Palatinose	0.94	0.37	86.2	1.02	104
Maltose	5.02	4.76	79.8	12.6	88.7

Table 9. Spike recovery results for honey sample HS9.

HS9		Spike Level 1		Spike Level 2	
Carbohydrate	Found (mg/L)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Trehalose	0.07	1.01	82.3	2.6	101
Glucose	179	40.3	84.7	54.1	84.1
Fructose	189	50.6	82.9	75.4	82.9
Sucrose	6.68	2.06	107	3.86	106
Raffinose	0	0.48	89.6	1.23	98.4
Gentiobiose	0.17	0.38	107	1.00	105
Turanose	7.13	5.02	96.9	12.6	96
Palatinose	0.81	0.37	89.8	1.02	92.2
Maltose	11.2	4.76	102	12.6	96.7

Table 10. Spike recovery results for honey sample HS10.

HS10		Spike Level 1		Spike Level 2	
Carbohydrate	Found (mg/L)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Trehalose	1.59	1.01	105	2.6	100
Glucose	175	40.3	81.1	54.1	78.5
Fructose	196	50.6	84.5	75.4	78.2
Sucrose	4.25	2.06	79.1	3.86	84.4
Raffinose	0.75	0.48	103	1.23	103
Gentiobiose	0.26	0.38	106	1	101
Turanose	6.87	5.02	96.5	12.6	95.2
Palatinose	1.11	0.37	96.5	1.02	83.9
Maltose	11.4	4.76	101	12.6	94.3

Adulteration of honey samples with sugar syrups

Honey has been considered a valuable product since ancient times. It possesses prebiotic, antioxidant, and antimicrobial properties. The cost of honey is much greater than that of any other sweetener, and it can, therefore, be a target of adulteration. Adulteration by sweeteners is the most important authenticity issue. The following sweeteners have been detected in adulterated honeys: sugar syrups produced by acids or enzymes from corn, sugar cane, and sugar beets, and syrups of natural origin such as maple. HPAE-PAD is a useful technique for detecting honey adulteration by sugar syrups.

For adulteration experiments we used five different sugar syrup samples (Table 11). All five syrups are commercial syrups purchased from a grocery store. SS1, SS2 and SS3 are commercial pancake syrups containing high amounts glucose and maltose and low amounts of fructose.

The sucrose content is relatively low in SS2 and SS3. The sugar profiles of SS4 (beet syrup) and SS5 (maple syrup) are quite different from the three corn syrups. SS4 has high amounts of glucose, fructose, sucrose and zero maltose. SS5 has very low amounts of glucose and fructose, but has high amounts of sucrose, and zero maltose.

Honey samples and sugar samples were diluted 1:3000 with DI water. Then, the diluted sugar syrup was added to the diluted honey sample in a ratio of 20:80 or 10:90.

Table 11. Sugar profile of different sugar syrup samples.

	Type	Glucose (mg/L)	Fructose (mg/L)	Sucrose (mg/L)	Maltose (mg/L)
SS_1	Corn syrup	51.49	<1	28.32	35.04
SS_2	Corn syrup	62.08	<5	6.10	42.84
SS_3	Corn syrup	65.76	<5	6.53	46.32
SS_4	Beet syrup	76.62	74.22	42.33	0.00
SS_5	Maple syrup	<5	<5	59.66	0.00

In this way, each of the six honey samples was adulterated with three sugar syrups at 20%/10% and then analyzed using HPAE-PAD. Additionally honey sample HS6 was adulterated with all five sugar syrups at 10%. Figure 9 represents the chromatographic profiles of 100% HS1 and HS1 adulterated with 20% SS1, SS2, and SS3. Tables 12, 13, and 14 list the adulteration parameters and their values for respective honey samples on addition of sugar syrups. For all six honey samples tested for adulteration, the amounts of fructose and glucose decreased on addition of the sugar syrup. The fructose/glucose ratio also decreased and is less than 1 for these samples, indicating adulteration. As discussed in the introduction, the amount of sucrose is a very important parameter in evaluating honey's authenticity. Upon addition of sugar syrups the amount of sucrose increased in all honey samples (HS1 through HS6). Similarly, maltose content increased, while turanose decreased upon addition of sugar syrup. Here, we have shown the detailed results for the addition of 20% sugar syrups, but we could also detect as low as 10% adulteration. Table 15 lists the adulteration parameters for honey sample HS6, adulterated with five sugar syrups at 10% level. For the 20% level, the amount of fructose and glucose decreased on addition of all sugar syrups except SS4. The F/G ratio decreased on addition of SS1, SS2, and SS3, but increased slilghtly with SS4 and SS5. This is due to the fact that SS4 has a higher amount of fructose than glucose. In SS5, both glucose and fructose are present in small amounts, thus the F/G ratio is due primarily to unadulterated honey. The amount of sucrose increased upon addition of sugar syrup samples and a significant increase was seen with SS4 and SS5. The S/T ratio increased, but the increase is almost 10 times higher upon addition of SS4 and SS5, in comparison to the corn syrups (SS1, SS2, and SS3). This is due to the high amounts of sucrose in SS4 and SS5. The S/M ratio also increased. A significant increase was seen with SS4 and SS5. This is due to the high amounts of sucrose and lack of maltose in these syrups.

