AOAC SMPR 2020.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Targeted Testing (TT) of Barley and Malt Extract, Beet Sugar Syrup, Corn and Cane Sugar Syrup, C-4 Plant Sugar and High Fructose Corn Sugar for Adulteration of Floral and Acacia Honey; Version 4; April 21, 2020

Intended Use

AOACI *SMPRs*® describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study.

SMPRs are written and adopted by AOACI using the consensus of stakeholders representing the industry, government, and academic and/or research institutions. AOACI SMPRs are used by AOACI expert review panels (ERPs) in their evaluation of validation study data for method being considered for *Performance Tested Methods*^{SM} or AOACI *Official Methods of Analysis*^{SM} and can be used as acceptance criteria for verification at user laboratories.

1. Applicability

This document contains assessment parameters on the performance of Targeted Testing methods to monitor honey for the detected presence of the following Economically Motivated Adulterants (EMA) ***barley and malt extract, beet sugar syrup, corn and cane sugar syrup, C-4 plant sugar and high fructose corn sugar in Floral and Acacia Honey***.

2. Analytical Technique

A Targeted Testing (TT) method(s) to monitor honey for the detected presence of the following EMAs: barley and malt extract, beet sugar syrup, corn and cane sugar syrup, C-4 plant sugar and high-fructose corn sugar in Floral and Acacia honey.

A Targeted method to be used to monitor and enforce regulatory requirements for honey for detected and identified EMAs.

Any method capable of detecting, identifying the presence of a defined adulterating ingredient in honey and using the method to quantify the amount (proportion/concentration) present in the food item will be considered.

The scope of the TT method will be defined by the authentic samples and or reference standard material (if available) that were used in validating the method.

3. Definitions

Applicability Statement – a general statement about the intended purpose and scope of the method entailing key aspects of expected achievements for the specific situation and circumstances. Key points to cover are the intended matrix, the purpose, and an indication of sensitivity, selectivity, enforcement and action levels where available.

Economically Motivated Adulteration – The fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller.

False Origin – Honeys containing mislabelled geographic and botanical sources.

Authentic Honey – The type(s) of honey used to generate the baseline fingerprint. The method's scope of authenticity is defined by the honey(s) used in generating the baseline fingerprint. The authentic samples and/or standard reference materials (RSMs) whenever available, used to validate the method will be used to properly define the TT method testing scope.

Non-authentic substance or adulterant - A known substance or adulterant used to adulterate honey for economic gain that can be targeted for analysis.

Lot of honey – means a discrete quantity of honey delivered for distribution at one time, and determined to have common characteristics, such as origin, variety, type of packing, packer or consignor, or markings, by the sampling official. ***(Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals"*** (Adopted 2009. Revision 2012, 2014)

Consignment of honey – means discrete quantity of honey as described on a particular contractor's shipping document. A consignment may be made up of different lots.

Primary honey sample – means a quantity of honey taken from one place in the lot, unless this quantity is inadequate for the residue analysis. When the quantity is inadequate for laboratory analysis, samples from more than one location can be combined for the primary sample.

Bulk sample – means the combined total of all the primary samples taken from the same lot.

Single Laboratory Validation – Demonstration by one laboratory of method performance on samples described according to internationally accepted validation guidelines contained in Guidance Documents such as AOAC'S Appendix D, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" the ***ISO/IEC 17025:2017 Document: "General requirements for the competence of testing and calibration laboratories",*** the ***Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals"*** (Adopted 2009. Revision 2012, 2014); the "***Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis CAC/GL-49-2003*** "***Harmonized Guidelines For Single-Laboratory Validation Of Methods Of Analysis***"; "***Guidelines on the use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative analysis of Residues CAC/GL 56-2005***"; "***Establishing the Fitness for Purpose of Mass Spectrometric methods."*** and ***SANTE/12682/2019. "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed"***- A Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.

Once the method has been demonstrated to meet the minimum requirements for validation and fit for purpose criteria, the method can be reviewed and considered by AOACI for classification as First Action Official Method of Analysis.

Multi-laboratory Validation – Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories according to guidelines described in the AOACI ***Appendix D***, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" be considered for classification as AOAC Final Action Method; "***Protocol for the design, conduct and interpretation of method performance studies***". Pure and Applied Chemistry, Horwitz, W. 1995. 67:331-343; "***Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food"- CAC/GL 27-1997***; "***Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement" - CAC/GL 37-2001***; and "***Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement"***

The Predicted ($PRSD_R$) of REPRODUCIBILITY is calculated from the Horwitz equation

$PRSD_R = 2C^{-0.15}$ Where C is expressed as a mass fraction

For Quantitative methods undergoing MLV 10 –12 labs must be recruited to provide at least 8 valid data sets; two blind duplicate replicates at five concentration levels for each analyte/matrix combination to each collaborator.

