

## Standard Method Performance Requirements (SMPRs®) for Determination of Phospholipids in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

### 1 Purpose

AOAC 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, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholders composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are 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

Quantitative determination of nutritionally relevant total and individual classes of phospholipids (PL), including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (SM), in infant formula and adult nutritionals.

### 3 Analytical Technique

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

### 4 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, 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.

*Limit of quantitation (LOQ).*—Minimum concentration or mass of analyte in each matrix that can be reported as a quantitative result.

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

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

*Total phospholipids.*—For the purposes of this SMPR, total phospholipids for nutritional purposes are calculated as the sum of PC, PE, PI, PS, SM.

### 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 Reference Material(s)

No infant formula certified reference materials available.

### 8 Standard Material(s)

As part of validation studies, the source of PL 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>.

### 10 Maximum Time-to-Result

No maximum time.

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**Table 1. Method performance requirements**

	PC	PE	PI	PS	SM	Total
Analytical range <sup>a</sup> , mg/100g RFP	0.4–50	0.4–44	0.4–44	0.4–44	0.4–44	0.4–226
Limit of quantitation <sup>a</sup> (LOQ)	0.4	0.4	0.4	0.4	0.4	NA
Recovery, %	90–110	90–110	85–115	85–115	90–110	90–110
Repeatability (RSD <sub>r</sub> , %)	7	7	7	7	7	7
Reproducibility (RSD <sub>R</sub> , %)	14	14	14	14	14	14

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