Standard Method Performance Requirements (SMPRs®) for Determination of 3′-Galactosyllactose, 4′-Galactosyllactose, and 6′-Galactosyllactose in Galactooligosaccharides Ingredients and 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 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*℠ and AOAC *Performance Tested Methods*℠ 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

Determination of 3′-galactosyllactose (3′-GL), 4′-galactosyllactose (4′-GL), and 6′-galactosyllactose (6′-GL) individually (Table 1) in galactooligosaccharides (GOS) ingredients and all forms of infant and adult/pediatric formulas as outlined in Table 2. Methods capable of analyzing all three analytes are preferred over those that target single components.

2 Analytical Technique

Any analytical technique that meets method performance requirements is acceptable.

3 Definitions

Accuracy.—Closeness of agreement between the average of an infinite number of replicates measured quantity values and a reference quantity value (corresponds to the VIM definition for "trueness").

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein. [*Official Methods of Analysis of AOAC INTERNATIONAL* (2023) "Appendix L: AOAC Recommended Guidelines for Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation." DOI: https://doi.org/10.1093/9780197610145.005.012]

Drink [product] for young children.—With added nutrients or product for young children with added nutrients or drink for young children or product for young children is a product manufactured for use as a liquid part of the diversified diet of young children. The term "young child" means a person from the age of more than 12 months up to the age of three years (36 months). (Codex Standard for Follow-up Formula for Older Infants and Product for Young Children CXS 156-1987 Rev. 2023)

Follow-up formula.—A product, manufactured for use as a breast milk substitute, as a liquid part of a diet for older infants when progressively diversified complementary feeding is introduced. The term "older infants" means a person from the age of 6 months and not more than 12 months of age. Essential composition: Based on milk of cows or other animals or a mixture thereof and/or other ingredients, which have been proven to be safe and suitable for the feeding of older infants. The nutritional safety and adequacy of follow-up formula shall be scientifically demonstrated to support growth and development of older infants. Due to the similarity of compositions, follow-up formulas can be included in the general infant formula matrix category for method validation and method applicability purposes. Therefore, methods applicable to infant formula are also applicable to follow-up formula. [*Official Methods of Analysis of AOAC INTERNATIONAL* (2023) "Appendix L: AOAC Recommended Guidelines for Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation." DOI: https://doi.org/10.1093/9780197610145.005.012]

Foods for special medical purposes (FSMP).—Category of foods for special dietary uses that are specially processed or formulated and presented for the dietary management of patients and may be used only under medical supervision. They are intended for the exclusive or partial feeding of patients with limited or impaired capacity to take, digest, absorb, or metabolize ordinary foodstuffs or certain nutrients contained therein, or who have other special medically-determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, by other foods for special dietary uses, or by a combination of the two. (Codex Standard for Labeling of and Claims for Foods for Special Medical Purposes CXS 180-1991)

Galactooligosaccharides (GOS).—Also known as oligogalactosyllactose, oligogalactose, or transgalactooligosaccharides (TOS) produced by transgalactosylation of lactose.

Table 1. Target analytes

Analyte (abbreviation)	IUPAC name	CAS No.
3′-Galactosyllactose (3′-GL)	β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose	32694-82-91
4′-Galactosyllactose (4′-GL)	β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-D-glucopyranose	6587-31-1
6′-Galactosyllactose (6′-GL)	β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-D-glucopyranose	32581-31-0

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Table 2. Target matrices

Mandatory matrix category	Representative matrix subcategory
GOS ingredient	Syrup
	Powder
Milk-based infant and adult/ pediatric nutritional formula products (powder, liquid, liquid concentrates) of bovine origin	Adult nutritionals
	FSMPs
	Follow-up formula
	Infant formula [including hypoallergenic formula (HA)]
	Drink [product] for young children
Optional matrix category	**Representative matrix subcategory**
Milk-based infant and adult/ pediatric nutritional formula products (powder, liquid, liquid concentrates) of caprine origin	Adult nutritionals
	FSMPs
	Follow-up formula
	Infant formula (including HA)
	Drink [product] for young children
Other finished products	Supplements
	Pet food
	Medical care products (e.g., electrolyte solution)

Infant formula.—Breastmilk substitute 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. The term "infant" means a person not more than 12 months of age. (Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants CXS 72-1981 amd. 2023)

Limit of quantitation (LOQ).—Lowest concentration of analytes that can be measured with a 95% level of confidence.

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 time. Expressed as repeatability standard deviation (SD_r) or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—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, mid-range, and highest points of the analytical range.

6 Reference Material(s)

No certified reference materials containing the analyte of interest are currently available.

7 Validation Guidance

Validation must be conducted on each of the representative matrix subcategories under the mandatory matrices category specified in Table 2. Method authors are encouraged to complement their validation with additional representative matrices.

Recommended level of validation: *Official Methods of Analysis*^{SM}:

Single-laboratory validation studies are described in *Official Methods of Analysis of AOAC INTERNATIONAL* (2023) "Appendix L: AOAC Recommended Guidelines for Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation." DOI: https://doi.org/10.1093/9780197610145.005.012

Multi-laboratory validation studies are described in *Official Methods of Analysis of AOAC INTERNATIONAL* (2023) "Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis." DOI: https://doi.org/10.1093/9780197610145.005.004

Table 3. Method performance requirements[a]

	GOS ingredients[b]	Finished products[c]
Analytical range, mg/100 g	10–2500[d]	0.3–500
Limit of quantitation (LOQ), mg/100 g	≤10	≤0.3
Recovery, %	85–110 (<20 mg/100 g) 90–110 (20–100 mg/100 g) 95–105 (>100 mg/100 g)	85–110 (<20 mg/100 g) 90–110 (≥20 mg/100 g)
Repeatability (RSD_r), %	≤4	≤6
Reproducibility (RSD_R), %	≤8	≤10

[a] Method performance requirements are related to each analyte individually.

[b] Concentrations apply to syrups and powders "as-is."

[c] Concentrations for infant formula and adult nutritionals apply to: (*1*) "ready-to-feed" liquids "as-is"; (*2*) reconstituted powders (25 g into 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight. Other finished products (from Table 2) may exceed analytical range but need to meet requirements for recovery, repeatability (RSD_r), and reproducibility (RSD_R).

[d] For analytes with low overall concentration, products at the higher end values of proposed range may not be available.

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Validation studies of the analytical method should provide sufficient evidence to demonstrate method specificity with the following potential interferences:

(a) Potential interferences include other nontargeted mono-, di-, and oligosaccharides and their derivatives that may be present in these matrices and co-elute with the analytes, thereby affecting quantification. Particular attention should be paid to other oligosaccharides, especially other GOS components.

(b) Probiotic activity that may be present in the product that can influence the concentration of the analyte of interest.

8 Maximum Time-to-Result

None.

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