Proficiency test for pyrrolizidine alkaloids in food matrices

EURLPT-MP07 (2022)

D.P.K.H. Pereboom, I. Elbers, M. de Nijs, P.P.J. Mulder

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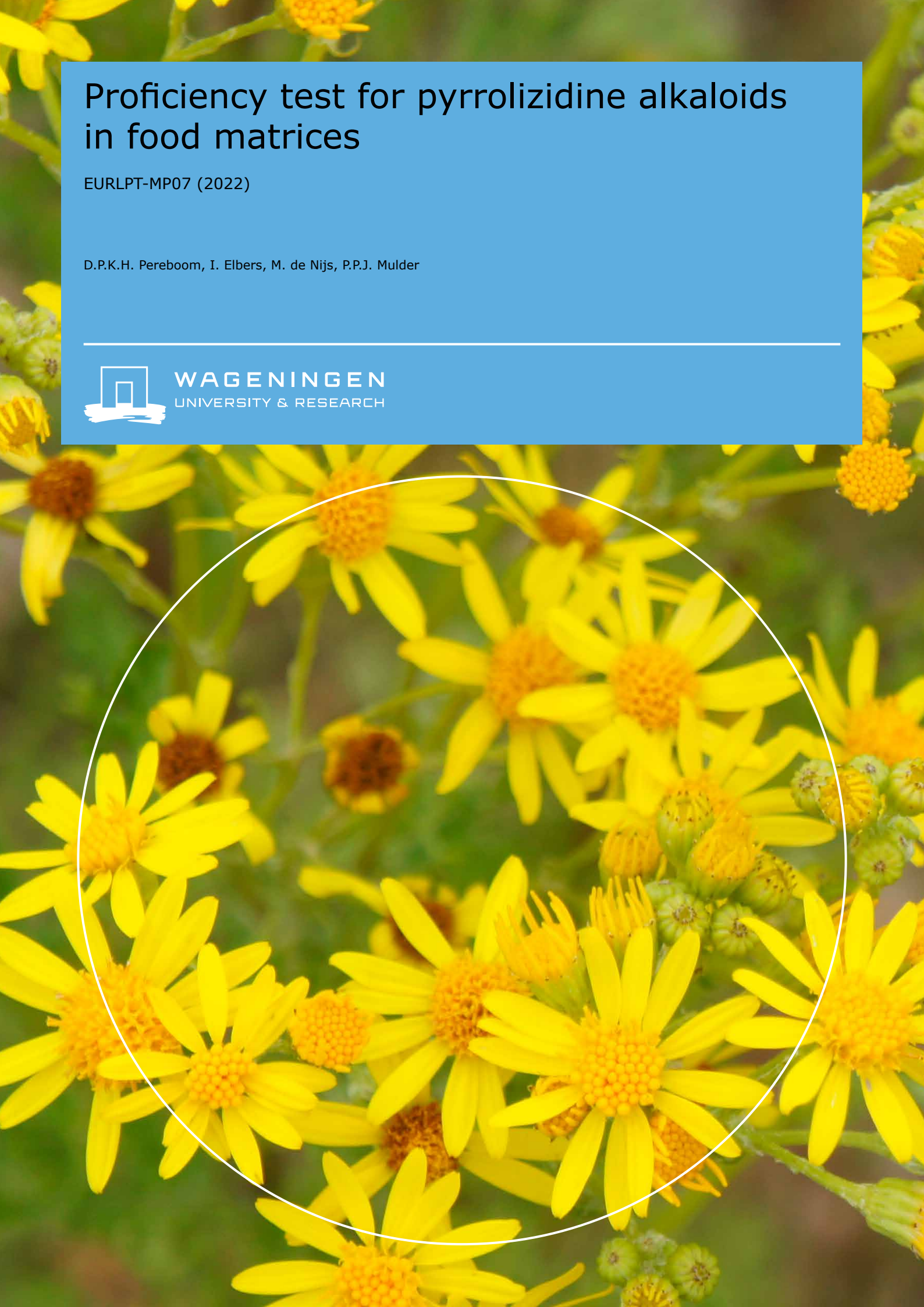

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Distribution list:
- Participating laboratories
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Contents

Summary

A proficiency test (PT) for the quantitative analysis of pyrrolizidine alkaloids (PAs) in the food matrices black tea and the culinary herb marjoram was organised by the European Union Reference Laboratory for mycotoxins & plant toxins in food and feed (EURL-MP) between December 2021 and March 2022. This PT was carried out by Wageningen Food Safety Research (WFSR) under accreditation (R013, Dutch Accreditation Council RvA, ISO/IEC 17043:2010. In December 2020 Commission Regulation (EU) 2020/2040 on maximum levels of PAs in certain foodstuffs was published and this has come into effect from July 1, 2022. The primary goal of this PT was to assess the proficiency of the National Reference Laboratories for mycotoxins & plant toxins in food and feed (NRLs) with respect to the quantitative determination of the 35 PAs mentioned in Commission Regulation (EU) 2020/2040.

The participants were asked to quantify PAs in the 2 materials and to report for each material 18 results, comprised of 7 individual PAs, 10 PA-isomer groups and the sum of 35 PAs mentioned in the legislation. The participants' performance was assessed as z-score in both materials for the individual PAs and the PA-isomer groups (maximum score 27 out of 27) and for the sum of the PAs in the samples (maximum score 2 out of 2).

Twenty-four laboratories, of which 22 National Reference Laboratories for mycotoxins and/or plant toxins in food (from 17 EU Member States plus Iceland and Norway) and 2 Official Laboratories (from one EU Member State plus Switzerland) participated in the PT.

Two materials, black tea (material A) and marjoram (material B), were prepared. Material A was artificially spiked with a solution containing a mix of PA standards. Material B was artificially spiked with a solution consisting of a mixture of extracts obtained from different PA-containing plants and some PA standards. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of 25 gram of each material. The participants were requested to report their results within 6 weeks after the dispatch of the samples.

For the identification and quantification of the PAs all participants used LC-MS/MS (23), except one participant who applied LC-HRMS (High Resolution Mass Spectrometry). Out of 24 participants, one did not report results for lasiocarpine-N-oxide and for the retrorsine-N-oxide PA-isomer group. Twenty participants reported Limit of Quantification (LOQ) values of 10 µg/kg or less for individual PAs and PA-isomer groups. Three participants reported LOQs >10 µg/kg for part of the individual PAs and/or PA-isomer groups and one participant did not report LOQs.

In this PT the robust mean based on the results of the participants that reported results before the deadline was used as consensus value and assigned value. Results were calculated for 7 individual PAs, 10 PA-isomer groups and the sum of 35 PAs. For material A, of the individual PAs, only senkirkine was present and the assigned value was 37.5 µg/kg and for the 10 PA-isomer groups the assigned values ranged from 15 to 73 µg/kg. For material B, the assigned values of the 6 individual PAs that were present ranged from 44 to 999 µg/kg and for the 10 PA-isomer groups it ranged from 131 to 789 µg/kg. For material A, none of the RSD_R of the reported results (ranging between 28-56%) were below the target standard deviation of 25%. For material B, for the individual PAs, 4 out of 6 RSD_R (ranging between 9.8–42%) were below the target standard deviation of 25%. For the PA-isomer groups in material B, 2 out of 10 RSD_R (ranging between 14-45%) were below the target standard deviation. For the sum of 35 PAs mentioned in legislation the RSD_R values were 37% and 17% for material A and B, respectively.

The proficiency of the participants was assessed as z-scores in both materials. Two participants reported their results after the deadline and for these participants the z-scores were calculated separately using the assigned values calculated from the data submitted by the other participants. For both materials (A and B) combined, 83% of the results for the 7 individual PAs and 10 PA-isomer groups were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 9% of the results fell

into the unsatisfactory range with $|z| \geq 3$. Four participants achieved optimal performance for both materials by detecting all individual PAs and all PA-isomer groups with correct quantification and the absence of false positive and false negative results. With respect to the sum of 35 PAs, for both materials combined, 84% of submitted results were satisfactory. Sixteen participants showed satisfactory performance for both materials. In this PT, 12 false negatives and 3 false positive results were reported.

From the results obtained in this PT on PAs it can be concluded that the large majority of participants has an analytical method available that includes the 35 PAs mentioned in legislation and with sufficiently low LOQs. Nevertheless, the results also reveal that for many individual PAs as well as PA-isomer groups relatively high robust RSD_R values are obtained due to a wide variation in the reported results. In this respect there has been not so much progress since the Research study on PAs (EURLPT-MP02) which was conducted in 2019. Continued efforts need to be made by the EURL-NRL network to improve the robustness of the implemented methods, in order to produce reliable data.

Table 1a *Summary of proficiency materials parameters and participants' performance – number of laboratories reporting quantitative values, <LOQ and false negative (FN).*

		Assigned value	Uncertainty	Robust RSD_R[1)]	No of labs out of 22 reporting		
PAs and PA-isomer groups	**Matrix**	**(µg/kg)**	**(µg/kg)**	**(%)**	**Quant. value**	**<LOQ**	**FN**
Europine	B	56.7	3.36	22	21	1	1
Europine-N-oxide	B	663	73.3	42	22		
Heliotrine	B	103	4.09	15	22		
Heliotrine-N-oxide	B	999	86.1	32	22		
Lasiocarpine	B	44.4	1.19	9.8	21	1	1
Lasiocarpine-N-oxide	B	516	31.9	23	21		
Senkirkine	A	37.5	4.05	39	20	2	2
Echimidine group	A	16.4	1.36	30	20	2	
	B	84.7	6.46	29	22		
Echimidine-N-oxide group	A	24.0	2.90	44	21	1	1
	B	256	18.4	27	22		
Intermedine group	A	37.4	5.62	56	22		
	B	325	23.4	27	22		
Intermedine-N-oxide group	A	60.4	4.45	28	22		
	B	463	43.9	36	22		
Retrorsine group	A	14.7	1.71	37	16	6	1
	B	131	4.82	14	22		
Retrorsine-N-oxide group	A	29.4	2.61	33	21		
	B	440	33.7	28	21		
Senecionine group	A	45.2	4.73	37	20	2	2
	B	216	19.4	34	22		
Senecionine-N-oxide group	A	73.1	7.55	39	22		
	B	789	93.6	45	22		
Seneciphylline group	A	24.8	2.04	29	19	3	2
	B	212	9.04	16	21	1	1
Seneciphylline-N-oxide group	A	39.7	3.63	34	21	1	1
	B	608	48.8	30	22		
Sum of 35 PAs	A	421	38.3	37	22		
	B	5964	278	17	22		

Matrix: A= black tea, B= marjoram.

[1)] robust relative standard deviation (interlaboratory RSD based on participants' results).

Table 1b *Summary of proficiency materials parameters and participants' performance – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

		Assigned	z-scores[2)]			Labs out of 22 with	
		Value	Satisfact.	Quest.	Unsatisf.	Accept. z or z'-score	
PAs and PA-isomer groups	**Matrix**	**(µg/kg)**	**(% of z or z'-scores)**	**(% of z or z'-scores)**	**(% of z or z'-scores)**	**No[3)]**	**%[3)]**
Europine	B	56.7	86.4	9.1	4.5	19	86.4
Europine-N-oxide	B	663	77.3	9.1	13.6	17	77.3
Heliotrine	B	103	90.9	9.1	0.0	20	90.9
Heliotrine-N-oxide	B	999	86.4	4.5	9.1	19	86.4
Lasiocarpine	B	44.4	86.4	4.5	9.1	19	86.4
Lasiocarpine-N-oxide	B	516	90.5	9.5	0.0	19	86.4
Senkirkine	A	37.5	72.7	18.2	9.1	16	72.7
Echimidine group	A	16.4	85.0	5.0	10.0	17	77.3
	B	84.7	90.9	0.0	9.1	20	90.9
Echimidine-N-oxide group	A	24.0	81.8	4.5	13.6	18	81.8
	B	256	77.3	13.6	9.1	17	77.3
Intermedine group	A	37.4	72.7	13.6	13.6	16	72.7
	B	325	77.3	13.6	9.1	17	77.3
Intermedine-N-oxide group	A	60.4	81.8	4.5	13.6	18	81.8
	B	463	77.3	13.6	9.1	17	77.3
Retrorsine group	A	14.7	82.4	5.9	11.8	14	63.6
	B	131	86.4	4.5	9.1	19	86.4
Retrorsine-N-oxide group	A	29.4	81.0	4.8	14.3	17	77.3
	B	440	85.7	9.5	4.8	18	81.8
Senecionine group	A	45.2	86.4	4.5	9.1	19	86.4
	B	216	81.8	9.1	9.1	18	81.8
Senecionine-N-oxide group	A	73.1	86.4	4.5	9.1	19	86.4
	B	789	63.6	31.8	4.5	14	63.6
Seneciphylline group	A	24.8	81.0	4.8	14.3	17	77.3
	B	212	90.9	0.0	9.1	20	90.9
Seneciphylline-N-oxide group	A	39.7	81.8	4.5	13.6	18	81.8
	B	608	86.4	9.1	4.5	19	86.4
Sum of 35 PAs for legislation	A	421	90.9	4.5	4.5	20	90.9
	B	5964	77.3	18.2	4.5	17	77.3

Matrix: A= black tea, B= marjoram.

[1)] robust relative standard deviation (interlaboratory RSD based on participants' results).

[2)] calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted here as unsatisfactory z-score.

[3)] the number and percentage here means: analyte determined, method with a sufficiently low LOQ to allow quantification, and obtaining a satisfactory z-score.

1 Introduction

Pyrrolizidine alkaloids (PAs) are toxic secondary plant metabolites produced by several plants from the families of Asteraceae *(e.g. Senecio spp.),* Boriginaceae (e.g. *Heliotropium spp*.) and Fabaceae *(e.g. Crotalaria spp.)*. PAs occur in plants as free base or as N-oxide (NO). Isomer forms do occur for several groups of PAs and PA-N-oxides. Many of these PAs have been shown to be toxic, causing hepatic veno-occlusive disease (VOD), liver cirrhosis and ultimately death. The European Food Safety Authority (EFSA) has concluded in their opinions [3, 14, 15] that PAs are potentially genotoxic carcinogenic compounds that can have long-term effects on human health even at low doses. PA-containing plants can be present as contaminants in all types of plant-based food and feed materials, including (herbal) teas, herbal food supplements, honey, fodder and feedstuffs. The European Commission (EC) and the Member States (MS) want to protect the health of consumers by regulating the presence of 1,2-unsaturated PAs in certain food products, such as herbal teas, infusions, food supplements, and spices. It has established maximum limits (MLs) for the total concentration of PAs in these commodities as described in Commission Regulation (EU) 2020/2040 [14]. The MLs have come into effect from July 1st, 2022.

The following 35 PAs are included in Commission Regulation (EU) 2020/2040: echimidine (Em), echimidine-N-oxide (EmNO), echinatine (En), echinatine-N-oxide (EnNO), europine (Eu), europine-N-oxide (EuNO), heliosupine (Hs), heliosupine-N-oxide (HsNO), heliotrine (Ht), heliotrine-N-oxide (HtNO), indicine (Id), indicine-N-oxide (IdNO), integerrimine (Ir), integerrimine-N-oxide (IrNO), intermedine (Im), intermedine-N-oxide (ImNO), lasiocarpine (Lc), lasiocarpine-N-oxide (LcNO), lycopsamine (Ly), lycopsamine-N-oxide (LyNO), retrorsine (Rt), retrorsine-N-oxide (RtNO), rinderine (Rn), rinderine-N-oxide (RnNO), senecionine (Sn), senecionine-N-oxide (SnNO), seneciphylline (Sp), seneciphylline-N-oxide (SpNO), senecivernine (Sv), senecivernine-N-oxide (SvNO), senkirkine (Sk), spartioidine (St), spartioidine-N-oxide (StNO), usaramine (Us) and usaramine-N-oxide (UsNO). These PAs and the respective PA-isomer groups are presented in Table 2. The limit of quantification (LOQ) requirement for individual PAs will be specified at 10 µg/kg. This requirement will be laid down in the regulation on methods of sampling and analysis for the control of plant toxins in food. However, this regulation is not yet formally endorsed or published.

Analysis of PAs is typically conducted by liquid chromatograpy coupled with tandem mass spectrometry (LC-MS/MS), which can be used to determine the sum of the PAs and their isomers or the isomers separately, depending on the chromatographic method applied. In practice no method is capable to chromatographically separate all isomers in a single analysis and (partial) co-elution of isomers will occur. Furthermore, it is known that chromatographic conditions and the brand or type of analytical column can have a great impact on the separation of the different PA isomers. This can lead for individual compounds to ambiguities in the data evaluation and consequently to uncertainties in the calculation of z-scores for individual laboratories. For this reason, isomeric PAs with similar chemical structure, are assessed as groups of isomers, in which the sum of all individual isomers is considered.

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and is demanded by ISO/IEC 17025:2017 [5]. Organisation of proficiency tests (PT) is one of the tasks of European Union Reference Laboratories (EURLs) [6]. Here the primary goal is to assess the proficiency of the National Reference Laboratories (NRLs). To facilitate NRLs in their task, official laboratories (OLs) can also participate, in consultation with their NRL.

2 PT material

2.1 Scope of the PT

This proficiency test (PT) focused on PAs in food matrices, using black tea and the culinary herb marjoram as representative matrices. The scope includes the 35 PAs, as mentioned in Regulation (EU) 2020/2040, and comprised a set of 7 individual PAs and 10 PA-isomer groups as presented in Table 2.

Table 2 *Scope of Pas.*

Individual PAs	PA-isomer group	
Europine (Eu)	Echimidine (Em) group	Echimidine (Em) Heliosupine (Hs)
Europine-N-oxide (EuNO)	Echimidine-N-oxide (EmNO) group	Echimidine-N-oxide (EmNO) Heliosupine-N-oxide (HsNO)
Heliotrine (Ht)	Intermedine (Im) group	Echinatine (En) Indicine (Id) Intermedine (Im) Lycopsamine (Ly) Rinderine (Rn)
Heliotrine-N-oxide (HtNO)	Intermedine-N-oxide (ImNO) group	Echinatine-N-oxide (EnNO) Indicine-N-oxide (IdNO) Intermedine-N-oxide (ImNO) Lycopsamine-N-oxide (LyNO) Rinderine-N-oxide (RnNO)
Lasiocarpine (Lc)	Retrorsine (Rt) group	Retrorsine (Rt) Usaramine (Us)
Lasiocarpine-N-oxide (LcNO)	Retrorsine-N-oxide (RtNO) group	Retrorsine-N-oxide (RtNO) Usaramine-N-oxide (UsNO)
Senkirkine (Sk)	Senecionine (Sn) group	Integerrimine (Ir) Senecionine (Sn) Senecivernine (Sv)
	Senecionine-N-oxide (SnNO) group	Integerrimine-N-oxide (IrNO) Senecionine-N-oxide (SnNO) Senecivernine-N-oxide (SvNO)
	Seneciphylline (Sp) group	Seneciphylline (Sp) Spartioidine (St)
	Seneciphylline-N-oxide (SpNO) group	Seneciphylline-N-oxide (SpNO) Spartioidine-N-oxide (StNO)

2.2 Material preparation

For preparation of the two materials A and B, black tea and marjoram were used respectively. The materials were milled using a centrifugal mill (Retsch ZM 200) to obtain a particle size of 500 µm. Material A was prepared by adding a 10 mL mixture of pyrrolizidine standards in methanol to 2000 grams of black tea. The black tea was slurried with 2100 mL methanol.
For material B, an extract was prepared in the following way: samples of 5 species of PA-containing plants were selected based on their PA profile and were ground to 1 mm. From each species two different samples were selected (A and B). *Echium vulgare* (2 * 2 g), *Eupatorium cannabinum* (2 * 2 g), *Heliotropium europaeum* (2 * 2 g), *Senecio inaequidens* (2 * 2 g) and *Senecio vulgaris* (2 * 2 g), were extracted with 30 mL methanol/water/formic acid (80:20:0.2) on a rotary tumbler for 30 minutes followed by centrifugation

(15 min, 3500 *g*). The extraction was repeated with 20 mL methanol. From these extracts a final mixture for spiking was prepared, by taking 10 mL of the extract A of *E. vulgare*, 25 mL of the extract A of *E. cannabinum*, 10 ml of the extract A of *H. europaeum*, 5 mL of the extract B of *S. inaequidens* and 25 mL of the extracts A and B of *S. vulgaris*. To the final mixture standards of heliosupine, heliosupine-N-oxide, usaramine and usaramine-N-oxide were added. Material B was fortified by adding the final mixture of extracts (100 ml) to 2000 grams of marjoram. The marjoram material was slurried with 5000 mL methanol.

