Application Note

Food Testing & Agriculture

Detection and Estimation of Special Marker for Rice Syrup (SMR) in Honey

Using the Agilent 1260 Infinity II LC system with Ultivo triple quadrupole LC/MS

Authors

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Abstract

This application note demonstrates the use of the Agilent 1260 Infinity II LC system coupled with the Agilent Ultivo triple quadrupole LC/MS (LC/TQ) to achieve low nanogram quantities of 2-AFGP in honey samples. The method was developed on an Ultivo LC/TQ which provides uncompromising results, despite the miniaturized form factor. This method is ideal for routine analysis in the food industry during the manufacturing, processing, and commercial testing of honey samples, or for academic research purposes. Using a simple liquid-liquid extraction (LLE) based sample preparation, a limit of quantitation (LOQ) of 0.005 mg/kg 2-AFGP can be successfully quantified in matrix.

Introduction

Honey, a flavorful and nutritious food produced by honeybees, is widely consumed due to its antioxidant, antimicrobial, and anti-inflammatory properties. However, its relatively high price, limited production, and complex composition make it vulnerable to adulteration, impacting consumers and manufacturers. The most common honey adulteration is by addition of sugar syrups, including rice syrup to pure honey. Additionally, feeding sugar syrups to honeybees to improve yield and profit represents indirect honey adulteration.

Figure 1. Honey product.

AFGP (2-acetylfuran-3-glucopyranoside) is the special marker for rice syrup also known as SMR that can be detected using stable carbon isotopic ratio, pulsed amperometric detection, LC-IRMS (liquid chromatography–isotopic ratio mass spectrometry), infrared spectroscopy (IR), TLC, GC/MS, and NMR just like other sugar markers. However, such traditional methods are time-consuming and laborious or sometimes have conflicting results or false negatives.

The triple quadrupole LC/MS system is the gold standards in U.S., EU, FSSAI, and other country guidelines for unambiguous confirmation of contaminants and adulterants in honey. The Ultivo LC/TQ, the ultimate evolution of triple quadrupole LC/MS systems has been used in this application whereby the obtained sensitivity exceeds the safety limits established by food regulation authorities.

Experimental conditions

This experiment used acetonitrile (Honeywell, LC/MS, 34967), methanol (Honeywell, LC/MS 34966), water (Millipore), formic acid (Honeywell, LC/MS 56302), 2-acetylfuran-3-glucopyranoside (TRC Canada, part number G596874) and an Agilent 0.2 µm PVDF syringe filter (part number 5191-5924). The stock solution was prepared using methanol and working dilutions of 2-AFGP were prepared in water.

Extraction

The sample preparation used 1 g of honey. The steps involved dilution with water as the diluent, centrifugation, and injecting the filtered supernatant into an LC/MS (accounting for the minimal cost of extraction). The detailed protocol is shown in Figure 2.

Table 1. HPLC gradient method.

Parameter	Value		
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 4 µm		
Mobile Phase	Water (0.1% FA): acetonitrile (0.1% FA); 500 µL/min		
Injection Volume	2 µL		
Column Temperature	40 °C		
Gradient	Time (Min)	Water (0.1% FA)	Acetonitrile (0.1% FA)
	0.0	98	2
	5.0	98	2
	8.0	90	10
	10.0	5	95
	12.0	5	95
	12.1	98	2

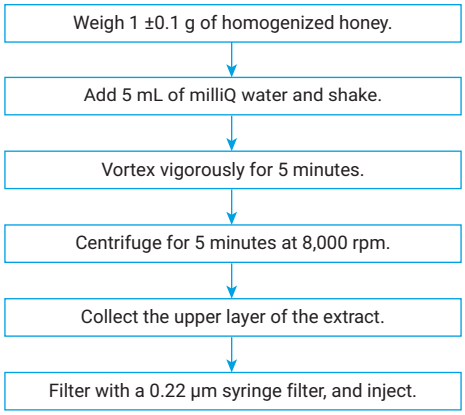


Figure 2. LLE-based sample preparation.

Instrumentation

The instruments used in this experiment included an Agilent 1260 Infinity II quaternary pump (G7104C), Agilent 1260 Infinity II vialsampler (G7129C), Agilent 1260 Infinity II multicolumn thermostat (G7116A), Ultivo LC/TQ with AJS ion source (G6465B). The LC and MS parameters are showcased in Tables 1 and 2.

Table 2. Ultivo LC/TQ conditions.

