AOAC SMPR 2020.XXX; Version 3.1, August 5, 2020

Method Name: **Determination of lacto-*N*-tetraose (LNT) in Infant and Adult/ Pediatric Nutritional Formula**

Approved by:
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. Applicability:

A quantitative determination of free lacto-*N*-tetraose (LNT) in all forms of infant, and adult, and/or pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates). The 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[1]

The 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[2], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)

The 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)

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

[1] Corresponds to the VIM definition for “trueness”.
[2] Codex Standard 72 – 1981.

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
The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

Lacto-*N*-tetraose (LNT)
β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose. CAS number: 14116-68-8

4. Method Performance Requirements:
See table 1

Table 1: Method Performance Requirements[a]	
Analytical range	2 – 300 mg/100g
Limit of Quantitation (LOQ)	≤ 1.6 mg/100g
Recovery	85-110% (2-20 mg/100g)
	90-110% (>20 mg/100g)
Repeatability (% RSD_r)	≤ 5%
Reproducibility (% RSD_R)	≤ 10%
[a] Concentrations apply to: i) "ready-to-feed' liquids "as is"; ii) reconstituted powders (25 g into 200 g of water); and iii) liquid concentrates diluted 1:1 by weight.	

5. System suitability tests and/or analytical quality control:
Suitable methods will include blank check samples, and check standards at the lowest, midrange, and highest 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.

List of potential interferants:

- Other non-targeted, mono-, di-, and oligosaccharides and/or derivatives that may be formed as side products during production or intentionally added.
- Probiotic activity that may influence the concentration of the analyte of interest.