The fortified slurries of the material A and B were homogenised using a kitchen mixer according to an in-house standard operating procedure [9], air dried in a fume hood and subsequently homogenised using a Stephan UM 12 mixer and stored in a freezer at -20°C until use.

2.3 Sample identification

After homogenisation, materials A and B were divided into sub-portions of approximately 25 grams and stored in polypropylene, airtight closed containers at 4-6°C until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was "2021/EURL PT MP/PAs/xxx", in which the three-digit number of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each participant. Each sample set consisted of one randomly selected sample of material A and one of material B. The codes of the samples for each sample set are shown in Annex 2. The samples for homogeneity and stability testing were also randomly selected out of materials A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, ten containers of materials A and B were analysed in duplicate for the PAs.

Method in brief, PAs were extracted from the homogenised sample (2 g) by addition of water containing 0.2% formic acid (40 mL), using a rotary tumbler. After centrifugation of the sample extract, an aliquot (5 mL) was purified using solid phase extraction (SPE) (Phenomenex StrataX, 200 mg/6 ml). The SPE eluate was evaporated to dryness using a Turbovap, reconstituted in methanol/water 1/9 (v/v) (500 µL) and filtered. Analysis was performed by LC-MS/MS, using reversed phase chromatography under alkaline conditions. The chromatography does not allow to separate the isomers lycopsamine and indicine of the intermedine group.

The homogeneity of both materials was evaluated according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [10] and ISO 13528:2015 [11]. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) were compared with the standard deviation for proficiency assessment (σ_P). The method applied for homogeneity testing is considered suitable if $s_w < 0.5 \times \sigma_P$ and a material is considered adequately homogeneous if $s_s < 0.3 \times \sigma_P$. Both materials proved to be sufficiently homogeneous for this PT.
The results of the homogeneity study (grand means with the corresponding RSD_r), are presented in Table 3. The statistical evaluation of materials A and B is presented in Annex 3.

Table 3 *Concentrations of PAs in materials A and B obtained during homogeneity testing.*

PA-isomer group	PAs	Material A: black tea		Material B: marjoram	
		Conc. (µg/kg)	RSD %	Conc. (µg/kg)	RSD %
	Europine			56.2	4.05
	Europine-N-oxide			674	2.17
	Heliotrine			90.4	2.98
	Heliotrine-N-oxide			917	2.25
	Lasiocarpine			45.6	4.38
	Lasiocarpine-N-oxide			545	4.42
	Senkirkine	45.9	5.78	-	-
Echimidine group	Echimidine	-	-	47.1	5.36
	Heliosupine	19.1	5.14	32.1	5.21
Echimidine-N-oxide group	Echimidine-N-oxide	-	-	261	4.21
	Heliosupine-N-oxide	32.3	4.69	35.2	4.55
Intermedine group	Echinatine	-	-	111	5.38
	Indicine	-	-	-	-
	Intermedine	16.2	8.49	62.4	7.64
	Lycopsamine	22.6	7.36	49.3	7.98
	Rinderine	-	-	132	2.44
Intermedine-N-oxide group	Echinatine-N-oxide	-	-	126	2.95
	Indicine-N-oxide	-	-	-	-
	Intermedine-N-oxide	23.5	9.44	85.6	5.34
	Lycopsamine-N-oxide	28.4	6.81	75.7	8.07
	Rinderine-N-oxide	-	-	217	2.75
Retrorsine group	Retrorsine	17.5	4.29	105	3.42
	Usaramine	-	-	35.0	5.15
Retrorsine-N-oxide group	Retrorsine-N-oxide	32.5	4.64	423	3.48
	Usaramine-N-oxide	-	-	43.7	3.92
Senecionine group	Integerrimine	19.8	3.35	34.1	3.73
	Senecionine	14.7	4.77	143	2.96
	Senecivernine	12.5	5.03	43.5	3.68
Senecionine-N-oxide group	Integerrimine-N-oxide	33.6	3.21	149	4.58
	Senecionine-N-oxide	26.8	8.16	531	5.10
	Senecivernine-N-oxide	20.1	7.75	149	6.82
Seneciphylline group	Seneciphylline	15.6	3.65	207	2.42
	Spartioidine	12.8	4.56	31.0	4.52
Seneciphylline-N-oxide group	Seneciphylline-N-oxide	30.5	6.35	5.97	3.41
	Spartioidine-N-oxide	20.9	3.73	108	4.02

2.5 Stability of the materials

The stability of the PAs in the materials was assessed according to [10, 11]. On January 17th, 2022, the day of distribution of the PT samples, six randomly selected containers of material A and B were stored in a freezer at -20°C. Under these conditions it is assumed that the PAs are stable in the materials. In addition, six samples of each material were stored in a refrigerator at 4-6°C.

On the 3rd of March 2022, 50 days after distribution of the samples, 6 samples of materials A and B, stored in the refrigerator and freezer, were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes had occurred [10,11] in the materials stored in the refrigerator. A consequential instability is observed when the average value of an analyte in the samples stored in the refrigerator is more than $0.3\sigma_p$ below the average value of the analyte in the samples stored in the freezer. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 4. None of the tested storage conditions caused a consequential difference for the analytes in both materials. The PAs in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

This PT focused on the determination of PAs in food, using black tea and the culinary herb marjoram. Invitations to the NRL network were sent out on December 14th, 2021 (Annex 5). Twenty-eight laboratories registered for the PT and 24 participants (Annex 1) reported their results of which 2 reported their results after the deadline. One participant was unable to report results due to problems with their method, t2 laboratories were unable to report results due to instrument problems and one participant did not report results, without providing a reason. Out of 24 participating laboratories, 22 were NRLs from 17 EU Member States plus Iceland and Norway and 2 were Official Laboratories (one from an EU Member State plus Switzerland). Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report the results through a web application designed for proficiency tests as well as to fill in a questionnaire, where it was asked to provide detailed information on the analytical method used for detection and quantification of PAs (extraction solvent/procedure, clean-up, detection technique, limit of detection and limit of quantification).

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding numbers, consisting of 2 coded samples (Annex 2) were sent to the participants on January 17th, 2022. The sample sets were dispatched immediately by courier to the participants in insulation boxes containing dry ice. The participants were asked to store the samples at 4-6°C and to analyse the samples according to their routine method. As reported by participants, all parcels were received in good order.

The samples were accompanied by a letter describing the requested analysis (Annex 6) and an acknowledgement of receipt form. In addition, each participant received instructions by e mail on how to use the web application to report the results. The questionnaire was intended to gather additional information on Limits of Quantification (LOQ), method recovery estimates (%) and other method-related aspects (e.g. extraction and clean-up, chromatographic and detection conditions, calibration strategy) to investigate individual and/or general patterns on the submitted results.

A single analysis result for the individual PAs and the PA-isomer groups in each sample was requested. The deadline for submitting the quantitative results was March 2nd, 2022, allowing the participants six weeks for analysis of the test samples. All results, except from 2 participants, were submitted within the deadline.

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [10], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [11] in combination with the insights published by the Analytical Methods Committee [12, 13] regarding robust statistics.

The evaluation of results was based on assigned values and the standard deviation for proficiency assessment (σ_P). From this, z-scores were calculated to classify the participants' performance. Detailed information on the methods used for the statistical evaluation can be found in the background document 'EURL-MP-background doc_001 (v1) Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed' available from the EURL mycotoxins & plant toxins website [4].

4.1 Calculation of the assigned value

The robust mean was used as consensus value in this PT. The consensus value based on the participants' results (all participants, both NRLs and OLs) was used as the assigned value. The values and their uncertainties are summarised in Table 1 in the Summary section. Assigned values were established for all the individual PAs, if present in the test material, all 10 PA-isomer groups and for the sum of 35 PAs in both materials.

4.2 Standard deviation for proficiency assessment (σ_P)

A fixed relative target standard deviation for proficiency assessment of 25% was used, irrespective of the plant toxin, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and best practises for mycotoxin and plant toxin determination in food and feed. The rationale behind this is provided in the background document 'EURL-MP PT performance assessment' on the EURL-MP website [4].

4.3 Quantitative performance (z-scores)

For evaluation of numerical results submitted by the participant, z-scores are calculated based on the assigned value, its uncertainty, and the standard deviation for proficiency assessment. When the uncertainty of the assigned value is negligible and no instability of the analytes in the material is observed, z-scores are calculated by:

$$z = \frac{x - C}{\sigma_p}$$ Equation 1

where:

z = z-score;
x = the result of the laboratory;
C = assigned value, here the consensus value;
σ_P = standard deviation for proficiency assessment.

The z-score compares the participants' deviation from the assigned value, taking the target standard deviation accepted for the proficiency test into account, and is interpreted as indicated in Table 4.

Table 4 *Classification of z-scores.*

$\|z\| \leq 2$	Satisfactory
$2 < \|z\| < 3$	Questionable
$\|z\| \geq 3$	Unsatisfactory

If the uncertainty of the assigned value and, if applicable, instability of the analyte in the PT material, is not negligible, this is taken into account in the determination of the z-score. If applicable, this is indicated by assigning a z'-, z_i-, or z_i'-score. For details see the background document 'EURL-MP PT performance assessment' on the EURL-MP website [4].

In this PT, the uncertainty of the assigned value for a number of individual PAs in both materials A and B were not negligible and this was taken into account in the assignment of the z-score (z'):
- material A: senkirkine.
- material B: europine-N-oxide, heliotrine-N-oxide.

For the PA-isomer groups, the uncertainty of the assigned value in both materials, except for the intermedine-N-oxide group in material A and the echimidine-N-oxide group, intermedine group, retrorsine group, seneciphylline group in material B, were not negligible and this was taken into account in the assignment of the z-score (z').

For the summed results of the 35 PAs in material A, the uncertainty of the assigned value has been taken into account for in the assignment of the z-score (z').

In all other cases, the uncertainty of the assigned value was negligible.

4.4 Evaluation of non-quantified results

In cases, where participant(s) reported '<[value]', 'detected' or 'not detected' (nd) (i.e. below their LOQ), 'proxy-z-scores' were calculated to assess possible false negatives and to benchmark the LOQ relative to the assigned value and the LOQ of the other participants.

A proxy-z-score was calculated by using equation IV and equation V of the background document 'EURL-MP-background doc_001' (for details see the EURL-MP website), using the reported LOQ value as a result [4]. Proxy-z-scores are for information only and indicated as a value between brackets.

Proxy-z-score values [z<-2] were considered as false negatives (see 4.5). Proxy-z-score values [z>2] indicate that the LOQ is high in relation to the assigned value and high in comparison to other participants.

Reported results, e.g. 'detected' or 'not detected', without specification of LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the specific analyte or analyte group/matrix. Non reported results for analytes or analyte groups are to be interpreted as unsatisfactory performance.

4.5 False positive and false negative results

A false positive is a quantitative result reported by the participant while the analyte is not detected in the PT material by the organiser, and/or not detected by most of the other participants. A threshold is then applied, above which results are considered false positives, indicated as FP. False positives are to be interpreted as unsatisfactory performance.

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', 'detected' or 'not detected', an assessment is made to judge whether such results should be classified as a false negative. This is the case when the proxy-z-score value (see 4.4) is <-2. False negatives are indicated as 'FN'. False negatives are to be interpreted as unsatisfactory performance.

5 Performance assessment

5.1 Scope and LOQ

This PT was dedicated to the quantification of PAs in black tea and the culinary herb marjoram. Annex 7 summarises the quantitative scope of each participant, with an indication of the LOQ for each PA and for each PA-isomer group. One participant provided no details on the LOQs of the individual PAs and 3 participants provided no details on their LOQs of the PA-isomer groups.

Twenty-one participants reported for both material A and B a total of 18 results, comprised of: 7 individual PAs, 10 PA-isomer groups and the sum of total PAs, as was requested. One participant reported a total of 16 results for both materials, as this participant did not have lasiocarpine-N-oxide and the retrorsine-N-oxide group in their scope.
In addition, participants PT8584 en PT8594 reported for both materials a total of 18 results, but their results were received after the deadline and for these participants the z-scores were calculated separately using the assigned values calculated from the data submitted by the other participants.

With respect to the LOQs for individual PAs and PA-isomer groups provided by the participants, one participant reported LOQs in the range of 0.22 – 0.27 µg/kg, 2 participants reported LOQs in the range of 2 – 2.5 µg/kg, 1 participant reported an LOQ of 4 µg/kg for all analytes, 6 participants reported an LOQ of 5 µg/kg for all analytes, 9 participants reported an LOQ of 10 µg/kg for all analytes, and 1 participant reported variable LOQs in the range of 1.5 – 10 µg/kg. All these participants reported LOQs that are in line with the upcoming regulation on methods of sampling and analysis of plant toxins which states that the LOQ should at least be 10 µg/kg (EC working document SANTE 14494/R2) [15]. Two participants reported LOQs in the range of 10 – 20 µg/kg, 1 laboratory reported LOQs in a wider range, namely 2.5 - 100 µg/kg and 1 laboratory provided no LOQs. It can be concluded that most participants are able to achieve LOQs of 10 µg/kg or lower, which is in line with the requirements of the (future) legislation. Some laboratories need to improve the sensitivity of their method for one or more PAs, in order to comply with the upcoming regulation on the methods of sampling and analysis of plant toxins in food. One participant needs to expand the scope of the method with a number of PAs to comply with Commission Regulation (EU) 2020/2040.

5.2 Analytical methods

All participating laboratories were asked to fill in a questionnaire addressing their accreditation, conditions used for sample preparation, chromatographic separation, detection, quantification and calibration (Annex 8). Two participants provided no information on the retention time of the PAs.

Out of the 24 laboratories, 8 participants reported their analytical method covered by ISO 17025 accreditation.

Based on the information provided on the laboratory sample preparation procedure, the median sample intake by the participants was 2 g, which was also the most often reported intake (19 participants). Three participants used 1 g and 2 participants used 5 g. The samples were extracted with 40 mL (median volume) of extraction solvent for approximately 30 min (median extraction time). The volumes most often used were 40 mL (15) and 20 mL (3). Most participants (17) reported an extraction time of 30 min, 4 participants used an extraction time between 2 and 15 min and 1 participant used 45 min. Two participants used a double extraction with 20 mL in combination with an extraction time of 15 min and 1 participant used a double extraction with 10 mL in combination with an extraction time of 15 min.

As extraction solvent participants often used an aqueous acidic solvent: 0.2% formic acid (13), 1-3% formic acid (4), or 0.05 M sulphuric acid (5). One participant used a mixture of methanol, water and formic acid and one used a mixture of acetonitrile with water.
Solid phase extraction (SPE) was used by 19 participants for sample extract purification. Three participants used no clean-up but only diluted the extract and 2 participants used another clean-up, without providing details. The following clean-up cartridges were reported: polymeric SPE sorbent (Stata X (10), Oasis HLB (2), Oasis MCX (2), Discovery DSC (2), Bond Elut (1), Chromabond HR-X (1), C18 (unspecified) (1).

For the identification and quantification of the PAs all participants used LC-MS/MS (23), except one participant who applied LC-HRMS (High Resolution Mass Spectrometry).

Participants used either acetonitrile (18) or methanol (7) as an organic mobile phase modifier. One participant used 2 different methods for the quantification of the PAs (acidic as well alkaline conditions), so in total 25 methods were reported. Thirteen participants indicated that acidic chromatography had been used. Five participants used only formic acid in their mobile phase, while 8 used an ammonium formate buffer in combination with formic acid to acidify the mobile phase. Twelve participants employed an alkaline mobile phase, in all cases ammonium carbonate buffer was used for this purpose.

A wide variety of columns from different suppliers was used for chromatography with acidic and alkaline conditions. Two participants used 2 different columns for the quantification of the PAs, so in total 26 columns were reported. For methods applying acidic conditions, mostly columns with a C18 based stationary phase were used: Waters: Acquity UPLC BEH (4), Xbridge BEH (1); Thermo Scientific: Hypersil Gold (2); Phenomenex: Gemini (1), Luna (2), Kinetex XB (1); Agilent: Poroshell SB (1). A few methods used a different stationary phase: Phenomenex Kinetex biphenyl or phenyl-hexyl (2). For methods applying alkaline conditions mostly C18 type stationary phases were used as well, mostly from one supplier: Waters Acquity BEH C18 (7), CSH (1), Atlantis T3 (1); Phenomenex: Gemini-NX (1), Kinetex EVO (1), Agilent: Zorbax Eclipse Plus (1). The column length mostly used was either 150 mm (18) or 100 mm (6). The total run time reported varied between 12 and 37 min, and typically was around 15 min (12).

The quantification approach followed by the participants is summarised in Table 5. Out of 24 participants, 18 used multi-level standard addition: 3 of them performed multi-level calibration with standards in a pure solvent, 8 used multi-level standard addition to the sample, 4 used multi-level standard addition before extraction and 3 after extraction. Six participants used a single-point standard addition approach; 5 of them added the standards before extraction and 1 added the standards after extraction. On questions about the correction the results for recovery all participants except one participant replied. Eleven participants (46%) have corrected their results for recovery while 12 (50%) reported that they didn’t.

Table 5 *Analytical strategies followed by the participants.*

Quantification approach	Calibration/ quantification	No. of participants	Recovery	
			Corrected	Not corrected
standard addition before extraction	single point	5	1	4
standard addition after extraction	single point	1	1	
standards in pure solvent	multi-level	3	3	
matrix-matched standards	multi-level	8	4	4
standard addition before extraction	multi-level	4	2	1
standard addition after extraction	multi-level	3		3

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for the PAs in materials A (black tea) and B (marjoram) are summarised in Annex 9 and 10, respectively. A summary of the performance of the participants in this PT is provided in Annex 11.

A summary of the statistical evaluation of the PT results is presented in Tables 6 to 8. These tables include all relevant parameters: the assigned value (A), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results. In case the uncertainty of the assigned value did not comply with the criterion $u>0.3\sigma_p$, the uncertainty of the assigned value was taken into account in the evaluation of the z-scores (by calculating the z'-score).

Table 6 *Summary of statistical evaluation of the PT results on the individual PAs, PA-isomer groups and the sum of PAs in material A.*

	Individual PAs	**PA-isomer group**										**All**
	Sk	**Em**	**Em NO**	**Im**	**Im NO**	**Rt**	**Rt NO**	**Sn**	**Sn NO**	**Sp**	**Sp NO**	**Total sum**
A (µg/kg)	37.5	16.4	24.0	37.4	60.4	14.7	29.4	45.2	73.1	24.8	39.7	421
u (µg/kg)	4.05	1.36	2.90	5.62	4.45	1.71	2.61	4.73	7.55	2.04	3.63	38.3
σ_p (µg/kg) (25%)	9.37	4.10	5.99	9.34	15.1	3.69	7.35	11.3	18.3	6.20	9.93	105
$u>0.3\sigma_p$	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
robust σ (µg/kg)	14.5	4.88	10.6	21.1	16.7	5.46	9.57	16.9	28.3	7.12	13.3	144
robust σ (%)	39	30	44	56	28	37	33	37	39	29	34	37
# reported	22	22	22	22	22	22	21	22	22	22	22	22
"<", nd, detected	2	2	1			6		2		3	1	
# quantitative results	20	20	21	22	22	16	21	20	22	19	21	22
$\|z\|\leq 2$	16	17	18	16	18	14	17	19	19	17	18	20
$2<\|z\|<3$	4	1	1	3	1	1	1	1	1	1	1	1
$\|z\|\geq 3$	0	2	2	3	3	1	3		2	1	2	1
FN	2		1			1		2		2	1	
S z-scores (%)	73	85	82	73	82	82	81	86	86	81	82	91

S z-scores: satisfactory z-scores.

FN= False negative.

nd= not detected.