Parameter	Setting	
Ionization Mode	AJS (+ve)	
Nebulizer Gas	50 psi	
Drying Gas	10 L/min at 300 °C	
Sheath Gas	11 L/min at 300 °C	
Capillary Voltage	4,000 V	
Nozzle Voltage	1,500 V	
Fragmentor Voltage	80 V	
Dwell Time	100 ms	
Resolution	Unit/unit	
Analyte	**MRM Transition**	**CE (V)**
2-AFGP	311.1/185	9
2-AFGP	311.1/148.9	13

Results and discussion

SMR is an easily detected analyte as seen in the MRM profile of prespike SMR at 0.05 mg/kg (50 ppb) level vs blank extracted honey (Figure 3). The method LOQ using the Ultivo LC/TQ was characterized to be 0.005 mg/kg against 1 mg/kg as the desired minimum concentration of detection according to food safety guidelines (FSSAI) of India. Additionally, a reproducible elution profile was obtained by injecting various concentrations of SMR in honey, as seen in Figure 4.

Calibration, RSD, and recovery

A calibration curve linearity plot was generated for pre-extracted SMR in honey across concentration levels from 0.005 to 0.25 mg/kg using 148.9 as the quantifier ion and 185.0 as the qualifier ion. For evaluating robustness, six replicates were obtained at low and high QC levels, each. The RSD (%CV) at both QC levels were within <10% and recoveries were within 70 to 120%, as shown in Figure 5. The screenshot of the calibration table with quantifier, qualifiers, and ion ratio is shown in Figure 6, in accordance with SANTE 2019 regulations.

Figure 3. SMR response on an Agilent Ultivo LC/TQ (blank versus 0.05 mg/kg).

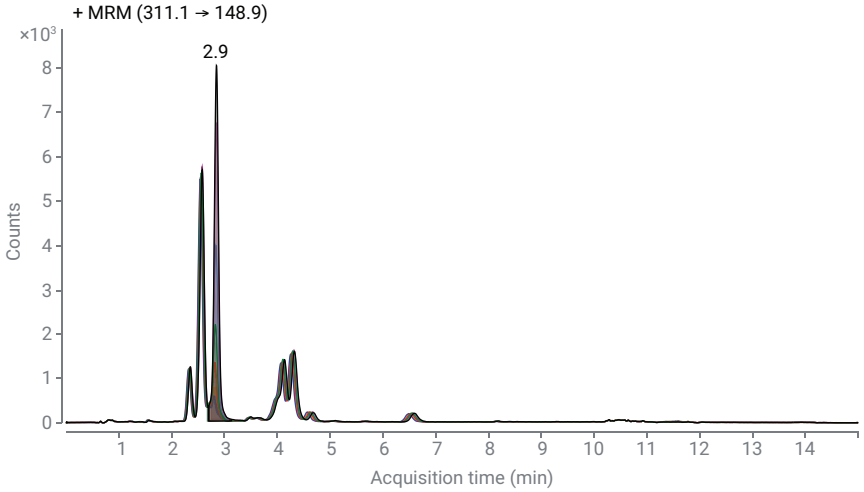
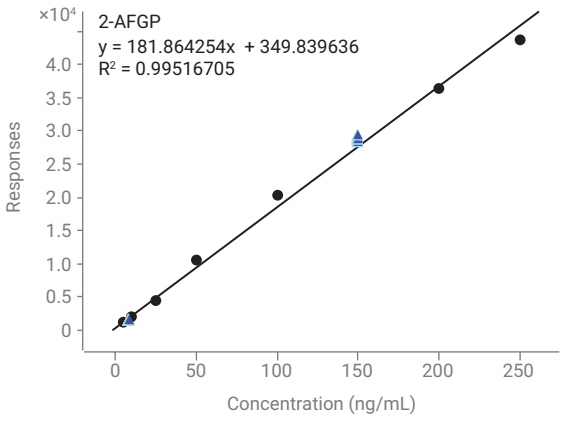


Figure 4. Overlay of various SMR concentrations at 2.9 minutes.



Sample	Conc.	Sample	Conc.
LQC 1	6.90	HQC 1	154.95
LQC 2	7.08	HQC 2	156.48
LQC 3	7.24	HQC 3	157.52
LQC 4	6.63	HQC 4	157.46
LQC 5	6.84	HQC 5	156.86
LQC 6	7.16	HQC 6	160.43
Avg	6.98	Avg	157.28
Stdev	0.224	Stdev	1.803
CV	3.209	CV	1.146
Recovery	87.25	Recovery	104.86

Figure 5. Linearity plot from 5 to 250 ppb (R^2 = 0.9952) and recovery calculation at QC levels.

Quantitation of SMR in various honey samples

The suggested method was extended to various honey samples. All samples were extracted and analyzed using the developed methodology. The initial sample was diluted five times in water, meaning a dilution factor of five was used and obtained values were reported. Across the total 10 samples, three were found to contain SMR. Of the positive samples, two found SMR higher than the calibration range, while SMR was either absent or it was lower than calibration range in rest of the samples. The quantitation data for all 10 samples can shown in Figure 6. Figure 7 shows the qualifier and quantifier MRM chromatogram profile for blank honey, SMR spiked in honey, a SMR-negative sample, and a SMR-positive sample.

