AOAC SMPR® 2017.021

Standard Method Performance Requirements (SMPRs®) for Quantitation of Wheat, Rye, and Barley Gluten in Oats

Intended Use: Quantitation of Gluten in the Context of Food Manufacturing

1 Purpose

AOAC *Standard Method Performance Requirements* (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 laboratory collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels (ERPs) in their evaluation of validation study data for a method(s) being considered to determine if it meets the requirements for *Performance Tested Methods*^{SM} or AOAC *Official Methods of Analysis*^{SM}, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Quantitation of total wheat, rye, and barley gluten in groats, rolled oats, steel cut oats, oat flour, oat bran, and extruded/cooked/finished oat products.

3 Analytical Technique

Enzyme-linked immunosorbent assay (ELISA) or related binding-based technologies.

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" [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, http://www.eoma.aoac.org/app_m.pdf; *J. AOAC Int*. **93**, 442–450(2010)]. This definition is not meant to be restrictive and encompasses other related binding-based technologies.

Gluten.—Protein fraction from wheat, rye, barley, or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5 M NaCl. [Codex Stan 118-1979: *Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten*, Codex Stan 118E_1979: Adopted in 1979. Amendment: 1983 and 2015. Revision: 2008]

Limit of detection (LOD; Appendix M).—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; Appendix M).—Lowest level of analyte in a test sample that can be quantified at a specified level of precision.

Recovery.—The fraction or percentage of analyte that is recovered when the test sample is analyzed using the entire method. [Appendix F: *Guidelines for Standard Method Performance Requirements (Section A2)*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)]

Repeatability (ISO 5725-1).—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 (ISO 5725-1).—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).

5 Method Performance Requirements

See Table 1.

6 System Suitability

See information on antibodies, cross reactivity, and calibrators in Appendix M.

7 Reference Material(s)

Samples of oat flour spiked with wheat, rye, and barley for validation studies are available from U.S. Pharmacopeia, Rockville, MD, USA.

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 (http://www.eoma.aoac.org/app_f.pdf)

8 Validation Guidance

For all candidate methods, developers must:

(*1*) Provide antibody information, cross reactivity data, and information on calibrators according to Appendix M

Table 1. Method performance requirements

Parameter	Acceptance criteria
Analytical range, ppm	≤5 to ≥15
LOQ, ppm	≤5
LOD, ppm	≤5
Recovery, %[a]	50 to 200[b]

[a] For validation purposes, individually measured as gluten from wheat, rye, and barley spiked individually in the prepared oat flour test samples, calculated from the slope of the dose response curve.
A sample series shall consist of one sample of unspiked oat flour; two samples spiked with wheat; two samples spiked with rye; and two samples spiked with barley.
Gluten content for wheat, rye, and barley used for spiking will be estimated as proteins that are insoluble in water and 0.5 M NaCl [*J. AOAC Int.* (future issue)].
See Estimating Recovery of a Gluten ELISA Kit Method for details on procedures for spiking the flour samples [*J. AOAC Int.* (future issue)].
Sample providers shall develop an SOP for producing, storing, and shipping the materials.

[b] Criteria for recovery are larger than traditionally used for SMPRs. There are two reasons for this. First, method results for gluten in oats are highly variable due to sample inhomogeneity. This lack of homogeneity may result in a wider range of recovery estimates than would normally be expected at ppm levels. Second, the different relative specificities of the antibodies against wheat, rye, and barley make balancing the response difficult.

(*2*) Wherever possible, identify peptide sequences or target epitopes for all antibodies used

(*3*) Determine and submit estimates for recovery for each gluten source (wheat, rye, and barley) using the oat flour testing materials (*see Reference Material(s)* section for source information)

For all claimed matrices, developers must submit:

(*1*) A sample processing procedure for homogenization, particle size reduction, and test portion size

(*2*) Data and estimates for LOD and LOQ

(*3*) Precision estimates (RSD_r and/or RSD_R)

Method developers may use spiked samples to determine the recovery performance of candidate methods for specific claimed matrices. Guidance on spiking for recovery evaluation are provided at:

(*1*) Koerner et al. (2013) *J. AOAC Int*. **96**(5), 1033. doi.org/10.5740/jaoacint.13-043 (*see* section on "*Spiking Methods"*)

(*2*) Estimating Recovery of a Gluten ELISA Kit Method, *J. AOAC Int.* (future issue)

(*3*) 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 (http://www.eoma.aoac.org/app_d.pdf)

(*4*) Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Approved by the International Stakeholder Panel on Alternative Methods (ISPAM) on September 24, 2017. Revised August 25, 2018 to update reference material(s) source. Revised March 12, 2019 to provide better calculations used to determine gluten content for wheat, rye, and barley used for spiking samples (wet chemical analysis vs Dumas nitrogen content method) in Table 1.

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