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Method Name: **Determination of Phospholipids 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 dispute resolution.

1. 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.

2. Analytical Technique:

Any analytical technique that meets the following Method Performance Requirements is acceptable.

3. Definitions:

Total Phospholipids

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

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, 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.

[1] Corresponds to the VIM definition for “trueness”.

[2] Codex Standard 72 – 1981.

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in each matrix that can be reported as a quantitative result.

Recovery
The 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 ($\%RSD_r$).

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).

4. Method Performance Requirements:

	PC	PE	PI	PS	SM	Total
Analytical range* (mg/100g RFP)	0.4–50	0.4–44	0.4–44	0.4–44	0.4–44	0.4–226
Limit of Quantitation* (LOQ)	0.4	0.4	0.4	0.4	0.4	N/A
Recovery (%)	90–110	90–110	85–115	85–115	90–110	90–110
Repeatability (RSD, %)	7	7	7	7	7	7
Reproducibility (RSD_R, %)	14	14	14	14	14	14

* 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 × 25mL/(25 + 200 mL).

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):
No infant formula certified reference materials available.

7. 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.

8. Validation Guidance:

Recommended level of validation: *Official Methods of Analysis*^{SM}.

9. Maximum Time-To-Result:

No maximum time.