Table 7 *Summary of statistical evaluation of the PT results on the individual PAs in material B.*

	Individual PAs					
	Eu	**EuNO**	**Ht**	**HtNO**	**Lc**	**LcNO**
A (µg/kg)	56.7	663	103	999	44.4	516
u (µg/kg)	3.36	73.3	4.09	86.1	1.19	31.9
σ_p (µg/kg) (25%)	14.2	166	25.8	250	11.1	129
$u>0.3\sigma_p$	No	Yes	No	Yes	No	No
robust σ (µg/kg)	12.3	275	15.3	323	4.37	117
robust σ (%)	22	42	15	32	9.8	23
# reported	22	22	22	22	22	21
"<", nd, detected	1				1	
# quantitative results	21	22	22	22	21	21
$\|z\|\leq 2$	19	17	20	19	19	19
$2<\|z\|<3$	2	2	2	1	1	2
$\|z\|\geq 3$		3		2	1	
FN	1				1	
s z-scores (%)	86	77	91	86	86	91

S z-scores: satisfactory z-scores.

FN= False negative.

nd= not detected.

Table 8 *Summary of statistical evaluation of the PT results on the PA-isomer groups and the sum of PAs in material B.*

	PA-isomer group										All
	Em	**EmNO**	**Im**	**ImNO**	**Rt**	**RtNO**	**Sn**	**SnNO**	**Sp**	**SpNO**	**Total sum**
A (µg/kg)	84.7	256	325	463	131	440	216	789	212	608	5964
u (µg/kg)	6.46	18.4	23.4	43.9	4.82	33.7	19.4	93.6	9.04	48.8	278
σ_p (µg/kg) (25%)	21.2	64.0	81.2	116	32.6	110	54.1	197	53.0	152	1491
$u>0.3\sigma_p$	Yes	No	No	Yes	No	Yes	Yes	Yes	No	Yes	No
robust σ (µg/kg)	24.2	69.2	87.8	165	18.1	124	72.9	351	33.1	183	1042
robust σ (%)	29	27	27	36	14	28	34	45	16	30	17
# reported	22	22	22	22	22	21	22	22	22	22	22
"<", nd, detected									1		
# quantitative results	22	22	22	22	22	21	22	22	21	22	22
$\|z\| \leq 2$	20	17	17	17	19	18	18	14	20	19	17
$2<\|z\|<3$	0	3	3	3	1	2	2	7		2	4
$\|z\| \geq 3$	2	2	2	2	2	1	2	1	1	1	1
FN									1		
S z-scores (%)	91	77	77	77	86	86	82	64	91	86	77

S z-scores: satisfactory z-scores.

FN= False negative.

nd= not detected.

For the 7 individual PAs and 10 PA-isomer groups in material A, 81% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 7% of the results fell into the questionable range with $2<|z|<3$ and 12% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). For material B was this respectively 83%, 9% and 7%. Overall, 83% percent of the results obtained for both materials (A and B) were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 9% of the results fell into the unsatisfactory range with $|z| \geq 3$.

In case of the sum of the 35 PAs mentioned in legislation, for material A, 91% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 5% of the results fell into the questionable range with $2<|z|<3$ and 5% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). For the sum of 35 PAs in material B was this respectively 77%, 18% and 5% (Table 8). In case of the sum of PAs, for both materials, 84% of submitted results were satisfactory.

In Annex 11 an overview of the overall performance for each participant in this PT is summarized. For the 2 materials combined, a maximum of 27 satisfactory z-scores, comprised of: 7 individual PA and 10 PA-isomer groups could be obtained, and '27 out of 27' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. Out of 22 participants, 4 participants achieved optimal performance for both materials by detecting all individual PAs and all PA-isomer groups with correct quantification, the absence of false positive and false negative results. For the other 18 participants either false negative or false positive results were reported, an incomplete scope of compounds was used, or one or more non-satisfactory z-scores were obtained. With respect to the sum of PAs mentioned in legislation, 16 participants showed satisfactory performance.

A total of 3 FP results were reported by participants for material A. One participant reported a FP result for europine N-oxide (95 µg/kg) and one participant reported the presence of heliotrine (34.9 µg/kg) and lasiocarpine (11.5 µg/kg).

A total of 9 false negative (FN) results was reported for material A. Two FNs were reported for senkirkine, 1 FN for the echimidine-N-oxide group, 1 FN for the retrorsine group, 2 FNs for the senecionine group, 2 FNs for the seneciphylline group and 1 for the seneciphylline-N-oxide group. For material B 3 FN results were reported: FN for europine, FN for lasiocarpine and FN for the seneciphylline group. These FN could not be explained based on the LOQs provided by the participants, which were in the range of 5 to 10 µg/kg.

5.4 Robust relative standard deviation

The robust standard deviation (RSD_R) was calculated according to ISO13528:2015 [12]. This provides a good estimation of the interlaboratory variability. The RSD_R values for each PA in both materials are shown in Annex 9, 10, in Tables 6 to 9 and in Table 1 (Summary section).

For material A, none of the RSD_R of the reported results (ranging between 28-56%) were below the target standard deviation (25%). For material B, for the individual PAs, 4 out of 6 RSD_R (ranging between 9.8–42%) were below the target standard deviation. For the PA-isomer groups in material B, 2 out of 10 RSD_R (ranging between 14-45%) were below the target standard deviation.

The RSD_R values for the sum of 35 PAs mentioned in legislation was for material A 37%, which is above the target standard deviation (25%) and for material B it was 17%.

6 Conclusions

Twenty-four laboratories, of which 22 National Reference Laboratories for mycotoxins and/or plant toxins in food (from 17 EU Member States plus Iceland and Norway) and 2 Official Laboratories (one from an EU Member State plus Switzerland) participated in the PT on quantitative determination of the 35 PAs, as mentioned in Regulation (EU) 2020/2024, in black tea and culinary herb marjoram.

Out of 22 participants, 21 reported a total of 18 results, comprised of 7 individual PAs, 10 PA-isomer groups and the sum of all PAs in the two samples material A and material B as was requested. Concerning the individual PAs included in the scope of the participants, one participant did not have lasiocarpine-N-oxide, retrorsine-N-oxide and usaramine-N-oxide in the scope of its method. Twenty participants used a method with a reported LOQ for individual PAs and PA-isomer groups of 10 µg/kg or lower. Three participants reported LOQs >10 µg/kg for part of the individual PAs and/or PA-isomer groups and one did not report LOQs.

Participants PT8584 en PT8594 reported their results after the deadline and for these participants the z-scores were calculated separately using the assigned values calculated from the data submitted by the other participants.

Most of the participants used methods based on LC-MS/MS (96%) and used SPE for clean-up (79%).

For material A, of the individual PAs only senkirkine was present. For this compound 73% of the participants obtained a satisfactory result. All 10 PA-isomer groups were present and the satisfactory results varied from 73 to 86%. The RSD_R of the reported results ranged between 28-56% and consequently none were below the target standard deviation of 25%. Combined, for the individual PAs and the PA-isomer groups, 81% of the results were satisfactory.

For the 6 individual PAs present in material B, satisfactory results varied from 77 to 91% and 4 out of 6 RSD_R (ranging between 9.8–42%) were below the target standard deviation of 25%. For the 10 PA-isomer groups the satisfactory results varied from 77 to 91%, except for the senecionine-N-oxide group for which only 64% of the participants obtained satisfactory results. Two out of 10 RSD_R (ranging between 14-45%) of these PA-isomer groups were below the target standard deviation. Combined, for the individual PAs and the PA-isomer groups, 83% of the results were satisfactory.

With respect to the sum of 35 PAs considered in legislation, for material A and B, respectively, 91% and 77% of the results were satisfactory. The RSD_R for material A and B was 37% and 17%, respectively.

Overall, for the individual PAs and the PA-isomer groups in both materials combined (27 results), 83% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 8% of the results fell into the questionable range with $2<|z|<3$ and 9% of the results fell into the unsatisfactory range with $|z| \geq 3$. Four participants had a satisfactory performance. With respect to the sum of the 35 PAs, in both materials combined (2 results), 84% of submitted results were satisfactory and 16 participants had a satisfactory performance.

From the results obtained in this PT on PAs it can be concluded that the large majority of participants has an analytical method available that includes the 35 PAs mentioned in legislation and with sufficiently low LOQs. Nevertheless, the results also reveal that for many individual PAs as well as PA-isomer groups relatively high robust RSD_R values are obtained due to a wide variation in the reported results. In this respect there has been not so much progress since the Research study on PAs (EURLPT-MP02) which was conducted in 2019. Continued efforts need to be made by the EURL-NRL network to improve the robustness of the implemented methods, in order to produce reliable data.

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Annex 1 List of participants

Country	Organisation
AUSTRIA*	Austrian Agency for Health and Food Safety
BELGIUM*	ILVO-T&V
CROATIA*	A. Stampar Teaching Institute of Public Health
CYPRUS*	Feeding Stuffs Quality Control Laboratory - Analytical Laboratories Section
CYPRUS*	State General Laboratory
CZECH REPUBLIC*	Central Institute for Supervising and Testing in Agriculture
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
DENMARK*	Danish Veterinary and Food Administration
FINLAND*	Finnish Food Authority
FRANCE*	Laboratoire SCL de Strasbourg
GERMANY***	CVUA-Mel
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GERMANY**	Eurofins WEJ Contaminants
GREECE*	General Chemical State Laboratory
IRELAND*	The Public Analyst's Laboratory
ITALY*	IZSLER
LATVIA*	Institute of Food Safety, Animal Health and Environment "BIOR"
LUXEMBOURG*	Laboratoire National de Sante
NORWAY*	Norwegian Institute of Bioeconomy Research (NIBIO)
POLAND*	National Institute of Public Health - National Institute of Hygiene
POLAND*	National Veterinary Research Institute
SLOVENIA*	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
SPAIN*	Centro Nacional de Alimentacion
SWITZERLAND**	Kantonales Laboratorium

* National Reference Laboratory (NRL) of EU Member State.

** National Reference Laboratory (NRL) of the European Free Trade Association (Eurofins WEJ Contaminants = Iceland).

*** Official Laboratory (OL).

Annex 2 Codification of the samples

Participant's code	Material A*	Material B*
PT8583	347	999
PT8584	912	231
PT8587	275	832
PT8588	984	960
PT8589	953	877
PT8592	693	614
PT8593	918	905
PT8594	594	859
PT8595	163	298
PT8596	656	279
PT8597	156	292
PT8598	371	159
PT8599	771	418
PT8600	226	301
PT8601	501	370
PT8602	277	106
PT8603	225	529
PT8604	490	317
PT8605	392	123
PT8606	360	763
PT8607	413	411
PT8608	943	376
PT8609	117	378
PT8610	460	783
PT8611	402	989

* All sample codes start with 2022/EURL PT MP/PAs/.

Annex 3 Statistical evaluation of homogeneity data

	Senkirkine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	46.9	41.9
Hom/A002	48.4	46.6
Hom/A003	47.3	45.6
Hom/A004	48.7	43.6
Hom/A005	46.3	44.9
Hom/A006	44.8	48.2
Hom/A007	46.5	44.7
Hom/A008	46.4	45.5
Hom/A009	45.2	47.7
Hom/A010	44.2	44.8
Grand mean	45.9	
Cochran's test		
C	0.320	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.5	
s_x	0.940	
s_w	2.02	
s_s	0.000	
Critical= 0.3 σ_P	3.44	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Heliosupine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	20.6	17.9
Hom/A002	20.8	18.0
Hom/A003	20.7	18.3
Hom/A004	19.0	18.8
Hom/A005	19.8	19.5
Hom/A006	19.3	19.6
Hom/A007	19.8	19.0
Hom/A008	20.3	19.1
Hom/A009	18.2	18.2
Hom/A010	18.1	17.6
Grand mean	19.1	
Cochran's test		
C	0.333	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.78	
s_x	0.625	
s_w	1.06	
s_s	0.000	
Critical= 0.3 σ_P	1.43	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

sx = Standard deviation of the sample averages.
sw = Within-sample standard deviation.
ss = Between-sample standard deviation.

Sample No.	Heliosupine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	30.4	30.2
Hom/A002	33.6	34.1
Hom/A003	33.2	32.1
Hom/A004	32.4	32.8
Hom/A005	32.4	30.9
Hom/A006	33.5	33.7
Hom/A007	33.9	31.7
Hom/A008	35.0	31.2
Hom/A009	31.7	31.4
Hom/A010	28.9	32.0
Grand mean	32.3	
Cochran's test		
C	0.437	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.06	
s_x	1.22	
s_w	1.29	
s_s	0.812	
Critical= 0.3 σ_P	2.42	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Intermedine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	15.4	17.0
Hom/A002	14.7	15.3
Hom/A003	15.6	17.7
Hom/A004	16.9	17.6
Hom/A005	14.5	15.0
Hom/A006	18.0	16.6
Hom/A007	17.5	17.9
Hom/A008	17.0	17.4
Hom/A009	14.0	14.1
Hom/A010	17.1	14.7
Grand mean	16.2	
Cochran's test		
C	0.347	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.05	
s_x	1.24	
s_w	0.903	
s_s	1.07	
Critical= 0.3 σ_P	1.22	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Lycopsamine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	24.1	20.8
Hom/A002	25.1	22.3
Hom/A003	24.3	20.6
Hom/A004	22.4	20.1
Hom/A005	23.5	23.5
Hom/A006	20.7	23.2
Hom/A007	22.0	22.4
Hom/A008	22.5	21.0
Hom/A009	23.2	26.6
Hom/A010	21.0	21.9
Grand mean	22.6	
Cochran's test		
C	0.235	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	5.64	
s_x	1.14	
s_w	1.71	
s_s	0.000	
Critical= 0.3 σ_P	1.69	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Intermedine-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	25.9	20.4
Hom/A002	25.1	24.1
Hom/A003	23.2	21.3
Hom/A004	22.2	26.7
Hom/A005	20.4	25.8
Hom/A006	25.7	20.6
Hom/A007	21.9	21.6
Hom/A008	24.3	24.5
Hom/A009	20.6	24.9
Hom/A010	24.8	26.7
Grand mean	23.5	
Cochran's test		
C	0.224	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	5.88	
s_x	1.23	
s_w	2.58	
s_s	0.000	
Critical= 0.3 σ_P	1.76	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Lycopsamine-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	30.0	28.4
Hom/A002	28.1	24.9
Hom/A003	29.0	28.7
Hom/A004	30.6	28.3
Hom/A005	31.7	27.9
Hom/A006	25.9	30.6
Hom/A007	29.3	31.7
Hom/A008	27.8	28.0
Hom/A009	29.2	25.5
Hom/A010	26.3	26.6
Grand mean	28.4	
Cochran's test		
C	0.293	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.11	
s_x	1.38	
s_w	1.92	
s_s	0.226	
Critical= 0.3 σ_P	2.13	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Retrorsine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	18.0	16.0
Hom/A002	17.4	18.2
Hom/A003	17.6	18.2
Hom/A004	18.5	17.3
Hom/A005	18.1	17.2
Hom/A006	16.5	17.8
Hom/A007	17.5	17.6
Hom/A008	16.3	17.9
Hom/A009	18.4	18.0
Hom/A010	16.2	17.0
Grand mean	17.5	
Cochran's test		
C	0.335	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.37	
s_x	0.507	
s_w	0.780	
s_s	0.000	
Critical= 0.3 σ_P	1.31	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Retrorsine-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	32.4	34.2
Hom/A002	32.0	31.1
Hom/A003	33.5	33.3
Hom/A004	31.0	32.7
Hom/A005	33.9	30.9
Hom/A006	33.6	31.5
Hom/A007	36.7	31.0
Hom/A008	32.9	32.3
Hom/A009	32.1	33.2
Hom/A010	31.1	30.4
Grand mean	32.5	
Cochran's test		
C	0.586	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.12	
s_x	0.934	
s_w	1.66	
s_s	0.000	
Critical= 0.3 σ_P	2.44	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Integerrimine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	20.5	20.5
Hom/A002	21.0	19.6
Hom/A003	20.7	18.3
Hom/A004	19.9	20.4
Hom/A005	19.4	19.4
Hom/A006	19.4	19.6
Hom/A007	20.2	19.3
Hom/A008	20.2	19.2
Hom/A009	19.3	20.1
Hom/A010	18.9	19.4
Grand mean	19.8	
Cochran's test		
C	0.519	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.94	
s_x	0.418	
s_w	0.720	
s_s	0.000	
Critical= 0.3 σ_P	1.48	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Senecionine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	16.1	14.4
Hom/A002	15.3	14.1
Hom/A003	14.5	15.4
Hom/A004	16.0	14.7
Hom/A005	14.2	13.7
Hom/A006	14.8	15.8
Hom/A007	14.8	13.9
Hom/A008	14.6	14.2
Hom/A009	15.1	14.3
Hom/A010	14.5	14.0
Grand mean	14.7	
Cochran's test		
C	0.304	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.68	
s_x	0.486	
s_w	0.715	
s_s	0.000	
Critical= 0.3 σ_P	1.10	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecivernine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	12.4	13.0
Hom/A002	12.2	13.2
Hom/A003	12.3	13.5
Hom/A004	12.6	13.1
Hom/A005	12.4	11.4
Hom/A006	12.4	12.2
Hom/A007	13.8	12.7
Hom/A008	12.7	11.7
Hom/A009	12.6	13.0
Hom/A010	11.4	12.2
Grand mean	12.5	
Cochran's test		
C	0.208	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.13	
s_x	0.464	
s_w	0.607	
s_s	0.177	
Critical= 0.3 σ_P	0.940	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Integerrimine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	33.4	34.0
Hom/A002	35.1	33.0
Hom/A003	36.1	35.0
Hom/A004	33.1	32.8
Hom/A005	34.8	34.0
Hom/A006	32.3	32.0
Hom/A007	33.6	32.7
Hom/A008	33.5	34.3
Hom/A009	32.5	33.3
Hom/A010	34.1	32.4
Grand mean	33.6	
Cochran's test		
C	0.383	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.40	
s_x	0.941	
s_w	0.784	
s_s	0.760	
Critical= 0.3 σ_P	2.52	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecionine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	29.6	24.1
Hom/A002	27.8	25.9
Hom/A003	25.8	31.5
Hom/A004	25.2	26.6
Hom/A005	28.1	28.2
Hom/A006	27.4	29.3
Hom/A007	26.6	25.3
Hom/A008	29.2	26.2
Hom/A009	23.6	23.6
Hom/A010	28.2	24.0
Grand mean	26.8	
Cochran's test		
C	0.317	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.70	
s_x	1.51	
s_w	2.24	
s_s	0.000	
Critical= 0.3 σ_P	2.01	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Senecivernine-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	18.8	22.4
Hom/A002	21.5	21.0
Hom/A003	23.8	19.7
Hom/A004	20.1	19.4
Hom/A005	18.7	18.1
Hom/A006	20.1	17.5
Hom/A007	21.1	20.0
Hom/A008	18.4	20.1
Hom/A009	21.4	21.5
Hom/A010	18.7	20.5
Grand mean	20.1	
Cochran's test		
C	0.380	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	5.03	
s_x	1.15	
s_w	1.50	
s_s	0.445	
Critical= 0.3 σ_P	1.51	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Seneciphylline in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	16.4	15.3
Hom/A002	15.8	16.2
Hom/A003	16.2	15.5
Hom/A004	15.7	15.6
Hom/A005	14.9	15.8
Hom/A006	14.8	15.6
Hom/A007	16.0	14.9
Hom/A008	16.6	14.5
Hom/A009	15.1	16.1
Hom/A010	15.6	15.3
Grand mean	15.6	
Cochran's test		
C	0.453	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.90	
s_x	0.254	
s_w	0.707	
s_s	0.000	
Critical= 0.3 σ_P	1.17	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Spartioidine in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	12.9	11.8
Hom/A002	12.9	13.2
Hom/A003	14.1	12.6
Hom/A004	13.3	12.9
Hom/A005	13.2	12.3
Hom/A006	12.2	12.6
Hom/A007	12.9	12.3
Hom/A008	14.1	12.2
Hom/A009	13.0	12.9
Hom/A010	12.5	12.8
Grand mean	12.8	
Cochran's test		
C	0.427	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.21	
s_x	0.347	
s_w	0.660	
s_s	0.000	
Critical= 0.3 σ_P	0.963	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Seneciphylline-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	30.7	32.1
Hom/A002	32.4	30.2
Hom/A003	33.8	32.0
Hom/A004	31.7	29.7
Hom/A005	30.8	30.3
Hom/A006	30.0	30.5
Hom/A007	24.2	29.4
Hom/A008	30.2	31.5
Hom/A009	30.1	32.2
Hom/A010	29.7	28.5
Grand mean	30.5	
Cochran's test		
C	0.539	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.63	
s_x	1.62	
s_w	1.56	
s_s	1.18	
Critical= 0.3 σ_P	2.29	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Spartioidine-N-oxide in A (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/A001	21.6	20.2
Hom/A002	21.6	20.9
Hom/A003	22.3	22.6
Hom/A004	20.8	20.6
Hom/A005	21.0	20.7
Hom/A006	20.0	20.1
Hom/A007		
Hom/A008	20.4	21.0
Hom/A009	20.9	21.7
Hom/A010	19.9	20.3
Grand mean	20.9	
Cochran's test		
C	0.533	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	5.23	
s_x	0.722	
s_w	0.476	
s_s	0.639	
Critical= 0.3 σ_P	1.57	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Europine in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	57.9	55.9
Hom/B002	56.4	52.3
Hom/B003	56.7	56.7
Hom/B004	54.1	52.6
Hom/B005	59.4	56.2
Hom/B006	57.2	56.0
Hom/B007	55.6	59.2
Hom/B008	57.4	54.1
Hom/B009	54.6	60.8
Hom/B010	58.4	53.4
Grand mean	56.2	
Cochran's test		
C	0.308	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	14.1	
s_x	1.44	
s_w	2.47	
s_s	0.000	
Critical= 0.3 σ_P	4.22	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Europine-N-oxide in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	677	693
Hom/B002	694	657
Hom/B003	678	680
Hom/B004	638	671
Hom/B005	689	653
Hom/B006	674	673
Hom/B007	675	667
Hom/B008	669	665
Hom/B009	670	687
Hom/B010	698	681
Grand mean	674	
Cochran's test		
C	0.294	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	169	
s_x	9.82	
s_w	15.3	
s_s	0.000	
Critical= 0.3 σ_P	50.58	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Heliotrine in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	93.4	91.8
Hom/B002	91.9	89.6
Hom/B003	90.8	93.7
Hom/B004	86.2	85.5
Hom/B005	92.9	90.0
Hom/B006	89.0	93.2
Hom/B007	88.3	90.6
Hom/B008	89.7	86.3
Hom/B009	86.8	94.7
Hom/B010	92.1	90.9
Grand mean	90.4	
Cochran's test		
C	0.507	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	22.6	
s_x	2.06	
s_w	2.48	
s_s	1.09	
Critical= 0.3 σ_P	6.78	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Heliotrine-N-oxide in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	935	915
Hom/B002	935	868
Hom/B003	929	937
Hom/B004	874	906
Hom/B005	928	895
Hom/B006	912	917
Hom/B007	931	898
Hom/B008	911	913
Hom/B009	936	922
Hom/B010	947	922
Grand mean	917	
Cochran's test		
C	0.501	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	229	
s_x	14.1	
s_w	21.2	
s_s	0.000	
Critical= 0.3 σ_P	68.74	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between sample standard deviation.