Sample				2-AFGP ...	2-AFGP Results					Qualifier ...	
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	MI
MATRIX_BLANK_3.d	MatrixBlank		5/17/2021 6:10 PM		2.85	77.0	☑	0.00		238.0	☑
MATRIX_5PPB.d	Cal	1	5/17/2021 6:26 PM	5	2.81	1205.0	☐	4.70	94.0	58.6	☐
MATRIX_10PPB.d	Cal	2	5/17/2021 6:42 PM	10	2.82	2102.0	☐	9.63	96.3	89.0	☐
MATRIX_25PPB.d	Cal	3	5/17/2021 6:57 PM	25	2.82	4570.0	☐	23.21	92.8	77.8	☐
MATRIX_50PPB.d	Cal	4	5/17/2021 7:13 PM	50	2.83	10595.0	☐	56.33	112.7	73.6	☐
MATRIX_100PPB.d	Cal	5	5/17/2021 7:29 PM	100	2.84	20323.0	☐	109.82	109.8	68.4	☐
MATRIX_200PPB.d	Cal	6	5/17/2021 7:44 PM	200	2.85	36321.0	☐	197.79	98.9	66.7	☐
MATRIX_250PPB.d	Cal	7	5/17/2021 8:00 PM	250	2.85	43726.0	☐	238.51	95.4	66.4	☐
LQC_1.d	QC	8	5/17/2021 8:16 PM	8	2.86	1604.0	☐	6.90	86.2	106.7	☐
LQC_2.d	QC	8	5/17/2021 8:31 PM	8	2.86	1637.0	☐	7.08	88.5	104.3	☐
LQC_3.d	QC	8	5/17/2021 8:47 PM	8	2.86	1667.0	☐	7.24	90.5	96.8	☐
LQC_4.d	QC	8	5/17/2021 9:03 PM	8	2.87	1556.0	☐	6.63	82.9	105.7	☐
LQC_5.d	QC	8	5/17/2021 9:18 PM	8	2.87	1599.0	☐	6.87	85.9	102.2	☐
LQC_6.d	QC	8	5/17/2021 9:34 PM	8	2.87	1652.0	☐	7.16	89.5	98.5	☐
HQC_1.d	QC	9	5/17/2021 9:50 PM	150	2.87	28529.0	☐	154.95	103.3	67.8	☐
HQC_2.d	QC	9	5/17/2021 10:05 P...	150	2.88	28808.0	☐	156.48	104.3	67.4	☐
HQC_3.d	QC	9	5/17/2021 10:21 P...	150	2.87	28998.0	☐	157.52	105.0	68.2	☐
HQC_4.d	QC	9	5/17/2021 10:37 P...	150	2.88	28986.0	☐	157.46	105.0	67.8	☐
HQC_5.d	QC	9	5/17/2021 10:52 P...	150	2.87	28877.0	☐	156.86	104.6	67.7	☐
HQC_6.d	QC	9	5/17/2021 11:08 P...	150	2.88	29527.0	☐	160.43	107.0	67.3	☐
MATRIX_BLANK_4.d	MatrixBlank		5/17/2021 11:24 P...		2.77	355.0	☐	0.03		90.1	☐
Sample_V_13.d	Sample		5/17/2021 11:40 P...		2.78	746.0	☐	2.18		84.2	☐
Sample_V_14.d	Sample		5/17/2021 11:55 P...		2.77	616.0	☐	1.46		95.1	☐
Sample_V_15.d	Sample		5/18/2021 12:11 A...		2.77	429.0	☐	0.44		89.5	☐
Sample_V_16.d	Sample		5/18/2021 12:27 A...		2.78	481.0	☐	0.72		80.0	☐
Sample_V_245.d	Sample		5/18/2021 12:42 A...		2.77	152.0	☐	0.00		119.7	☐
Sample_K_231.d	Sample		5/18/2021 12:58 A...		2.76	202.0	☐	0.00		55.9	☐
Sample_K_198.d	Sample		5/18/2021 1:14 AM		2.86	79206.0	☐	433.60		62.2	☐
Sample_J_215.d	Sample		5/18/2021 1:29 AM		2.86	13160.0	☐	70.44		68.3	☐
Sample_J_221.d	Sample		5/18/2021 1:45 AM		2.87	49042.0	☐	267.74		63.7	☐
Sample_N_5390.d	Sample		5/18/2021 2:01 AM		2.76	228.0	☐	0.00		81.6	☐
MATRIX_BLANK_5.d	Sample		5/18/2021 2:16 AM		2.78	335.0	☐	0.00		91.9	☐

Figure 6. Calibration table for SMR in honey from 5 to 250 ppb (0.005 to 0.25 mg/kg).

