AOAC SMPR® 2026.004

Standard Method Performance Requirements (SMPRs®) for Characterization of Molecular Weight Distribution in Dairy Protein Hydrolysates

Intended Use: Method for Routine Analysis by Trained Technicians

Purpose

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*SM and AOAC *Performance Tested Methods*SM programs. AOAC SMPRs also may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Determination of molecular weight distribution of proteins, peptides, and amino acids in hydrolyzed dairy protein ingredients and finished products formulated with hydrolyzed dairy proteins (as outlined in Table 1).

Identification of the species and/or origin of the proteins, peptides, and amino acids is outside the scope of this SMPR.

2 Analytical Technique

Any analytical technique(s) that meets the method performance requirements.

3 Definitions

Hydrolyzed.—Obtained by intentional cleavage of peptide bonds of proteins.

Table 1. Target matrices[a]

Mandatory matrix category	Representative matrix subcategory	AOAC DPH Validation Materials Kit samples
Hydrolyzed bovine milk protein ingredients	Hydrolysates derived from whey: e.g., hydrolysates of whey isolate, hydrolysates of whey concentrate	DPH01: Hydrolysate of whey isolate A DPH02: Hydrolysate of whey isolate B DPH03: Hydrolysate of whey concentrate A DPH04: Hydrolysate of whey concentrate B DPH05: Hydrolysate of whey concentrate C
	Hydrolysates derived from casein: e.g., hydrolysates of casein isolate, hydrolysates of casein concentrate, hydrolysates of caseinate	DPH06: Hydrolysate of casein concentrate DPH07: Hydrolysate of caseinate A DPH08: Hydrolysate of caseinate B
Finished products formulated with hydrolyzed dairy proteins from bovine milk	Infant formula	DPH09: Infant formula with hydrolyzed whey DPH10: Infant formula with hydrolyzed casein DPH11: Infant formula with hydrolyzed dairy proteins
	Follow-up formula	
	Drink [product] for young children	
	Foods for special medical purposes (FSMP)	DPH12: FSMP
	Formulated sports foods	
Supplementary matrix category	**Representative matrix subcategory**	**AOAC DPH Validation Materials Kit samples**
Other hydrolyzed bovine milk protein ingredients (not included in the mandatory matrix category)		DPH13: Whey protein hydrolysate DPH14: Combo whey/casein hydrolysate
Hydrolyzed other mammalian milk protein ingredients		
Finished products formulated with hydrolyzed dairy proteins from other mammalian milk	Infant formula	
	Follow-up formula	
	Drink [product] for young children	
	FSMP	
	Formulated sports foods	

[a] Refer to Section 7 Validation Guidance for requirements on matrices to be validated.

Table 2. Molecular weight categories

	Molecular weight (Da)[a]					Total
	>10000	>3000–10 000	>1000–3000	>200–1000	≤200	
Relative portion[b]	Result in %	Result in %	Result in %	Result in %	Result in %	100%

[a] Molecular weight categories can be modified depending on the sample of interest (e.g., boundaries can be set to 10 000, 3000, 1000, and 200 Da for a given sample, but 10 000, 5000, 2000, 1000, and 200 Da for another sample). When doing so, it is important that the relative portion of each category remains between the allowed range (relative portion %).

[b] Percentage contribution of each molecular weight category to the total.

Hydrolyzed dairy protein ingredients.—Protein ingredients derived from milk proteins that have undergone peptide bond hydrolysis. This category includes, but is not limited to, whey protein hydrolysates, casein protein hydrolysates, and milk protein hydrolysates.

Finished products formulated with hydrolyzed dairy proteins.—Includes infant formula, follow-up formula, food for special medical purposes, formulated sports foods, and drinks for young children that contain hydrolyzed dairy protein ingredients.

Infant formula.—Breastmilk 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. The term "infant" means a person not more than 12 months of age. (Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants CXS 72-1981 amd. 2023)

Follow-up formula.—Product for older infants, manufactured for use as a breastmilk-substitute, as a liquid part of a diet for older infants when progressively diversified complementary feeding is introduced. The term "older infant" means a person from the age of 6 months and not more than 12 months. (Codex Standard for Follow-up Formula for Older Infants and Product for Young Children CXS 156-1987 Rev. 2023).

Drink [product] for young children.—With added nutrients or product for young children with added nutrients or drink for young children or product for young children is a product manufactured for use as a liquid part of the diversified diet of young children. The term "young child" means a person from the age of more than 12 months up to the age of three years (36 months). (Codex Standard for Follow-up Formula for Older Infants and Product for Young Children CXS 156-1987 Rev. 2023)

Foods for special medical purposes (FSMP).—Category of foods for special dietary uses which are specially processed or formulated and presented for the dietary management of patients and may be used only under medical supervision. They are intended for the exclusive or partial feeding of patients with limited or impaired capacity to take, digest, absorb, or metabolize ordinary foodstuffs or certain nutrients contained therein, or who have other special medically-determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, by other foods for special dietary uses, or by a combination of the two. (Codex Standard for Labeling of and Claims for Foods for Special Medical Purposes CXS 180-1991)

Formulated sports foods.—Product that is specifically formulated to assist sports people in achieving specific nutritional or performance goals. (Australia New Zealand Food Standards Code–Standard 2.9.4–Formulated supplementary sports foods)

Soluble material.—Fraction of total protein-derived material recovered during sample preparation that will be submitted for instrumental analysis.

