AOAC SMPR® 2024.006

Standard Method Performance Requirements (SMPRs®) for Determination of Milk Fat Globule Membrane Proteins in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

Purpose:

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*SM and AOAC *Performance Tested Methods*SM programs. AOAC SMPRs also may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Quantify all, or as many as possible, bovine milk fat globule membrane (MFGM) proteins described in Table 1. Quantify additional bovine MFGM proteins using the same method when possible. The method must distinguish intact MFGM proteins from their hydrolyzed forms.

The minimum requirement as a target matrix for validating these bovine proteins is infant, adult, and/or pediatric formulas. In addition to these matrices, the method may also cover other milk and milk products (Table 2).

2 Analytical Technique

Any analytical technique(s) that measures the analyte(s) of interest and meets the following method performance requirements is/are acceptable.

3 Definitions

Adult/pediatric formula.—Nutritionally complete, specially formulated food consumed in liquid form, which may constitute the sole source of nourishment. It is made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula.—Breast-milk substitutes specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72–1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of quantitation (LOQ).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Milk fat globule membrane (MFGM) proteins.—For this SMPR, seven MFGM proteins were selected (Table 1). The method result does not represent total MFGM ingredient or total MFGM protein content. For molecular weight of the protein, only the mature protein weight should be used as listed in Table 1 (excluding signal peptide or initiator methionine where applicable and excluding post-translational modifications).

Milk and milk products.—Milk is defined as the normal mammary secretion of a milk animal, intended for consumption as liquid milk or further processing. Milk product is defined as a product obtained by any processing of milk. Although a milk product shall be made from milk, the definition does not hinder the milk from being subjected to various processing steps before it becomes an intermediate- or end-product [Bulletin of IDF 397 (2005) *Codex General Standard for the Use of Dairy Terms, Its Nature, Intent and Implications*].

Recovery.—Fraction or percentage of spiked analyte that is recovered when test sample is analyzed using entire method.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short period. Expressed as repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Table 1. Target analytes (seven milk fat globule membrane proteins)

Protein	UniProtKB-reviewed (Swiss-Prot) accession Nos.[a]	Amino acids chain	MW of protein chain, average mass/monoisotopic mass (Da)
Perilipin-2	PLIN2, Q9TUM6	2-450	49236.88/49206.29
Butyrophilin subfamily 1 member A1	BTN1A1, P18892	27-526	56474.23/56438.22
Mucin-1 (may be cleaved into its two subunits)	MUC1, Q8WML4	23-580	55709.88/55676.03
Xanthine dehydrogenase/oxidase	XDH, P80457	2-1332	146659.28/146565.29
Lactadherin	MFGE8, Q95114	19-427	45531.51/45502.10
Fatty acid-binding protein, heart	FABP3, P10790	2-133	14647.71/14638.56
Glycosylation-dependent cell adhesion molecule 1	GLYCAM1, P80195	19-153	15304.25/15294.96

[a] UniProtKB (www.uniprot.org) reviewed (Swiss-Prot) accession numbers listed for the seven selected *Bos taurus* (bovine) MFGM proteins should be included in the validation report. If additional proteins are validated, similar information must be included.

Table 2. Target matrices

Matrix category	Matrix subcategory	Representative matrices
	Minimum required target matrix	
Infant/adult formulas	Bovine milk-based	Powders
		Ready-to-feed liquids
		Liquid concentrates
	Bovine milk-based, MFGM-enriched	Powders
		Ready-to-feed liquids
		Liquid concentrates
	Additional matrices that may be included	
Infant/adult formula ingredients	Bovine milk-based, MFGM-enriched	Whey-based ingredient
		Cream-based ingredient
	Bovine milk-based	Whey protein concentrate
		Whey protein isolate
		Cream
Bovine dairy products	Milk	Raw milk
		Milk with varying fat content (e.g., skimmed, full-fat)
		Buttermilk
	Cream	
	Cheese	Soft cheese
		Hard cheese
	Yogurt	
	Quark	

[a] Number of matrices must be at least one for each representative matrix. Matrix sources should cover expected concentrations of analyte(s) of interest. If only a single matrix is studied, then ≥3 sources are recommended, preferably with different attributes (e.g., maturity, varieties, age).

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratories by different operators on different instruments. Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

4 Method Performance Requirements

See Table 3.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples and check standards at the lowest point and midrange point of the analytical range.

6 Reference Material(s)

NIST Standard Reference Material® (SRM) 1849b Infant/Adult Nutritional Formula I (milk-based) should be included as a matrix blank to enable method comparison. Also, NIST SRM 1869 Infant/Adult Nutritional Formula II (milk-/whey-/soy-based) must be included to determine innate levels in such matrix. However, levels of proteins of interest are not certified for these materials. If various matrices with different physical and chemical properties are included in the validation (Table 2), then a CRM of each matrix type shall be included. Use of a CRM, when available, is recommended to assess method accuracy as bias. The CRM should be accompanied by documentation (certificate) issued by an authoritative body.

Table 3. Method performance requirements[a]

	PLIN2, MUC1, FABP3		MFGE8		GLYCAM1, BTN1A1, XDH	
Limit of quantitation (LOQ)[b]	0.1		0.4		1	
Analytical range[b]	0.1–50	>50–500	0.4–200	>200–2000	1–500	>500–5000
Recovery, %	80–120	85–115	80–120	85–115	80–120	85–115
Repeatability (RSD_r), %	10	7	10	7	10	7
Reproducibility (RSD_R), %	20	14	20	14	20	14

[a] For proteins with low overall concentration, higher end values of proposed range may not be achieved.
[b] mg/100 g reconstituted final product. Concentrations apply to (*1*) milk and "ready-to-feed" liquid formula "as is;" (*2*) reconstituted powders (25 g into 200 g water); (*3*) liquid concentrates diluted 1:1 by weight; and (*4*) other milk products as mg/100 g.

Any reference standard used must be accompanied by a certificate of analysis, stating supplier, identity, batch number, purity, and basis for the purity statement in the SLV report. Purity of reference standards used (including moisture levels if relevant) should be established, understood, and fit for purpose. If noncommercial reference standard is used, its origin must be identified along with all pertinent information demonstrating purity and/or analyte concentration and how these were determined.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*℠ and "Appendix L: AOAC Recommended Guidelines for Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation" [*Official Methods of Analysis of AOAC INTERNATIONAL* (2023) 22nd Ed., G.W. Latimer, Jr. (Ed), Oxford University Press, New York, NY, USA; https://doi.org/10.1093/9780197610145.005.012 (accessed January 2023)]

Method must distinguish intact MFGM proteins from their hydrolyzed forms. When LC-MS/MS is considered, method should target conserved regions of protein that are not susceptible to post-translational modifications and are unique to bovine species (*Bos taurus)*.

For molecular weight of protein, only the mature protein weight should be used (excluding signal peptide or initiator methionine where applicable and excluding post-translational modifications). Method should provide UniProtKB-reviewed (Swiss-Prot) accession numbers for correct protein identification when additional bovine MFGM proteins are included in the validation.

8 Maximum Time-to-Result

No maximum time.

Developed by AOAC Working Group on Milk Fat Globule Membrane Protein. Approved by AOAC Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) on October 25, 2024. Final version date and version number: May 6, 2024, Version 3. Effective date: January 6, 2025.