

**Standard Method Performance Requirements (SMPRs®) for Acrylamide in Potato Products, Baby Food, Bread, Other Cereal and Bakery Products, Cocoa Products, Coffee, Tea, Herbs and Spices (Including Their Extracts and Mixtures), Dry Pet Food, and Nuts**

Intended Use: Surveillance and Monitoring by Trained Technicians

**1 Purpose**

AOAC SMPRs are consensus standards developed in accordance with AOAC policy, “AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents.” SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, single-laboratory validation, multi-site collaborative study, or another AOAC-approved study design for method characterization and validation. SMPRs are written and adopted by AOAC through its stakeholder-based integrated science programs and projects, which are composed of representatives and experts from the academic, government, industry, and nonprofit sectors. AOAC SMPRs may be used to develop validation studies (AOAC Consulting Service) along with validation guidance to validate and optimize methods. They are also used by AOAC method review experts, including expert review panels in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods*<sup>SM</sup>, *Reviewed and Recognized*<sup>SM</sup>, or *Official Methods of Analysis*<sup>SM</sup>, and can be used as acceptance criteria for verification at user laboratories.

**2 Applicability**

Quantitative analysis of acrylamide in potato products, baby food, bread, other cereal and bakery products, cocoa products, coffee, tea, herbs and spices (including their extracts and mixtures), dry pet food, and nuts.

**3 Analytical Technique**

Chromatographic separation with mass spectrometric detection.

**4 Definitions**

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

*Recovery*.—Fraction or percentage of analyte that is measured when 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 repeatability standard deviation (SD)<sub>r</sub>; or % repeatability relative standard deviation (%RSD)<sub>r</sub>.

*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

reproducibility standard deviation (SD)<sub>R</sub>; or % reproducibility relative standard deviation (%RSD)<sub>R</sub>.

*Selectivity*.—Ability of the extraction, cleanup, separation system, and (especially) detector to discriminate between analyte and other compounds.

**5 Method Performance Requirements**

See Tables 1 and 2.

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

Suitable methods will include analysis of blanks and appropriate check standards. Solvent blanks should be below the limit of detection (LOD = 0.3 × LOQ). Whenever possible, quality control/reference materials should also be included (see *Validation Guidance*).

**7 Reference Materials**

Examples of currently available (as of May 2022) proficiency test, reference, and quality control materials for acrylamide may be sourced from Fapas (Sand Hutton, York, United Kingdom), Federal Institute for Materials Research and Testing (BAM; Berlin, Germany), Deutsches Referenzbüro für Ringversuche und Referenzmaterialien (DRRR; Kempten, Germany), and/or BIPEA (Paris, France) (see below and Table 3):

*French fries (precooked)*.—e.g., Fapas FCCP3-PRO25, PT-30127; FCCP3-PRO25QC, T30115QC; and FCCP3-PRO25RM, TFV013RM

*Potato crisps*.—e.g., Fapas FCCP3-PRO31, PT-30133; and BIPEA PT 94, Code 0394

*Biscuit (cookie)*.—e.g., Fapas FCCP3-PRO2, PT-30126; and FCCP3-PRO2QC, T30104QC

*Crispbread*.—e.g., Fapas FCCP3-PRO15, PT-30131; Fapas FCCP3-PRO15QC, T30118QC; and BAM ERM®-BD272

*Toasted bread*.—e.g., BAM ERM®-BD273

*Rusk*.—e.g., BAM ERM®-BD274; and BIPEA PT 94, Code 0194

*Instant coffee*.—e.g., Fapas FCCP3-DRH12, PT-30130; and FCCP3-DRH12QC, T30117QC

*Infant biscuits*.—e.g., Fapas FCCP3-INF8, PT-30124

*Infant cereals*.—e.g., BIPEA PT 94, Code 0494

*Cocoa and chocolate*.—e.g., BAM ERM®-BD513, ERM®-BD513, ERM®-BD514; and DRRR No. 2010339RR

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* (2023) 22nd Ed.

**8 Validation Guidance**

Validation must be conducted on at least one representative from each matrix category listed in Table 3. Preference will be given to methods applicable to as many matrix categories as possible and including at least one of the more challenging matrix categories, i.e., cocoa products, coffee, tea, and/or herbs and spices.

For each validated matrix category, replicate analysis of at least one representative reference material (to determine trueness and repeatability) must be included in the validation if available. Replicate analysis of representative reference materials with relevant levels of acrylamide is the preferred option to assess method performance, in which case spiking experiments described below may not be necessary for the given matrix category.

For matrices with low levels of acrylamide, validation should be conducted at the target LOQ and 5x 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 matrices that naturally contain higher levels of acrylamide and where suitable matrix blanks (with acrylamide concentration  $\leq 30\%$  of the target LOQ) are not available, the matrix should be analyzed as such and spiked at least at one concentration level in the range of 3–5x the acrylamide level in the evaluated matrix. In this case, LOQ can be estimated based on extrapolation of signal-to-noise ratio (S/N) obtained for a concentration level naturally present in the evaluated matrix to a concentration level that would correspond to  $S/N = 10$ .

Selectivity of the method should be evaluated to demonstrate that known interferences, including *N*-acetyl- $\beta$ -alanine, lactamide, and 3-aminopropanamide, do not coelute with the acrylamide peak. Baseline separation of the acrylamide peak and peaks of these compounds should be achieved, and/or it should be demonstrated that these interferences are removed prior to the chromatographic separation (during extraction and/or cleanup steps). See Table 4 for acrylamide, isotopically labeled internal standard(s), and compounds for selectivity evaluation.