Sample No.	Lasiocarpine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	46.3	47.4
Hom/B002	48.6	44.9
Hom/B003	48.1	45.3
Hom/B004	44.3	41.5
Hom/B005	47.4	45.2
Hom/B006	45.8	47.2
Hom/B007	45.6	46.6
Hom/B008	45.5	43.6
Hom/B009	43.6	45.9
Hom/B010	47.4	41.2
Grand mean	45.6	
Cochran's test		
C	0.447	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.4	
s_x	1.35	
s_w	2.08	
s_s	0.000	
Critical= 0.3 σ_P	3.42	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Lasiocarpine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	533	538
Hom/B002	581	510
Hom/B003	589	538
Hom/B004	507	521
Hom/B005	559	526
Hom/B006	529	557
Hom/B007	557	521
Hom/B008	585	524
Hom/B009	562	555
Hom/B010	542	562
Grand mean	545	
Cochran's test		
C	0.331	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	136	
s_x	13.9	
s_w	27.5	
s_s	0.000	
Critical= 0.3 σ_P	40.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Echimidine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	48.7	48.6
Hom/B002	47.7	42.9
Hom/B003	43.6	46.2
Hom/B004	45.9	43.0
Hom/B005	51.4	45.6
Hom/B006	48.0	48.3
Hom/B007	47.1	50.9
Hom/B008	45.0	45.5
Hom/B009	51.3	48.8
Hom/B010	48.0	45.9
Grand mean	47.1	
Cochran's test		
C	0.350	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.8	
s_x	2.01	
s_w	2.21	
s_s	1.26	
Critical= 0.3 σ_P	3.53	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Heliosupine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	34.3	32.9
Hom/B002	30.1	30.6
Hom/B003	34.5	31.8
Hom/B004	29.5	29.8
Hom/B005	34.8	29.9
Hom/B006	30.8	33.0
Hom/B007	32.2	31.2
Hom/B008	33.6	32.7
Hom/B009	33.1	34.0
Hom/B010	32.1	31.5
Grand mean	32.1	
Cochran's test		
C	0.582	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.03	
s_x	1.35	
s_w	1.43	
s_s	0.891	
Critical= 0.3 σ_P	2.41	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Echimidine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	255	263
Hom/B002	276	248
Hom/B003	274	257
Hom/B004	245	245
Hom/B005	268	254
Hom/B006	254	268
Hom/B007	267	244
Hom/B008	278	254
Hom/B009	268	269
Hom/B010	270	269
Grand mean	261	
Cochran's test		
C	0.302	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	65.3	
s_x	7.23	
s_w	11.6	
s_s	0.000	
Critical= 0.3 σ_P	19.6	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Heliosupine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	32.1	34.1
Hom/B002	36.2	32.1
Hom/B003	36.6	34.8
Hom/B004	33.3	35.1
Hom/B005	36.2	35.6
Hom/B006	34.7	37.5
Hom/B007	36.0	33.4
Hom/B008	37.3	35.8
Hom/B009	37.2	34.5
Hom/B010	35.1	36.3
Grand mean	35.2	
Cochran's test		
C	0.327	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.80	
s_x	1.10	
s_w	1.64	
s_s	0.000	
Critical= 0.3 σ_P	2.64	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Intermedine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	65.6	64.8
Hom/B002	61.2	55.8
Hom/B003	59.1	61.0
Hom/B004	64.4	62.4
Hom/B005	62.7	58.8
Hom/B006	73.4	57.8
Hom/B007	66.3	62.9
Hom/B008	71.4	55.9
Hom/B009	57.0	60.1
Hom/B010	67.0	60.3
Grand mean	62.4	
Cochran's test		
C	0.402	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	15.6	
s_x	2.70	
s_w	5.49	
s_s	0.000	
Critical= 0.3 σ_P	4.68	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Lycopsamine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	46.9	45.2
Hom/B002	52.8	52.8
Hom/B003	49.7	56.0
Hom/B004	42.9	44.6
Hom/B005	53.2	50.4
Hom/B006	43.1	49.9
Hom/B007	45.3	51.4
Hom/B008	46.0	50.7
Hom/B009	48.5	56.2
Hom/B010	50.4	50.5
Grand mean	49.3	
Cochran's test		
C	0.273	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	12.3	
s_x	3.21	
s_w	3.31	
s_s	2.20	
Critical= 0.3 σ_P	3.70	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Echinatine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	112	111
Hom/B002	114	108
Hom/B003	108	115
Hom/B004	108	105
Hom/B005	114	110
Hom/B006	110	109
Hom/B007	112	113
Hom/B008	111	106
Hom/B009	108	121
Hom/B010	116	110
Grand mean	111	
Cochran's test		
C	0.512	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	27.7	
s_x	2.31	
s_w	4.14	
s_s	0.000	
Critical= 0.3 σ_P	8.32	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Rinderine in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	135	134
Hom/B002	132	126
Hom/B003	128	134
Hom/B004	128	129
Hom/B005	137	132
Hom/B006	131	130
Hom/B007	132	134
Hom/B008	132	127
Hom/B009	130	138
Hom/B010	136	132
Grand mean	132	
Cochran's test		
C	0.333	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	33.0	
s_x	2.31	
s_w	3.19	
s_s	0.495	
Critical= 0.3 σ_P	9.90	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Intermedine-N-oxide in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	84.2	83.3
Hom/B002	85.7	83.2
Hom/B003	91.7	88.5
Hom/B004	78.6	84.3
Hom/B005	82.1	76.0
Hom/B006	85.4	90.0
Hom/B007	84.5	80.7
Hom/B008	94.2	86.7
Hom/B009	84.8	91.6
Hom/B010	85.5	90.7
Grand mean	85.6	
Cochran's test		
C	0.224	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	21.4	
s_x	3.88	
s_w	3.56	
s_s	2.95	
Critical= 0.3 σ_P	6.42	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

	Lycopsamine-N-oxide in B (µg/kg)	
Sample No.	**Replicate 1**	**Replicate 2**
Hom/B001	81.5	76.2
Hom/B002	79.7	68.6
Hom/B003	68.0	69.8
Hom/B004	73.8	77.2
Hom/B005	81.6	85.1
Hom/B006	75.4	69.6
Hom/B007	78.3	83.5
Hom/B008	66.6	71.5
Hom/B009	84.0	71.2
Hom/B010	83.2	69.7
Grand mean	75.7	
Cochran's test		
C	0.300	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	18.9	
s_x	4.75	
s_w	5.52	
s_s	2.71	
Critical= 0.3 σ_P	5.68	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Echinatine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	131	126
Hom/B002	130	120
Hom/B003	131	126
Hom/B004	119	126
Hom/B005	130	121
Hom/B006	126	128
Hom/B007	128	129
Hom/B008	126	123
Hom/B009	130	124
Hom/B010	129	124
Grand mean	126	
Cochran's test		
C	0.313	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	31.6	
s_x	2.03	
s_w	4.35	
s_s	0.000	
Critical= 0.3 σ_P	9.47	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Rinderine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	219	214
Hom/B002	228	209
Hom/B003	216	218
Hom/B004	206	209
Hom/B005	217	209
Hom/B006	215	218
Hom/B007	220	217
Hom/B008	217	211
Hom/B009	228	220
Hom/B010	225	216
Grand mean	217	
Cochran's test		
C	0.553	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	54.2	
s_x	4.46	
s_w	5.60	
s_s	2.05	
Critical= 0.3 σ_P	16.3	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Retrorsine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	108	102
Hom/B002	110	98
Hom/B003	112	107
Hom/B004	103	107
Hom/B005	106	102
Hom/B006	108	99
Hom/B007	101	105
Hom/B008	107	103
Hom/B009	104	108
Hom/B010	106	105
Grand mean	105	
Cochran's test		
C	0.373	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	26.3	
s_x	1.78	
s_w	4.34	
s_s	0.00	
Critical= 0.3 σ_P	7.88	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Usaramine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	35.1	34.9
Hom/B002	36.5	32.6
Hom/B003	38.2	36.5
Hom/B004	32.0	36.1
Hom/B005	36.1	33.4
Hom/B006	36.1	31.2
Hom/B007	33.8	36.2
Hom/B008	33.3	35.0
Hom/B009	35.7	36.3
Hom/B010	36.5	33.6
Grand mean	35.0	
Cochran's test		
C	0.287	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.74	
s_x	1.06	
s_w	2.04	
s_s	0.000	
Critical= 0.3 σ_P	2.62	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Retrorsine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	437	424
Hom/B002	420	391
Hom/B003	440	411
Hom/B004	419	415
Hom/B005	435	414
Hom/B006	420	413
Hom/B007	449	406
Hom/B008	408	416
Hom/B009	423	436
Hom/B010	447	427
Grand mean	423	
Cochran's test		
C	0.378	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	106	
s_x	9.61	
s_w	15.7	
s_s	0.000	
Critical= 0.3 σ_P	31.7	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Usaramine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	44.8	42.6
Hom/B002	43.9	39.8
Hom/B003	46.0	41.8
Hom/B004	43.8	44.1
Hom/B005	42.9	42.9
Hom/B006	44.5	42.1
Hom/B007	45.7	42.4
Hom/B008	42.6	43.1
Hom/B009	43.2	47.3
Hom/B010	45.6	44.0
Grand mean	43.7	
Cochran's test		
C	0.232	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.9	
s_x	0.978	
s_w	1.96	
s_s	0.000	
Critical= 0.3 σ_P	3.27	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Integerrimine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	34.1	36.0
Hom/B002	34.9	33.0
Hom/B003	34.6	34.8
Hom/B004	34.1	32.0
Hom/B005	33.2	33.5
Hom/B006	34.3	33.7
Hom/B007	34.0	33.4
Hom/B008	36.8	34.3
Hom/B009	33.4	35.1
Hom/B010	31.6	35.8
Grand mean	34.1	
Cochran's test		
C	0.448	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.53	
s_x	0.782	
s_w	1.41	
s_s	0.000	
Critical= 0.3 σ_P	2.56	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecionine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	139	145
Hom/B002	146	144
Hom/B003	142	140
Hom/B004	139	134
Hom/B005	149	137
Hom/B006	148	140
Hom/B007	144	143
Hom/B008	145	144
Hom/B009	146	151
Hom/B010	139	140
Grand mean	143	
Cochran's test		
C	0.471	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	35.7	
s_x	3.30	
s_w	3.79	
s_s	1.93	
Critical= 0.3 σ_P	10.7	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecivernine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	41.3	44.8
Hom/B002	42.1	42.4
Hom/B003	43.3	43.4
Hom/B004	41.5	43.0
Hom/B005	44.5	40.4
Hom/B006	46.0	45.4
Hom/B007	42.4	45.6
Hom/B008	44.1	43.3
Hom/B009	45.1	43.0
Hom/B010	42.7	45.8
Grand mean	43.5	
Cochran's test		
C	0.293	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.9	
s_x	1.07	
s_w	1.67	
s_s	0.00	
Critical= 0.3 σ_P	3.26	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Integerrimine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	153	142
Hom/B002	157	140
Hom/B003	142	146
Hom/B004	145	152
Hom/B005	149	144
Hom/B006	146	149
Hom/B007	163	142
Hom/B008	153	147
Hom/B009	146	162
Hom/B010	162	151
Grand mean	149	
Cochran's test		
C	0.342	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	37.4	
s_x	3.70	
s_w	8.04	
s_s	0.000	
Critical= 0.3 σ_P	11.2	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecionine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	528	533
Hom/B002	569	501
Hom/B003	509	527
Hom/B004	498	521
Hom/B005	540	532
Hom/B006	542	526
Hom/B007	535	464
Hom/B008	517	519
Hom/B009	546	583
Hom/B010	572	547
Grand mean	531	
Cochran's test		
C	0.394	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	133	
s_x	20.4	
s_w	25.3	
s_s	9.90	
Critical= 0.3 σ_P	39.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Senecivernine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	161	135
Hom/B002	147	147
Hom/B003	135	153
Hom/B004	153	157
Hom/B005	144	140
Hom/B006	149	136
Hom/B007	167	161
Hom/B008	165	139
Hom/B009	141	158
Hom/B010	142	148
Grand mean	149	
Cochran's test		
C	0.310	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	37.2	
s_x	6.72	
s_w	10.7	
s_s	0.000	
Critical= 0.3 σ_P	11.2	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Seneciphylline in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	204	210
Hom/B002	209	203
Hom/B003	206	208
Hom/B004	202	200
Hom/B005	214	195
Hom/B006	213	204
Hom/B007	206	205
Hom/B008	210	209
Hom/B009	211	209
Hom/B010	203	216
Grand mean	207	
Cochran's test		
C	0.518	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	51.7	
s_x	2.76	
s_w	5.81	
s_s	0.000	
Critical= 0.3 σ_P	15.5	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Spartioidine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	29.5	30.3
Hom/B002	30.0	33.2
Hom/B003	32.6	29.0
Hom/B004	29.5	31.0
Hom/B005	31.1	28.0
Hom/B006	31.7	31.8
Hom/B007	31.0	32.4
Hom/B008	33.1	30.5
Hom/B009	32.8	30.5
Hom/B010	30.9	30.8
Grand mean	31.0	
Cochran's test		
C	0.269	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.75	
s_x	0.839	
s_w	1.57	
s_s	0.00	
Critical= 0.3 σ_P	2.32	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Seneciphylline-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	634	564
Hom/B002	614	569
Hom/B003	571	574
Hom/B004	591	596
Hom/B005	607	586
Hom/B006	624	598
Hom/B007	601	573
Hom/B008	593	590
Hom/B009	615	624
Hom/B010	615	604
Grand mean	597	
Cochran's test		
C	0.544	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	149	
s_x	13.5	
s_w	21.4	
s_s	0.00	
Critical= 0.3 σ_P	44.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Sample No.	Spartioidine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	112	104
Hom/B002	113	102
Hom/B003	104	107
Hom/B004	106	108
Hom/B005	109	106
Hom/B006	112	102
Hom/B007	113	99
Hom/B008	109	104
Hom/B009	106	112
Hom/B010	113	111
Grand mean	108	
Cochran's test		
C	0.342	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	26.9	
s_x	1.88	
s_w	5.39	
s_s	0.000	
Critical= 0.3 σ_P	8.07	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.
s_w = Within-sample standard deviation.
s_s = Between-sample standard deviation.

