AOAC SMPR® 2018.012

Standard Method Performance Requirements (SMPRs®) for Quantitation of Peanut by ELISA-Based Methods

Intended Use: Method for Quantitation of Peanut in the Context of Food Manufacturing

1 Purpose

AOAC 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, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Quantitation of peanut in one or more food(s), such as chocolate, cookies, baked goods, ice cream, salad dressings, sauces, noodles/pasta, spices, and trail mixes.

3 Analytical Technique

Enzyme-linked immunosorbent assay (ELISA)-based assays.

4 Definitions

Enzyme-linked immunosorbent assay (ELISA).—For the purposes of this document, ELISA is defined as an analytical procedure characterized by the recognition and binding of specific antigens by antibodies. This definition is not meant to be restrictive and encompasses other related binding based technologies.

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. (ISO 5725-1:1994)

Limit of quantitation (LOQ).—The lowest level of analyte in a test sample that can be quantified at a specified level of precision. (ISO 5725-1:1994)

Peanut.—A legume and classified as *Arachis hypogaea.* Peanut is usually consumed roasted, boiled, or fried. For the purposes of this SMPR, peanut is represented by NIST SRM 2387 (Peanut Butter).

Peanut butter is a food paste or spread made from ground dry roasted peanuts. It often contains additional seasoning and stabilizing ingredients that modify the taste or texture (Standard of Identity, 21 CFR 164.150 Peanut Butter).

Recovery.—The fraction or percentage of 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 (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 laboratories by different operators on different instruments. The 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). (ISO 5725-1:1994)

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

See information on antibody, cross reactivity, calibrators, and matrices in the section "Required Allergen-Specific Information to Be Provided on the ELISA Method" of Appendix M (http://www.eoma.aoac.org/app_m.pdf).

Method developers should provide (*1*) conversion factor for peanut and total peanut protein relative to NIST SRM 2387 and (*2*) applicability statement for intended use and claimed matrices.

7 Reference Material(s)

NIST SRM 2387 Peanut Butter

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* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at http://www.eoma.aoac.org/app_f.pdf

8 Validation Guidance

Method developers must provide:

(*1*) Data for method performance in all the claimed matrices. Must specify if samples used for validation are incurred or spiked, processed (baked), or unprocessed (raw).

(*2*) Recovery data using incurred samples for all claimed matrices.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at http://www.eoma.aoac.org/app_d.pdf

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

Table 1. Method performance requirements

Parameter	Minimum acceptance criteria for target matrix	
Analytical range, ppm[a]	Lower limit	≤10
	Upper limit	≥10
LOQ, ppm[b]	≤10	
LOD, ppm[b]	≤10	
Recovery, %[c]	50–150	
RSD_r, %	≤20	
RSD_R, %	≤30	

[a] ppm in peanut.

[b] *See* "Annex: Choice of LOD/LOQ for Quantitation of Peanut by ELISA-Based Methods" at the end of this SMPR for rationale for setting lower limit of range.

[c] Use incurred samples as per Appendix M in OMA.

INTERNATIONAL (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at http://www.eoma.aoac.org/app_f.pdf

Appendix M: *Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at http://www.eoma.aoac.org/app_m.pdf

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ANNEX:
Choice of LOD/LOQ for Quantitation of Peanut by ELISA-Based Methods
(March 13, 2018)

Limit of detection (LOD) and limit of quantification (LOQ) are selected based on user requirements.

The proposed limits in the SMPR constitute minimum requirements for food allergen testing in the targeted matrices as part of a food processing control. Assay users or developers may want to consider assays with a broader performance range, e.g., more sensitive and/or broader range, than the minimum acceptance criteria, as needed by their applications. For example, some users may seek the lower bound of the analytical range to correspond with either a regulatory or a health-driven threshold limit.

Only a few jurisdictions such as Japan have set a regulatory limit of 10 ppm protein for all their priority allergens. Other jurisdictions attempt to rely on risk-based thresholds for the various priority allergens. Nonetheless, recent developments in reference doses (1) have been used by food manufacturers and others as part of risk management approaches that are developed by the food industry sector in Australia and New Zealand (the Allergen Bureau was established in 2005 and is funded by membership from the Australian and New Zealand food industry). Even with this new information, the food safety risk assessment community has not adopted a validated food allergen reference or benchmark doses, which can be applied consistently by food regulators and food manufacturers in allergen-related health risk assessments and the management of precautionary allergen labeling.

The Voluntary Incidental Trace Allergen Labeling (VITAL; 2) initiative of the Allergen Bureau in Australia and New Zealand developed a scientific approach (1) using reference doses for allergen risk characterization, taking into account of clinical food allergen challenge studies. For each priority allergen targeted, a reference dose is defined as the milligram protein level (total protein from an allergenic food) below which only the most sensitive individuals (between 1 and 5%, depending on the quality of the data set available) in the allergic population are likely to experience an adverse reaction. For example, in VITAL 2.0 the reference doses are currently set at 0.2 mg protein for peanut, 0.1 mg protein for milk, and 0.03 mg protein for egg (2).

These reference doses may be used to generate action levels for food allergen control, taking into account the serving size of the food in an eating occasion. Analytical targets may therefore be set at such action levels or lower. For example, LOQ or action level for peanut protein potentially present in a food consumed at a 100 g serving size would be 2 ppm peanut protein (0.2 mg protein in 100 g).

References

(1) Taylor, S.L., Baumert, J.L., Kruizinga, A.G., Remington, B.C., Crevel, R.W.R., Brooke-Taylor, S., Allen, K.J., The Allergen Bureau of Australia & New Zealand, & Houben, G. (2014) *Food Chem. Toxicol*. **63**, 9–17. https://doi.org/10.1016/j.fct.2013.10.032

(2) http://allergenbureau.net/vital/ (accessed on August 25, 2017)