AOAC SMPR® 2024.005

Standard Method Performance Requirements (SMPRs®) for Determination of Ethylene Oxide and Its Marker Residue 2-Chloroethanol in Oil Seeds and Nuts, Grains, Pulses, Dried Herbs, Spices and Extracts Thereof, Food Additives, Dietary Supplement Products and Ingredients, Dried Fruits, Essential Oils, Coffee, Cocoa and Composite Foods

Intended Use: Global Reference Method for Surveillance and Monitoring by Trained Technicians

1 Purpose

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*℠ and AOAC *Performance Tested Methods*℠ programs. AOAC SMPRs also may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

2 Applicability

Determination of ethylene oxide and its marker residue 2-chloroethanol (analyzed individually and reported individually and as a sum expressed as ethylene oxide) with limits of quantification meeting the European Union (EU) and other global regulations in target matrices provided in oil seeds and nuts, grains, pulses, dried herbs, spices and extracts thereof, food additives, dietary supplement products and ingredients, dried fruits, essential oils, coffee, cocoa and composite foods (*see* Table 1). Methods employing separate analytical workflows for ethylene oxide and 2-chloroethanol are acceptable.

3 Analytical Technique

Chromatographic separation with mass spectrometric detection.

4 Definitions

Limit of quantification (LOQ).—Level selected by laboratory as lowest concentration that can be reported with adequate accuracy and precision considering practical aspects, such as regulatory limits, customer needs, and performance fluctuations of instrumentation in routine use. LOQ must be equal or higher than lowest successfully validated concentration for which recovery, precision, and identification criteria are met.

Recovery.—Fraction or percentage of analyte that is measured when test sample is analyzed using entire method.

Repeatability.—Variation arising when results are generated using same method on same sample material in one laboratory by same operator, with same instrument, within short interval of time (one day or one sequence). Expressed as % repeatability relative standard deviation (%RSD_r).

Reproducibility.—Variation arising when results are generated using same method on same sample material in different laboratories. Expressed as % reproducibility relative standard deviation (%RSD_R). %RSD_R can be derived from collaborative studies and proficiency tests.

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

4 Method Performance Requirements

See Tables 2–4.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include analysis of (procedural) blank injected after the highest calibration standard and check standards (continuing calibration verification). Positive control sample(s), such as spiked (blank) matrices, incurred matrices, and quality control (QC) or reference materials, should also be included.

6 Reference Material(s)

Examples of available (as of April 2024) proficiency test (PT) and QC materials are provided in Table 5 and may be sourced from FAPAS (Sand Hutton, York, United Kingdom), LGC (Bury, Lancashire, United Kingdom), PROOF-ACS (Bremen, Germany), Deutsches Referenzbüro für Ringversuche und Referenzmaterialien (DRRR; Kempten, Germany), and DLA Proficiency Tests (Oering, Germany). Other quality control materials and (certified) reference materials, preferably from an ISO-accredited producer, are acceptable as they become available. Currently available materials and PT schemes cover 2-chloroethanol only.

7 Validation Guidance

Candidate methods should include description of procedures that allow obtaining homogeneous test material while minimizing analyte losses. Additionally, sample storage conditions should be recommended to avoid potential cross-contamination of blank samples from highly contaminated samples.

Validation should be conducted on at least one representative matrix to demonstrate method performance in the matrix category listed in Table 1. Preference will be given to methods covering as many matrix categories as possible.

Suitable matrix blanks should be selected that are free or do not contain the target analytes at a level higher than 30% of the target LOQ level. The LOQ cannot be lower than the lowest spiking level that meets recovery, precision, and identification criteria.

Validation of target analytes should be performed at least at two concentration levels, including LOQ and 10x LOQ level, with at least five replicates evaluated per concentration level. For 2-chloroethanol, accuracy is determined as recovery from spiked matrix samples or via analysis of reference materials. Accuracy for ethylene oxide is determined as recovery from spiked matrix samples. Given its high volatility, fortifications with ethylene oxide may be done after matrix soaking with water or after adding the extraction solvent to the sample matrix. Selectivity of method should be evaluated to demonstrate that acetaldehyde (a known

isobaric interference) is baseline separated from ethylene oxide, removed during extraction/cleanup steps and/or not of concern due to selective ethylene oxide conversion to another analytical form.

For analyte MS identification criteria, refer to Part D in SANTE/11312/2021 guidelines or current version of document.

Additional (optional) data to be generated in the method validation may include evaluation of matrix effects (analyte signal suppression and enhancement) and data supporting stability of reference standard solutions and sample extracts under defined storage conditions.

See Table 6 for relevant target analytes and isotopically labeled internal standards.

Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods of Analysis of AOAC INTERNATIONAL* (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at: https://academic.oup.com/aoac-publications/book/45491/chapter/392387882

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at: https://academic.oup.com/aoac-publications/book/45491/chapter/392389499

SANTE guidelines on "Analytical quality control and method validation procedures for pesticide residues analysis in food and feed," issued by European Commission Directorate General for Health and Food Safety (SANTE/11312/2021 version 2 or current version). Available at: https://eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

8 Maximum Time-to-Result

None.