Annex 4 Statistical evaluation of stability data

Stability evaluation for senkirkine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	41.8	42.0
	44.6	43.9
	44.0	44.2
	44.5	41.8
	44.2	40.4
	43.6	41.1
Average amount (µg/kg)	43.8	42.3
n	6	6
st. dev (µg/kg)	1.02	1.51
Difference		1.54
$0.3*\sigma_P$		3.28
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echimidine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	49.8	54.8
	52.4	51.8
	57.2	49.6
	53.4	54.3
	52.5	54.1
	54.7	53.6
Average amount (µg/kg)	53.3	53.0
n	6	6
st. dev (µg/kg)	2.49	1.98
Difference		0.325
$0.3*\sigma_P$		4.00
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliosupine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	18.0	18.8
	19.3	18.6
	18.5	18.9
	19.6	18.1
	19.9	19.2
	20.5	19.6
Average amount (µg/kg)	19.3	18.9
n	6	6
st. dev (µg/kg)	0.924	0.504
Difference		0.462
$0.3*\sigma_P$		1.45
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echimidine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	259	265
	255	247
	264	246
	260	252
	246	266
	274	245
Average amount (µg/kg)	260	254
n	6	6
st. dev (µg/kg)	9.32	9.49
Difference		6.24
$0.3*\sigma_P$		19.5
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliosupine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	31.2	30.1
	32.4	32.3
	33.0	32.5
	32.7	33.4
	34.0	37.0
	35.2	35.3
Average amount (µg/kg)	33.1	33.4
n	6	6
st. dev (µg/kg)	1.37	2.43
Difference		-0.339
$0.3*\sigma_P$		2.48
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for intermedine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	13.7	11.0
	12.9	11.6
	11.4	14.3
	13.4	12.1
	11.5	13.3
	11.2	13.5
Average amount (µg/kg)	12.4	12.6
n	6	6
st. dev (µg/kg)	1.11	1.26
Difference		-0.283
$0.3*\sigma_P$		0.926
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lycopsamine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	21.1	24.9
	22.1	19.9
	22.0	22.3
	22.5	25.1
	21.3	22.3
	26.1	23.2
Average amount (µg/kg)	22.5	23.0
n	6	6
st. dev (µg/kg)	1.86	1.92
Difference		-0.430
$0.3*\sigma_P$		1.69
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for intermedine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	23.9	24.2
	25.6	21.6
	23.7	22.1
	20.0	24.3
	22.2	24.0
	21.9	22.5
Average amount (µg/kg)	22.9	23.1
n	6	6
st. dev (µg/kg)	1.96	1.18
Difference		-0.231
$0.3*\sigma_P$		1.72
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lycopsamine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	29.1	33.4
	29.8	31.6
	32.7	32.3
	39.3	31.7
	36.3	29.9
	34.6	35.5
Average amount (µg/kg)	33.6	32.4
n	6	6
st. dev (µg/kg)	3.90	1.89
Difference		1.22
$0.3*\sigma_P$		2.52
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	15.1	14.6
	15.8	16.6
	17.3	13.7
	16.1	15.1
	15.9	15.7
	14.8	14.6
Average amount (µg/kg)	15.8	15.1
n	6	6
st. dev (µg/kg)	0.903	1.01
Difference		0.783
$0.3*\sigma_P$		1.19
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	31.1	32.2
	32.7	31.7
	31.7	31.4
	32.1	30.8
	33.2	31.8
	33.5	30.0
Average amount (µg/kg)	32.4	31.3
n	6	6
st. dev (µg/kg)	0.903	0.815
Difference		1.06
$0.3*\sigma_P$		2.43
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for integerrimine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	18.4	17.8
	18.0	19.2
	18.4	18.2
	17.8	18.1
	19.8	18.5
	19.6	18.5
Average amount (µg/kg)	18.7	18.4
n	6	6
st. dev (µg/kg)	0.831	0.473
Difference		0.300
$0.3*\sigma_P$		1.40
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecionine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	13.4	15.3
	14.8	15.5
	15.1	15.3
	14.9	14.6
	16.1	15.3
	16.1	14.8
Average amount (µg/kg)	15.1	15.1
n	6	6
st. dev (µg/kg)	0.996	0.367
Difference		-0.075
$0.3*\sigma_P$		1.13
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecivernine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	13.9	13.1
	13.8	14.3
	12.4	12.7
	13.8	13.1
	15.0	14.4
	13.4	13.4
Average amount (µg/kg)	13.7	13.5
n	6	6
st. dev (µg/kg)	0.838	0.689
Difference		0.230
$0.3*\sigma_P$		1.03
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for integerrimine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	33.7	32.8
	33.4	35.4
	35.1	33.9
	33.8	30.3
	36.0	33.4
	37.0	31.3
Average amount (µg/kg)	34.8	32.9
n	6	6
st. dev (µg/kg)	1.46	1.83
Difference		1.97
$0.3*\sigma_P$		2.61
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecionine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	25.6	25.7
	28.6	29.2
	27.1	25.5
	29.3	25.4
	26.3	25.0
	27.7	27.2
Average amount (µg/kg)	27.5	26.3
n	6	6
st. dev (µg/kg)	1.39	1.58
Difference		1.14
$0.3*\sigma_P$		2.06
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecivernine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	19.5	17.8
	20.2	18.9
	19.0	18.9
	21.8	22.9
	24.9	20.6
	18.9	22.0
Average amount (µg/kg)	20.7	20.2
n	6	6
st. dev (µg/kg)	2.30	1.98
Difference		0.549
$0.3*\sigma_P$		1
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	17.6	17.2
	18.5	19.1
	17.8	18.8
	18.8	17.3
	19.8	20.3
	19.5	18.6
Average amount (µg/kg)	18.6	18.6
n	6	6
st. dev (µg/kg)	0.874	1.14
Difference		0.080
$0.3*\sigma_P$		1.40
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for spartioidine in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	14.3	13.0
	13.3	14.6
	12.5	14.3
	13.4	12.8
	14.6	14.1
	13.9	14.3
Average amount (µg/kg)	13.7	13.8
n	6	6
st. dev (µg/kg)	0.753	0.725
Difference		-0.166
$0.3*\sigma_P$		1.03
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	30.6	32.3
	29.9	31.5
	31.7	32.3
	30.3	29.9
	32.8	31.2
	32.4	30.5
Average amount (µg/kg)	31.3	31.3
n	6	6
st. dev (µg/kg)	1.19	0.963
Difference		0.012
$0.3*\sigma_P$		2.35
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for spartioidine-N-oxide in material A.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	22.0	21.7
	21.0	22.8
	22.9	22.5
	22.4	21.2
	24.6	22.5
	21.0	22.0
Average amount (µg/kg)	22.3	22.1
n	6	6
st. dev (µg/kg)	1.36	0.593
Difference		0.205
$0.3*\sigma_P$		1.67
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for europine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	59.1	61.4
	60.4	56.0
	61.4	56.2
	58.3	56.7
	60.2	60.7
	58.6	58.0
Average amount (µg/kg)	59.7	58.2
n	6	6
st. dev (µg/kg)	1.19	2.34
Difference		1.50
$0.3*\sigma_P$		4.48
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for europine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	624	675
	684	646
	679	602
	667	637
	638	685
	677	674
Average amount (µg/kg)	662	653
n	6	6
st. dev (µg/kg)	24.6	31.1
Difference		8.24
$0.3*\sigma_P$		49.6
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliotrine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	91.0	94.1
	95.4	87.9
	97.9	88.7
	94.5	88.0
	93.0	98.9
	94.6	89.6
Average amount (µg/kg)	94.4	91.2
n	6	6
st. dev (µg/kg)	2.34	4.43
Difference		3.16
$0.3*\sigma_P$		7.08
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliotrine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	897	960
	967	920
	948	881
	940	923
	907	933
	956	953
Average amount (µg/kg)	936	928
n	6	6
st. dev (µg/kg)	28.1	28.2
Difference		7.49
$0.3*\sigma_P$		70.2
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lasiocarpine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	44.7	46.8
	45.5	42.0
	49.8	39.1
	43.8	45.1
	44.6	45.5
	46.6	42.2
Average amount (µg/kg)	45.8	43.4
n	6	6
st. dev (µg/kg)	2.19	2.88
Difference		2.38
$0.3*\sigma_P$		3.44
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lasiocarpine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	534	572
	549	545
	548	527
	552	558
	523	573
	585	542
Average amount (µg/kg)	548	553
n	6	6
st. dev (µg/kg)	21.1	18.1
Difference		-4.35
$0.3*\sigma_P$		41.1
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echimidine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	49.8	54.8
	52.4	51.8
	57.2	49.6
	53.4	54.3
	52.5	54.1
	54.7	53.6
Average amount (µg/kg)	53.3	53.0
n	6	6
st. dev (µg/kg)	2.49	1.98
Difference		0.325
$0.3*\sigma_P$		4.00
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliosupine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	32.2	32.0
	31.7	29.9
	33.8	32.3
	30.3	31.6
	32.6	32.3
	32.5	31.7
Average amount (µg/kg)	32.2	31.6
n	6	6
st. dev (µg/kg)	1.17	0.881
Difference		0.552
$0.3*\sigma_P$		2.41
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echimidine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	259	265
	255	247
	264	246
	260	252
	246	266
	274	245
Average amount (µg/kg)	260	254
n	6	6
st. dev (µg/kg)	9.32	9.49
Difference		6.24
$0.3*\sigma_P$		19.5
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliosupine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	34.5	35.5
	37.0	35.7
	33.2	34.9
	36.3	38.6
	32.8	39.8
	35.9	33.7
Average amount (µg/kg)	35.0	36.3
n	6	6
st. dev (µg/kg)	1.73	2.33
Difference		-1.38
$0.3*\sigma_P$		2.62
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for intermedine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	56.6	57.6
	56.5	55.0
	68.7	66.5
	62.9	63.4
	67.7	54.9
	64.3	60.2
Average amount (µg/kg)	62.8	59.6
n	6	6
st. dev (µg/kg)	5.28	4.67
Difference		3.20
$0.3*\sigma_P$		4.71
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lycopsamine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	53.5	52.5
	64.9	52.8
	48.3	59.2
	51.6	47.3
	50.5	58.6
	52.8	54.1
Average amount (µg/kg)	53.6	54.1
n	6	6
st. dev (µg/kg)	5.84	4.40
Difference		-0.463
$0.3*\sigma_P$		4.02
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echinatine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	117	119
	119	118
	124	114
	119	112
	117	130
	121	116
Average amount (µg/kg)	119	118
n	6	6
st. dev (µg/kg)	2.72	6.19
Difference		0.987
$0.3*\sigma_P$		8.94
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for rinderine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	144	148
	143	139
	152	139
	141	138
	142	149
	145	144
Average amount (µg/kg)	144	143
n	6	6
st. dev (µg/kg)	3.92	5.04
Difference		1.51
$0.3*\sigma_P$		10.8
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for intermedine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	79.4	75.3
	71.3	75.3
	87.6	96.7
	82.3	88.2
	88.6	73.6
	91.3	85.3
Average amount (µg/kg)	83.4	82.4
n	6	6
st. dev (µg/kg)	7.37	9.22
Difference		1.02
$0.3*\sigma_P$		6.26
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lycopsamine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	72.8	72.8
	93.2	86.5
	68.2	66.9
	77.2	75.8
	80.0	91.5
	75.4	80.5
Average amount (µg/kg)	77.8	79.0
n	6	6
st. dev (µg/kg)	8.54	9.04
Difference		-1.21
$0.3*\sigma_P$		5.84
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for echinatine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	116	126
	132	125
	126	114
	122	122
	120	125
	128	125
Average amount (µg/kg)	124	123
n	6	6
st. dev (µg/kg)	5.76	4.39
Difference		1.13
$0.3*\sigma_P$		9.30
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for rinderine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	195	220
	223	216
	215	198
	211	206
	205	215
	221	222
Average amount (µg/kg)	212	213
n	6	6
st. dev (µg/kg)	10.7	9.21
Difference		-1.13
$0.3*\sigma_P$		15.9
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	85.2	97.0
	95.6	96.5
	106	89.0
	96.6	93.0
	92.3	93.6
	100	93.1
Average amount (µg/kg)	96.1	93.7
n	6	6
st. dev (µg/kg)	7.18	2.90
Difference		2.35
$0.3*\sigma_P$		7.20
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for usaramine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	29.8	32.4
	32.6	30.1
	38.9	30.1
	37.5	36.0
	33.3	30.1
	35.4	35.6
Average amount (µg/kg)	34.6	32.4
n	6	6
st. dev (µg/kg)	3.35	2.81
Difference		2.20
$0.3*\sigma_P$		2.59
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	393	427
	421	399
	434	387
	420	401
	394	445
	429	411
Average amount (µg/kg)	415	412
n	6	6
st. dev (µg/kg)	17.4	21.4
Difference		3.42
$0.3*\sigma_P$		31.1
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for usaramine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	38.9	45.0
	45.2	40.3
	44.4	37.2
	42.9	40.7
	38.9	45.9
	44.6	42.1
Average amount (µg/kg)	42.5	41.9
n	6	6
st. dev (µg/kg)	2.90	3.20
Difference		0.630
$0.3*\sigma_P$		3.19
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for integerrimine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	32.3	35.6
	36.1	33.8
	35.6	30.1
	34.4	33.5
	33.0	36.9
	37.0	32.8
Average amount (µg/kg)	34.7	33.8
n	6	6
st. dev (µg/kg)	1.82	2.35
Difference		0.967
$0.3*\sigma_P$		2.61
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecionine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	135	150
	145	143
	155	143
	146	144
	141	148
	143	144
Average amount (µg/kg)	144	145
n	6	6
st. dev (µg/kg)	6.59	2.79
Difference		-1.38
0.3*σ_P		10.8
Consequential difference? Diff < 0.3*σ_P		No

Stability evaluation for senecivernine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	41.0	46.3
	45.0	45.5
	45.4	45.3
	45.7	41.5
	42.5	48.1
	45.5	43.1
Average amount (µg/kg)	44.2	45.0
n	6	6
st. dev (µg/kg)	1.95	2.33
Difference		-0.765
0.3*σ_P		3.32
Consequential difference? Diff < 0.3*σ_P		No

Stability evaluation for integerrimine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	132	146
	144	134
	145	134
	146	141
	134	148
	145	135
Average amount (µg/kg)	141	140
n	6	6
st. dev (µg/kg)	6.22	6.23
Difference		1.60
0.3*σ_P		10.6
Consequential difference? Diff < 0.3*σ_P		No

Stability evaluation for senecionine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	447	444
	498	459
	420	472
	474	464
	470	463
	482	458
Average amount (µg/kg)	465	460
n	6	6
st. dev (µg/kg)	27.6	8.95
Difference		5.07
0.3*σ_P		34.9
Consequential difference? Diff < 0.3*σ_P		No

Stability evaluation for senecivernine-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	163	172
	153	175
	175	180
	163	148
	145	146
	152	153
Average amount (µg/kg)	159	162
n	6	6
st. dev (µg/kg)	10.5	14.7
Difference		-3.90
$0.3*\sigma_P$		11.9
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	200	219
	215	208
	231	212
	220	208
	212	222
	214	208
Average amount (µg/kg)	215	213
n	6	6
st. dev (µg/kg)	10.2	6.37
Difference		2.32
$0.3*\sigma_P$		16.1
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for spartioidine in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	32.0	34.9
	33.3	29.9
	35.8	31.6
	33.9	31.9
	33.0	32.4
	33.2	31.2
Average amount (µg/kg)	33.5	32.0
n	6	6
st. dev (µg/kg)	1.26	1.67
Difference		1.53
$0.3*\sigma_P$		2.51
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline-N-oxide in material B.

Storage temperature	freezer	fridge
Time (days)	0	50
Calculated amounts (µg/kg)	532	598
	592	574
	589	570
	606	607
	553	602
	606	576
Average amount (µg/kg)	580	588
n	6	6
st. dev (µg/kg)	30.4	16.4
Difference		-7.92
$0.3*\sigma_P$		43.5
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for spartioidine-N-oxide in material B.

Storage temperature	**freezer**	**fridge**
Time (days)	0	50
Calculated amounts (µg/kg)	92.3	104
	101	98.8
	106	96.6
	101	101
	95.6	106
	104	98.1
Average amount (µg/kg)	100	101
n	6	6
st. dev (µg/kg)	5.08	3.52
Difference		-0.683
$0.3*\sigma_P$		7.50
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

NRLs mycotoxins & plant toxins

Wageningen Food Safety Research

Natural toxins

DATE
December 14, 2021

SUBJECT
Invitation EURL mycotoxins & plant toxins proficiency test pyrrolizidine alkaloids in food matrices 2022 (EURLPT-MP07)

YOUR REFERENCE
NR

OUR REFERENCE
2135438/WFSR

POSTAL ADDRESS
P.O. Box 230
6700 AE Wageningen
The Netherlands

VISITORS' ADDRESS
Wageningen Campus
Building 123
Akkersmaalsbos 2
6708 WB Wageningen

INTERNET
www.wur.nl

CoC NUMBER
09098104

HANDLED BY
Ingrid Elbers

TELEPHONE
+31(0) 317 481451

EMAIL
pt.wfsr@wur.nl

Dear colleague,

The EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), will organize a proficiency test regarding pyrrolizidine alkaloids in food matrices in 2022 (EURLPT-MP07). This proficiency test will focus on the quantification of 35 pyrrolizidine alkaloids as mentioned in Regulation (EU) 2020/2040 and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013).

I would like to invite you to participate in this proficiency test.

Harmonised EU regulation for pyrrolizidine alkaloids in food is laid down in Regulation (EU) 2020/2040 and will apply from July 1, 2022. The primary goal of this proficiency test is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of these compounds in food and feed matrices.

According to Regulation (EU) 2017/625, it is mandatory for all EU National Reference Laboratories (NRLs) mycotoxins & plant toxins in food and/or feed to participate.

The following matters are important for participation in this proficiency test:

1. Test materials
   One black tea test material and one kitchen herb test material will be provided. The test amounts sent will be approximately 25 gram.

2. Shipment of the test materials
   Test materials will be send in the week of January 17, 2022. The distribution of the test materials will be announced by e-mail. The deadline for reporting will be six weeks after the shipment of the samples.

3. Scope of the analysis
   The materials contain one or more the following analytes, as defined in Regulation (EU) 2020/2040:
   - Echimidine (Em) and Echimidine-N-oxide (EmNO)
   - Europine (Eu) and Europine-N-oxide (EuNO)
   - Heliotrine (Ht) and Heliotrine-N-oxide (HtNO)
   - Intermedine (Im) and Intermedine-N-oxide (ImNO)
   - Lasiocarpine (Lc) and Lasiocarpine-N-oxide (LcNO)
   - Lycopsamine (Ly) and Lycopsamine-N-oxide (LyNO)
   - Retrorsine (Rt) and Retrorsine-N-oxide (RtNO)

Senecionine (Sn) and Senecionine-N-oxide (SnNO)
Seneciphylline (Sp) and Seneciphylline-N-oxide (SpNO)
Senecivernine (Sv) and Senecivernine-N-oxide (SvNO)
Senkirkine (Sk)
Indicine (Id) and Indicine-N-oxide (IdNO)
Echinatine (En) and Echinatine-N-oxide (EnNO)
Rinderine (Rn) and Rinderine-N-oxide (RnNO)
Integerrimine (Ir) and Integerrimine-N-oxide (IrNO)
Heliosupine (Hs) and Heliosupine-N-oxide (HsNO)
Spartioidine (St) and Spartioidine-N-oxide (StNO)
Usaramine (Us) and Usaramine-N-oxide (UsNO)

The participants should provide their own analytical standards.

4. Questionnaire

A questionnaire will be sent electronically. In this questionnaire the particants will be asked to provide information about the laboratory method(s) used. This information is necessary to conduct a more in depth analysis of the results obtained in this proficiency test.

2. Report

- Preliminary results of this proficiency test will be reported to the participants in May 2022.
- The report is expected to be dispatched in September 2022.
- Results of the proficiency test will be presented anonymously.
- Disclosure of the results of the NRLs to the representative of the European Commission is foreseen after the report is published.
- The follow-up protocol on proficiency test from DG Santé will be applied.

6. Additional information

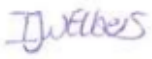
- WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
- WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

4. Costs

- Participation is free of charge for NRLs.
- Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee for OLs is €270,- (ex. VAT) as a compensation for the preparation and transportation of the samples.
- If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back **before January 10th 2022**: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiciency test,



Ingrid Elbers
Proficiency tests

EURL mycotoxins & plant toxins in food and feed
Wageningen Food Safety Research

Annex 6 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory mycotoxins & plant toxins

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

Dear Madam, Sir,

Thank you very much for your interest in the proficiency test regarding pyrrolizidine alkaloids in food.

The parcel shipped to you should contain:
- One black tea test material and one kitchen herb test material.
- Each test material unit contains approximately 25 grams of homogenised test material.

Instructions:
- After arrival the samples should be stored at +4°C.
- Please fill in the accompanying 'acknowledgement of receipt' form and return it immediately upon receipt of the samples, by e-mail to pt.wfsr@wur.nl.
- Before analysis, homogenise the samples according to your laboratory's procedure.
- Treat the test material as a sample for routine analysis. Report one result, and not an average of multiple measurements.
- Quantify the 35 PA's as defined in Regulation (EU) 2020/2040.
- **Report a total of 18 results, comprised of: 7 individual PA, 10 groups of isomeric PA's and the sum of total PA's:**

1. Europine	
2. Europine-N-oxide	
3. Heliotrine	
4. Heliotrine-N-oxide	
5. Lasiocarpine	
6. Lasiocarpine-N-oxide	
7. Senkirkine	
8. Echimidine group[1]	Echimidine Heliosupine
9. Echimidine N-oxide group	Echimidine-N-oxide Heliosupine-N-oxide
10. Intermedine group	Echinatine Indicine Intermedine Lycopsamine Rinderine)

[1] Report the sum of the PA's belonging to each group.

Wageningen Food Safety Research

Natural toxins

DATE
January 17, 2022

SUBJECT
EURLPT-MP07 Instruction PT pyrrolizidine alkaloids in food matrices

YOUR REFERENCE
-

OUR REFERENCE
NR

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P.O. Box 230
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CUC NUMBER
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eurl.mycotoxins-planttoxins@wur.nl

11. Intermedine N-oxide group	Echinatine-N-oxide Indicine-N-oxide Intermedine-N-oxide Lycopsamine-N-oxide Rinderine-N-oxide
12. Retrorsine group	Retrorsine Usaramine
13. Retrorsine N-oxide group	Retrorsine-N-oxide Usaramine-N-oxide
14. Senecionine group	Integerrimine Senecionine Senecivernine
15. Senecionine N-oxide group	Integerrimine-N-oxide Senecionine-N-oxide Senecivernine-N-oxide
16. Seneciphylline group	Seneciphylline Spartioidine
17. Seneciphylline N-oxide group	Seneciphylline-N-oxide Spartioidine-N-oxide
18. Sum of 35 PAs	

- Reporting:
  - Report all analytical results in **µg/kg**
  - Report other results as '**<[value µg/kg]**' or '**nt**' as follows:
    - '**<[value in µg/kg]**': When the result for the analyte is below the LOQ of the method, report the result as below the LOQ value in µg/kg, e.g. as '<10 µg/kg'.
    - '**nt**': If an analyte is not included in the scope of the method, report the result as not tested, 'nt'.
- Results reported in any other format (e.g. nd, <LOQ etc) will be regarded as not tested, 'nt'.
- Please use the web application to submit the results for the test materials (https://crlwebshop.wur.nl/apex/f?p=107:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- Provide detailed information in the questionnaire on the analysis of the multiple mycotoxins and the analytical method used and send it back to us by e-mail pt.wfsr@wur.nl.
- The deadline for submitting test-results for this test is **March 2nd 2022.**

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

Please contact me if you have any questions or need any assistance.

