Application note 013

Detecting adulteration of honey – ^{13}C analysis with the LC-I

Introduction

Honey is a naturally sweet substance loved by humans throughout history and is also renowned for its health benefits. Many early civilisations recognised its unique properties; in ancient Egypt, Queen Cleopatra was thought to use mixtures containing honey as part of her beauty regime. Thousands of years later, the de-mand for honey has risen significantly.

Manuka honey, which is produced by honey bees that feed on the Manuka bush which grows is New Zealand and South Eastern Australia, is highly regarded in the west for its antibacterial and healing properties.

In the Middle East, some honey types are regarded as sa-cred, including those produced by bees who feed on the Al Sidr tree, as it is mentioned in the Quran as one of the plants found in paradise.

As demand for honey grows, bee populations are in de-cline, affected badly by intense farming methods, the use of pesticides, ecosystem damage due to climate change, and the effects of war in counties like Yemen which previ-ously had large bee colonies. All of these factors have led to a huge rise in adulteration of honey – mainly by the ad-dition of cheap artificial sugars to the premium product.

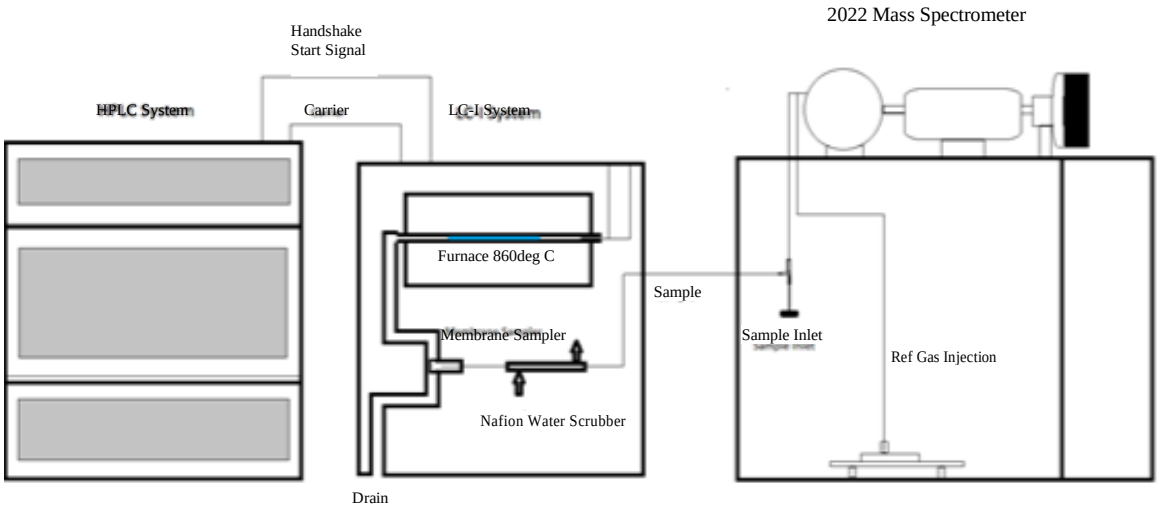
Tests have been developed to detect this adulteration and the SCIRA test has been the industry standard for decades. This test involves placing the honey sample in an elemental analyser (EA) to convert the carbon in the sample into CO_2 and the subsequent analysis of the ^{13}C ratio via isotope ratio mass spectrometry (IRMS). Adulterers of honey have become wise to the technique and have begun to add sugars which have a similar ^{13}C ratio.

