

1 **Standard Method Performance Requirements (SMPRs) for Glyphosate, its Metabolites,**
2 **and Trimesium in Fruits and Vegetables, Cereals, Food of Animal Origin, Pet food and**
3 **Baby Food**

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5 Intended Use: Surveillance and Monitoring by Trained Technicians
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7 **1. Purpose**

8 AOAC SMPRs describe the minimum recommended performance characteristics to be
9 used during the evaluation of a method. The evaluation may be an on-site verification, a
10 single-laboratory validation, or a multi-site collaborative study. SMPRs are written and
11 adopted by AOAC stakeholder panels composed of representatives from the industry,
12 regulatory organizations, contract laboratories, test kit manufacturers, and academic
13 institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of
14 validation study data for method being considered for *Performance Tested MethodsSM* or
15 *AOAC Official Methods of AnalysisSM*, and can be used as acceptance criteria for
16 verification at user laboratories.

17 **2. Applicability**

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19 Determination of glyphosate, and its metabolites (N-acetylglyphosate,
20 aminomethylphosphonic acid (AMPA), and N-acetyl-aminomethylphosphonic acid (N-
21 acetyl AMPA)), and/or trimesium in matrices listed in Table 3.
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23 Methods for determination of trimesium can be accepted separately from methods for
24 analysis of glyphosate and its metabolites.
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26 **3. Analytical Technique**

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28 Any analytical technique(s) that measures the analyte(s) of interest and meets the
29 following method performance requirements is/are acceptable.
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31 **4. Definitions**

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33 Glyphosate.—The residue definition of glyphosate is dependent on regulatory
34 requirements. Metabolites should be expressed as per the residue definition.
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36 Trimesium.—Trimethyl-sulfonium cation, resulting from the use of glyphosate.
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38 *Limit of quantitation (LOQ).*—LOQ is the lowest level of analyte in a test sample that can
39 be quantified at a specified level of precision.
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43 *Repeatability*.—Variation arising when all efforts are made to keep conditions constant
44 by using the same instrument and operator (in the same laboratory) and repeating
45 during a short time period. Expressed as the repeatability standard deviation (SDr); or
46 % repeatability relative standard deviation (%RSDr).

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48 *Reproducibility*.—Variation arising when identical test materials are analyzed in different
49 laboratory by different operators on different instruments. The standard deviation or
50 relative standard deviation calculated from among-laboratory data. Expressed as the
51 reproducibility standard deviation (SDR); or % reproducibility relative standard deviation
52 (% RSDR).

53
54 *Recovery*.—The fraction or percentage of analyte that is measured when the test sample
55 is analyzed using the entire method.

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57 *Baby Food*.— Food intended for use by infants when they are weaned and by young
58 children as a supplement to their diet and/or for their progressive adaptation to ordinary
59 food. These include cereal-based products sold as dry, to be reconstituted before
60 consumption or wet meals ready to eat, sweet or savory.¹

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62 *Pet Food*.—Material consumed or intended to be consumed by animals other than
63 humans that contributes nutrition, taste, or aroma or has a technical effect on the
64 consumed material. This includes raw materials, ingredients, and finished product.
65 (AAFCO)

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67 **5. Method Performance Requirements**

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69 **Table 1. Limit of quantitation (LOQ)**

	Individual analytes*	Sum**
Pet food	≤ 10 mg/kg	≤ 50 mg/kg
Baby food and all other matrices in Table 3	≤ 0.01 mg/kg	≤ 0.05 mg/kg

71 * glyphosate, N-acetylglyphosate, AMPA, N-acetyl AMPA, trimesium

72 ** sum of glyphosate, N-acetylglyphosate, AMPA, and N-acetyl AMPA, expressed as
73 glyphosate

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75 **Table 2. Recovery, repeatability and reproducibility parameters**

Recovery, %	70-120*
RSDr, %	≤ 20%
RSDR, %	≤ 25%

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¹ Reference for the Baby food definition: European Commission Food Labeling Guidelines.
https://ec.europa.eu/food/safety/labelling_nutrition/special_groups_food/children_en

77 *Recoveries between 30%-140% are acceptable if the repeatability requirement is met.

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80 **6. System Suitability Tests and/or Analytical Quality Control**

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82 Suitable methods will include blanks and appropriate check standards.

