AOAC SMPR® 2021.008

Standard Method Performance Requirements (SMPRs®) for Glyphosate, Its Metabolites, and Trimesium in Fruits and Vegetables, Cereals, Food of Animal Origin, Pet Food, and Baby Food

Intended Use: Surveillance and Monitoring by Trained Technicians

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 drafted by AOAC working groups composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. Approved by AOAC, AOAC SMPRs may be used for method development, are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠ and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Determination of glyphosate, and its metabolites [*N*-acetylglyphosate, aminomethylphosphonic acid (AMPA), and *N*-acetyl-aminomethylphosphonic acid (*N*-acetyl AMPA)], and/or trimesium in matrices listed in Table 1.

Methods for determination of trimesium can be accepted separately from methods for analysis of glyphosate and its metabolites.

3 Analytical Technique

Any analytical technique(s) that measures any of the analyte(s) of interest (*see* Table 2) and meets the following method performance requirements is/are acceptable.

4 Definitions

Baby food.—Food intended for use by infants when they are weaned and by young children as a supplement to their diet and/or for their progressive adaptation to ordinary food. These include cereal-based products sold as dry, to be reconstituted before consumption or wet meals ready to eat, sweet or savory (reference for 'baby food' definition: *European Commission Food Labeling Guidelines*, https://ec.europa.eu/food/safety/labelling_nutrition/special_groups_food/children_en).

Glyphosate.—Residue definition of glyphosate is dependent on regulatory requirements. Metabolites should be expressed as per the residue definition.

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

Pet food.—Material consumed or intended to be consumed by animals other than humans that contributes nutrition, taste, or aroma or has a technical effect on the consumed material. This includes raw materials, ingredients, and finished product.

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 (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).

Trimesium.—Trimethyl-sulfonium cation.

5 Method Performance Requirements

See Tables 3 and 4.

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 <30% LOQ.

7 Validation Guidance

Validation should be conducted at least at the target LOQ and 10x LOQ levels. LOQ is determined as the lowest spiking level that meets the 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. Method developers should select at least one matrix from each commodity group. For pet food, both dry and wet pet food samples should be included. For baby food, each of the following should be included: vegetable- and/or fruit-based baby food, vegetable-based baby food with meat, and infant cereals.

For matrices that contain higher levels of incurred glyphosate and its metabolites and where suitable matrix blanks are not available (for all or certain analytes), spiking experiments should be conducted for the affected analytes at two concentration levels in the range of 3–10x the analyte level in the evaluated matrix. In this case, LOQ can be estimated based on extrapolation of signal-to-noise (S/N) ratio obtained for a concentration level naturally present in the evaluated matrix to a concentration level that would correspond to S/N = 10. S/N ratio can be calculated based on peak-to-peak basis for the quantifier obtained by selected reaction monitoring (SRM).

During validation, method developers are encouraged to use incurred matrices when available to evaluate method performance and repeatability.

To reduce sample heterogeneity during sampling process, method developers should consult the following guidance for sample preparation:

https://www.aafco.org/Publications/GoodTestPortions

For additional information about validations, refer to following resources:

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).

Appendix D: *Guidelines for Collaborative Study Procedures To Validate Characteristics of a Methods 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).

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 the recent version).

Validation guidance including LOQ estimation criteria for nonliquid chromatography methods, such as quantitative ELISA, can be found in:

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).

8 Reference Materials

To evaluate the method performance, method developers are encouraged to use certified reference material (CRM) during the method validation. The CRM should contain the target analytes included in the submitted method.

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)

9 Maximum Time-to-Results

None.

Developed and approved by AOAC Analytical Solutions Forum (ASF) Working Group on Glyphosates and interested stakeholders. Approved on June 18, 2021. Final Version Date: June 18, 2021

Posted: June 2021

Table 1. Target matrices

Commodity group[a]	Representative matrices
High-water content	Stone fruits, leafy vegetables, potatoes, watermelon, beets
High-acid and -water content	Citrus, berries
High-sugar content	Honey, molasses, sugar cane
High-oil and low-water content	Tree nuts: Almond, walnut Oil seeds: Soybean, peanut, canola/rapeseed, flaxseed
High-starch and/or -protein content and low-water and -fat content	Dry legume vegetables/pulses: Lentils, peas, chickpeas Cereal grains and products: Wheat, corn, barley, oats, rice, quinoa High protein: Milk powder, whey protein concentrate, soy protein isolate
Difficult or unique matrices	Green and roasted coffee beans, teas (black, green, and herbal teas), carobs, hops, spices (cinnamon), herbals (raw material and infusions), ginger powder, turmeric powder
Pet food	Dry and wet pet foods (canned, kibbles, frozen, fresh)
Baby food	Vegetable- and/or fruit-based baby food, vegetable-based baby food with meat, infant cereals

[a] Method developers should select at least one matrix from each commodity group. For pet food, both dry and wet pet food samples should be included. For baby food, each of the following should be included: vegetable- and/or fruit-based baby food, vegetable-based baby food with meat, and infant cereals.

Table 2. Analytes of interest, isotopically labeled internal standards, and known vendors

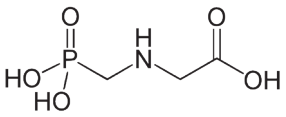
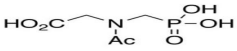
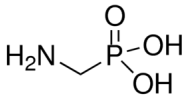
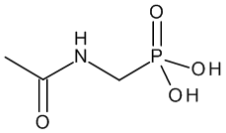
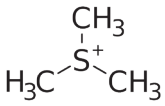
Common name	CAS No.	Molecular structure	Isotopically labeled internal standard: Name and CAS No. (if available)	Vendor and product No.
Glyphosate	1071-83-6		Glyphosate-13C2, 15N 285987-24-7	Cambridge Isotope Laboratories CNLM-4666-10
N-Acetyl Glyphosate	129660-96-4		N-Acetyl Glyphosate-13C2, 15N 1346598-31-9	Santa Cruz Biotechnology sc-479502
AMPA	1066-51-9		AMPA-13C, 15N	Santa Cruz Biotechnology sc-479588
N-Acetyl AMPA	57637-97-5		N-acetyl-AMPA-D_3	Toronto Research Chemicals A168257
Trimesium	676-84-6		Trimethyl-d_9-sulfonium Iodide 106776-17-4	Medical Isotopes D2677

Table 3. Limit of quantitation (LOQ)

	Individual analytes[a], mg/kg	Sum, mg/kg[b]
Pet food	≤10	≤50
Baby food and all other matrices in Table 1	≤0.01	≤0.05

[a] Glyphosate, *N*-acetylglyphosate, AMPA, *N*-acetyl AMPA, and trimesium.

[b] Sum of glyphosate, *N*-acetylglyphosate, AMPA, and *N*-acetyl AMPA, expressed as glyphosate.

Table 4. Recovery, repeatability, and reproducibility parameters

Recovery, %	70–120[a]
RSD_r, %	≤20
RSD_R, %	≤25

[a] Recoveries between 30–140% are acceptable if repeatability requirement in Table 1 is met. For recoveries outside of 80-120%, a correction factor for recovery must be included in the method calculations.