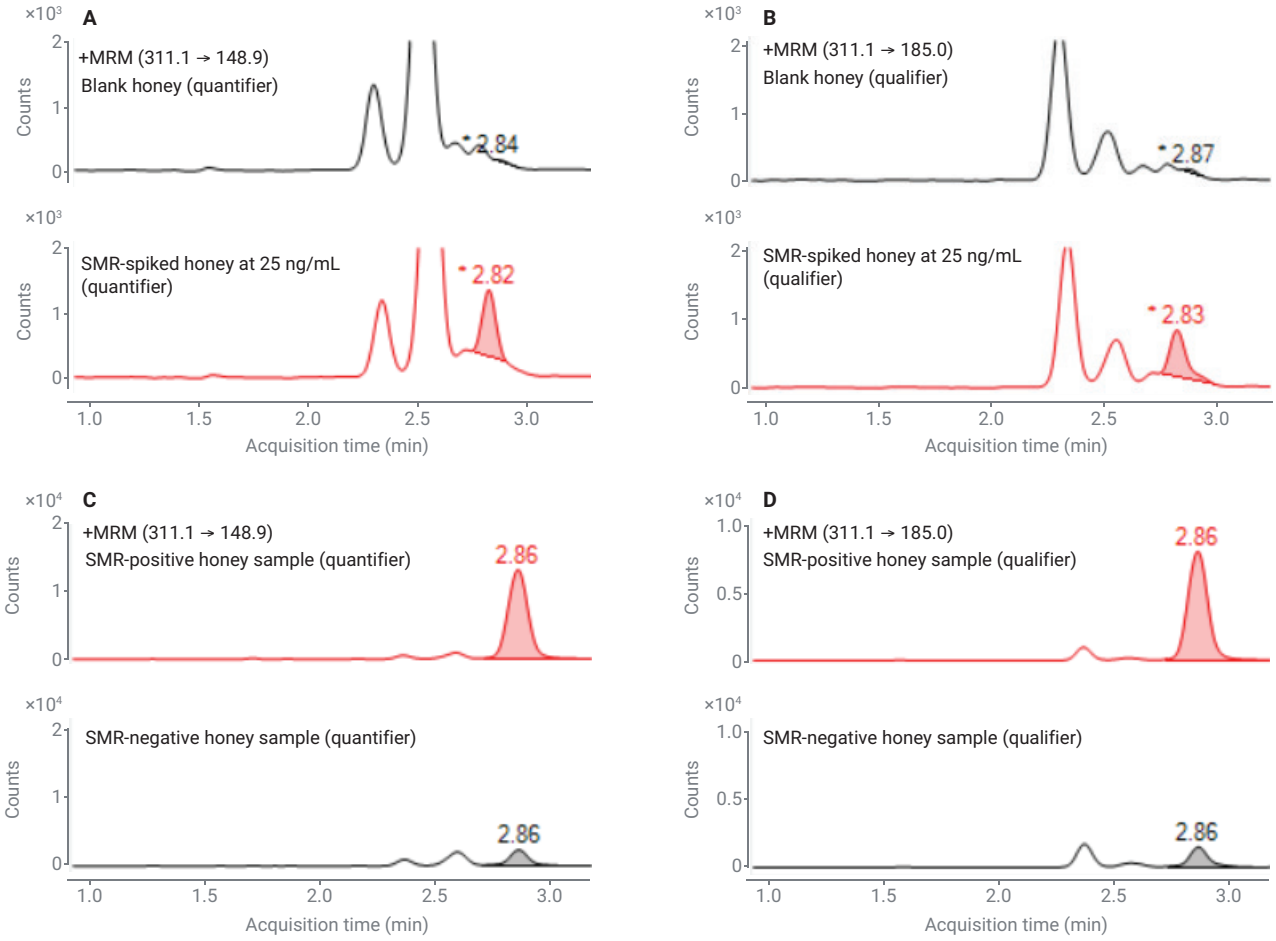


Figure 7. (A) Quantifier MRM for SMR in blank matrix versus SMR in honey at 25 ng/mL. (B) Qualifier MRM for SMR in bank matrix versus SMR in honey at 25 ng/mL. (C) Quantifier MRM for SMR in honey, a positive sample versus a negative sample. (D) Qualifier MRM for SMR in honey, a positive sample versus a negative sample.

Conclusion

Using a rigorous validation approach, the LOQ for the method described in this application note is 200 times lower than FSSAI norms for SMR in honey. Based on six replicates from two QC levels, %CV values are less than 5%, and the percentage recovery values at QC levels are within 70 to 120%.

In conclusion, true honey samples can be successfully analyzed for SMR as per EU norms by making use of the Ultivo LC/TQ system coupled to an 1260 Infinity II. The sample preparation method defines a dilute-and-shoot LLE-based protocol through easy, quick, and cost-effective steps.

References

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