AOAC SMPR® 2020.004

Standard Method Performance Requirements (SMPRs®) for Determination of Lacto-*N*-Neotetraose (L*N*nT) in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

1 Applicability

Quantitative determination of free lacto-*N*-neotetraose (L*N*nT) in all forms of infant, adult, and/or pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates. Analytical method should account for potential interferences in these matrices (list of interferences to consider at end of document).

2 Analytical Technique

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

3 Definitions

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

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.

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.

Lacto-N-neotetraose (LNnT).—β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose (CAS No. 13007-32-4).

Limit of detection (LOD).—Minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

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 ($\%RSD_r$).

Table 1. Method performance requirements[a]

Analytical range	5–100 mg/100 g
Limit of quantitation (LOQ)	≤4 mg/100 g
Recovery, %	85–110 (5–20 mg/100 g)
	90–110 (>20 mg/100 g)
Repeatability ($\%RSD_r$)	≤5 (5–100 mg/100 g)
Reproducibility ($\%RSD_R$)	≤10 (5–100 mg/100 g)

[a] Concentrations 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.

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 ($\%RSD_R$).

4 Method Performance Requirements

See Table 1.

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)

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

7 Validation Guidance

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

8 Maximum Time-to-Result

No maximum time.

9 Potential Interferants

(*1*) Other nontargeted, mono-, di-, and oligosaccharides and/or derivatives that may be formed as side products during production or intentionally added.

(*2*) Probiotic activity that may influence the concentration of the target analyte.

Approved by AOAC Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) on April 27, 2020.

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