Ring test
Pyrrolizidine alkaloids and tropane alkaloids in dried nettle
P2203-RT

Summary

The entire report is available to participants only.

The 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
4 January 2023

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 dried nettle. 21 pyrrolizidine alkaloids as well as 14 co-eluting pyrrolizidine alkaloids according to Commission Regulation 2020/2040 are within the scope of the test.

Dried nettle with incurred residues of PAs was used as raw material for the preparation of the test material. The nettle is milled to a fine powder.

The nettle contains incurred residues of several PAs at trace levels. Two incurred PAs are considered for evaluation:

heliosupine-N-oxide and seneciphylline.

Seven PAs are spiked to the raw material to prepare the test material:

heliosupine, intermedine, integerrimine, lasiocarpine-N-oxide, retrorsine, senecionine, and senkirkine.

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 and incurred PAs mentioned above as follows:

- heliosupine, echimidine,
- intermedine, lycopsamine,
- integerrimine, senecionine, senecivernine,
- retrorsine, usaramine,
- heliosupine-N-oxide, echimidine-N-oxide,
- seneciphylline-N-oxide, spartioidine-N-oxide.

The PAs were evaluated with respect to the individual PAs, except for retrorsine and seneciphylline-N-oxide. With respect to integerrimine and senecionine, the individual PAs as well as the sum of integerrimine, senecionine and senecivernine are considered for evaluation.

11 laboratories across seven countries (Croatia, France, Germany, Netherlands, South Africa, Switzerland, and Vietnam) took part in the test. All laboratories reported results and are considered for evaluation.

The performance of laboratories is evaluated according to:

- the correct *identification* of 9 PAs (7 spiked and 2 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 9 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	27	27.3	8	7	7
Scopolamine	13	11.9	10	9	9
Heliosupine	29	26.3	10	10	9
Intermedine	17	14.4	10	8	9
Integerrimine	36	32.7	7	6	5
Senecionine	65	60.0	7	6	6
Sum integerrimine/ senecionine *	101	79.7	11	9	7
Lasiocarpine-NO	44	39.1	10	10	8
Retrorsine**	14	11.1	10	9	6
Senkirkine	18	16.5	11	10	9
Heliosupine-NO	incurred	10.3	7	6	Not applicable
Sum seneciphylline-NO/ spartioidine-NO	incurred	8.06	8	7	Not applicable

* Sum of integerrimine and senecionine: the sum of integerrimine, senecionine and senecivernine is considered for evaluation.

** Retrorsine: the sum of retrorsine and usaramine is considered for evaluation.