With kind regards,

Ingrid Elbers

Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research
Netherlands

Annex 7 Scope and LOQ

LOQs reported for individual PAs

	LOQ (µg/kg)																				
								Em Group		EmNO Group		Im Group					ImNO Group				
Lab code	**Eu**	**EuNO**	**Ht**	**HtNO**	**Lc**	**LcNO**	**Sk**	**Em**	**Hs**	**EmNO**	**HsNO**	**En**	**Id**	**Im**	**Ly**	**Rn**	**EnNO**	**IdNO**	**ImNO**	**LyNO**	**RnNO**
PT8583	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8584	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8588	10	10	10	10	10	10	10	10	10	11	11	12	12	12	12	12	13	13	13	13	13
PT8589																					
PT8592	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8593	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8594	5	5	5	5	5	5	5	5		5				5	5				5	5	
PT8595	0.27	0.27	0.23	0.26	0.22	0.23	0.24	0.27	0.27	0.23	0.23	0.24		0.22	0.26		0.26		0.27	0.26	
PT8596	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8597	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8598	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8599	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8600	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	-	2.5	2.5	2.5	2.5		2.5	2.5	2.5
PT8601	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8602	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8603	2.12	1.81	1.51	1.5	4.29	4.16	4.18	3.74		9.98				2.22	1.84				2.68	2.7	
PT8604	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT8605	5	5	2.5	2.5	5	2.5	20	5	5	2.5	10	10	2.5	5	2.5		2.5	2.5	2.5	2.5	
PT8606	10	10	10	10	10	-	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8607	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8608	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8609	4	4	4	4	4	4	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4
PT8610	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8611	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

	LOQ (µg/kg)													
	Rt Group		RtNO Group		Sn Group			SnNO Group			Sp Group		SpNO Group	
Lab code	Rt	Us	RtNO	UsNO	Ir	Sn	Sv	IrNO	SnNO	SvNO	Sp	St	SpNO	StNO
PT8583	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8584	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8588	10	10	11	11	12	12	12	13	13	13	10	10	11	11
PT8589														
PT8592	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8593	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8694	5	-	5	-	5	5	5	5	5	5	5	5	5	
PT8595	0.27	-	0.26	-	0.27	0.26	0.27	0.22	0.27	0.27	0.23		0.22	
PT8596	5	-	5	-	5	5	5	5	5	5	5		5	
PT8597	20	20	20	20	10	10	10	10	10	10	20	20	20	20
PT8598	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8599	5	5	10	10	10	10	5	5	5	5	5	5	5	5
PT8600	2.5	2.5	2.5			2.5	2.5		2.5	2.5	2.5		2.5	
PT8601	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8602	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8603	2.02		6.86			3.43	5.83		7.95	5.69	2.75		7.28	
PT8604	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT8605	100	100	10		25	10	25	25	25	10	10		5	
PT8606	10	10			10	10	10	10	10	10	10	10	10	
PT8607	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8608	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8609	4	4	4	4	4	4	4	4	4	4	4		4	
PT8610	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT8611	10	10	10	10	10	10	10	10	10	10	10	10	10	10

LOQs reported for PA-isomer groups

	LOQ (µg/kg)										
Lab code	**Em Group**	**EmNO Group**	**Im Group**	**ImNO Group**	**Rt Group**	**RtNO Group**	**Sn Group**	**SnNO Group**	**Sp Group**	**SpNO Group**	**Sum of 35 PAs**
PT8583	5	5	5	5	5	5	5	5	5	5	5
PT8584											
PT8588	10 each compound	11 each compound	12 each compound	13 each compound	10 each compound	11 each compound	12 each compound	13 each compound	10 each compound	11 each compound	10 each compound
PT8589											
PT8592	10	10	10	10	10	10	10	10	10	10	10
PT8593	10	10	10	10	10	10	10	10	10	10	10
PT8594	5	5	5	5	5	5	5	5	5	5	5
PT8595	0.54	0.46	0.72	0.79	0.25	0.26	0.8	0.76	0.23	0.22	6.75
PT8596	5	5	5	5	5	5	5	5	5	5	5
PT8597	10	10	10	10	20	20	10	10	20	20	20 (10)
PT8598	10	10	10	10	10	10	10	10	10	10	
PT8599	5	5	5	5	5	10	10	5	5	5	
PT8600	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
PT8601	5	5	5	5	5	5	5	5	5	5	5
PT8602	10	10	25	25	10	10	15	15	10	10	5
PT8603	3.74	9.98	1.84	2.68	2.02	6.86	3.43	5.69	2.75	7.28	
PT8604	2	2	2	2	2	2	2	2	2	2	2
PT8605	10	12.5	15	10	200	10	60	60	10	5	435
PT8606											
PT8607	10	10	10	10	10	10	10	10	10	10	10
PT8608	10	10	10	10	10	10	10	10	10	10	10
PT8609	4	4	4	4	4	4	4	4	4	4	4
PT8610	10	10	10	10	10	10	10	10	10	10	10
PT8611	10	10	10	10	10	10	10	10	10	10	10

Annex 8 Analytical method details

Lab code	Method	Column	Column Length (mm)	Total run Time (min)	Retention time (min)										
												Em Group		EmNO Group	
					Eu	EuNO	Ht	HtNO	Lc	LcNO	Sk	Em	Hs	EmNO	HsNO
PT8593	Acid	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.1	2.25	2.46	4.15	4.44	8.45	9.10	6.41	6.68	6.53	6.55	7.29
PT8594	Acid	Phenomenex Kinetex, XB-C18, 2.1x150 mm, 1.7 µm	150	20	5.79	6.87	10.87	11.87	16.87	17.23	15.52	15.45		15.30	
PT8596	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	30	6.76	7.49	11.05	11.88	18.28	18.62	15.61	15.71	15.43	15.56	17.18
PT8597	Acid	Phenomenex Gemini C18 110Å, 3x150 mm, 3 µm	150	21	4.74	5.85	9.33	11.58	15.55	16.43	14.31	14.33	14.33	14.65	15.24
PT8598	Acid	Thermo Hypersil Gold C18, 2.1x150 mm, 1.8 µm	150	15		5.4	6.4	6.7	9.0	9.1	8.0	7.2	7.3	7.25	7.89
PT8599	Acid	ThermoFisher Hypersil GOLD C18, 2.1x150 mm, 3 µm	150	15.5	4.134	4.681	6.384	7.127	10.637	10.92	10.059	9.828	9.828	9.567	10.43
PT8600	Acid	Phenomenex Luna C18, 2.0x150 mm; Phenomenex Kinetex Phenylhexyl, 2.1x150 mm	150	25; 37	8.18	8.94	10.76	11.79	16.84	17.06	15.38	14.89	15.18	15.40	16.75
PT8601	Acid	Waters Acquity UPLC BEH C8 2.1x100 mm, 1.7µm	100	17	6.2		9.5		14.5		13.3	13.2	13.2	12.4	13.4
PT8602	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	16											
PT8603	Acid	Waters Xbridge BEH C18 XP, 2.1x100 mm, 2.5 µm	100	12	4.05	4.52	5.42	5.70	7.03	7.25	6.62	6.51		6.58	
PT8605	Acid	Phenomenex Luna Omega C18 100 Å, 2.1x100 mm, 1.6 µm	100	32	9.22	6.63	8.84	9.23	14.16	15.20	12.09	12.06	11.93	11.83	12.91
PT8607	Acid	Phenomenex Kinetex Biphenyl, 2.1x150 mm, 1.7 µm	150	26	7.52	7.85	9.94	10.38	14.22	14.90	12.79	12.21	12.39	12.47	13.41
PT8610	Acid	Agilent Poroshell 120 SB C8, 2.1x100 mm, 2.7 µm	100	22	7.9	8.6	10.2	10.8	14.3	15.2	12.7	12.7	12.8	12.7	13.7
PT8583	Alkaline	Waters Atlantis T3, 100 Å, 3x150 mm, 3 µm	150	15	7.45	5.52	9.70	6.68	13.33	9.41	8.32	12.37	12.37	8.35	8.35
PT8584	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	15	6.16	3.46	8.12	5.44	11.27	8.21	7.01	10.54	10.4	7.34	7.05
PT8588	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm		15											
PT8589	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.20	3.64	8.16	5.58	11.23	8.30	7.14	10.61	10.61	7.49	7.19
PT8592	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.71	4.02	8.75	5.98	11.64	8.70	7.58	10.96	10.86	7.86	7.55
PT8595	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.08	3.62	8.05	5.58	11.32	8.30	7.12	10.69	10.43	7.40	7.16
PT8604	Alkaline	Phenomenex Gemini-NX C18, 2x150 mm, 5 µm	150	24.2	9.93	6.04	12.32	8.66	18.46	12.56	10.81	16.34	16.03	11.4	11.08
PT8606	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1,7 µm	150	14.2	4.5	2.4	5.85	4.3	10.1		4.9	9.4	9.05	4.9	4.7
PT8607	Alkaline	Phenomenex Kinetex C18 EVO, 2.1x150 mm, 1.7 µm	150	18	6.54	3.65	8.44	5.53	11.66	8.41	7.21	10.82	10.70	7.58	7.31
PT8608	Alkaline	Zorbax Eclipse Plus C18, 2.1x100 mm, 1.8 µm	100	14.20	6.99	4.23	8.89	6.18	11.78	8.86	7.77	11.05	10.96	8.05	7.77
PT8609	Alkaline	Waters Acquity UPLC CSH C18, 2.1x150 mm, 1.7 µm	150	20	8.31	4.77	11.05	7.31	13.92	11.06	9.41	13.02	12.93	9.96	9.53
PT8611	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.20	6.32	3.92	8.18	5.84	11.56	8.35	7.14	10.98	10.85	7.39	7.11

Lab code	Method	Column	Column Length (mm)	Total run Time (min)	Retention time (min)									
					Im Group					ImNO Group				
					En	Id	Im	Ly	Rn	EnNO	IdNO	ImNO	LyNO	RnNO
PT8593	Acid	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.1	2.35	2.33	2.22	2.29	2.36	2.59	2.77	2.74	2.83	2.57
PT8594	Acid	Phenomenex Kinetex, XB-C18, 2.1x150 mm, 1.7 µm	150	20			4.79	5.22				7.5	8.15	
PT8596	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	30	7.14	6.98	6.68	6.98	7.14	8.03	8.31	8.31	8.60	7.85
PT8597	Acid	Phenomenex Gemini C18 110Å, 3x150 mm, 3 µm	150	21	4.51	4.51	4.25	4.51	4.51	6.19	5.83	6.19	6.67	6.19
PT8598	Acid	Thermo Hypersil Gold C18, 2.1x150 mm, 1.8 µm	150	15	4.64	4.65	4.51	4.65	4.59	5.03	5.13	5.08	5.22	4.94
PT8599	Acid	ThermoFisher Hypersil GOLD C18, 2.1x150 mm, 3 µm	150	15.5	4.318	4.318	4.017	4.318	4.318	5.063	5.063	5.063	5.357	4.769
PT8600	Acid	Phenomenex Luna C18, 2.0x150 mm; Phenomenex Kinetex Phenylhexyl, 2.1x150 mm	150	25; 37	12.53		10.54	11.22	12.23	13.74		13.92	14.47	13.36
PT8601	Acid	Waters Acquity UPLC BEH C8 2.1x100 mm, 1.7µm	100	17	6.1	6.1	5.8	6.1	6.1	5.6	5.8	5.8	6.1	5.4
PT8602	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	16										
PT8603	Acid	Waters Xbridge BEH C18 XP, 2.1x100 mm, 2.5 µm	100	12			3.99	4.25				4.75	4.87	
PT8605	Acid	Phenomenex Luna Omega C18 100 Å, 2.1x100 mm, 1.6 µm	100	32	6.25	6.05	5.85	6.05		6.70	6.91	6.91	7.13	
PT8607	Acid	Phenomenex Kinetex Biphenyl, 2.1x150 mm, 1.7 µm	150	26	7.43	6.95	6.66	6.83	7.42	7.78	7.98	7.89	8.04	7.82
PT8610	Acid	Agilent Poroshell 120 SB C8, 2.1x100 mm, 2.7 µm	100	22	7.6	7.5	7.1	7.5	7.7	8.3	8.8	8.8	9.1	8.5
PT8583	Alkaline	Waters Atlantis T3, 100 Å, 3x150 mm, 3 µm	150	15	7.67	7.07	7.07	7.07	7.67	5.47	5.47	5.47	5.47	5.47
PT8584	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	15	6.43	5.61	5.49	5.53	6.55	3.69	3.49	3.34	3.38	3.79
PT8588	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm		15										
PT8589	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.46		5.45	5.50		3.82		3.42	3.44	
PT8592	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.98	6.08	6.08	6.08	7.13	4.20	4.00	3.83	3.83	4.31
PT8595	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	6.35		5.45	5.50		3.83		3.42	3.48	
PT8604	Alkaline	Phenomenex Gemini-NX C18, 2x150 mm, 5 µm	150	24.2	10.28	9.10	9.04	9.19	10.35	6.24	5.74	5.45	5.63	6.24
PT8606	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	4.3	4.65	4.35	4.35	4.6	2.45	2.45	2.35	2.35	2.7
PT8607	Alkaline	Phenomenex Kinetex C18 EVO, 2.1x150 mm, 1.7 µm	150	18	6.79	5.85	5.85	5.93	6.90	3.77	3.53	3.39	3.46	3.83
PT8608	Alkaline	Zorbax Eclipse Plus C18, 2.1x100 mm, 1.8 µm	100	14.20	7.26	6.37	6.35	6.43	7.40	4.50	4.04	4.02	4.12	4.52
PT8609	Alkaline	Waters Acquity UPLC CSH C18, 2.1x150 mm, 1.7 µm	150	20	8.73	7.5	7.45	7.55	8.93	5.13	4.72	4.47	4.62	5.2
PT8611	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.20	6.54	5.85	5.85	5.85	6.67	4.08	3.89	3.74	3.77	4.17

Lab code	Method	Column	Column Length (mm)	Total run Time (min)	Retention time (min)									
					Rt Group		RtNO Group		Sn Group			SnNO Group		
					Rt	Us	RtNO	UsNO	Ir	Sn	Sv	IrNO	SnNO	SvNO
PT8593	Acid	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.1	3.76	3.62	3.88	3.77	5.31	5.56	5.49	5.59	5.84	5.66
PT8594	Acid	Phenomenex Kinetex, XB-C18, 2.1x150 mm, 1.7 µm	150	20	10.08		10.58		13.09	13.57	13.28	13.68	14.30	14.16
PT8596	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	30	10.61		11.01		13.44	13.90	13.90	14.23	14.66	14.23
PT8597	Acid	Phenomenex Gemini C18 110Å, 3x150 mm, 3 µm	150	21	9.03	9.03	11.35	11.35	13.07	13.23	12.87	14.11	14.11	13.83
PT8598	Acid	Thermo Hypersil Gold C18, 2.1x150 mm, 1.8 µm	150	15	5.55	5.78	5.72	5.78	6.47	6.53	6.43	6.78	6.78	6.65
PT8599	Acid	ThermoFisher Hypersil GOLD C18, 2.1x150 mm, 3 µm	150	15.5	5.975	5.975	6.32	6.32	7.929	7.929	7.594	8.426	8.426	8.004
PT8600	Acid	Phenomenex Luna C18, 2.0x150 mm; Phenomenex Kinetex Phenylhexyl, 2.1x150 mm	150	25; 37	10.08		10.75			12.51	12.19		13.63	13.22
PT8601	Acid	Waters Acquity UPLC BEH C8 2.1x100 mm, 1.7µm	100	17	8.7	8.7	7.6	7.6	11.2	11.6	11.2	10.6	10.8	10.1
PT8602	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	16										
PT8603	Acid	Waters Xbridge BEH C18 XP, 2.1x100 mm, 2.5 µm	100	12	5.14		5.32			5.93	5.85		6.24	6.12
PT8605	Acid	Phenomenex Luna Omega C18 100 Å, 2.1x100 mm, 1.6 µm	100	32	8.48	8.39	8.62		10.29	10.29	10.52	10.98	11.18	10.81
PT8607	Acid	Phenomenex Kinetex Biphenyl, 2.1x150 mm, 1.7 µm	150	26	9.8	9.99	10.74	10.99	11.56	11.56	11.56	12.66	12.53	12.52
PT8610	Acid	Agilent Poroshell 120 SB C8, 2.1x100 mm, 2.7 µm	100	22	9.75	9.75	10.2	10.5	11.4	11.3	11.2	12.4	12.2	12.0
PT8583	Alkaline	Waters Atlantis T3, 100 Å, 3x150 mm, 3 µm	150	15	9.78	9.78	6.44	6.44	12.34	12.34	12.34	7.74	7.74	7.74
PT8584	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	15	8.48	8.20	5.24	5.09	9.97	10.24	10.40	6.42	6.60	6.64
PT8588	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm		15										
PT8589	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	8.62		5.42		10.14	10.28	10.36	6.56	6.80	6.75
PT8592	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	9.05	8.76	5.78	5.64	10.57	10.76	10.88	6.93	7.12	7.12
PT8595	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	8.59		5.44		10.14	10.36	10.52	6.58	6.80	6.75
PT8604	Alkaline	Phenomenex Gemini-NX C18, 2x150 mm, 5 µm	150	24.2	13.12	12.83	8.62	8.50	15.4	15.75	16.06	10.16	10.34	10.34
PT8606	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1,7 µm	150	14.2	6.2	5.8			8.45	9.9	9.1	4.55	4.65	4.65
PT8607	Alkaline	Phenomenex Kinetex C18 EVO, 2.1x150 mm, 1.7 µm	150	18	8.74	8.53	5.41	5.29	10.35	10.6	10.81	6.64	6.78	6.85
PT8608	Alkaline	Zorbax Eclipse Plus C18, 2.1x100 mm, 1.8 µm	100	14.20	9.20	8.98	6.04	5.92	7.22	11.00	11.16	7.22	7.38	7.47
PT8609	Alkaline	Waters Acquity UPLC CSH C18, 2.1x150 mm, 1.7 µm	150	20	11.48	11.20	7.17	7.01	12.78	12.94	13.11	8.74	8.97	9.11
PT8611	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.20	8.70	8.34	5.69	5.56	10.34	10.66	10.83	6.60	6.75	6.80

