AOAC SMPR® 2020.009

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

Intended Use:

AOAC 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 AOAC using the consensus of stakeholders representing the industry, government, and academic and/or research institutions. AOAC SMPRs are used by AOAC expert review panels (ERPs) in their evaluation of validation study data for method being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠ 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 (TT) 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 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 and 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.—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.

Authentic honey.—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. Authentic samples and/or standard reference materials (SRMs), whenever available, used to validate the method will be used to properly define the TT method testing scope.

Bulk sample.—Combined total of all the primary samples taken from the same lot.

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

Economically motivated adulteration (EMA).—Fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller.

False origin.—Honeys containing mislabeled geographic and botanical sources.

Lot of honey.—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)]

Multilaboratory validation (MLV).—Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories according to guidelines described in AOAC *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" [Horwitz, W. (1995) *Pure Appl. Chem.* **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 *Harmonized Guidelines for the Use of Recovery Information in Analytical Measurement.*

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 laboratories must be recruited to provide at least eight valid data sets; two blind duplicate replicates at five concentration levels for each analyte/matrix combination to each collaborator.

$$\text{HorRat (repeatability, r)} = RSD_r/PRSD_R$$

$$\text{HorRat (reproducibility, R)} = RSD_R/PRSD_R$$

For interlaboratory 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.

Nonauthentic substance or adulterant.—Known substance or adulterant used to adulterate honey for economic gain that can be targeted for analysis.

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

Single-laboratory validation (SLV).—Demonstration by one laboratory of method performance on samples described according

to internationally accepted validation guidelines contained in guidance documents, such as AOAC *Appendix D*: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study*; ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories;* Codex Alimentarius Committee Guidance Document CAC/GL 71-2009, *Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Program Associated with the Use of Veterinary Drugs in Food Producing Animals* (Adopted 2009, Revision 2012, 2014); 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 minimum requirements for validation and fit-for-purpose criteria, the method can be reviewed and considered by AOAC for classification as First Action *Official Method of Analysis*℠.

4 Method Performance Requirements

Specific sample preparation instructions for honey [Codex Alimentarius Committee Guidance Document CAC/GL 71-2009, *Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Program Associated with the Use of Veterinary Drugs in Food Producing Animals* (Adopted 2009, Revision 2012, 2014)]

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

(b) Liquidize 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 x 0.500 mm (ISO 565-1990).

(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 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.

See Tables 1–5.

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 in this SMPR.

(c) For SLV studies, the method will be evaluated using prescribed adulterated materials as shown in Tables 1–5. Methods approved at this level will proceed to a second level of evaluation (MLV), 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 Program 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/

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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 (1989) *J. Assoc. Off. Anal. Chem.* **72**, 694–704

Appendix F: Guidelines for Standard Method Performance Requirements (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, Rockville, MD, USA, www.eoma.aoac.org

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Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P., & Stein, S. (2003) "Establishing the Fitness for Purpose of Mass Spectrometric Methods," *J. Am. Soc. Mass Spectrom*. **14**, 528–541

Horwitz, W. (1995) "Protocol for the Design, Conduct, and Interpretation of Method Performance Studies," *Pure Appl. Chem*. **67**, 331–343

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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	18

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	18

Table 3. Method performance requirements for corn and cane 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	18

Table 4. Method performance requirements for C-4 plant 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	18

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	18