Application note | AN002003

Isotope ratio MS

LC-IRMS: carbon isotope fingerprints in routine honey fraud analysis

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Introduction

Honey adulteration is typically defined as diluting honey with cheaper sugar syrups. The authentication of honey can be a major challenge as there can be a wide range of geographical origins, botanical types, and environmental factors which lead to the large compositional variation of honey. In addition, honey can also be a commercial blend of different honeys; further complicating the differentiation between honeys produced naturally and honey adulteration. The analytical methods selected to detect honey adulteration need to be able to compensate for the natural variations in honey composition while being robust enough to detect disparities due to adulteration. The Thermo Scientific™ LC IsoLink™ II IRMS System is designed to streamline honey fraud analysis and offers a reliable, robust tool for compound specific isotope analysis of individual sugars. Here we present 20 honey samples (10 adulterated, 10 authentic) that were analyzed using the new Thermo Scientific LC-IRMS System.

The isotope fingerprint of honey

Carbon isotope fingerprints can be used to identify adulteration of honey that results from the addition of exogenous sugars. The carbon isotope fingerprints ($\delta^{13}C$) of plants are different because of photosynthetic processes. Authentic honey is derived from C3 plants that utilize the Calvin photosynthetic pathway to fix CO_2 and have a carbon isotope fingerprint between -33 ‰ to -22 ‰. Typical honey adulterants, such as sugar cane and corn syrups, are C4 plants that utilize the Hatch-Slack photosynthetic pathway and have a carbon isotope fingerprint between-16 ‰ to -8 ‰. Honey adulteration with C4 sugars can be detected at 1 % levels using the LC-IRMS System. Other groups of honey adulterants such as rice, sugar beet and wheat are C3 plants and such adulterations can be detected at 10 % levels using the LC-IRMS System[1]. Additionally, bulk honey and honey protein carbon isotope analysis using the Thermo Scientific™ EA IsoLink™ IRMS System can be performed for the detection of > 7 % of C4 sugars addition[2].

Analytical configuration

All measurements are performed using the LC IsoLink II IRMS System (Figure 1), consisting of the Thermo Scientific™ LC IsoLink™ II Conversion Interface that is integrated in the Thermo Scientific™ Vanquish™ Core HPLC system. A liquid sample is injected with mobile phase and all organic compounds eluting from an HPLC column are analyzed while maintaining the chromatographic resolution. The sample is oxidized within the aqueous solution, and the resulting CO_2 is removed from the liquid phase in a downstream degassing unit and entrained into a stream of Helium for admission to the Thermo Scientific™ DELTA™ Q IRMS via ConFlo IV™ Universal Interface. Full LC IsoLink II IRMS System operation is driven by the Thermo Scientific™ Qtegra™ ISDS Software that features integration with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software capabilities.

Samples

A total of 20 honey samples were prepared for this study by dissolving 1 g of honey in 4.2 ml water and shaking until fully dissolved. The solution is diluted 1:100 and filtered through a 0.45 µm syringe filter directly into an autosampler vial. After preparation, the vials are kept in the autosampler at 10 °C to avoid microbial degradation of the samples.

Figure 1. Thermo Scientific LC IsoLink II IRMS System

Analysis setup

Separation of the sugar fractions is carried out using an isocratic HPLC method with water as an eluent, as described in Table 1.

Table 1. LC IsoLink II IRMS setup for honey analysis

Collumn	
Type	Shodex™ Sugar SC-1011
Operating temperature	60 °C
Injection volume	10 µL
Total run time	40 min
Eluent	
Type	H_2O
Flow	0.3 mL/min
Pressure	15 – 18 bar
Reagents	
Type	$Na_2S_2O_8$ (0.3 mol L^{-1}), H_3PO_4 (8 %)
Flow	0.1 mL/min, 50:50

To ensure quality control of measurements, a honey laboratory standard is analyzed in each sequence, in addition to glucose and fructose-glucose (1:1) standards. The glucose standard with certified carbon isotope value is used for data normalization. The sample list is set up as follows:

Blank
Glucose standard
Glucose standard
Glucose standard
Fructose-Glucose standard
Honey laboratory standard (QC)
Samples block
Honey laboratory standard (QC)
Glucose standard
Glucose standard
Blank **with backflush functionality**

New, patented backflush functionality simplifies routine maintenance, minimizing flow path blockage and maximizing system uptime and productivity. It is fully controlled by the Qtegra ISDS Software and easily implemented in day-to-day system operation.

