AOAC SMPR® 2022.005

Standard Method Performance Requirements (SMPRs®) for A1- and A2-Type β-Casein in Infant Formulas and Adult Nutritionals

Intended Use: Reference Method for 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*SM, *Reviewed and Recognized*SM, or AOAC *Official Methods of Analysis*SM, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Measure the amount of bovine A1- and A2-type β-casein found in all forms of infant, adult, and/or pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates).

3 Analytical Technique

Any analytical technique(s) that measures the analyte(s) of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

A1-type β-casein.—Full-length variants of β-casein that contain histidine at amino acid position 67, including, e.g., phosphorylated and glycated proteoforms.

A2-type β-casein.—Full-length variants of β-casein that contain proline at amino acid position 67, including, e.g., phosphorylated and glycated proteoforms.

Bovine β-casein.—Sequence that represents the secreted, full-length bovine A2 β-casein after signal peptide removal. Multiple variants are known for β-casein.

—>sp|P02666|16-224|CASB_BOVIN Beta-casein OS=Bos taurus OX=9913 GN=CSN2 PE=1 SV=2

RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTE DELQDKIHPFAQTQSLVY PFPGPIPNSLPQNIPPLTQTPVVVP PFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSL TLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLS QSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPI IV

Follow-up formula.—From 6 to 12 months and for young children.

Foods for special medical purposes.—Nutritionally complete, specially formulated food for adults, consumed in liquid form, which may constitute the sole source of nourishment (AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals; 2010).

Infant formula.—Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infant 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.

Infant formula and formulas for special medical purposes.—0–12 months of age.

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).—Lowest level of analyte in a test sample that can be quantified at a specified level of precision.

Recovery.—Fraction or percentage of analyte that is measured 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 (in the same laboratory) and repeating during a short time period. Expressed as the repeatability standard deviation (s_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 (s_R); or reproducibility relative standard deviation (RSD_R).

Young children.—12–36 months of age.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks and appropriate check standards.

7 Reference and Harmonization Materials

Refer to "Annex F: Development and Use of In-House Reference Materials" in "Appendix F: Guidelines for *Standard Method Performance Requirements*." Available at http://www.eoma.aoac.org/app_f.pdf.

Table 1. Method performance requirements[a]

Analytical range	3–1000 mg/100 g[b]	
Limit of quantitation (LOQ)	3 mg/100 g (0.003%)[b]	
Recovery, %	80–110	
Concentrations	3–50 mg/100 g[b]	>50 mg/100 g[b]
Repeatability (RSD_r), %	15	10
Reproducibility (RSD_R), %	20	15

[a] Concentrations apply to: (a) "ready-to-feed" liquids "as is"; (b) reconstituted powders (25 g into 200 g water).
[b] mg/100 g reconstituted final product.

8 Validation Guidance

Recommended level of validation: AOAC *Official Methods of Analysis*^{SM}. Method developers are responsible for assessing interferences with the method. Protein or peptide standard material characterization, by appropriate methodology, should be described within the method submission. Examples may include protein content, protein purity (PAGE, other), and isoform characterization for A1- and A2-type β-casein.

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_l.pdf.

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

9 Maximum Time-to-Results

None.

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