Lab code	Method	Column	Column Length (mm)	Total run Time (min)	Retention time (min)			
					Sp Group		SpNO Group	
					Sp	St	SpNO	StNO
PT8593	Acid	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.1	4.33	4.20	4.65	4.48
PT8594	Acid	Phenomenex Kinetex, XB-C18, 2.1x150 mm, 1.7 µm	150	20	10.96	10.72	12.15	
PT8596	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	30	11.75		12.64	
PT8597	Acid	Phenomenex Gemini C18 110Å, 3x150 mm, 3 µm	150	21	9.91	9.91	12.21	12.21
PT8598	Acid	Thermo Hypersil Gold C18, 2.1x150 mm, 1.8 µm	150	15	5.77	5.78	6.03	6.03
PT8599	Acid	ThermoFisher Hypersil GOLD C18, 2.1x150 mm, 3 µm	150	15.5	6.34	6.34	6.913	6.913
PT8600	Acid	Phenomenex Luna C18, 2.0x150 mm; Phenomenex Kinetex Phenylhexyl, 2.1x150 mm	150	25; 37	10.66		11.68	
PT8601	Acid	Waters Acquity UPLC BEH C8 2.1x100 mm, 1.7µm	100	17	9.3	9.3	8.6	8.6
PT8602	Acid	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	16				
PT8603	Acid	Waters Xbridge BEH C18 XP, 2.1x100 mm, 2.5 µm	100	12	5.29		5.59	
PT8605	Acid	Phenomenex Luna Omega C18 100 Å, 2.1x100 mm, 1.6 µm	100	32	9.15		9.63	
PT8607	Acid	Phenomenex Kinetex Biphenyl, 2.1x150 mm, 1.7 µm	150	26	10.38	10.39	11.59	11.59
PT8610	Acid	Agilent Poroshell 120 SB C8, 2.1x100 mm, 2.7 µm	100	22	10.2	10.2	11.1	11.1
PT8583	Alkaline	Waters Atlantis T3, 100 Å, 3x150 mm, 3 µm	150	15	11.23	11.23	6.94	6.94
PT8584	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	15	9.22		5.69	
PT8588	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm		15				
PT8589	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	9.39		5.88	
PT8592	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	9.88	9.61	6.25	6.14
PT8595	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	9.37		5.89	
PT8604	Alkaline	Phenomenex Gemini-NX C18, 2x150 mm, 5 µm	150	24.2	14.23	13.88	9.15	9.04
PT8606	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1,7 µm	150	14.2	7.4	6.95	4.4	
PT8607	Alkaline	Phenomenex Kinetex C18 EVO, 2.1x150 mm, 1.7 µm	150	18	9.64	9.38	5.94	5.81
PT8608	Alkaline	Zorbax Eclipse Plus C18, 2.1x100 mm, 1.8 µm	100	14.20	10.18	9.90	6.55	6.42
PT8609	Alkaline	Waters Acquity UPLC CSH C18, 2.1x150 mm, 1.7 µm	150	20	12.29	12.06	7.78	7.61
PT8611	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.20	9.57	9.27	6.06	5.97

Lab. code	Sample weight (g)	Extraction solvent	Extr. solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Mobile phase	Detection technique
PT8583	2	2% formic acid	40	mechanical shaking	30	SPE	Strata-X 33 µm 200 mg/6 mL	5	0.05	A: 10 mM ammonium carbonate in water; B: acetonitrile	MS/MS
PT8584	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Strata-X 200 mg/6 ml	6	2	A: 10 mM ammonium carbonate pH 9; B: acetonitrile	MS/MS
PT8588	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Strata-X 200 mg/6 ml	6	2	A: 10 mM ammonium carbonate pH 9; B: acetonitrile	MS/MS
PT8589	2	0.2% formic acid	40	mechanical shaking	30	SPE	Phenomenex, Strata Polymeric SPE, 200 mg/6ml	5	0.05	A: 10 mM ammonium carbonate in water; B: acetonitrile	MS/MS
PT8592	2	0.2% formic acid (aq)	40	mechanical shaking	30	SPE	Strata-X 33 µm Polymeric Reversed Phase 200 mg/6mL	6	4	A: 10 mM ammonium carbonate in water, pH 9; B: acetonitrile	MS/MS
PT8593	2	0.05M H_2SO_4	40	ultrasonic	30	SPE	Discovery DSC-18, 500mg/6mL	10	0.5	A: H_2O + 0.1% HCOOH + 5 mM $HCOONH_4$; B: acetonitrile	MS/MS
PT8594	5	0.05 M H_2SO_4	40	mechanical shaking	30	SPE	MCX	15	1.87	A: 0.3% formic acid; B: MeOH:ACN (3:2, v/v)	MS/MS
PT8595	2	0.2% formic acid	40	mechanical shaking	30	SPE	Bond Elut C18, 500 mg/6 ml	6	1.2	A: 10 mM ammonium carbonate in water; B: acetonitrile	MS/MS
PT8596	2	2% formic acid	40	mechanical shaking	30	SPE	Macherey-Nagel; Chromabond HR-X, 45 µm, 3 ml/60 mg	5	0.5	A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile	MS/MS
PT8597	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Oasis HLB 200 mg/6cc	5	0.5	A: 0.1% formic acid in water; B: acetonitrile	MS/MS
PT8598	2	0.05 M H_2SO_4	2x20	ultrasonic	2x15	dilution			0.005	A: 0.005 mol ammonium formate, 0.1% formic acid in water; B: 0.005 mol ammonium formate 0.1% formic acid in 95% MeOH	MS/MS
PT8599	2	MeOH/H_2O/formic acid	20	ultrasonic	2	dilution	-	-	0.05	A: H_2O/formic acid/ammonium formate; B: methanol	MS/MS
PT8600	5	aqueous sulfuric acid solution	50	ultrasonic	15	SPE	C18	5	0.5	A: 5 mmol/l ammonium formate and 0.1% formic acid in water; B: 5 mmol/l ammonium formate and 0.1% formic acid in methanol	MS/MS

Lab. code	Sample weight (g)	Extraction solvent	Extr. solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)	Mobile phase	Detection technique
PT8601	1	0.2% formic acid in water	25	mechanical shaking	45	SPE	OASIS, MCX 3cc	10	0.4	A: ammonium formate 5 mM 0.1% formic acid H_2O; B: ammonium formate 5 mM 0.1% formic acid MeOH	MS/MS
PT8602	2	H_2O + 0.2% formic acid	40	mechanical shaking	30	SPE	Strata-X 200mg	5	0.5	A: H_2O + 0.1% formic acid; B: acetonitrile + 0.1% formic acid	MS/MS
PT8603	1	3% formic acid	2x10	mechanical shaking	15	dilution			0.05	A: 10 mM $HCOONH_4$ + 10 mM HCOOH in H_2O B: 10 mM $HCOONH_4$ + 10 mM HCOOH in MeOH	MS/MS
PT8604	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Phenomenex Strata X	5	0.5	A: 10 mM ammonium carbonate in water; B: acetonitrile	MS/MS
PT8605	2	1% formic acid in acetonitrile:water (1:1, v/v)	20	mechanical shaking	10	other				A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile	MS/MS
PT8606	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Strata-X	5	0.5	A: 10 mM ammonium carbonate in water, pH 9; B: acetonitrile	MS/MS
PT8607	2	H_2SO_4 0.05M	2x20	ultrasonic	2x15	SPE	Supelco DSC-C18 6 ml 500 mg	10	0.5	A: 10 mM ammonium carbonate; B: acetonitrile	MS/MS
										A: water; HCOOH 0.1%; NH_4+$HCOO^-$: 315 mg/L; B: methanol; HCOOH 0.1%; NH_4+$HCOO^-$: 315 mg/L	MS/MS
PT8608	2	0.2% formic acid in water	10	mechanical shaking	30	SPE	Strata-X	6	0.05	A: water, 10 mM ammonium carbonate; B: acetonitrile	MS/MS
PT8609	1	acetontrile:water (10+2)	12	mechanical shaking	30	other			0.1	A: 10 mM ammonium carbonate; B = acetonitrile	HRMS
PT8610	2	0.2% formic acid	40	mechanical shaking	30	SPE	Strata-X 200 mg	5	0.5	A: 0.1% formic acid, 2.5 nM NH_3; B: methanol	MS/MS
PT8611	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Waters OASIS HLB Sep-pak	5	0.05	A: 10 mM ammonium carbonate: B: acetonitrile	MS/MS

ACN = acetonitrile; MeOH = methanol; FA = HCOOH = formic acid; H_2SO_4 = sulfuric acid; $HCOONH_4$ = NH_4+$HCOO^-$ = ammonium formate; NH_3 = ammonia.

Annex 9 Results material A (black tea)

Lab code	Europine A: nr u: nr σ_p: nr robust σ: nr		Europine-N-oxide A: nr u: nr σ_p: nr robust σ: nr		Heliotrine A: nr u: nr σ_p: nr robust σ: nr		Heliotrine-N-oxide A: nr u: nr σ_p: nr robust σ: nr	
	Result (µg/kg)	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**
		No statistical evaluation possible		No statistical evaluation possible		No statistical evaluation possible		No statistical evaluation possible
PT8583	nd		nd		34.9	**FP**	nd	
PT8588	nd		nd		nd		nd	
PT8589	<10		<10		<10		<10	
PT8592	nd		nd		nd		nd	
PT8593	<10		<10		<10		<10	
PT8595	nd		nd		nd		nd	
PT8596	nd		nd		nd		nd	
PT8597	<10		<10		<10		<10	
PT8598	nd		nd		nd		nd	
PT8599	<5		<5		<5		<5	
PT8600	nd		nd		nd		nd	
PT8601	nd		nd		nd		nd	
PT8602	nd		nd		nd		nd	
PT8603	nd		nd		nd		nd	
PT8604	<2		<2		<2		<2	
PT8605	<5		<5		<2.5		<2.5	
PT8606	<10		<10		<10		<10	
PT8607	nd		nd		nd		nd	
PT8608	nd		nd		nd		nd	
PT8609	nd		95	**FP**	nd		nd	
PT8610	<10		<10		<10		<10	
PT8611	nd		< 10		<10		<10	
PT8584#	nd		nd		nd		nd	
PT8594#	nd		nd		nd		nd	

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nr = not relevant.

FP = false positive.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

Lab code	Lasiocarpine A: nr u: nr σ_p: nr robust σ: nr		Lasiocarpine-N-oxide A: nr u: nr σ_p: nr robust σ: nr		Senkirkine A: 37.5 µg/kg u: 4.05 µg/kg σ_p: 9.37 µg/kg (25%) robust σ: 14.5 µg/kg (39%)	
	Result (µg/kg)	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**
		No statistical evaluation possible		No statistical evaluation possible		
PT8583	11.5	**FP**	nd		<5	**[-3.18] FN**
PT8588	nd		nd		50.6	1.29
PT8589	<10		<10		43.7	0.61
PT8592	nd		nd		19.2	-1.79
PT8593	<10		<10		<10	**[-2.69] FN**
PT8595	nd		nd		61	**2.31**
PT8596	nd		nd		63.1	**2.51**
PT8597	<10		<10		32.2	-0.52
PT8598	nd		nd		22	-1.52
PT8599	<5		<5		43	0.54
PT8600	nd		nd		17.2	-1.99
PT8601	nd		nd		35.5	-0.19
PT8602	nd		nd		46.8	0.92
PT8603	nd		nd		42.7	0.51
PT8604	<2		<2		43.2	0.56
PT8605	<5		<2.5		35.7	-0.17
PT8606	<10		nt		40	0.25
PT8607	nd		nd		11.6	**-2.53**
PT8608	nd		nd		34.1	-0.33
PT8609	nd		nd		29.6	-0.77
PT8610	<10		<10		23	-1.42
PT8611	<10		<10		58	**2.01**
PT8584#	nd		nd		41.64	0.41
PT8594#	nd		nd		29.5	-0.78

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nd = not detected.

nr = not relevant.

nt = not tested.

FP = false positive.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

Lab code	Echimidine group A: 16.4 µg/kg u: 1.36 µg/kg σ_p: 4.10 µg/kg (25%) robust σ: 4.88 µg/kg (30%)		Echimidine-N-oxide group A: 24.0 µg/kg u: 2.90 µg/kg σ_p: 5.99 µg/kg (25%) robust σ: 10.6 µg/kg (44%)		Intermedine group A: 37.4 µg/kg u: 5.62 µg/kg σ_p: 9.34 µg/kg (25%) robust σ: 21.1 µg/kg (56%)		Intermedine-N-oxide group A: 60.4 µg/kg u: 4.45 µg/kg σ_p: 15.1 µg/kg (25%) robust σ: 16.7 µg/kg (28%)	
	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z-score
PT8583	27.0	**2.45**	<5	**[-2.85] FN**	172.2	**12.37**	272.4	**14.03**
PT8588	18.3	0.44	31.2	1.09	50.1	1.17	69.7	0.61
PT8589	13	-0.79	22.3	-0.25	34.9	-0.23	55.9	-0.30
PT8592	<10	[-1.48]	14.5	-1.42	12.2	**-2.31**	23.3	**-2.46**
PT8593	14.99	-0.33	24.1	0.02	11.01	**-2.42**	48.4	-0.80
PT8595	18.5	0.48	30.1	0.92	34.7	-0.25	55.8	-0.31
PT8596	21.5	1.18	15.3	-1.30	18.2	-1.76	46.6	-0.92
PT8597	<10	[-1.48]	12.9	-1.66	58.2	1.91	55.4	-0.33
PT8598	10	-1.48	10	**-2.10**	27	-0.95	35	-1.68
PT8599	12	-1.02	25	0.16	37	-0.03	60	-0.03
PT8600	18.5	0.48	29.9	0.89	33	-0.40	69.9	0.63
PT8601	11.1	-1.23	20.1	-0.58	48.2	0.99	63.4	0.20
PT8602	11.5	-1.14	12	-1.80	19.5	-1.64	84.9	1.62
PT8603	14.2	-0.51	11.7	-1.84	36.9	-0.04	57	-0.23
PT8604	30	**3.14**	32.4	1.27	40.7	0.30	48.2	-0.81
PT8605	44.7	**6.54**	59.1	**5.28**	72.2	**3.19**	124	**4.21**
PT8606	20	0.83	50	**3.91**	160	**11.25**	140	**5.27**
PT8607	14	-0.56	27.4	0.52	15.9	-1.97	60.5	0.00
PT8608	17.8	0.32	23.4	-0.08	11.3	**-2.39**	43.9	-1.09
PT8609	12.6	-0.88	27.6	0.55	31.2	-0.57	65.2	0.32
PT8610	14	-0.56	17	-1.05	51	1.25	52	-0.56
PT8611	17.1	0.16	37.3	2.00	48.1	0.98	78.5	1.20
PT8584#	20.36	0.91	39.84	**2.39**	17.8	-1.80	98.69	**2.53**
PT8594#	26.2	**2.26**	19.7	-0.64	51.4	1.29	59.6	-0.06

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Retrorsine group A: 14.7 µg/kg u: 1.71 µg/kg σ_p: 3.69 µg/kg (25%) robust σ: 5.5 µg/kg (37%)		Retrorsine-N-oxide group A: 29.4 µg/kg u: 2.61 µg/kg σ_p: 7.35 µg/kg (25%) robust σ: 9.57 µg/kg (33%)		Senecionine group A: 45.2 µg/kg u: 4.73 µg/kg σ_p: 11.3 µg/kg (25%) robust σ: 16.9 µg/kg (37%)		Senecionine-N-oxide group A: 73.1 µg/kg u: 7.55 µg/kg σ_p: 18.3 µg/kg (25%) robust σ: 28.3 µg/kg (39%)	
Lab code	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**
PT8583	<5	**[-2.40] FN**	85.0	**7.13**	<5	**[-3.28] FN**	260.9	**9.49**
PT8588	14.6	-0.03	34	0.59	48.4	0.26	81.9	0.44
PT8589	10.3	-1.09	35.3	0.76	70.3	**2.05**	91.1	0.91
PT8592	<10	[-1.17]	15.9	-1.73	<10	**[-3.59] FN**	23	**-2.53**
PT8593	<10	[-1.17]	18.6	-1.38	28.9	-1.33	56.3	-0.85
PT8595	16.2	0.36	35.2	0.75	67.3	1.81	105.1	1.62
PT8596	33.6	**4.64**	66.3	**4.73**	26.5	-1.52	37.5	-1.80
PT8597	<20	[1.29]	21.3	-1.04	53.7	0.70	51.2	-1.11
PT8598	11	-0.92	34	0.59	27	-1.48	52	-1.07
PT8599	16	0.31	37	0.98	47	0.15	80	0.35
PT8600	14.3	-0.11	30.1	0.09	33.4	-0.96	67	-0.31
PT8601	11.6	-0.77	24.9	-0.58	32.6	-1.03	51.3	-1.10
PT8602	24.6	**2.43**	96.4	**8.60**	53.8	0.70	90	0.85
PT8603	13.4	-0.33	29.1	-0.04	45.4	0.02	77.4	0.22
PT8604	15.9	0.29	30.1	0.09	63.2	1.47	94.3	1.07
PT8605	<200	**[45.6]**	24.9	-0.58	63	1.46	168	**4.80**
PT8606	20	1.29	nt		50	0.39	80	0.35
PT8607	10	-1.17	24.4	-0.64	29.3	-1.30	64.3	-0.45
PT8608	<10	[-1.17]	11.8	**-2.26**	38.5	-0.54	53	-1.02
PT8609	7	-1.91	23.4	-0.77	30.8	-1.17	79.4	0.32
PT8610	10	-1.17	16	-1.72	32	-1.08	36	-1.88
PT8611	20.3	1.37	34.2	0.62	62.7	1.43	98.1	1.26
PT8584#	18.14	0.84	24.64	-0.61	43.14	-0.17	39.85	-1.68
PT8594#	12.6	-0.53	31.2	0.23	48.5	0.27	78.1	0.25

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nt = not tested.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Seneciphylline group A: 24.8 µg/kg u: 2.04 µg/kg σ_p: 6.20 µg/kg (25%) robust σ: 7.12 µg/kg (29%)		Seneciphylline-N-oxide group A: 39.7 µg/kg u: 3.63 µg/kg σ_p: 9.93 µg/kg (25%) robust σ: 13.3 µg/kg (34%)		Sum of 35 PAs A: 421 µg/kg u: 38.3 µg/kg σ_p: 105 µg/kg (25%) robust σ: 144 µg/kg (37%)	
Lab code	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**
PT8583	<5	**[-3.03] FN**	142.8	**9.75**	979.6	**4.99**
PT8588	19.2	-0.86	29.9	-0.93	447.9	0.24
PT8589	18.9	-0.90	31.3	-0.80	427	0.05
PT8592	<10	**[-2.27] FN**	24.3	-1.46	132.4	**-2.58**
PT8593	16.1	-1.33	36.5	-0.30	254.9	-1.48
PT8595	20.1	-0.72	34.6	-0.48	479	0.52
PT8596	36.2	1.75	53.1	1.27	418	-0.03
PT8597	<20	[-0.74]	36.3	-0.32	321	-0.89
PT8598	22	-0.43	41	0.12	293	-1.14
PT8599	28	0.49	48	0.78	430	0.08
PT8600	24.2	-0.09	44	0.41	381.5	-0.35
PT8601	23.2	-0.25	30.1	-0.91	352	-0.62
PT8602	67.1	**6.48**	83.8	**4.17**	606	1.65
PT8603	32.6	1.19	40.6	0.08	401	-0.18
PT8604	34.7	1.52	51.4	1.11	484.1	0.56
PT8605	20.4	-0.67	31.1	-0.81	643	1.98
PT8606	30	0.80	<10	**[-2.81] FN**	590	1.51
PT8607	18.6	-0.95	44.5	0.45	320.5	-0.90
PT8608	26.4	0.24	22.3	-1.65	283	-1.23
PT8609	15.9	-1.36	39.6	-0.01	457	0.32
PT8610	23	-0.28	14	**-2.43**	288	-1.19
PT8611	39.5	**2.25**	59.7	1.89	553.5	1.18
PT8584#	17.52	-1.12	34.46	-0.50	375.71	-0.40
PT8594#	41.7	**2.59**	51.2	1.09	449.7	0.26

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

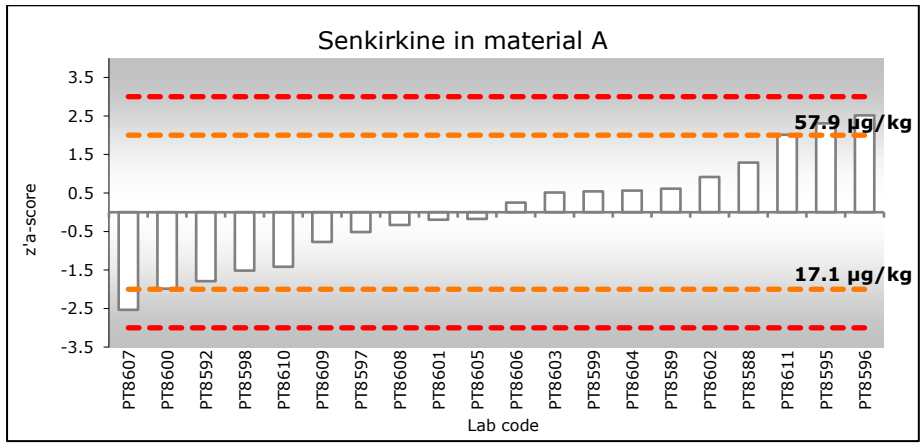

Figure 1 *Graphical representation of the z'-scores for senkirkine in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