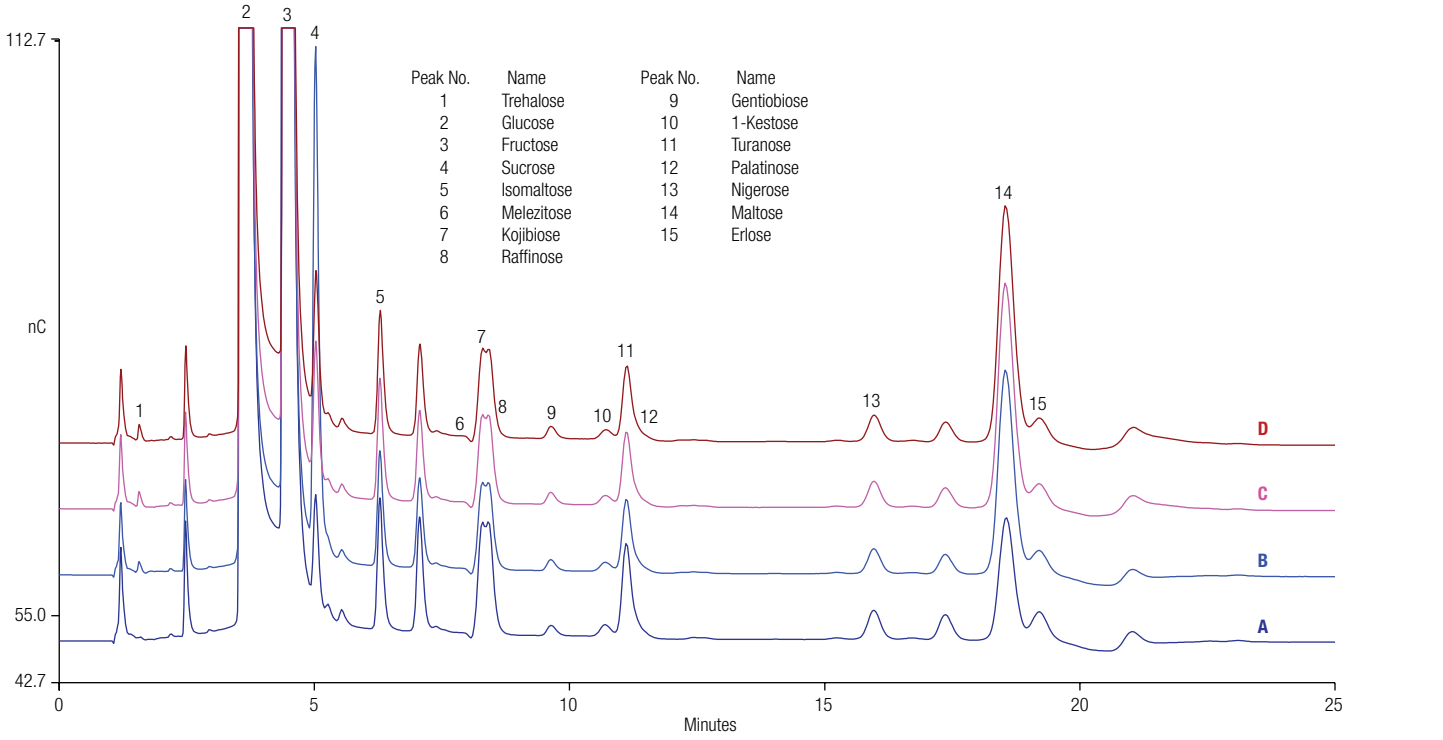

Figure 9. Chromatogram of (A), 100% honey sample (HS1); (B), 80% HS1 adulterated with sugar syrup 1 (+ 20% SS1); (C), 80% HS1+ 20% SS2; and (D), 80% HS1+ 20% SS3.

Table 12. Adulteration parameters for honey sample HS1 (left) and HS2 (right).

