File No. 11023/53/2018-QA

Food Safety and Standards Authority of India

(A statutory Authority established under the Food Safety and Standards Act, 2006)

(Quality Assurance Division)

FDA Bhawan, Kotla Road, New Delhi – 110002

Dated, the 6th July, 2020

CORRIGENDUM

Subject: **Method for Detection of 2-Acetylfuran-3-Glucopyranoside (AFGP)/ 3-O-α-D-Glucosyl Isomaltol, in honey by LC-MS/MS - reg.**

In suppression of FSSAI Order of even number dated 04.06.2020, please find attached the amended Method for Detection of 2-Acetylfuran-3-Glucopyranoside (AFGP)/ 3-O-α-D-Glucosyl Isomaltol, in honey by LC-MS/MS **(Annexure – I)**.

2. The food testing laboratories are hereby requested to use the aforesaid amended method, with immediate effect

Encl: Method

6/7/2020

(Bhaskar N)
Advisor (QA)

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories

Copy to:

1. Executive Director (Regulatory Compliance), FSSAI
2. Advisor (Science & Standards), FSSAI
3. Head (Regulations), FSSAI

fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food	**Method for Detection of 2-Acetylfuran-3-Glucopyranoside (AFGP)/ 3-O-α-D-Glucosyl Isomaltol, in honey by LC-MS/MS**		
Method No.	**01**	**Revision No. & Date**	**06.07.2020**
Introduction	The presence of 2-AFGP in honey is as indicative marker of rice syrup. The minimum concentration of detection 1 mg AFGP/kg honey.		
Abbreviations:	LC/MS/MS: Liquid Chromatography Mass Spectrometry EI: Electron Ionization MRM: Multiple Reaction Monitoring CE: Collision Energy		
Caution	Always wear gloves and mask while doing sample analysis and standard handling.		
Principle	The method involves dilution of honey with water; a clean-up through HLB cartridge and subsequent analysis by Liquid Chromatography – Mass Spectrometry (LC-MS/MS).		
Equipment	High performance LC or Ultra-high-performance LC (UHPLC) system, consisting of a dual pump system, sample injector unit, degasser unit, and column oven. Column: Agilent Eclipse plus C18 (100 mm × 4.6 mm, 3.5 µm)/Waters Acquity UPLC HSS PFP (100×2.1 mm, 1.8 µm) or equivalent. Mass spectrometer: Triple-quadrupole mass spectrometer or equivalent MS/MS instrument. Centrifuge tubes (15 mL) Analytical balance (0.0001 g) Vortex mixer Micro pipettes 20 – 200 µL and 100-1000 µL **Glassware & Others:** a) Injection vials b) Volumetric flask Class A, 10 mL and 1 mL c) Glass tubes 15 mL Capacity d) Hydrophilic syringe filters (0.22 µm) e) Hydrophilic-Lipophilic-Balanced (HLB) water-wettable, reversed-phase sorbent cartridge or equivalent should be used for sample preparation		
Chemicals	a) Acetonitrile (MS Grade) b) Methanol (MS Grade) c) Milli Q Water/HPLC grade: Electrical Resistivity, min, 18.2 MΩ.cm (at 25°C) d) Standard2-acetylfuran-3- glucopyranoside (AFGP) (Synonym: 3-O-α-D-Glucosyl Isomaltol TRC Canada; Catalogue no. - G596875		
Preparation of standards	a) **Stock Solution**: Accurately weigh standard AFGP and add methanol as solvent make a stock solution of approximate 1.0 g/L (1000 mg/L		

which is same as 1 mg/mL) in a volumetric flask.

b) Intermediate Standard Solution: Prepare the intermediate standards of concentration of 10.0 mg/L (10000 µg/L) and 1.0 mg/L (1000 µg/L) by subsequent dilution with water.

Concentration of stock standard. (g/L)	Vol. of stock standard (µL)	Vol. of water (µL)	Final conc. (g/L)
1.0	100	900	0.1
0.1	100	900	0.01
0.01	100	900	0.001

c) Working Standard (WS) Solution for calibration curve: Prepare the working standards from the intermediate standard (0.001 g/L) by dilution with water as shown below.

Working standard concentration (µg/L (ppb))	**Volume of Intermediate standard(µL)**	**Volume of water (µL)**	**Total volume (µL)**
100	100	900	1000
200	200	800	1000
300	300	700	1000
400	400	600	1000
800	800	100	1000
1000	100	0	1000
Note If sample preparation is carried out using HLB cartridge the dilution must be carried out with methanol			

Sample preparation

By dilution

1. Weigh 1 g±0.01 g of honey sample in a 15 mL centrifuge tube.
   Note (If the honey sample has particles centrifuge it at 5000 g for 5 mins or pass through a nylon mesh (100-150 micron)
2. Add 1 ml water and shake vigorously.
3. Dilute 1:5 if necessary.
4. Vortex the tubes for 5 minutes and roto spin for 5 minutes.
5. Centrifuge the tubes at 7000 × g for 5 minutes.
6. Collect upper clean extract and filter it through syringe filter (0.22µm)
7. Use for LC-MS/MS

Using HLB cartridge

1. Take 1 g of honey sample in a 15 mL centrifuge tube.
   (If the honey sample has particles centrifuge it at 5000 g for 5 mins or pass through a nylon mesh (100-150 micron)
2. Add 5 mL MilliQ water and mix in a vortex for 3 minutes.
3. Make the volume up to 10 mL with water.
4. Take a 500 mg/6 cc HLB cartridge, condition it with methanol first