Molecular weight category.—Segment of the total molecular weight profile defined by its molecular weight boundaries (e.g., from 3000 to 10000 Da) (*see* Table 2).

Relative portion.—Percentage contribution of each molecular weight category to the total.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating within short intervals of time. Expressed as repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratories by different operators on different instruments. Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

4 Method Performance Requirements

See Table 3.

5 System Suitability Tests and/or Analytical Quality Control

Each analytical sequence must include a set of calibration standards and at least one quality control sample as described below.

Calibration standards.—A set of calibration standards must comprehensively span the molecular weight range relevant to the analysis and may be distributed throughout the analytical sequence as appropriate to demonstrate consistent system performance. The following criteria must be met prior to sample analysis and periodically verified throughout the sequence to confirm continued system suitability:

- The coefficient of determination (r^2) for the calibration curve must be ≥ 0.995.
- The measured molecular weight of each calibration standard must be within ±10% of its theoretical value.

Quality control samples.—A suitable quality control sample may consist of a representative protein hydrolysate or other matrix-appropriate material, selected in accordance with best practice guidelines. At least one quality control sample must be used to verify system suitability and serve as for ongoing monitoring of system performance as described below.

- *System suitability precision requirement*.—Triplicate injections of the quality control sample must demonstrate RSD of selected molecular weights (e.g., major chromatographic peaks) below 5%.

Table 3. Performance requirements

Molecular weight accuracy		
Calibration curve r^2	≥0.995	
Calibration curve residuals[a]	Within ±10%	
Calibration range	200–20 000 Da	
Molecular weight check	200–10 000 Da	<200 and >10 000 Da
	Within ±15%	Within ±20%
Relative portion precision		
Relative portion, %	RSD_r, %	RSD_R, %
0–5	≤20	≤25
>5–15	≤15	≤20
>15	≤10	≤15

[a] Difference between measured (calculated using calibration curve) and theoretical molecular weight of each calibration standard.

- *Ongoing monitoring.*—The quality control sample must be injected at appropriate intervals throughout the sequence (e.g., using a bracketing approach) to monitor for analytical drift or system deviations.

6 Reference Material(s)

No certified reference material is available at this time. AOAC INTERNATIONAL collaborated with the U.S. National Institute of Standards and Technology (NIST) to create a Dairy Protein Hydrolysates (DPH) Validation Materials Kit, which includes samples DPH01–DPH14 listed in Table 1. These materials must be included in the validation of corresponding mandatory representative matrix subcategories, as available, and can be obtained by contacting AOAC INTERNATIONAL at SciencePrograms@aoac.org.

7 Validation Guidance

Matrices to be validated.—For the mandatory matrix categories, all corresponding AOAC DPH Validation Materials Kit samples listed in Table 1 shall be included in the single-laboratory validation (SLV), when such materials exist and are reasonably available for use. This includes DPH01–DPH08 for hydrolyzed bovine milk protein ingredients derived from whey and casein and DPH09–DPH12 for finished products formulated with hydrolyzed dairy proteins from bovine milk.

For mandatory representative matrix subcategories included in Table 1 for which no corresponding AOAC DPH Validation Materials Kit sample currently exists, validation shall include at least one representative matrix from each applicable representative matrix subcategory. This includes, at a minimum, one Follow-up Formula matrix, one Drink [Product] for Young Children matrix, and one Formulated Sports Foods matrix.

Method developers are encouraged to include matrices within the supplementary matrix category to demonstrate additional method applicability. However, it is optional and not required for method acceptability.

Calibration.—A minimum of six calibration levels that span the desired molecular weight range is required. If the number of calibration levels used is <6, the report must substantiate reasons to be fit for purpose, based on the response of the instrumentation and methodology used.

Molecular weight accuracy.—Validation studies must encompass evaluation of molecular weight accuracy of calibration standards and check standards. For the molecular weight check, include at least six individual compounds (different than the compounds used for molecular weight calibration), referred to as "check standards," with molecular weights that reasonably span the molecular weight range of the calibrants. If the number of check standards used is <6, the report must substantiate reasons to be fit for purpose, based on the response of the instrumentation and methodology used.

Relative portion precision.—All samples selected for precision studies shall be analyzed in duplicate on each of 6 days using multiple analysts and instruments as practical for the different days. Fresh reagents and working standards must be used each day. Reports must include information on the number of analysts, instruments, etc.

Proportion of soluble protein-derived material.—For each validated matrix, the proportion of soluble protein-derived material recovered during sample preparation, which shall be submitted for instrumental analysis, must be determined. This proportion can be determined, for example, by comparing the nitrogen and/or amino acid content of the original sample (total nitrogen and/or amino acids) with that of the prepared sample extract (soluble nitrogen and/or amino acids). If the proportion of nitrogen present in the prepared sample extract is determined relative to the total nitrogen in the original sample, the conversion from nitrogen to protein is not necessary. Methods with higher recovery of soluble protein-derived material in the analyzed sample extract may be preferred. Sample preparation should not change the molecular weight of the protein-derived material in the sample through chemical or enzymatic modification, such as hydrolysis, cleavage, or covalent rearrangement (e.g., disulfide bond breakage and peptide bond breakage).

Potential interferences.—Validation studies shall include the assessment of potential matrix interferences, which may include but are not limited to lipids, phenolic compounds, and non-protein nitrogen components (e.g., urea and polyamines).

8 Maximum Time-to-Result

None.

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