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Standard Method Performance Requirements (SMPRs®) for A1-type and A2-type beta casein in Infant Formulas and Adult Nutritionals;

Intended Use: Method Dispute Resolution

1. Applicability

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

2. Analytical Technique

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

3. Definitions

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) Infant Formula and Formulas for Special Medical Purposes – 0–12 month of age; Follow-Up Formula – from 6–12 months and for young children; Young Children – 12–36 months of age; 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). Made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch and amino acids, with and without intact protein.

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

This sequence represents the secreted, full-length bovine A2 β-casein after signal peptide removal. Multiple variants are known for β-casein.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.

Limit of detection (LOD). — The 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). — The 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).

4. Method Performance Requirements See Table 1.

Table 1. Method performance requirements[a]

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

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

6. 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, 21st Ed. of the Official Methods of Analysis of AOAC INTERNATIONAL (2019). Available at http:// www.eoma.aoac.org/app_f.pdf

7. Validation Guidance

Recommended level of validation: AOAC Official Methods of Analysis℠. 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-type β-casein and A2-type β-casein.

8. Maximum Time-to-Results

None