83 Method (procedural) and solvent blanks should be below 30% of LOQ.

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85 **7. Validation Guidance**

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87 For method validation procedure, refer to appendix A in SANTE guidelines for pesticide
88 residue analysis.

89 ([https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkd
90 oc_2019-12682.pdf](https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkd
90 oc_2019-12682.pdf))

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92 Validation should be conducted at least at the target LOQ and 10xLOQ levels. The LOQ is
93 determined as the lowest spiking level that meets the recovery and repeatability
94 requirements. Suitable matrix blanks should be selected that do not contain more than
95 30% of the target LOQ level for each analyte. Method developers should select at least
96 one matrix from each commodity group. For pet food, both dry and wet pet food samples
97 should be included. For baby food, each of the following should be included: Vegetable
98 and/or Fruit-based baby food, Vegetable-based baby food with meat, and Infant cereals.

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

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109 For MS identification criteria refer to Part D in SANTE/12682/2019 guidelines

110 ([https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkd
111 oc_2019-12682.pdf](https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkd
111 oc_2019-12682.pdf))

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113 Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods
114 of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville,
115 MD, USA (http://www.eoma.aoac.org/app_f.pdf).

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117 Method developers should consult the following guidance for sample preparation:

118 <https://www.aafco.org/Publications/GoodTestPortions>

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120 *Add formula*

121 Method developers are encouraged to use incurred matrices when available to
122 evaluate repeatability.

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127 **8. Reference materials**

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129 If available, certified reference materials containing incurred levels of glyphosate and its
130 metabolites can be used during validation to evaluate the method performance.

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132 Method developers are encouraged to use reference materials, which are available from
133 NIST in various matrices.

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135 Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F:
136 *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC
137 INTERNATIONAL Official Methods of Analysis (2012). Available at:

138 http://www.eoma.aoac.org/app_f.pdf

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140 **9. Maximum Time-to-Results**

141 None.

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168 **Table 3: Target Matrices**

169 Method developers should select at least one matrix from each commodity group. For
170 pet food, both dry and wet pet food samples should be included. For baby food, each of
171 the following should be included: Vegetable and/or Fruit-based baby food, Vegetable-
172 based baby food with meat, and Infant cereals.

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174 **Pet foods**

175 Dry and wet pet foods (canned, kibbles, frozen, fresh)

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177 **Baby food**

178 Vegetable and/or Fruit-based baby food, Vegetable-based baby food with meat, Infant
179 cereals

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181 **Other matrices**

Commodity Group	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, rapeseed
High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses: Lentils, yellow peas chickpeas Cereal grains and products: Wheat, corn, barley, oats, rice, flax seed, quinoa High protein: Milk powder, whey protein concentrate, soy protein isolate
Difficult or unique matrices	Green coffee beans and roasted coffee beans, teas (black/green tea and other herbal tea), carobs, hops, spices (cinnamon), and; herbals (raw material and infusions), ginger powder, turmeric powder

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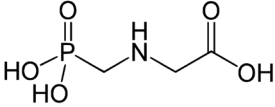
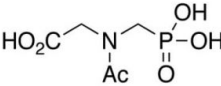
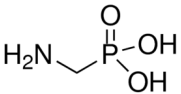
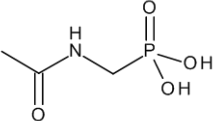
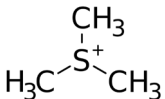
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Table 4: Analytes of interest, isotopically labeled internal standards and known vendors

Common Name	CAS Number	Molecular Structure	Isotopically Labeled Internal Standard: Name and CAS Number (if available)	Vendor and Product Number
Glyphosate	1071-83-6		Glyphosate-13C2, 15N 285987-24-7	Cambridge Isotope Laboratories Product No: CNLM-4666-10
N-Acetyl Glyphosate	129660-96-4		N-Acetyl Glyphosate-13C2, 15N 1346598-31-9	Santa Cruz Biotechnology Product No: sc-479502
AMPA	1066-51-9		AMPA-13C, 15N	Santa Cruz Biotechnology Product No: sc-479588
N-Acetyl AMPA	57637-97-5		N-acetyl-AMPA-D3	Toronto Research Chemicals Product No: A168257
Trimesium	676-84-6		Trimethyl-d9-sulfonium Iodide 106776-17-4	Medical Isotopes Product No: D2677

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