

Standard Method Performance Requirements for Fatty Acids, Including LCPUFAs, in Infant Formula and Adult/Pediatric Nutritional Formula

Intended Use: Global dispute resolution method

1 Applicability

Determination of individual fatty acids, including long-chain, saturated, mono-, poly-unsaturated, *trans* fatty acids, C4–C24, in all forms of infant/adult/pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2 Analytical Technique

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

3 Definitions

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed and/or intact protein, starch, and amino acids.

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 and/or intact protein, starch, and amino acids.

Limit of quantitation (LOQ).—The 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.—The 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).

Recovery.—The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

Analytical range	≤0.001–8.00 ^b	
Limit of quantitation (LOQ)	≤0.001 ^b	
Repeatability (RSD _r)	≥3.0 ^b	≤2
	<3.0 and ≥0.5 ^b	≤4.0
	<0.5 ^b	≤7.0
Recovery	90 to 110% for labeled fatty acids 80 to 110% of mean spiked recovery over the range of the assay	
Reproducibility (RSD _R)	>3.0 ^b	≤4
	<3.0 and ≥0.5 ^b	≤8
	<0.5 ^b	≤15
^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.		
^b Grams of fatty acids/100 g reconstituted final product.		

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)

NIST Standard Reference Material® (SRM) 1849a Infant/Adult Nutritional Formula, or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849a consists of 10 packets, each containing approximately 10 g material. See Annex A for the certified values of fatty acids in NIST SRM 1849a.

7 Validation Guidance

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

8 Maximum Time-to-Result

Time to determine must be 12 h or less.

Approved by the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) on September 29, 2012. Final Version Date: September 29, 2012.

ANNEX A

Table A1. Certified mass fraction values for fatty acids as free fatty acids and cholesterol in SRM 1849a

	Mass fraction, %	Coverage factor (<i>k</i>)
Octanoic acid (C8:0) ^{a-c} (caprylic acid)	0.588 ± 0.049	2.00
Dodecanoic acid (C12:0) ^{a,b} (lauric acid)	2.877 ± 0.092	2.00
Tetradecanoic acid (C14:0) ^{a,b} (myristic acid)	0.968 ± 0.032	2.00
(Z)-9-Tetradecenoic Acid (C14:1) ^{a,b} (myristoleic acid)	0.0023 ± 0.0001	2.00
Hexadecanoic acid (C16:0) ^{a-c} (palmitic acid)	1.99 ± 0.22	2.00
(Z)-9-Hexadecenoic acid (C16:1 n-7) ^{a-c} (palmitoleic acid)	0.0221 ± 0.0014	2.00
Octadecanoic acid (C18:0) ^{a-c} (stearic acid)	0.835 ± 0.017	2.00
(Z)-9-Octadecenoic acid (C18:1 n-9) ^{b,c} (oleic acid)	10.2 ± 1.1	2.00
(Z)-11-Octadecenoic acid (C18:1 n-7) ^{a-c} (vaccenic acid)	0.2034 ± 0.0068	2.00
(Z,Z)-9,12-Octadecadienoic acid (C18:2 n-6) ^{a-c} (linoleic acid)	5.24 ± 0.42	2.00
(Z,Z,Z)-9,12,15-Octadecatrienoic acid (C18:3 n-3) ^{a-c} (α-linolenic acid)	0.516 ± 0.050	2.00
Eicosanoic acid (C20:0) ^{a-c} (arachidic acid)	0.0808 ± 0.0026	2.00
(Z)-11-Eicosenoic acid (C20:1 n-9) ^{a-c} (gondoic acid)	0.0589 ± 0.0037	2.00
Docosanoic acid (C22:0) ^{a-c} (behenic acid)	0.0642 ± 0.0024	2.00
Tetracosanoic acid (C24:0) ^{a-c} (lignoceric acid)	0.0333 ± 0.0016	2.00
(Z)-15-Tetracosenoic acid (C24:1 n-9) ^{b,c} (nervonic acid)	0.0207 ± 0.0015	2.00
Cholesterol ^d	0.1374 ± 0.0029 mg/g	2.00

^a NIST GC/MS.

^b NIST GC-FID.

^c Collaborating laboratories.

^d NIST ID GC/MS.