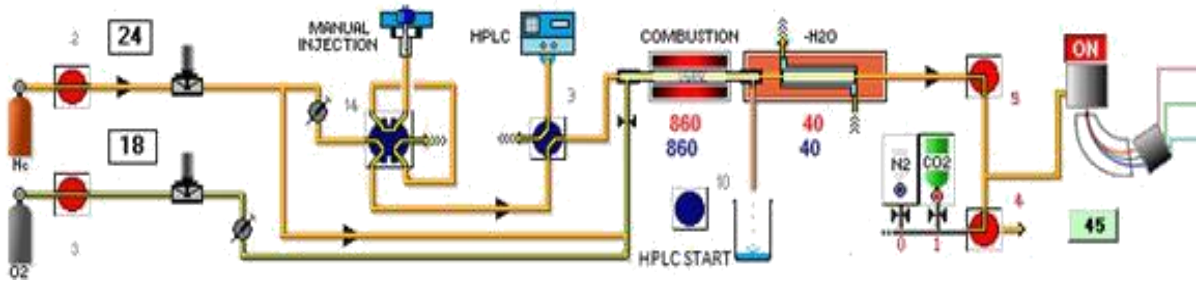
Interfacing the IRMS with a high performance liquid chromatography system (HPLC) allows the sugars to be separated prior to analysis and the adulteration of honey to be detected even when sugars of an identical ^{13}C ratio have been added. Sercon's LC-I is the in-terface between an HPLC and our high performance 20-22 IRMS. The interface contains a furnace with a catalyst, which combusts the sugars post HPLC separa-tion and transfers the CO_2 produced to the IRMS after purification.

Materials and methods

The Sercon LC-I interface allows any HPLC system to be connected to the 2022 Mass spectrometer. For this study, samples were injected into an UltiMate 3000 HPLC System. The aqueous solvent containing the separated compounds of interest from the HPLC are passed through a conversion furnace where the or-ganic molecules are combusted, with the use of a cat-alyst to CO_2 .

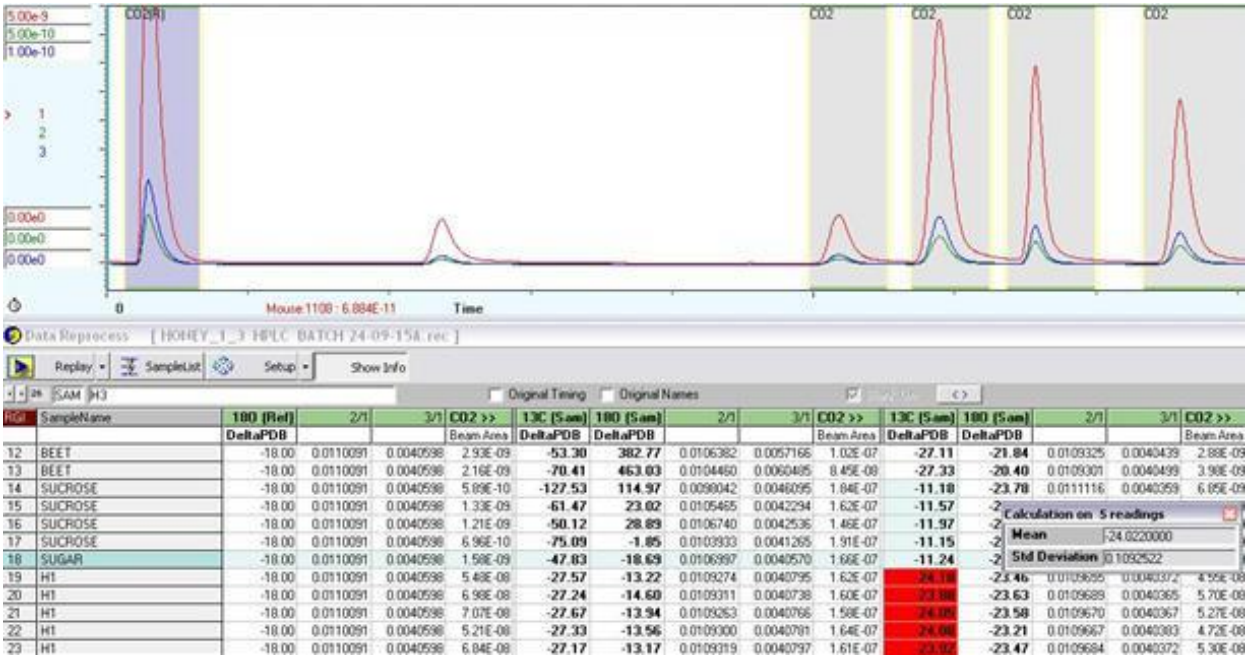
The resulting mixture of steam and CO_2 is cooled and the CO_2 gas sampled across a membrane, the other side of which is connected to the Sercon 2022 IRMS via a Nafion water scrubber. The peaks of interest were then analysed for the ^{13}C content of each of the com-pounds, via calibration with the Sercon Calisto soft-ware.





