AOAC SMPR® 2023.006

Standard Method Performance Requirements (SMPRs) for Vanillin, Ethyl Vanillin, Methyl Vanillin, and Coumarin in Infant Formulas and Their Ingredients

Intended Use: Surveillance and Monitoring by Trained Technicians

1 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 the 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 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.

2 Applicability

Quantitative analysis of vanillin, ethyl vanillin, methyl vanillin, and coumarin in infant formulas and their ingredients (Table 1).

3 Analytical Technique

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

4 Definitions

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, 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.

Coumarin.—$C_9H_6O_2$ (CAS 91-64-5).

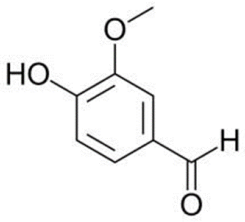
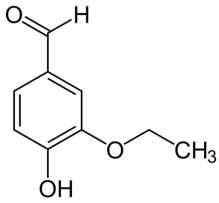
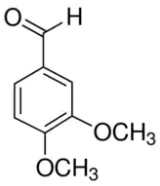
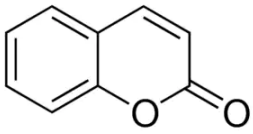
Ethyl vanillin.—$C_9H_{10}O_3$ (CAS 121-32-4).

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.

Ingredients.—Constituents of products used in their formulations.

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

Table 1. Analytes

Common name	CAS No.	Molecular structure
Vanillin	121-33-5	
Ethyl vanillin	121-32-4	
Methyl vanillin	120-14-9	
Coumarin	91-64-5	

Methyl vanillin.—$C_9H_{10}O_3$ (CAS 120-14-9).

Recovery.—Fraction or percentage of analyte that is measured when test sample is analyzed using 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 (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. 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_R).

Vanillin.—$C_8H_8O_3$ (CAS 121-33-5).

5 Method Performance Requirements

See Table 2.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks and appropriate check standards.

Method (procedural) and solvent blanks should be below the limit of detection (LOD = 0.3 × LOQ).

Table 2. Method performance requirements

Parameter	Requirement
Limit of quantitation (LOQ)	
Infant/adult formula[a], mg/kg	≤0.05
Infant formula ingredients[a], mg/kg	≤0.05
Recovery, %	80–110
RSD_r, %	≤15
RSD_R, %	≤20

[a] Results should be reported in products and ingredients as sold.

7 Validation Guidance

Validation should be conducted at the target LOQ and 10x LOQ levels. LOQ is determined as the lowest spiking level that meets recovery and repeatability requirements. Suitable matrix blanks should be selected that do not contain more than 30% of the target LOQ level for each analyte.

For matrices that contain incurred levels of vanillin and where suitable matrix blanks are not available, spiking experiments should be conducted at two concentration levels in the range of 3–10x the analyte level in the evaluated matrix. LOQ can then be estimated based on extrapolation of signal-to-noise ratio (S/N) obtained for a concentration level present in the evaluated matrix to a concentration level that would correspond to S/N = 10. To include the given matrix category (*see* Table 3) in the method applicability, the method validation must be conducted on all representative matrices listed as the minimum requirement for that matrix category.

Additional guidance can be found in the following resources:

SANTE guidelines on "Analytical quality control and method validation procedures for pesticide residues analysis in food and feed," issued by the European Commission Directorate General for Health and Food Safety (SANTE/12682/2019 or recent version)

For MS identification criteria, refer to Part D in SANTE/11813/2017 guidelines (https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf).

"Appendix F: Guidelines for *Standard Method Performance Requirements*," *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," *Official Methods of Analysis of AOAC INTERNATIONAL*

8 Reference Materials

Refer to "Annex F: Development and Use of In-House Reference Materials" in "Appendix F: Guidelines for *Standard Method Performance Requirements*," *Official Methods of Analysis of AOAC INTERNATIONAL*

9 Maximum Time-to-Results

None.

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Table 3. Target matrices[a]

Matrix category	Matrix subcategory	Minimum representative matrices[a]
Infant formula		Milk-based, soy-based, FSMP
Infant/adult formula ingredient	Animal-based milk powdered protein sources	Whole milk powder and whey protein concentrate
	Plant-based protein sources	Soy protein isolate
	Liquid milk	Bovine
	Carbohydrate-based	Lactose and maltodextrin

[a] In order to include the given matrix category in the method applicability, the validation must be conducted on all representative matrices listed as the minimum requirement for that matrix category.