Results

The results in Table 2 display combined LC-IRMS data ($\delta^{13}C$ Oligosaccharides, $\delta^{13}C$ Trisaccharides, $\delta^{13}C$ Disaccharides, $\delta^{13}C$ Glucose, $\delta^{13}C$ Fructose) and EA-IRMS data ($\delta^{13}C$ Protein, $\delta^{13}C$ Honey), and the calculated values ($\Delta\delta^{13}C$ F-G, $\Delta\delta^{13}C$ max., C4 Sugar). The authenticity of samples was assessed based on the following criteria for pure honey (Apidologie 39 (2008), 574-5879)[3]:

- $\Delta\delta^{13}C$ Fructose-Glucose ((F-G): not higher than ± 1.0 ‰
- $\Delta\delta^{13}C$ (max): not higher than ± 2.1 ‰
- C4 sugar (calculated): < 7 %
- Oligosaccharides (related to total sugar content): not detected (n.d.) < 0.7 %

Ten adulterated samples that do not meet specifications of pure honey are indicated in red, Table 2.

Table 2. LC-IRMS and EA-IRMS honey samples analysis results

Honey sample	$\delta^{13}C$ Oligo-saccharides ‰	$\delta^{13}C$ Trisaccharides ‰	$\delta^{13}C$ Disaccharides ‰	$\delta^{13}C$ Glucose ‰	$\delta^{13}C$ Fructose ‰	$\delta^{13}C$ Protein ‰	$\delta^{13}C$ Honey ‰	$\Delta\delta^{13}C$ F-G ‰	$\Delta\delta^{13}C$ max. ‰	C4 Sugar %	Assessment
1	n.d.	n.a.	-26.86	-26.62	-26.4	-25.9	-26.5	0.25	-0.96	0	Pass
2	n.d.	-25.32	-27.25	-26.98	-27	-26.1	-27	-0.06	-1.93	0	Pass
3	n.d.	-18.04	-21.67	-22.65	-22.5	-23.2	-22.4	0.15	-5.16	5.9	Fail
4	n.d.	n.a.	-25.5	-25.41	-25.4	-25.2	-25.4	0.04	-0.3	0	Pass
5	n.d.	-23.24	-25.93	-24.97	-24.9	-25.1	-25	0.04	-2.69	0.6	Fail
6	n.d.	-21.15	-24.67	-25.28	-25.2	-25.3	-25.2	0.04	-4.15	0.6	Fail
7	n.d.	n.a.	-26.57	-25.8	-25.9	-25.4	-25.9	-0.08	-1.17	0	Pass
8	n.d.	-23.46	-24.82	-24.46	-24.4	-25	-24.4	0.11	-1.54	3.9	Pass
9	n.d.	-26.27	-27.4	-26.44	-26.6	-26.3	-26.6	-0.15	-1.13	0	Pass
10	-25.63	-25.23	-24.67	-25.23	-26.3	-24.9	-25.8	-1.08	-1.64	0	Fail
11	n.d.	-25.73	-27.92	-26.85	-27	-27.1	-27	-0.14	-2.19	0.6	Fail
12	n.d.	n.a.	-25.18	-26.01	-25.8	-26.6	-25.9	0.17	-1.42	4.1	Pass
13	n.d.	n.a.	-24.51	-23.37	-23.7	-24.1	-23.6	-0.3	-1.14	3.5	Pass
14	n.d.	-19.89	-25.19	-24.91	-25	-25.2	-24.9	-0.06	-5.31	1.9	Fail
15	n.d.	-23.38	-24.51	-26.65	-26.7	-23.8	-26.5	-0.08	-3.35	n.a	Fail
16	n.d.	-25.58	-26.46	-25.28	-25.5	-25.7	-25.5	-0.23	-1.18	1.3	Pass
17	n.d.	-24.26	-26.42	-25.82	-25.9	-25.6	-25.9	-0.1	-2.16	0	Fail
18	n.d.	-25.91	-26.28	-25.16	-25.3	-24.8	-25.3	-0.13	-1.48	0	Pass
19	n.d.	-23.99	-26.71	-26.26	-26.6	-26	-26.4	-0.29	-2.72	0	Fail
20	n.d.	-26.61	-27.43	-26.31	-26.6	-25.2	-26.6	-0.31	-2.23	0	Fail

*n.a.: not analyzed, because n.d.

Figure 2 (a,b,c) shows chromatograms of three honey samples. Honey sample 8 (Figure 2a) is a pure honey sample, and honey sample 19 (Figure 2b) is an adulterated honey sample (see assessment in Table 2). The visual similarity of the chromatographic pattern of individual sugar fractions allows no assessment of honey purity, unlike the isotopic values of individual sugar fractions that are the decisive criteria in confirming adulteration of honey sample 19. In honey sample 10 (Figure 2c) oligosaccharides are detected which is unusual for flower honey.