Adulteration Parameters	HS1			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	132	121	125	126
Fructose(F), mg/L	140	115	120	120
F/G ratio	1.06	0.95	0.96	0.95
Sucrose(S), mg/L	2.22	10.9	2.68	2.7
Turanose(T), mg/L	4.95	3.45	3.81	3.33
S/T ratio	0.45	3.16	0.71	0.81
Maltose(M), mg/L	6.64	9.62	11.37	11.27
S/M ratio	0.33	1.13	0.24	0.24

Adulteration Parameters	HS2			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	124	115	119	117.5
Fructose(F), mg/L	137	115.8	120	120.8
F/G ratio	1.12	1.01	1.01	1.02
Sucrose(S), mg/L	6.09	14.06	6.05	5.75
Turanose(T), mg/L	8.12	5.18	5.49	5.27
S/T ratio	0.96	2.71	1.1	1.09
Maltose(M), mg/L	6.65	12.1	13.3	14
S/M ratio	0.71	1.15	0.45	0.41

Table 13. Adulteration parameters for honey sample HS3 (left) and HS4 (right).

Adulteration Parameters	HS3			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	127	118	121.1	119.5
Fructose(F), mg/L	133	110.2	114.7	110.3
F/G ratio	1.05	0.93	0.95	0.92
Sucrose(S), mg/L	0	9.05	0.86	0.88
Turanose(T), mg/L	4.84	3.04	3.09	2.82
S/T ratio	0.01	2.97	0.28	0.31
Maltose(M), mg/L	5.9	10.3	11.06	11.03
S/M ratio	0	0.88	0.08	0.08

Adulteration Parameters	HS4			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	114	107	107.1	109.5
Fructose(F), mg/L	126	104.7	102.1	105.9
F/G ratio	1.11	0.98	0.95	0.97
Sucrose(S), mg/L	1.17	9.82	1.64	1.72
Turanose(T), mg/L	6.56	3.98	4.17	4.17
S/T ratio	0.18	2.47	0.39	0.41
Maltose(M), mg/L	3.65	8.01	8.65	9.53
S/M ratio	0.32	1.23	0.19	0.18

Table 14. Adulteration parameters for honey sample HS5 (left) and HS6 (right).

Adulteration Parameters	HS5			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	119	110.7	114.1	114.7
Fructose(F), mg/L	126	108.6	108.9	108.9
F/G ratio	1.06	0.98	0.95	0.95
Sucrose(S), mg/L	5.69	14.81	6	6.07
Turanose(T), mg/L	5.95	3.98	4.05	3.94
S/T ratio	0.95	3.72	1.48	1.54
Maltose(M), mg/L	5.89	9.83	11.08	11.38
S/M ratio	0.97	1.51	0.54	0.53

Adulteration Parameters	HS6			
	100% honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G), mg/L	119	110.2	112	112.9
Fructose(F), mg/L	126	104.2	105.5	102.8
F/G ratio	1.07	0.95	0.94	0.91
Sucrose(S), mg/L	0.39	9.17	1.16	1.18
Turanose(T), mg/L	4.57	2.66	2.88	2.79
S/T ratio	0.09	3.44	0.4	0.42
Maltose(M), mg/L	5.95	9.42	9.84	10.61
S/M ratio	0.07	0.97	0.12	0.11

Table 15. Adulteration parameters for HS6 adulterated with SS1 through SS5 at 10% level.

Adulteration Parameters	HS6 (wild mountain honey)					
	100% honey	+ 10% SS1	+ 10% SS2	+ 10% SS3	+ 10% SS4	+ 10% SS5
Glucose(G), mg/L	121	115	116	117	119	107
Fructose(F), mg/L	127	115	115	116	126	116
F/G ratio	1.04	1.00	1.00	0.99	1.06	1.09
Sucrose(S), mg/L	0.37	5.08	0.68	0.90	9.23	11.85
Turanose(T), mg/L	4.99	4.43	4.50	4.40	4.44	4.41
S/T ratio	0.07	1.15	0.15	0.20	2.08	2.68
Maltose(M), mg/L	5.96	8.47	8.82	9.11	5.48	5.48
S/M ratio	0.06	0.60	0.08	0.10	1.69	2.16

Conclusion

An HPAE-PAD method was successfully developed and validated for the sugar analysis of 12 commercial honey samples using the Dionex CarboPac PA210-4µm column. This column allows the separation of 15 sugars in honey with minimal sample preparation and an overall cycle time of 45 min. PAD is sensitive, thus allowing the determination of low concentration carbohydrates in honey, while at the same time detecting the high concentrations of the major components, glucose and fructose. The method showed good precision and accuracy with recovery range of 80–120%. This method enabled us to detect the addition of industrial sugar syrups (adulteration) to honey samples.

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