AOAC SMPR® 2023.002

Standard Method Performance Requirements (SMPRs®) for Pyrrolizidine Alkaloids in Teas, Herbal Infusions, Dried Herbs, Seed Spices, Honey, and Botanical Dietary Supplements and Ingredients

Intended Use: 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 uses SMPRs in its core science programs in which they are 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. Additionally, AOAC SMPRs 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

Quantitative analysis of 35 pyrrolizidine alkaloids (PAs) in teas, herbal infusions, dried herbs, seed spices, honey, and botanical dietary supplements and ingredients (*see* Tables 1 and 2). In this document, the term 'food supplement' is synonymous with dietary supplement. Performance requirements pertain to the methods used for analyzing PAs as contaminants.

3 Analytical Technique

Chromatographic separation with mass spectrometric (MS) detection.

4 Definitions

Intermediate precision.—Variation arising when results are generated using the same method on the same sample material in one laboratory but on different days (preferably a longer time interval) and may include other conditions, e.g., involving different operators and/or different (equivalent) instruments. Expressed as % intermediate precision (within-lab reproducibility) relative standard deviation (%RSD_{INT}).

Limit of quantitation (LOQ).—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 the entire method.

Repeatability.—Variation arising when results are generated using the same method on the same sample material in one laboratory by the same operator, with the same instrument, within a 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 the same method on the same sample material in different laboratories. Expressed as % reproducibility relative standard deviation (%RSD_R). The %RSD_R can be derived from collaborative studies and proficiency tests.

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

5 Method Performance Requirements

See Tables 3 and 4.

6 System Suitability Tests and/or Analytical Quality Control

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

7 Reference Material(s)

Examples of available (as of June 2023) proficiency test and quality control materials for PAs are provided in Table 5 and may be sourced from FAPAS (Sand Hutton, York, United Kingdom), PROOF-ACS (Bremen, Germany), and Deutsches Referenzbüro für Ringversuche und Referenzmaterialien (DRRR; Kempten, Germany). Other (certified) reference materials, preferably from an ISO-accredited producer, are acceptable as they become available.

"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., AOAC INTERNATIONAL, Rockville, MD, USA. Available at: https://academic.oup.com/aoac-publications/book/45491/chapter/392387882

See Table 6 for available stable isotope-labeled PAs.

8 Validation Guidance

An initial and important stage in testing of PAs in materials of plant origin is the preparation of a homogeneous sample. Detailed and complete procedures for reproducible preparation of test samples should be addressed during method validation with generated data for incurred (naturally contaminated) samples included in the method validation submission. Guidance information on representative sample size and appropriate test material particle size is provided in *Ph. Eur.* chapter Contaminant Pyrrolizidine Alkaloids (2.8.26) (available at: https://pheur.edqm.eu/app/11-2/content/11-2/20826E.htm?highlight=on&terms=2.8.26).

Validation should be conducted at least at two concentration levels, including LOQ and 5–15x LOQ with at least five replicates evaluated for each concentration. LOQ is determined as the lowest spiking level that meets recovery, precision, and identification criteria. Accuracy is determined as recovery from spiked matrix samples or via analysis of reference materials. At minimum, a single representative matrix per matrix category should be included in the validation. Inclusion of seed oil matrix category in the validation is optional. Botanical food supplement dosage forms requiring sample preparation procedure should be validated separately. Chromatographic and MS conditions should be optimized by means of pure standards to provide analyte signals with sufficient sensitivity and selectivity. For MS identification criteria, refer to Part D in SANTE/11312/2021 guidelines (available at: https://food.

ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf). For isomers that cannot be separated chromatographically, problems may arise in meeting the ion ratio criteria for identification. In such cases, deviations from the relative ion ratio criterion of ±30% are considered acceptable.

"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

9 Maximum Time-to-Result

None.

Final Version: October 6, 2023. Approved by stakeholders associated with the AOAC Botanical Ingredients and Dietary Supplements Integrity (BIDSI) Program. Effective Date: October 31, 2023.

