AOAC SMPR® 2021.006

Standard Method Performance Requirements (SMPRs®) for Determination of Difucosyllactose (DFL) in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

1 Purpose

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 drafted by AOAC working groups composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. Approved by AOAC, AOAC SMPRs may be used for method development, are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠ and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Determination of free difucosyllactose (DFL) in all forms of infant and adult/pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates). The analytical method should account for potential interferences in these matrices (*see* section 8 *Validation Guidance* for potential interferences).

3 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

4 Definitions

Accuracy.—Closeness of agreement between the average of an infinite number of replicate 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.

Difucosyllactose (DFL).—α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl--(1→4)-[α-L-fucopyranosyl-(1→3)]-D-glucopyranose; lactodifucotetraose (LDFT); 2′,3-difucosyllactose (CAS No. 20768-11-0).

Infant formula.—Breast-milk 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 (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.

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 period. Expressed as the 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 the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

Table 1. Method performance requirements[a]

Analytical range	1.5–100 mg/100 g
Limit of quantitation (LOQ)	≤1.2 mg/100 g
Recovery	85–110% (1.5–20 mg/100 g)
	90–110% (>20 mg/100 g)
Repeatability (RSD_r)	≤5%
Reproducibility (RSD_R)	≤10%

[a] Concentrations apply to: (*1*) "ready-to-feed' liquids "as is"; (*2*) reconstituted powders (25 ginto 200 g water); and (*3*) liquid concentrates diluted 1:1 by weight.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

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

7 Reference Material(s)

No certified reference materials of infant and adult/pediatric nutritional formula containing the analyte of interest are currently available.

8 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*℠

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

Appendix L: *AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www. eoma.aoac.org/app_l.pdf).

List of interferences.—Validation studies should include challenging the method with the following potential interferences:

(**a**) Other nontargeted, mono-, di-, and oligosaccharides and/or derivatives that may be formed.

(**b**) Probiotic activity that may influence the concentration of the analyte of interest.

9 Maximum Time-to-Result

No maximum time.

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