<table>
<tr><td></td><td>then followed with water.
5. Pass the honey solution through the cartridge with constant speed and without applying any external pressure.
6. Elute the cartridge using 5.0 mL methanol.
7. Collect the elute in a clean tube.
8. Filter using 0.2 µm syringe filter prior to LC analysis</td></tr>
<tr><td>Liquid chromatographic instrument settings</td><td>HPLC/UPLC configuration
I. Set up the HPLC/UPLC system with the configuration shown below
a. Column: C18 (100 mm × 4.6 mm, 3.5µm)/(100×2.1 mm, 1.8 µm) or equivalent
b. Injection volume: 10 µL
c. Flow rate: 0.5 mL/min
d. Elution: Gradient
e. Solvent A: Water containing 0.1% Formic acid
f. Solvent B: Acetonitrile containing 0.1% Formic acid
II. Form gradients by high-pressure mixing of the two mobile phases, A and B, using the gradient programme shown below
<table><tr><td colspan="3">Gradient programme for HPLC/UPLC*</td></tr><tr><td>Time (min)</td><td>Solvent A (%)</td><td>Solvent B (%)</td></tr><tr><td>Start</td><td>95</td><td>5</td></tr><tr><td>7</td><td>10</td><td>90</td></tr><tr><td>7.01</td><td>5</td><td>95</td></tr><tr><td>10</td><td>5</td><td>95</td></tr><tr><td>11</td><td>95</td><td>5</td></tr><tr><td>13</td><td>Stop</td><td></td></tr><tr><td colspan="3">*Gradient can be suitably modified and optimized to obtain best peak shape and resolution</td></tr></table>
III. After verifying equilibration of the HPLC/UPLC system, inject the working standards followed by a reagent blank, control sample, and sample extracts. Injected working standards after the analysis of the last sample extract.</td></tr>
<tr><td>Mass spectrometer instrument settings</td><td>Set up the mass spectrometer with the instrument settings listed below
<table><tr><td>Gas temp.(°C)</td><td>300</td></tr><tr><td>Gas Flow (l/min)</td><td>10</td></tr><tr><td>Nebulizer (psi)</td><td>50</td></tr><tr><td>Sheath Gas Heater (°C)</td><td>300</td></tr><tr><td>Sheath Gas Flow (L/min)</td><td>10</td></tr><tr><td>Capillary (V)</td><td>3500</td></tr><tr><td>VCharging</td><td>500</td></tr></table>
Note: These settings are suitable for the 6460 triple-quadrupole (Agilent Technologies) mass spectrometer. Optimal tuning on alternative</td></tr>
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	instruments will differ. Tune the instrument to obtain the precursor and product ions. Follow the manufacturer's instruction or alter conditions to obtain the best resolution of AFGP peaks

Mass analysis parameters for AFGP#							
AFGP ion	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Dwell time (ms)	Fragmentor	CE (V)	Cell Acceleration	Polarity
Analyte qualifier	311.07	185	100	162	9	7	Positive
Analyte quantifier	311.07	148.9	100	162	13	7	Positive
CE: Collision Energy#							

Peak Identification

a. Peak shape and response ratio of extracted ion chromatograms of sample should be similar to those obtained from calibration standard
b. The retention time of the AFPG in the extract should correspond to that of the calibration standard with a tolerance of ±0.1min.
c. Identification in MRM mode largely relies on the correct selection of ions.
d. Chromatographic peaks of different selected ions for the analyte must fully overlap.
e. Ion ratio from sample should be within ±30% (relative) of average of calibration standards from same sequence

Calculation	Acquire the chromatograms and prepare the calibration curve. Calculate the regression by plotting peak height response r for each working standard vs AFGP concentration. Carry out a regression analysis R^2= 0.999 Calculate the concentration of AFGP in the sample using the equation y= mx+c Where, y=Area under the curve for AFGP in sample x= Concentration of Analyte m= slope of the calibration curve c= value of y intercept The curve can also be directly taken from instrumental software. If the analyte concentration in sample is greater than the calibration standards, the sample elute should be appropriately diluted and analyzed.
Reporting results	If concentration of AFGP is <1.0 mg/kg reported as negative/absent. If marker concentration is ≥ 1.0 mg/kg, to be reported as positive/present.
Quality control	Perform replicate analysis and recovery study for every batch of samples.
Reference	1. 2-Acetylfuran-3-Glucopyranoside as a Novel Marker for the detection of Honey adulterated with Rice syrup. Xue Xiaofeng, Wang Qiang, Li Yi, Wu Liming, Chen Lanzhen, Zhao Jing and Liu Fengmao. J. Agric. Food Chem., 2013, 61, 7488-7493p. 2. Rapid screening of multiclass syrup adulterants in honey by

	Ultrahigh - Performance Liquid Chromatography/Quadrupole Time of Flight Mass Spectrometry, Du Bing, Wu Liming, Xue Xiaofeng, Chen Lanzhen, Zhao Jing and Cao Wei. J. Agric. Food Chem, 2015, 63(29), 6614-6623. 3. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed; SANTE/11813/2017
Approved by	Scientific Panel on Methods of Sampling and Analysis