

**Standard Method Performance Requirements (SMPRs®) for Determination of Fatty Acids Which Are Esterified at the *sn*-2 Position (Beta) of the Triacylglycerol Molecules in Infant and Adult/ Pediatric Nutritional Formula**

Intended Use: Reference Method for Global Dispute Resolution

**1 Purpose**

AOAC SMPRs are consensus standards developed in accordance with AOAC policy, AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents. 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, a multi-site collaborative study, or another AOAC approved study design for method characterization and validation. SMPRs are written and adopted by AOAC through its stakeholder-based integrated science programs and projects, which are composed of representatives and experts from the academic, government, industry, and nonprofit sectors. AOAC SMPRs may be used to develop validation studies along with validation guidance to validate and optimized methods. They are also used by AOAC method review experts, including expert review panels, in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods*<sup>SM</sup>, *Reviewed and Recognized*<sup>SM</sup>, or AOAC *Official Methods of Analysis*<sup>SM</sup>, and can be used as acceptance criteria for verification at user laboratories.

**2 Applicability**

Quantitation of the fatty acids which are esterified at the *sn*-2 position (beta) of the triacylglycerol (TAG) 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.

**3 Analytical Technique**

Any analytical technique that meets the method performance requirements in this document is acceptable.

**4 Definitions**

*Accuracy (corresponds to the VIM definition for “trueness”).*—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, 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.

*Expression of results.*—Varies per country. Legislation exists where results must be expressed in mg fatty acid in *sn*-2 position/100 g powdered product. Alternatively, results may need to be expressed as % 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 below for guidance).

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

*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 detection (LOD).*—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).*—Lower LOQ below which the concentration of the analyte does not meet the SMPR for bias (recovery) or repeatability.

*Name of analyte.*—C4–C24 saturated and unsaturated fatty acids esterified at the *sn*-2 position of TAG.

*Repeatability.*—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<sub>r</sub>); or % repeatability relative standard deviation (%RSD<sub>r</sub>).

*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 (SD<sub>R</sub>); or % reproducibility relative standard deviation (%RSD<sub>R</sub>).

**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 point and midrange point of the analytical range.

**7 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 TAG, such as TAGs with palmitic acid or oleic acid on the *sn*-2 position.

**Table 1. Method performance requirements<sup>a</sup>**

Analytical range	0.003–2 <sup>b</sup>		
Limit of quantitation (LOQ), g/100 g	≤0.003 <sup>b</sup>		
Concentrations	0.003–0.1 <sup>b</sup>	0.03–0.1 <sup>b</sup>	>0.1–2 <sup>b</sup>
Recovery, %	85–115	—	90–110
Repeatability (RSD <sub>r</sub> ), %	≤20	≤15	≤7
Reproducibility (RSD <sub>R</sub> ), %	≤40	≤30	≤15

<sup>a</sup> Concentrations apply to: (a) “ready-to-feed” liquids “as is”; (b) reconstituted powders (25 g into 200 g water); (c) liquid concentrates diluted 1:1 by weight. 1 mg/100 g RFP ≈ 1 mg/100 g dry weight × 25 mL / (25 + 200 mL).

<sup>b</sup> g fatty acid in *sn*-2 position/100 g reconstituted product.

## **8 Standard Material(s)**

As part of validation studies, the source of TAG standard needs to be provided and its composition specified to address how the standard relates to forms measured in samples.

## **9 Validation Guidance**

Recommended level of validation: *Official Methods of Analysis*<sup>SM</sup>.

Total fatty acid profile that is needed to calculate % 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.

*Official Methods of Analysis of AOAC INTERNATIONAL*, “Appendix L: AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation.” Available at [http://www.eoma.aoac.org/app\\_1.pdf](http://www.eoma.aoac.org/app_1.pdf).

See other appendices of the *Official Methods of Analysis* for additional validation guidelines.

## **10 Maximum Time-To-Result**

No maximum time.

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