AOAC SMPR 2020.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Targeted Testing (TT) of formaline/formaldehyde, starch, soy protein as Adulterants for Evaluation of Liquid Raw Bovine Milk; Version 4; April 21, 2020

Intended Use

AOACI 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, 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 MethodsSM* or AOACI *Official Methods of AnalysisSM* 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 the viscosity and protein content respectively.

Any method capable of detecting, 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 — 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.

Authentic Samples — Samples representative of the genuine commodity. ~~Ideally~~ 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.

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.

Milk — is the 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.]***

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

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

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

Guidance on Sample Type and Minimum Quantity — of Whole liquid milk raw, pasteurised, UHT & 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 Programme 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.

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 Study", ***ISO/IEC 17025:2017 Guideline 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); and, the "***Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis CAC/GL-49-2003***; "***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." 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 (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 the AOACI ***Appendix D***, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study," to be considered for classification as AOAC Final Action Method ; "***Protocol for the design, conduct and interpretation of method performance studies***". "***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

(Table 1: Method Performance Requirements for formaline/formaldehyde in raw bovine milk)

Analytical Parameter	Acceptance Criteria
Analytical Range (mg/L (ppm))	0.01-0.40
LOQ (%)	≥ 0.01
Recovery %	80-120
Accuracy %	±20
Precision (Repeatability) RSD_r	21
Precision (Reproducibility) RSD_R	21

(Table 2: Method Performance Requirements for starch in raw bovine milk)

Analytical Parameter	Acceptance Criteria
Analytical Range (mg/L (ppm))	1.0-10
LOQ (%)	≥ 1
Recovery %	80-120
Accuracy %	±20
Precision (Repeatability) RSD_r	11
Precision (Reproducibility) RSD_R	11

(Table 3: Method Performance Requirements for soy protein in raw bovine milk)

Analytical Parameter	Acceptance Criteria
Analytical Range (mg/L (ppm))	1.0-5
LOQ (%)	≥ 1
Recovery %	80-120
Accuracy %	±20
Precision (Repeatability) RSD_r	11
Precision (Reproducibility) RSD_R	11

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 – 3 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:

Procedural Manual of the **Codex Alimentarius Commission** 26th edition. 2018. Place of publication: 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 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.