**AOAC SMPR® 2020.011**

## *Standard Method Performance Requirements* (SMPRs®) for Targeted Testing (TT) of Formaline/ Formaldehyde, Starch, and Soy Protein as Adulterants for Evaluation of Liquid Raw Bovine Milk

### Intended Use:

AOAC SMPRs® (Appendix F: "Guidelines for *Standard Method Performance Requirements*") 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 (SLV), or a multi-site collaborative study. SMPRs are written and adopted by AOAC using the consensus of stakeholders representing industry, government, 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*SM or AOAC *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 (TT) methods to monitor liquid raw bovine milk for the detected presence of economically motivated adulterants (EMAs), such as formalin/formaldehyde, starch, and soy protein.

#### *2 Analytical Technique*

A targeted method to be used to detect, identify, and quantify liquid raw bovine milk for the detected presence of EMAs, such as formalin/formaldehyde, starch, and soy protein. Formaldehyde has been used in certain regions of the world as a disinfectant/ preservative to increase the shelf life of milk, while starch and soy protein are added intentionally to increase viscosity and protein content, respectively.

Any method capable of detecting and identifying the presence of the defined adulterants and can be used for quantifying the amount (proportion/concentration) present in raw bovine milk will be considered.

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

#### *3 Definitions*

*Applicability statement.*—Method must be applicable to the analysis of formaline/formaldehyde, starch, and soy protein as adulterants in raw bovine liquid milk.

*Authentic samples.*—Samples representative of the genuine commodity. These samples should represent the food's or ingredient's variability seen naturally in the commodity. The authentic samples and or reference standard materials used to validate the method will be used to properly define the TT method testing scope.

*Control milk.*—Milk from animals not treated with veterinary drugs of the same species, sex, age, and physiological status as the target species.

*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.

*Guidance on sample type and minimum quantity.*—Of whole liquid milk raw, pasteurized, UHT, and sterilized milk classified as Group 033 in the Codex document [*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)] required for laboratory samples: Mix thoroughly and immediately take a sample by means of a dipper. In retail containers, take sufficient units to meet laboratory sample size requirements.

*Incurred milk*—Milk containing residues of an analyte arising by the route through which trace concentrations would normally be expected by treatment or dosing according to intended use, as opposed to residues from laboratory fortification of samples.

*Milk.*—Normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it; intended for consumption as liquid milk or for further processing. [*Codex Alimentarius Procedural Manual*]

*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 by AOAC Appendix D: "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis" to be considered for classification as AOAC Final Action Method; "Protocol for the design, conduct and interpretation of method performance studies;" CAC/GL 27-1997: "Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food;" and CAC/GL 37-2001: "Harmonized IUPAC 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, 10–2 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 0.5–2.

*For within-laboratory studies*.—Acceptable HorRat (r) of 0.3–1.3.

*Single-laboratory validation (SLV).*—Demonstration by one laboratory of method performance on samples described according to internationally accepted validation guidelines described in guidance documents, such as AOAC's Appendix D: "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis;" ISO/IEC 17025:2017 Guideline Document: "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); CAC/GL-49-2003: "Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis"; CAC/GL 56-2005: "Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation, and Quantitative Analysis of Residues;" SANTE/12682/2019: "Establishing the Fitness for Purpose of Mass Spectrometric Methods;" "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 AOAC for classification as First Action *Official Method of Analysis*$^{SM}$.

*Spiked or fortified milk.*—Milk containing known concentrations of an analyte added to a sample of control milk.

### *4 Method Performance Requirements*

*See* Tables 1–3.

### *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 SLV studies, the method will be evaluated using prescribed adulterated materials as shown in Tables 1–3. 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

**Table 1. Method performance requirements for formaline/formaldehyde in raw bovine liquid milk**

| Analytical parameter | Acceptance criteria |
|---|---|
| Analytical range, mg/L (ppm) | 0.01–0.40 |
| LOQ, ppm | ≤0.01 |
| Recovery, % | 80–120 |
| Accuracy, % | ±20 |
| Precision (repeatability) $RSD_r$ | 21 |
| Precision (reproducibility) $RSD_R$ | 30 |

**Table 2. Method performance requirements for starch in raw bovine milk**

| Analytical parameter | Acceptance criteria |
|---|---|
| Analytical range, mg/L (ppm) | 10–50 |
| LOQ, ppm, | ≤10 |
| Recovery, % | 80–120 |
| Accuracy, % | ±20 |
| Precision (repeatability) $RSD_r$ | 11 |
| Precision (reproducibility) $RSD_R$ | 20 |

**Table 3. Method performance requirements for soy protein in raw bovine liquid milk**

| Analytical parameter | Acceptance criteria |
|---|---|
| Analytical range, mg/L (ppm) | 10–50 |
| LOQ, ppm, | ≤10 |
| Recovery, % | 80–120 |
| Accuracy, % | ±20 |
| Precision (repeatability) $RSD_r$ | 11 |
| Precision (reproducibility) $RSD_R$ | 20 |

### *References*


*Procedural Manual of the Codex Alimentarius Commission* (2018) 26th Ed., Rome, Italy, https://thenhf.com/wp-content/uploads/2018/10/Codex-Procedural-Manual-26th-edition.pdf

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/

ISO/IEC 17025:2017 Guidance Document: "General requirements for the competence of testing and calibration laboratories" (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 Analysis" (1989) *J. Assoc. Off. Anal. Chem*. **72**, 694–704, https://doi.org/10.1093/jaoac/72.4.694

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

Thompson, M., Ellison, S.L.R., & Wood, R. (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

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

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*Developed by the AOAC Food Authenticity Methods (FAM) Working Group on Targeted Testing. Approved by stakeholders of AOAC FAM on August 5, 2020.*

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