For MS identification criteria, refer to Part D in SANTE/11312/2021 guidelines ([https://food.ec.europa.eu/system/files/2022-02/pesticides\\_mrl\\_guidelines\\_wrkdoc\\_2021-11312.pdf](https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf)).

“Appendix F: Guidelines for *Standard Method Performance Requirements*,” *Official Methods of Analysis of AOAC INTERNATIONAL* (2023) 22nd Ed.

### 9 Maximum Time-to-Results

None.

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*Approved by AOAC Acrylamide in Food stakeholders. Final Version Date: June 11, 2022 (version 5). Effective Date: November 1, 2022.*

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

| Matrix category | Targeted LOQ, $\mu\text{g}/\text{kg}$ |
|-----------------|---------------------------------------|
| Baby food       | $\leq 20$                             |
| Bread           | $\leq 20$                             |
| Other           | $\leq 50$                             |

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

| Parameter      | Criterion, %   |
|----------------|--|
| Recovery       | 75–110   |
| $\text{RSD}_r$ | $0.66 \times \text{RSD}_R$<br>as derived from (modified) Horwitz equation <sup>a</sup> |
| $\text{RSD}_R$ | As derived from (modified) Horwitz equation <sup>a</sup>                               |

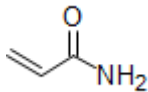
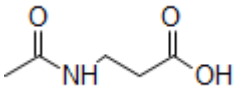
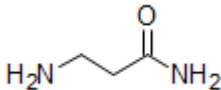
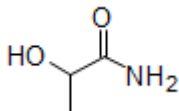
<sup>a</sup> Horwitz equation for predicted relative standard deviation of reproducibility:  $\text{PRSD}_R = 2C^{-0.15}$ , where C is analyte concentration expressed as mass fraction.

**Table 3. Target matrices<sup>a</sup>**

| Matrix category                                   | Typical representatives   | Reference material examples   |
|---|---|---|
| Potato-based products                             | French fries  | Precooked:<br>#1 (FCCP3-PRO25, PT-30127, Fapas)<br>#2 (FCCP3-PRO25QC, T30115QC, Fapas)<br>#3 (FCCP3-PRO25RM, TFV013RM, Fapas) |
|   | Crisps (chips)  | #1 (FCCP3-PRO31, PT-30133, Fapas)<br>#2 (PT 94, Code 0394, BIPEA)   |
|   | Seasoned potato based products                                      |   |
| Baby food   | Infant cereals  | PT94, Code 0494 (BIPEA)   |
|   | Baby jar foods  |   |
|   | Baby biscuits   | FCCP3-INF8, PT-30124 (Fapas)  |
| Bread   | Soft bread  |   |
| Other cereal and bakery products                  | Breakfast cereals   |   |
|   | Crispbread  | #1 (FCCP3-PRO15, PT-30131, Fapas)<br>#2 (FCCP3-PRO15QC, T30118QC, Fapas)<br>#3 (ERM@-BD272, BAM)                              |
|   |   | Toasted bread:<br>ERM@-BD273 (BAM)  |
|   | Cookies   |   |
|   | Biscuits  | #1 (FCCP3-PRO2, PT-30126, Fapas)<br>#2 (FCCP3-PRO2QC, T30104QC, Fapas)  |
|   | Rusks   | #1 (ERM@-BD274, BAM)<br>#2 (PT94, Code 0194, BIPEA)   |
|   | Wafers  |   |
|   | Crackers  |   |
|   | Seasoned snacks (e.g., popcorn, corn chips, pretzels)               |   |
|   | Cocoa products  | Cocoa powder  |
| Chocolate, including dark, milk, baking chocolate |   |   |
| Coffee  | Roasted coffee  |   |
|   | Instant coffee  | #1 (FCCP3-DRH12, PT-30130, Fapas)<br>#2 (FCCP3-DRH12QC, T30117QC, Fapas)  |
| Tea   | Instant tea, including green tea, black tea, herbal tea, oolong tea |   |
| Herbs and spices                                  | Herb/spice extracts and mixtures                                    |   |
|   | Botanical powdered extract (e.g., ginger powder)                    |   |
|   | Seasoning   |   |
| Dry pet food                                      | Dry dog or dry cat pet food (e.g., kibbles)                         |   |
| Nuts  | Roasted almonds, hazelnuts  |   |

<sup>a</sup> Validation must be conducted on at least one representative from each matrix category listed in table. Preference will be given to methods applicable to as many matrix categories as possible and including at least one of the more challenging matrix categories, i.e., cocoa products, coffee, tea, and/or herbs and spices. For each validated matrix category, replicate analyses of at least one reference material must be included in the validation if available (provided examples of reference material are available as of May 2022).

**Table 4. Acrylamide, isotopically labeled internal standard(s), and compounds for selectivity evaluation**

| Common name  | CAS No.      | Molecular structure   |
|--|--------------|---|
| Acrylamide   | 79-06-1      |  |
| <i>d</i> <sub>3</sub> -Acrylamide                                | 122775-19-3  | —   |
| <sup>13</sup> C <sub>3</sub> -Acrylamide                         | 287399-26-2  | —   |
| <sup>13</sup> C <sub>3</sub> , <i>d</i> <sub>3</sub> -Acrylamide | 2319788-93-5 | —   |
| <i>N</i> -acetyl-β-alanine                                       | 3025-95-4    |  |
| 3-Aminopropanamide   | 4726-85-6    |  |
| Lactamide  | 2043-43-8    |  |