Table 1. Target analytes

Name	Formula	CAS No.	Isomer information[a]	Example provider of standard material [order code/salt]
Echimidine	$C_{20}H_{31}NO_7$	520-68-3	Echimidine group	PhytoLab [86541/perchlorate]
Heliosupine	$C_{20}H_{31}NO_7$	32728-78-2	Echimidine group	PhytoLab [86678/sulfate]
Echimidine-*N*-oxide	$C_{20}H_{31}NO_8$	41093-89-4	Echimidine-*N*-oxide group	PhytoLab [83590/NA]
Heliosupine-*N*-oxide	$C_{20}H_{31}NO_8$	31701-88-9	Echimidine-*N*-oxide group	PhytoLab [84092/NA]
Europine	$C_{16}H_{27}NO_6$	570-19-4	NA	PhytoLab [83237/hydrochloride]
Europine-*N*-oxide	$C_{16}H_{27}NO_7$	65582-53-8	NA	PhytoLab [83238/NA]
Heliotrine	$C_{16}H_{27}NO_5$	303-33-3	NA	PhytoLab [80403/NA]
Heliotrine-*N*-oxide	$C_{16}H_{27}NO_5$	6209-65-0	NA	PhytoLab [83236/NA]
Intermedine	$C_{15}H_{25}NO_5$	10285-06-0	Intermedine group	PhytoLab [82424/NA]
Lycopsamine	$C_{15}H_{25}NO_5$	10285-07-1	Intermedine group	PhytoLab [89726/NA]
Indicine	$C_{15}H_{25}NO_5$	1195140-94-3	Intermedine group	PhytoLab [83234/hydrochloride]
Echinatine	$C_{15}H_{25}NO_5$	480-83-1	Intermedine group	PhytoLab [86118/sulfate]
Rinderine	$C_{15}H_{25}NO_5$	6029-84-1	Intermedine group	PhytoLab [84162/NA]
Intermedine-*N*-oxide	$C_{15}H_{25}NO_6$	95462-14-9	Intermedine-*N*-oxide group	PhytoLab [83446/NA]
Lycopsamine-*N*-oxide	$C_{15}H_{25}NO_6$	95462-15-0	Intermedine-*N*-oxide group	PhytoLab [83447/NA]
Indicine-*N*-oxide	$C_{15}H_{25}NO_6$	41708-76-3	Intermedine-*N*-oxide group	PhytoLab [83235/NA]
Echinatine-*N*-oxide	$C_{15}H_{25}NO_6$	20267-93-0	Intermedine-*N*-oxide group	PhytoLab [84093/NA]
Rinderine-*N*-oxide	$C_{15}H_{25}NO_6$	137821-16-0	Intermedine-*N*-oxide group	PhytoLab [84163/NA]
Lasiocarpine	$C_{21}H_{33}NO_7$	303-34-4	NA	PhytoLab [80412/NA]
Lasiocarpine-*N*-oxide	$C_{21}H_{33}NO_8$	127-30-0	NA	PhytoLab [83220/NA]
Retrorsine	$C_{18}H_{25}NO_6$	480-54-6	Retrorsine group	PhytoLab [89775/NA]
Usaramine	$C_{18}H_{25}NO_6$	15503-87-4	Retrorsine group	PhytoLab [84274/NA]
Retrorsine-*N*-oxide	$C_{18}H_{25}NO_7$	15503-86-3	Retrorsine-*N*-oxide group	PhytoLab [82630/NA]
Usaramine-*N*-oxide	$C_{18}H_{25}NO_7$	117020-54-9	Retrorsine-*N*-oxide group	PhytoLab [84703/NA]
Senecionine	$C_{18}H_{25}NO_5$	130-01-8	Senecionine group	PhytoLab [89789/NA]
Senecivernine	$C_{18}H_{25}NO_5$	72755-25-0	Senecionine group	PhytoLab [83436/NA]
Integerrimine	$C_{18}H_{25}NO_5$	480-79-5	Senecionine group	PhytoLab [83968/NA]
Senecionine-*N*-oxide	$C_{18}H_{25}NO_6$	13268-67-2	Senecionine-*N*-oxide group	PhytoLab [82631/NA]
Senecivernine-*N*-oxide	$C_{18}H_{25}NO_6$	101687-28-9	Senecionine-*N*-oxide group	PhytoLab [83437/NA]
Integerrimine-*N*-oxide	$C_{18}H_{25}NO_6$	85955-28-8	Senecionine-*N*-oxide group	PhytoLab [83969/NA]
Seneciphylline	$C_{18}H_{23}NO_5$	480-81-9	Seneciphylline group	PhytoLab [89275/NA]
Spartioidine	$C_{18}H_{23}NO_5$	520-59-2	Seneciphylline group	PhytoLab [86055/NA]
Seneciphylline-*N*-oxide	$C_{18}H_{23}NO_6$	38710-26-8	Seneciphylline-*N*-oxide group	PhytoLab [82632/NA]
Spartioidine-*N*-oxide	$C_{18}H_{23}NO_6$	121123-61-3	Seneciphylline-*N*-oxide group	PhytoLab [86056/NA]
Senkirkine	$C_{19}H_{27}NO_6$	2318-18-5	NA	PhytoLab [89274/NA]