Results

Good separation of the sugars was achieved via the HPLC and precision across 5 replicates of 2 honey samples was ≤ 0.2‰.



	SampleName	18O (Rel) DeltaPDB	2/1	3/1	CO2 >> Beam Area	13C (Sam) DeltaPDB	18O (Sam) DeltaPDB	2/1	3/1	CO2 >> Beam Area	13C (Sam) DeltaPDB	18O (Sam) DeltaPDB	2/1	3/1	CO2 >> Beam Area
12	BEET	-18.00	0.0110091	0.0040598	2.93E-09	-53.30	382.77	0.0106382	0.0057166	1.02E-07	-27.11	-21.84	0.0109325	0.0040439	2.88E-09
13	BEET	-18.00	0.0110091	0.0040598	2.16E-09	-70.41	463.03	0.0104460	0.0060485	8.45E-08	-27.33	-20.40	0.0109301	0.0040499	3.98E-09
14	SUCROSE	-18.00	0.0110091	0.0040598	5.89E-10	-127.53	114.97	0.0098042	0.0046095	1.84E-07	-11.18	-23.78	0.0111116	0.0040359	6.85E-09
15	SUCROSE	-18.00	0.0110091	0.0040598	1.33E-09	-61.47	23.02	0.0105465	0.0042294	1.62E-07	-11.57	-2			
16	SUCROSE	-18.00	0.0110091	0.0040598	1.21E-09	-50.12	28.89	0.0106740	0.0042536	1.46E-07	-11.97	-2			
17	SUCROSE	-18.00	0.0110091	0.0040598	6.96E-10	-75.09	-1.85	0.0103933	0.0041265	1.91E-07	-11.15	-2			
18	SUGAR	-18.00	0.0110091	0.0040598	1.58E-09	-47.83	-18.69	0.0106997	0.0040570	1.66E-07	-11.24	-2			
19	H1	-18.00	0.0110091	0.0040598	5.48E-08	-27.57	-13.22	0.0109274	0.0040795	1.62E-07		-23.46	0.0109695	0.0040372	4.55E-08
20	H1	-18.00	0.0110091	0.0040598	6.98E-08	-27.24	-14.60	0.0109311	0.0040738	1.60E-07		-23.63	0.0109689	0.0040365	5.70E-08
21	H1	-18.00	0.0110091	0.0040598	7.07E-08	-27.67	-13.94	0.0109263	0.0040766	1.58E-07		-23.58	0.0109670	0.0040367	5.27E-08
22	H1	-18.00	0.0110091	0.0040598	5.21E-08	-27.33	-13.56	0.0109300	0.0040781	1.64E-07		-23.21	0.0109667	0.0040383	4.72E-08
23	H1	-18.00	0.0110091	0.0040598	6.84E-08	-27.17	-13.17	0.0109319	0.0040797	1.61E-07		-23.47	0.0109684	0.0040372	5.30E-08

Summary

In order to detect adulteration of honey samples us-ing the isotope ratio of C in sugar, the sugars must be separated prior to IRMS analysis. The Sercon LC-I inter-face allows the connection of any HPLC with our 20-22 IRMS and the data gives precision of ≤ 0.2‰.

This technique allows researchers to be confident in detecting which honey samples have been adulterat-ed and consumers to be well informed when purchas-ing this exceptional product.

For more information on this technique and the other application areas in which HPLC-IRMS interfacing may detect adultera-tion of foodstuffs, please contact info@sercongroup.com.