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Method ring test
Pyrrolizidine alkaloids and tropane alkaloids in fennel seeds
P2202-MRT

Summary

The entire report is available to participants only.

The method ring test was designed, realised, evaluated and authorised on behalf of PROOF-ACS GmbH by

Dr. Birgit Schindler
Managing Director PROOF-ACS GmbH
Project coordinator

The report was approved by

Dr. Birgit Schindler
14 June 2022

Participants with any comments or concerns related to this ring test are invited to contact:

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PROOF-ACS GmbH does not have any analytical laboratory facilities of its own. Homogeneity testing and stability testing are subcontracted to laboratories, accredited according to DIN EN ISO 17025. The subcontracted laboratory may also participate in the ring tests. If so, the laboratory is treated in exactly the same way as other participants and the same rules of confidentiality apply.

The proficiency test evaluates the performances of laboratories with respect to their ability to quantify tropane alkaloids (TA) and pyrrolizidine alkaloids (PA) in fennel seeds. 21 pyrrolizidine alkaloids as well as 14 co-eluting pyrrolizidine alkaloids according to Commission Regulation 2020/2040 are within the scope of the test.

Ground fennel seeds (powder) with incurred residues of PAs was used as raw material for the preparation of the test material.

The fennel seeds contain incurred residues of five PAs:

europine, europine-N-oxide, heliotrine-N-oxide, lasiocarpine, and lasiocarpine-N-oxide.

Seven PAs are spiked to the raw materials in order to prepare the test material:

heliosupine-N-oxide, intermedine, lycopsamine, rinderine, senecionine, senecivernine, and usaramine-N-oxide.

Furthermore, the test material is spiked with two TAs:

hyoscyamine and scopolamine.

According to Commission Regulation (EU) 2020/2040 coelution is known for the spiked PAs as follows:

- Heliosupine-N-oxide, echimidine-N-oxide
- Integerrimine, senecionine, senecivernine
- Echinatine, intermedine, indicine, lycopsamine, rinderine
- Usaramine-N-oxide, retrorsine-N-oxide

The spiked PAs were evaluated with respect to the individual PAs, except for usaramine-N-oxide, where the sum of usaramine-N-oxide and retrorsine-N-oxide is considered for evaluation.

18 laboratories across seven European countries (Belgium, France, Germany, Greece, Italy, Poland, and Spain) took part in the test. All 18 laboratories reported results and are considered for evaluation.

The performance of laboratories is evaluated according to:

- the correct *identification* of 12 PAs (7 spiked and 5 incurred).
- the *comparability* of the results. The evaluation of the comparability is based on the z-score model. The z-score should be at least ≤ |2|. The comparability criterion is applied to all 12 PAs and to the 2 TAs.
- the *trueness* of the results. The trueness is expressed as the coverage of the spiked level in %. The coverage should be at least between 70 and 120 % of the spiked level. The trueness criterion is applied to the 7 spiked PAs and to the 2 spiked TAs.

Results

Pyrrolizidine alkaloid	Spiked level [µg/kg]	Assigned value [µg/kg]	Total number of results	**Comparability criterion:** no. of participants, which pass the criterion (z-score ≤ |2|)	**Trueness criterion:** no. of participants which pass the criterion (70-120 % recovery of the spiked level)
Hyoscyamine	12	12.1	8	7	6
Scopolamine	18	19.1	12	11	10
Heliosupine-N-oxide	8.0	7.40	12	11	11
Intermedine	10	8.82	15	13	12
Lycopsamine	15	14.7	12	10	9
Rinderine	33	28.9	8	7	7
Senecionine	19	15.9	14	13	9
Senecivernine	26	21.5	15	14	12
Usaramine-N-oxide*	14	13.7	15	11	9
Europine	incurred	24.6	18	17	Not applicable
Europine-N-oxide	incurred	84.8	18	17	Not applicable
Heliotrine-N-oxide	incurred	2.77	10	9	Not applicable
Lasiocarpine	incurred	4.41	14	12	Not applicable
Lasiocarpine-N-oxide	incurred	12.7	17	16	Not applicable

* Usaramine-N-oxide: sum of usaramine-N-oxide and retrorsine-N-oxide.