[a] Alkaloids from the same isomer group can be quantified as the sum.

Table 2. Target matrices

Matrix category	Examples
Herbal infusions (dry material and liquid infusions)	Rooibos (*Aspalathus linearis*) leaf, anise (*Pimpinella anisum*) seed, lemon balm (*Melissa officinalis*) leaf, chamomile (*Matricaria chamomilla*) flower, thyme (*Thymus vulgaris*) herb, peppermint (*Mentha* × *piperita*) leaf, lemon verbena (*Aloysia citrodora*) leaf, sage (*Salvia officinalis*) leaf, and mixtures
Tea (*Camellia sinensis*) leaf (dry material and liquid infusions)	Fermented and nonfermented tea
Botanical food supplements, including pollen-based supplements (tablets, capsules, soft gels, tinctures, powders, and other dosage forms[a])	Finished food supplement products containing botanical ingredients, such as St. John's wort (*Hypericum perforatum*) herb, dandelion (*Taraxacum officinale*) leaf/root, licorice (*Glycyrrhiza glabra*, *G. uralensis*, *G. inflata*) root, Lady's mantle (*Alchemilla vulgaris*) leaf, milk thistle (*Silybum marianum*) fruit, echinacea (*Echinacea purpurea*, *E. angustifolia*, *E. pallida*) root/aerial parts Finished food supplement products containing pollen, such as honey powder and/or bee pollen
Botanical ingredients (botanical powder and extracts)	Botanical ingredients listed above
Honey	Processed honey
Dried herbs	Lovage (*Levisticum officinale*) leaf, marjoram (*Origanum majorana*) herb, oregano (*Origanum vulgare*) herb, and parsley (*Petroselinum crispum*) leaf
Seed spices	Cumin (*Cuminum cyminum*) seed, fennel (*Foeniculum vulgare*) seed, anise (*Pimpinella anisum*) seed
Seed oil[b]	Sunflower (*Helianthus annuus*) oil

[a] List is nonexhaustive; other dosage forms may exist.

[b] PAs are known not to be transferred into oils during pressing/extraction, but oils may be contained as ingredients in certain supplement matrices. As such, inclusion of this matrix category in the validation is optional.

Table 3. Limit of quantification (LOQ)

Matrix	Individual analytes (µg/kg) or (µg/L)
Aqueous infusions	≤0.15
Honey	≤5.0
Other samples	≤10

Table 4. Recovery, repeatability, within-laboratory reproducibility, and reproducibility

Parameter (individual analyte)	Criterion, %
Recovery	70–120[a]
RSD_r	≤20
RSD_{INT}	≤20
RSD_R	≤25

[a] In exceptional cases, average recoveries outside the above range can be accepted, but shall lie within 50-130%, and only when the precision criteria for RSD_r and RSD_{INT} are met.