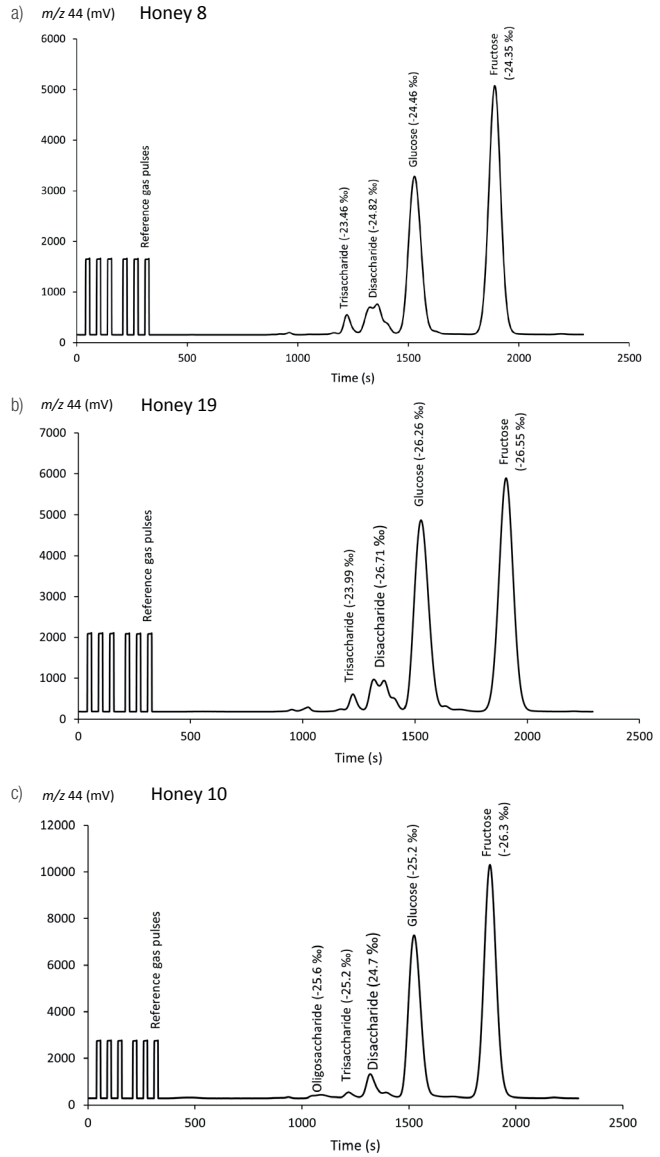

Figure 2. a) pure honey sample, b) and c) adulterated honey samples

The overall LC IsoLink II IRMS System performance and quality control of the acquired data, including the 20 honey samples presented in Table 2, was monitored over a period of 8 months using a honey laboratory standard. Over 120 measurements (Figure 3) demonstrate high precision and robustness of the LC IsoLink II IRMS System to deliver repeatable isotope measurements of honey samples. The average $\delta^{13}C$ of sugar fractions and corresponding SD is reported in Table 3.

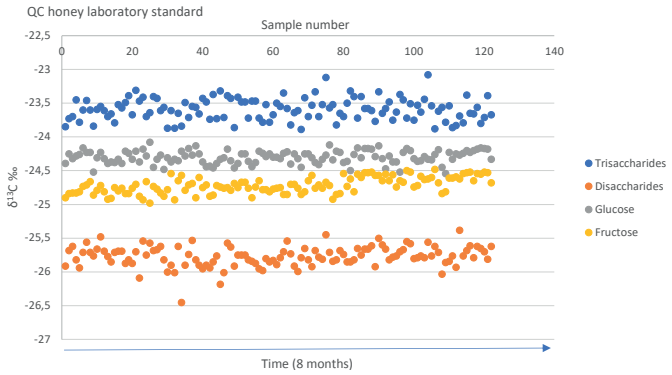

Figure 3. $\delta^{13}C$ of long-term precision measurements of the QC honey standard over an 8 month period

Table 3. $\delta^{13}C$ isotope ratios of sugar fractions of the QC honey standard over 8 an month period

	$\delta^{13}C$ average (‰)	SD
Trisaccharides	-23.59	0.16
Disaccharides	-25.77	0.15
Glucose	-24.30	0.10
Fructose	-24.72	0.12

Conclusion

The next generation LC IsoLink II IRMS System provides a highly robust platform to address honey fraud using a globally recognized analytical technique to gain isotope fingerprints and investigate the sugar profile of honey within a single HPLC run. The new design of the LC IsoLink II Conversion Interface provides exceptional instrument reliability, cost-efficient sample processing and high throughput. Full integration with the Vanquish Core HPLC system simplifies system operation, providing streamlined workflow for high sensitivity on-line determination of $^{13}C/^{12}C$ isotope ratios.

Reference

[1] Ana. I. Cabañero, Jose L. Recio and Mercedes Rupérez, J. Agric. Food Chem. 54 (2006), 9719-9727

[2] AOAC 998.12-1998, C-4 plant sugars in honey.

[3] Lutz Elflein and Kurt-Peter Raezke, Apidologie 39 (2008), 574–587

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