

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 04 June, 2020


**ORDER**

**Subject: Method for Detection of 2-Acetylfuran-3-Glucopyranoside (2-AFGP)/3-O- $\alpha$ -D-Glucosyl Isomaltol, the Specific Marker for Rice Syrup (SMR) by LC-MS/MS - reg.**

The Scientific Panel on Methods of Sampling and Analysis and Food Authority has approved the Method for Detection of 2-Acetylfuran-3-Glucopyranoside (2-AFGP)/3-O- $\alpha$ -D-Glucosyl Isomaltol, the Specific Marker for Rice Syrup (SMR) by LC-MS/MS (**Annexure - I**).

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

*Encl: Method*

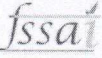
  
(Kumar Anil)  
Advisor (QA)  
4.6.2020

To:

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

Copy to:

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

 <small>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA</small> <small>Inspiring Trust, Assuring Safe &amp; Nutritious Food</small> <small>Ministry of Health and Family Welfare, Government of India</small>	<b>Method for Detection of 2-Acetylfuran-3-Glucopyranoside (2-AFGP)/3-O-<math>\alpha</math>-D-Glucosyl Isomaltol, the Specific Marker for Rice Syrup (SMR) by LC-MS/MS</b>		
Method No.	01	Revision No. & Date	04.06.2020
<b>Introduction</b>	<b>Scope:</b> Detection of 2-AFGP in honey. The minimum concentration of detection is 1 mg/Kg]		
<b>Abbreviations:</b>	LC/MS/MS : Liquid Chromatography Mass Spectrometry EI : Electron Ionization RT : Retention Time CV : Coefficient of Variance SD : Standard Deviation MRM : Multiple Reaction Monitoring CE : Collision Energy SS : Stock Solution IS : Intermediate Solution WS : Working Solution		
<b>Caution</b>	Always wear gloves and mask while doing sample analysis and standard handling.		
<b>Principle</b>	2-AFGP is extracted from honey sample by dissolving in water and analysed by LC-MS/MS.		
<b>Equipment</b>	<b>LC-MS/MS</b> a) Column: Agilent Eclipse plus C18 (100mm x 4.6 mm, 3.5 $\mu$ m) or equivalent b) Centrifuge tubes (15ml) c) Analytical balance (0.0001g) d) Vortex e) Micro pipettes 20 – 200 $\mu$ l and 100-1000 $\mu$ l capacity ranges  <b>Glassware &amp; Others:</b> a) Injection vials b) Volumetric flask Class A, 10 mL and 1 mL c) Glass tubes 15mL Capacity d) Hydrophilic syringe filters (0.22 $\mu$ m)		
<b>Chemicals</b>	<b>Reagents:</b> a) Acetonitrile (LCMSMS Grade) b) Formic Acid (LCMSMS Grade) c) Milli Q Water		

**Standard:**

Compounds	Catalogue No.	Source
Acetylfuran-3-Glucopyranoside/3-O- $\alpha$ -D-Glucosyl Isomaltol	G596874	TRC, Canada

**Preparation of standards**

- a) **Stock Solution:** Weigh standard using micro balance and add Methanol as solvent making the stock solution.
- b) **Intermediate Standard Solution:** Prepare the Intermediate standard according to the LOQ requirements and do the subsequent dilutions with water.
- c) **Working Standard (WS) Solution:** Prepare the working standard from Intermediate of variable concentration according to the LOQ requirements and do the subsequent dilutions with water.

**Example of stock concentration and intermediate concentration is given in the table below:**

**Table: Intermediate standard (IS) preparation**

Analyte	Stock conc. (in $\mu\text{g/mL}$ )	Volume of stock taken (in mL)	Final volume (in mL)	Final concentration of Intermediate standard [ $\mu\text{g/mL}$ (ppm)]
AFGP	1040	0.075	1	78

- d) **Preparation of Calibration Curve Standards:** Prepare the calibration curve by using the working standard as shown below

Final concentration ( $\mu\text{g/mL}$ )	Volume taken of Working standard ( $\mu\text{L}$ )	Final volume (mL)
5	5	1
10	10	1
20	20	1
40	40	1
80	80	1
100	100	1

**Instrument Conditions**

A summary of conditions is as follows:

Compound	Precursor Ion	Product Ion	Dwell	Fragmentor	CE	Cell Acceleration	Polarity
AFGP	311.07	185	100	162	9	7	Positive
	311.07	148.9	100	162	13	7	Positive

**Source parameters**

Gas temp.(°C)	300
Gas Flow (l/min)	10
Nebulizer (psi)	50
Sheath Gas Heater	300
Sheath Gas Flow	10
Capillary (V)	3500
VCharging	500

**Quaternary Pump**

Flow (ml/min)	0.5
Run Time (min)	15
Solvent mode	Gradient

**Gradient**

Time (min)	Acetonitrile (0.1% Formic acid) (%)	Water (0.1% Formic acid) (%)	Flow (ml/min)	Pressure (bar)
7	90	10	0.5	600
7.01	5	95	0.5	600
10	5	95	0.5	600

**Sample preparation**

Prepare the samples by adopting the following method:

- a) Weigh 1g±0.1 g honey sample in 15ml centrifuge tube.
- b) Add 1ml water and shake vigorously.

	<p>c) Vortex the tubes for 5 min and roto-spin for 5 minutes.</p> <p>d) Centrifuge the tubes at 8000rpm for 5 minutes.</p> <p>e) Transfer upper clean extract and filter it through syringe filter.</p> <p>f) Inject the extract in LC-MS/MS.</p>
<b>Data processing/ Calculation</b>	<p>Acquire the chromatograms and prepare the calibration curve. Carry out a regression analysis <math>R^2 = 0.999</math></p> <p>Calculate the concentration of AFGP in the sample Using the equation</p> $y = mx + c$ <p><b>Where,</b> <math>y</math> = Area under the curve for AFGP in sample  <math>x</math> = Concentration of Analyte  <math>m</math> = slope of the calibration curve  <math>c</math> = value of <math>y</math> intercept</p>
<b>Quality control</b>	<p>Perform replicate analysis and recovery study for every batch of samples. If recovery is less than 70%, then HLB (Hydrophilic-Lipophilic-Balanced) water-wettable, reversed-phase sorbent cartridge or equivalent should be used for sample preparation.</p>
<b>Reference</b>	<ol style="list-style-type: none"> <li>1. Xue Xiaofen, Wang Qiang, Li Yi, Wu Liming, Chen Lanzhen, Zhao Jing and Liu Fengmao. 2-Acetylfuran-3-Glucopyranoside as a Novel Marker for the detection of Honey adulterated with Rice syrup. J. Agric. Food Chem., 2013, 61, 7488-7493p.</li> <li>2. Du Bing, Wu Liming, Xue Xiaofeng, Chen Lanzhen, Zhao Jing and Cao Wei. Rapid screening of multiclass syrup adulterants in honey by Ultrahigh - Performance Liquid Chromatography/Quadrupole Time of Flight Mass Spectrometry. J. Agric. Food Chem., 2015, 63(29), 6614-6623.</li> <li>3. Eurachem/CITAC (2000). Quantifying uncertainty in analytical measurement.</li> </ol>
<b>Approved by</b>	Scientific Panel on Methods of Sampling and Analysis