Table 5. Reference materials

Source	Product
FAPAS (https://fapas.com/shop/product/pyrrolizidine-alkaloids-pas-in-ispaghula-husk-psyllium-husk-proficiency-test-22209/2243/8736)	Psyllium husk: 2023 PT Round 22209
FAPAS (https://fapas.com/shop/product/pyrrolizidine-alkaloids-pas-in-herb-proficiency-test-22212/2538/8739)	Herb (*Origanum* spp.): 2023 PT Round 22212
FAPAS (https://fapas.com/shop/product/pyrrolizidine-alkaloids-pas-in-spice-proficiency-test-22216/2539/8743)	Cumin: 2023 PT Round 22216
FAPAS (https://fapas.com/shop/product/pyrrolizidine-alkaloids-pas-in-ispaghula-husk-psyllium-husk-quality-control-t22194qc/2680/9467)	Psyllium husk: T22194QC
FAPAS (https://fapas.com/shop/product/pyrrolizidine-alkaloids-pas-in-honey-proficiency-test-22222/2610/8923)	Honey: 2024 PT Round 22222
PROOF-ACS (https://www.proof-acs.de/referencematerials/142/)	Dried nettle: P2203-RMNe
DRRR (https://odin.drrr.de/catalog/?lang=en)	Spices: 2023 and 2024 PT 2010347
DRRR (https://odin.drrr.de/catalog/?lang=en)	Tea: 2023 and 2024 PT 2010139
DRRR (https://odin.drrr.de/catalog/?lang=en)	Honey: 2023 and 2024 PT 2011006

Table 6. Available stable isotope-labeled pyrrolizidine alkaloids

Name	Formula	Example provider of standard material [order code]
Echimidine-D_3	$C_{20}H_{28}D_3NO_7$	Toronto Research Chemicals [E325582]
Echimidine-*N*-oxide-D_3	$C_{20}H_{28}D_3NO_8$	Toronto Research Chemicals [E325587]
Europine-D_6	$C_{16}H_{21}D_6NO_6$	Toronto Research Chemicals [E649942]
Europine-*N*-oxide-D_6	$C_{16}H_{21}D_6NO_6$	Toronto Research Chemicals [E649946]
Heliotrine-D_3	$C_{16}H_{24}D_3NO_5$	Toronto Research Chemicals [H235112]
Heliotrine-*N*-oxide-D_3	$C_{16}H_{24}D_3NO_6$	Toronto Research Chemicals [H235122]
Indicine-D_7	$C_{15}H_{18}D_7NO_5$	Toronto Research Chemicals [I521102]
Indicine-*N*-oxide-D_7	$C_{15}H_{18}D_7NO_6$	Toronto Research Chemicals [I521107]
Intermedine-D_7	$C_{15}H_{18}D_7NO_5$	Toronto Research Chemicals [I666662]
Intermedine-*N*-oxide-D_7	$C_{15}H_{18}D_7NO_6$	Toronto Research Chemicals [I666667]
Lasiocarpine-*N*-oxide-D_3	$C_{21}H_{30}D_3NO_8$	Toronto Research Chemicals [L177812]
Lycopsamine-D_7	$C_{15}H_{18}D_7NO_5$	Toronto Research Chemicals [L487527]
Lycopsamine-*N*-oxide-D_7	$C_{15}H_{18}D_7NO_6$	Toronto Research Chemicals [L487532]
Monocrotaline-D_4	$C_{16}H_{19}D_4NO_6$	Toronto Research Chemicals [M526002]
Monocrotaline-*N*-oxide-D_4	$C_{16}H_{19}D_4NO_7$	Toronto Research Chemicals [M526007]
Retrorsine-D_4	$C_{18}H_{21}D_4NO_6$	Toronto Research Chemicals [R279002]
Retrorsine-*N*-oxide-D_4	$C_{18}H_{21}D_4NO_7$	Toronto Research Chemicals [R279007]
Senecionine-D_3	$C_{18}H_{22}D_3NO_5$	Toronto Research Chemicals [S258652]
Senecionine-*N*-oxide-D_3	$C_{18}H_{22}D_3NO_6$	Toronto Research Chemicals [S258657]