AOAC SMPR 2021.XXX; Version 3; January 19, 2022

Method Name: **Determination of fatty acids which are esterified at the *sn*-2 position (beta) of the triacylglycerol molecules in Infant and Adult/ Pediatric Nutritional Formula**

Approved by: Stakeholder Program for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for global dispute resolution.

1. Applicability:

Quantitation of the fatty acids which are esterified at the *sn*-2 position (beta) of the triacylglycerol molecules in all forms of infant formula and adult/pediatric nutritionals.
The method is applicable to infant formula and adult/pediatric nutritionals containing C4-C24 saturated and unsaturated fatty acids.

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 replicates measured quantity values and a reference quantity value.

Adult/Pediatric Formula

Nutritionally complete, especially 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 lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level.

Limit of Quantitation (LOQ)

The lower limit of quantitation below which the concentration of the analyte does not meet the SMPR requirements for bias (recovery) or repeatability.

Repeatability

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

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

Reproducibility

Variation arising when identical test materials are analyzed in different laboratory by different operators on different instruments. The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SDR); or %reproducibility relative standard deviation (%RSDR).

Name of Analyte

C4-C24 saturated and unsaturated fatty acids esterified at the *sn*-2 position of triacylglycerol.

Expression of results

Expression of results is varying per country. Legislation exists where results need to be expressed in mg of fatty acid in *sn*-2 position/100g of powdered product.

Alternatively, the results may need to be expressed as % of fatty acid in *sn*-2 position on total amount of that fatty acid. Total fatty acid profile in the product is needed for this expression of results (see paragraph 7 for guidance).

For both ways of expressing the results the values in the table can be recalculated to either g of fatty acid in *sn*-2 position/100g of powdered product OR to % of fatty acid in *sn*-2 position of that fatty acid in total.

4. Method Performance Requirements:

Table 1: Method performance requirements[a]		
Analytical range (g/100g)	0.003-2[b]	
Limit of Quantitation (LOQ) (g/100g)	≤0.003[b]	
Recovery	0.003-0,1[b]	85-115%
	> 0.1-2[b]	90-110%
Repeatability (RSD_r)	0.003-0.03 [b]	≤20%
	0.03-0.1[b]	≤15%
	> 0.1-2[b]	≤7%
Reproducibility (RSD_R)	0.003-0.03 [b]	≤40%
	0.03-0.1[b]	≤30%
	> 0.1-2[b]	≤15%
[a] *Concentrations apply to:* *a) "ready-to-feed" liquids "as is";* *b) re-constituted powders (25 g into 200 g of water);* *c) liquid concentrates diluted 1:1 by weight.* *1 mg/100g RFP ≈ 1mg/100g dry weight x 25 mL/(25+200 mL)*		
[b] g of fatty acid in *sn*-2 position/100g reconstituted product		

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. **Test Material(s):**
No infant formula certified reference materials available. To allow method comparison it is suggested to include NIST 1849b 1869, and/or 1549a.
Recovery should be demonstrated using model triacylglycerol (TAG) such as TAGs with palmitic acid or oleic acid on the *sn*-2 position.

7. **Standard Material(s):**
As part of validation studies, the source of triacylglycerol standard needs to be provided and its composition specified to address how the standard relates to forms measured in samples.

8. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.
The total fatty acid profile that is needed to calculate % of fatty acid in *sn*-2 position on total amount of that fatty acid may be determined by ISO method 16958, AOAC 2012.13, IDF 231 or similar.

9. **Maximum Time-To-Result**: No maximum time.