HorRat (Repeatability, r) = $RSD_r/PRSD_R$
HorRat (Reproducibility, R) = $RSD_R/PRSD_R$

For Inter-laboratory studies: acceptable HorRat (R) of 1 with limits of acceptability of 0.5 to 2;
For Within-Laboratory studies: acceptable HorRat (r) of 0.3 – 1.3

4. Method Performance Requirements

Specific Sample Preparation Instructions for Honey (CAC/GL 71-2009 Adopted 2009.Revision 2012, 2014) (*Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals"* (Adopted 2009. Revision 2012, 2014)

(a) Collect 250 mL of liquid or strained honey after the following preparations as applicable;

(b) Liquidise comb honey: Cut across top of comb, if sealed, and separate completely from comb by straining through a sieve the meshes of which are made by so weaving wire as to form square opening of 0.500 mm by 0.500 mm (ISO 565-1990)17.

(c) If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40°C in water bath and strain through cheesecloth in hot-water-funnel before sampling.

When a sample is free from granulation, mix thoroughly by stirring or shaking; if granulated, place closed container in water-bath without submerging, and heat for 30 min at 60°C; then if necessary heat at 65°C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as the sample liquefies.

(Table 1: Method Performance Requirements for Barley and Malt extract in honey)

Analytical Parameter	**Acceptance Criteria**
Analytical Range (%)	**10-50**
LOQ (%)	**≥ 10**
Recovery %	**80-120**
Accuracy %	**±20**
Precision (Repeatability) RSD_r	**10**
Precision (Reproducibility) RSD_R	**10**

(Table 2: Method Performance Requirements for Beet Sugar Syrup in honey)

Analytical Parameter	**Acceptance Criteria**
Analytical Range (%)	**10-50**
LOQ (%)	**≥10**
Recovery (%)	**80-120**
Accuracy (%)	**±20**
Precision (Repeatability) RSD_r	**10**
Precision (Reproducibility) RSD_R	**10**

(Table 3: Method Performance Requirements for Corn and Cane Sugar Syrup in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	5-95
LOQ (%)	≥5
Recovery (%)	80-120
Accuracy (%)	±20
Precision (Repeatability) RSD_r	10
Precision (Reproducibility) RSD_R	10

(Table 4: Method Performance Requirements for C-4 Plant Sugar in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	0.20-50
LOQ (%)	≥0.38
Recovery (%)	80-120
Accuracy (%)	±20
Precision (Repeatability) RSD_r	15
Precision (Reproducibility) RSD_R	15

(Table 5: Method Performance Requirements for High Fructose Corn Sugar in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	10-50
LOQ (%)	≥10
Recovery (%)	80-120
Accuracy (%)	±20
Precision (Repeatability) RSD_r	10
Precision (Reproducibility) RSD_R	10

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

6. Reference Materials

A detailed description of the process used to obtain and evaluate authentic/reference standard materials (sources), and of the test protocol used for validating the method must be provided.

7. Validation Guidance

a. Data demonstrating method performance is required.

b. Samples: Complete documentation for the collection and use of authentic samples must be supplied by the method authors. The scope of "authentic" samples used to validate the method must be applicable to the defined scope of the TT method. Expansion of the scope is possible with the inclusion of additional authentic samples and abbreviated validation using the protocol listed in this SMPR.

c. For single lab validation studies, the method will be evaluated using prescribed adulterated materials as shown in Tables 1 – 5 above. Methods approved at this level will proceed to a second level of evaluation (multi-laboratory) where blinded samples containing unknown adulterants will be sent to participating laboratories.

d. Statistical analysis of interlaboratory studies. Sample size needed to meet performance requirement on proportion.

8. Maximum Time-to-Results

None.

References:

CAC/GL 27-1997 - "***Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food"***
http://www.fao.org/input/download/standards/355/CXG_027e.pdf

CAC/GL 37-2001 - "***Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement"*** http://www.fao.org/input/download/standards/376/CXG_037e.pdf

CAC/GL-49-2003 - "***Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis***
http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/
CAC/GL 56-2005 – CXG 56 "***Guidelines on the use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative analysis of Residues"***
http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals" (Adopted 2009. Revision 2012, 2014
http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

ISO/IEC 17025:2017 Guideline Document: "General requirements for the competence of testing and calibration laboratories", the ***Codex Alimentarius Committee Guidance Document***
https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:en

SANTE/12682/2019. "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed"- A Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.
https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

Appendix D, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" J. Assoc. Off. Anal. Chem. 72, 694–704(1989).

Appendix F: "Guidelines for Standard Method Performance Requirements"2016 AOAC Official Methods of Analysis

M. Thompson, S.L.R. Ellison and R. Wood, 2002. "***Harmonized Guidelines for Single-Laboratory Validation Of Methods Of Analysis***" Pure Appl. Chem., 74, (5) 835 – 855.
http://publications.iupac.org/pac/2002/pdf/7405x0835.pdf

Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P., and Stein, S. (2003). "***Establishing the Fitness for Purpose of Mass Spectrometric methods."*** Journal of the American Society for Mass Spectrometry 14: 528-541.

Horwitz, W. 1995 "***Protocol for the design, conduct and interpretation of method performance studies***". Pure and Applied Chemistry 67:331-343

Thompson, M., Ellison, S., Fajgelj, A., Willetts, P., & Wood, R. (1999) "***Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement"*** Pure Applied Chemistry, 71: 337-348.