Approved by AOAC stakeholders for Ethylene Oxide and Contaminants. Effective date: August 21, 2024.

Table 1. Validation matrices[a]

SANTE commodity group	Matrix category	Representative matrices
High sugar and low water content	Dried fruits	Apple, cranberry
High oil content and very low water content	Oil seeds and tree nuts	Sesame seeds, cashew nuts
High starch and/or protein content and low water and fat content	Grains, pulses	Barley, wheat, lentils
	Thickening agents	Locust bean gum, xanthan gum
Difficult or unique commodities	Dried herbs and spices	Turmeric, chili, oregano
	Herbal extracts	Boswellia extract, vanilla extract
	Dietary supplements	Psyllium husk, Ashwagandha powder, finished products containing the above, vitamin supplements
	Capsules used for dietary supplements	Hydroxypropyl methylcellulose (HPMC) or gelatine capsules
	Essential oils	Eucalyptus oil, orange oil
	Salt food additives	Calcium carbonate, choline bitartrate
	Coffee, cocoa, tea	Coffee, cocoa, tea
	Composite foods	Instant noodles (as consumed)

[a] Validation should be conducted on at least one representative matrix to demonstrate method performance in the matrix category. Preference will be given to methods covering as many matrix categories as possible.

Table 2. Limit of quantification (LOQ)

Matrix	LOQ, mg/kg[a,b]
Dried fruits, grains, pulses, and composite food	≤0.010
Oil seeds and tree nuts	≤0.025
Other matrices	≤0.050

[a] LOQ requirements apply individually to ethylene oxide and 2-chloroethanol (expressed as ethylene oxide) and are set to allow control of current EU maximum residue levels or maximum levels put in place to manage ethylene oxide contamination in matrices covered by this SMPR (if available). Maximum residue levels can be accessed via MRL-EU pesticide database available at: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls.

[b] Maximum LOQ (on mg/kg) of 2-chloroethanol is obtained by dividing values in table by factor of 0.55.

Table 3. Recovery, repeatability, and reproducibility for 2-chloroethanol

Parameter	Criterion, %
Recovery	70–120
RSD_r	≤20
RSD_R	≤30[a]

[a] RSD_R criterion applies to all evaluated concentrations and was calculated using modified Horwitz equation for 0.018 mg/kg level (corresponding to 0.010 mg/kg level for ethylene oxide) and rounded.

Table 4. Recovery and repeatability for ethylene oxide[a]

Parameter	Criterion, %
Recovery[b]	40–120
RSD_r	≤40

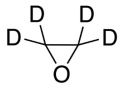
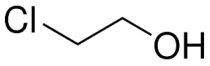
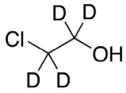
[a] Due to high volatility of ethylene oxide, conducting collaborative study to obtain reproducibility data is not feasible. Consequently, the RSD_R criterion is not provided for ethylene oxide.

[b] In fortification experiments with ethylene oxide, recoveries can be evaluated for sum of ethylene oxide and 2-chloroethanol (expressed as ethylene oxide) to cover potential conversion of spiked analyte into 2-chloroethanol.

Table 5. Reference materials

Source	Product
FAPAS (https://fapas.com/shop/product/ethylene-oxide-measured-as-2-chloro-ethanol-in-sesame-seeds-proficiency-test-19420/2237/10586)	Sesame seeds 2024 PT Round 19420
FAPAS (https://fapas.com/shop/product/ethylene-oxide-measured-as-2-chloro-ethanol-in-sesame-seeds-quality-control-t19383qc/2683/10987)	Sesame seeds Quality control material T19383QC
LGC AXIO (https://www.lgcstandards.com/GB/en/857-Ethylene-oxide-in-food-products/p/PT-FC-857)	Sesame product 2024 PT FC-857
LGC AXIO (https://www.lgcstandards.com/GB/en/868-Ethylene-oxide-in-spices/p/PT-FC-868)	Spices 2024 PT FC-868
PROOF-ACS (https://www.proof-acs.de/competenceschemes/136/)	Peppercorn (*Piper nigrum*) 2024 PT P2420-RT
PROOF-ACS (https://www.proof-acs.de/referencematerials/166/)	Green tea P2314-RMGt
DRRR (https://odin.drrr.de/catalog/?lang=en)	Spices 2024 and 2025 PT 2011095
DLA Proficiency Tests (https://www.dla-lvu.de/Formulare/Online%20Anmeldung%20DLA%202024%20en.html)	Sesame 2024 PT ptRE01
DLA Proficiency Tests (https://www.dla-lvu.de/Reference%20Material/DLA%20Reference%20Material.pdf)	Sesame (RM 2023-RE01) Sesame (RM 2022-RE01) Spice mixture (RM 2022-RE02)

Table 6. Analytes of interest, isotopically labeled internal standards, and compound for selectivity evaluation

Common name	CAS No.	Molecular structure
Ethylene oxide	75-21-8	
Ethylene-d_4 oxide	6552-57-4	
2-Chloroethanol	107-07-3	
2-Chloroethanol-d_4	117067-62-6	
Acetaldehyde	75-07-0