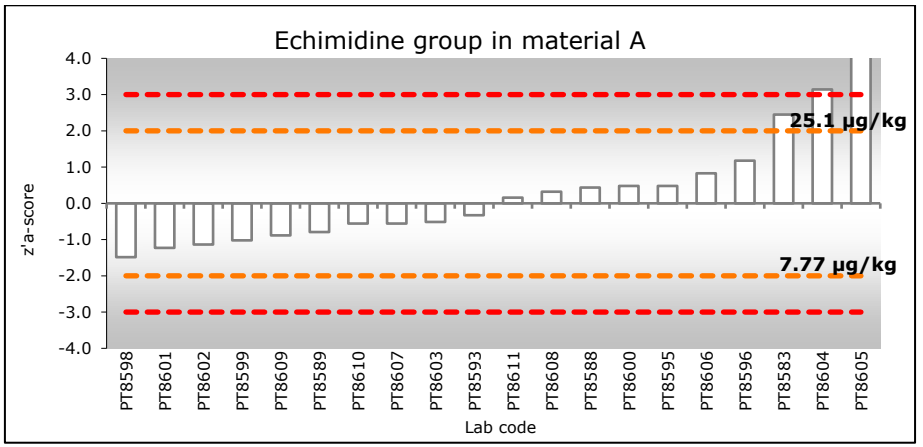

Figure 2 *Graphical representation of the z'-scores for the echimidine group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

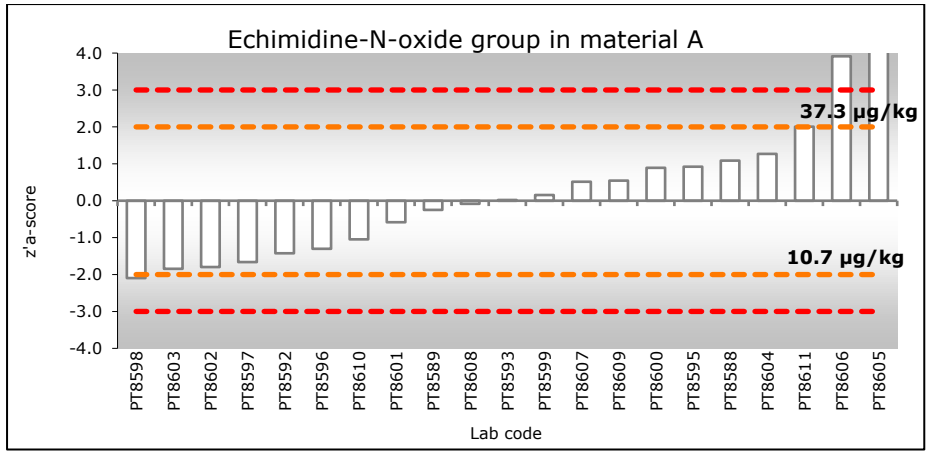

Figure 3 *Graphical representation of the z'-scores for the echimidine-N-oxide group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

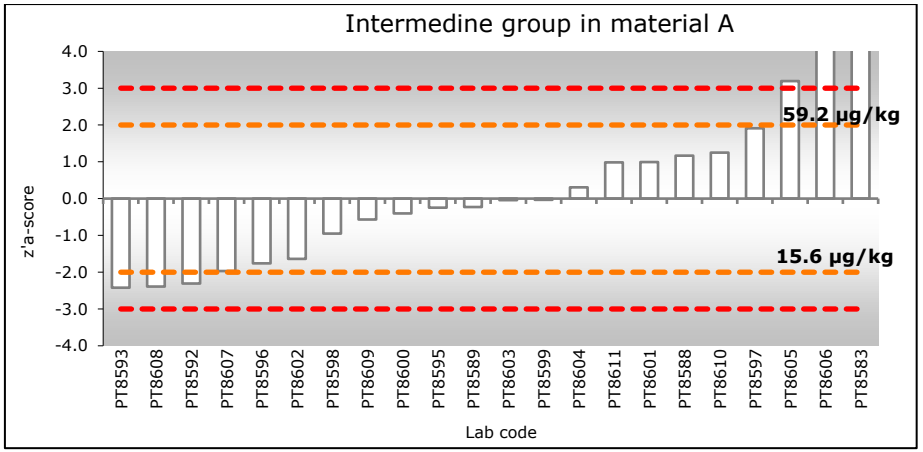

Figure 4 *Graphical representation of the z'-scores for the intermedine group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

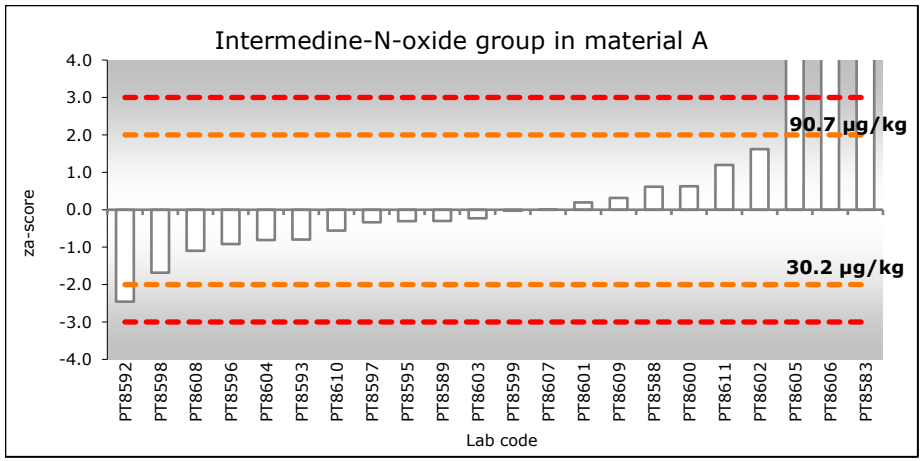

Figure 5 *Graphical representation of the z-scores for the intermedine-N-oxide group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

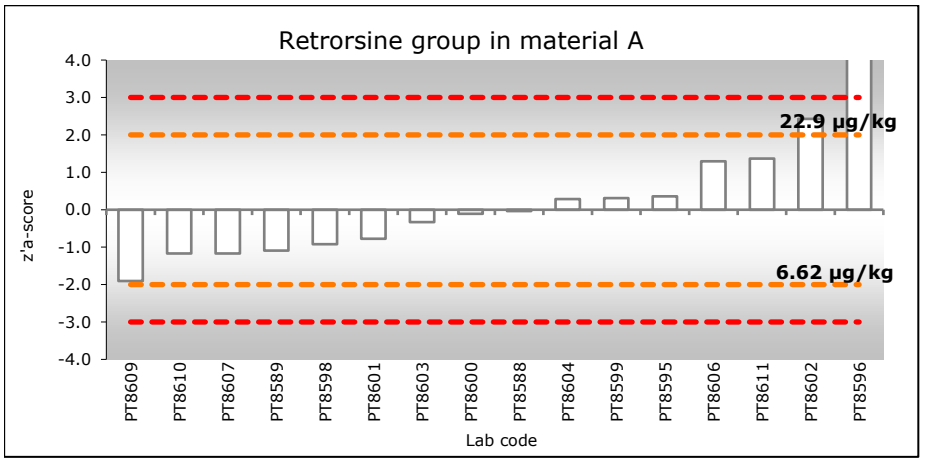

Figure 6 *Graphical representation of the z' -scores for the retrorsine group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

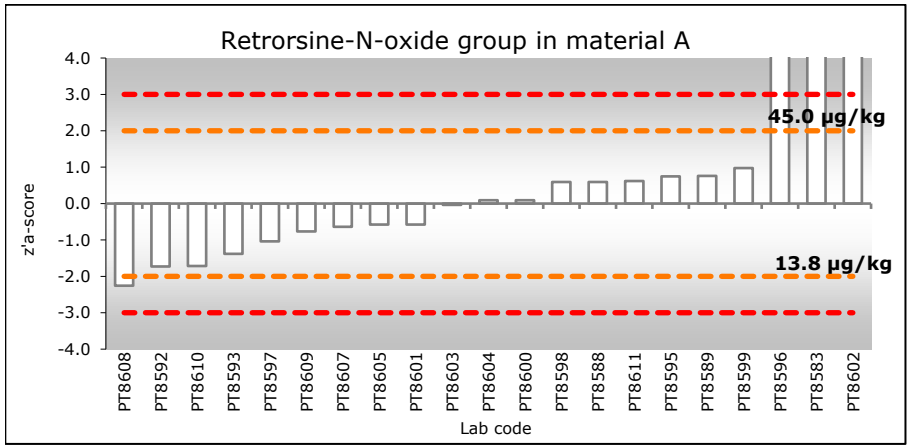

Figure 7 *Graphical representation of the z'-scores for the retrorsine-N-oxide group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

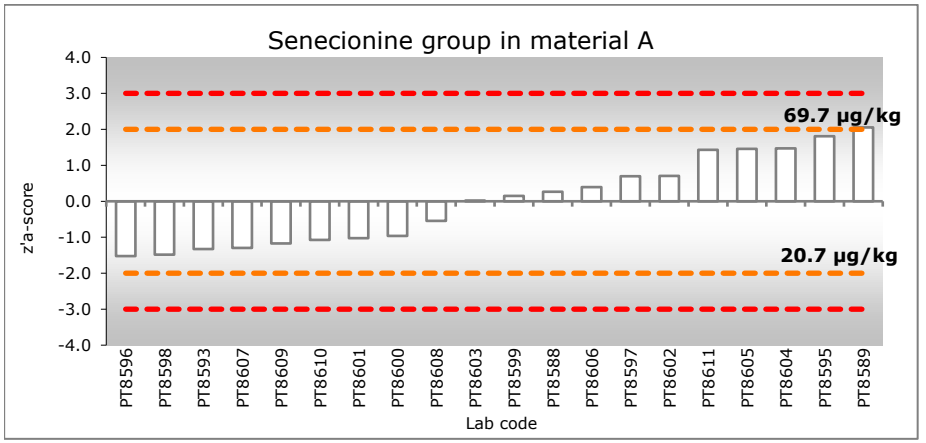

Figure 8 *Graphical representation of the z'-scores for the senecionine group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

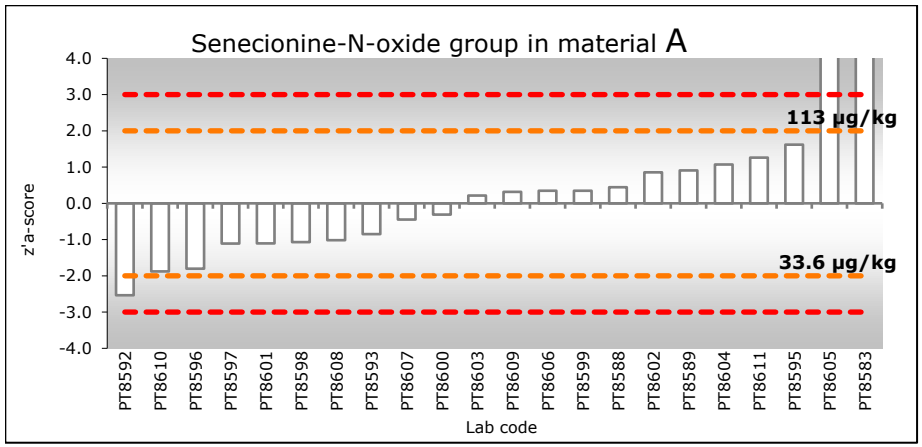

Figure 9 *Graphical representation of the z'-scores for the senecionine-N-oxide group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

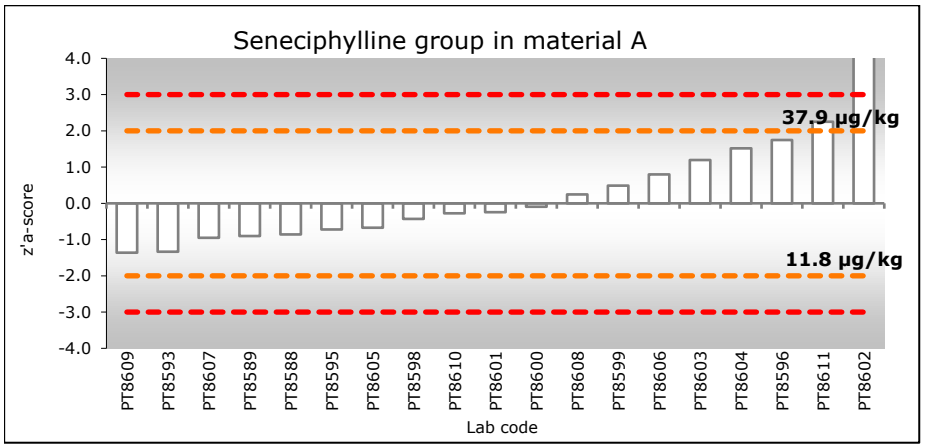

Figure 10 *Graphical representation of the z'-scores for the seneciphylline group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

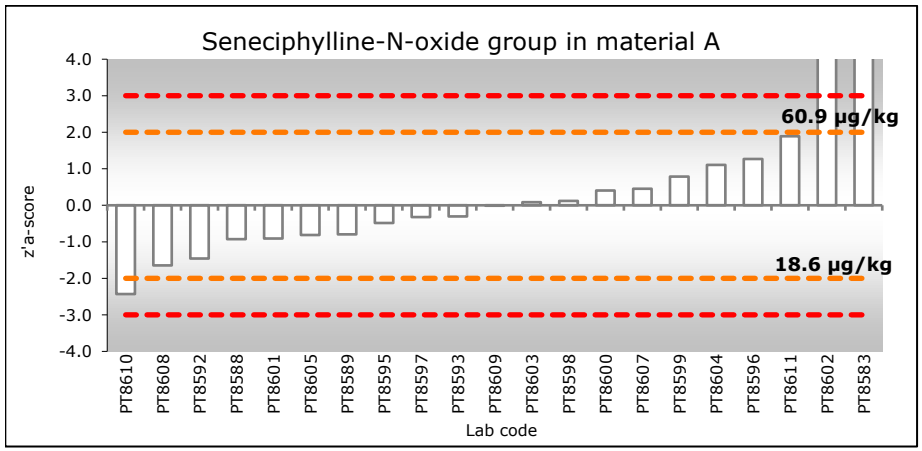

Figure 11 *Graphical representation of the z'-scores for the seneciphyline-N-oxide group in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg and ± 3.*

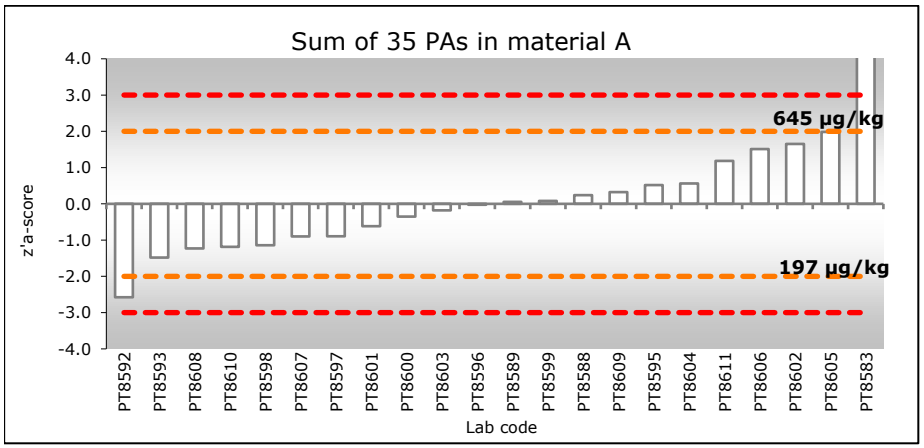

Figure 12 *Graphical representation of the z'-scores for the sum of 35 PAs in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 10 Results material B (marjoram)

	Europine A: 57.7 µg/kg u: 3.36 µg/kg σ_p: 14.2 µg/kg (25%) robust σ: 12.3 µg/kg (22%)		Europine-N-oxide A: 663 µg/kg u: 73.3 µg/kg σ_p: 166 µg/kg (25%) robust σ: 275 µg/kg (42%)		Heliotrine A: 103 µg/kg u: 4.09 µg/kg σ_p: 25.8 µg/kg (25%) robust σ: 15.3 µg/kg (15%)		Heliotrine-N-oxide A: 999 µg/kg u: 86.1 µg/kg σ_p: 250 µg/kg (25%) robust σ: 323 µg/kg (32%)	
Lab code	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**
PT8583	<5	**[-3.65] FN**	458.8	-1.13	84.6	-0.72	630.8	-1.39
PT8588	53.5	-0.22	918.7	1.41	100.7	-0.09	1425.8	1.61
PT8589	64.4	0.55	1265.9	**3.33**	107.2	0.16	1306.6	1.16
PT8592	68.3	0.82	594	-0.38	107	0.15	724	-1.04
PT8593	43.5	-0.93	496.9	-0.92	90.2	-0.50	1083	0.32
PT8595	65.3	0.61	731	0.38	99.8	-0.13	1144	0.55
PT8596	34	-1.60	387	-1.52	47	**-2.18**	659	-1.29
PT8597	54.3	-0.17	873	1.16	89.8	-0.52	1008	0.03
PT8598	49	-0.54	716	0.29	109	0.23	1055	0.21
PT8599	60	0.24	610	-0.29	90	-0.51	770	-0.87
PT8600	57.5	0.06	784	0.67	111	0.31	1210	0.80
PT8601	38.7	-1.27	499.6	-0.90	109.8	0.26	1072.6	0.28
PT8602	71.1	1.02	1293.7	**3.48**	157.2	**2.10**	1178.5	0.68
PT8603	55.9	-0.05	672	0.05	87.2	-0.62	992	-0.03
PT8604	57.4	0.05	700	0.20	93	-0.39	1071	0.27
PT8605	17.3	**-2.78**	777	0.63	115	0.46	1557	**2.11**
PT8606	80	1.65	210	**-2.50**	130	1.04	170	**-3.14**
PT8607	53.7	-0.21	718	0.30	98.6	-0.17	972.3	-0.10
PT8608	89	**2.28**	461	-1.11	138	1.36	841	-0.60
PT8609	47	-0.68	261	**-2.22**	97.9	-0.20	714	-1.08
PT8610	58	0.09	1504	**4.64**	104	0.04	3440	**9.24**
PT8611	61.2	0.32	431.1	-1.28	115.6	0.49	638.4	-1.37
PT8584#	34.94	-1.53	727.36	0.35	104.51	0.06	1117.78	0.45
PT8594#	66.4	0.69	831.7	0.93	89.6	-0.52	857.1	-0.54

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Lasiocarpine A: 44.4 µg/kg u: 1.19 µg/kg σ_p: 11.1 µg/kg (25%) robust σ: 4.37 µg/kg (9.8%)		Lasiocarpine-N-oxide A: 516 µg/kg u: 31.9 µg/kg σ_p: 129 µg/kg (25%) robust σ: 117 µg/kg (23%)		Senkirkine A: nr u: nr σ_p: nr robust σ: nr	
Lab code	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**
						No statistical
						evaluation
						possible
PT8583	16.8	**-2.49**	193.6	**-2.50**	nd	
PT8588	47.2	0.25	679.1	1.27	nd	
PT8589	48.3	0.35	687.7	1.33	<10	
PT8592	36.1	-0.75	273	-1.88	nd	
PT8593	30.2	-1.28	587.6	0.56	<10	
PT8595	46.5	0.19	584	0.53	nd	
PT8596	49	0.41	224	**-2.26**	detected*	
PT8597	43.6	-0.07	461	-0.42	<10	
PT8598	43	-0.13	533	0.13	nd	
PT8599	48	0.32	550	0.27	<5	
PT8600	45.4	0.09	519	0.03	nd	
PT8601	43.2	-0.11	541.3	0.20	nd	
PT8602	101.4	**5.13**	754.8	1.85	nd	
PT8603	44.6	0.02	502	-0.11	nd	
PT8604	42.7	-0.15	558	0.33	<2	
PT8605	44.7	0.03	511	-0.04	<20	
PT8606	<10	**[-4.0] FN**	nt		<10	
PT8607	33.8	-0.96	450.3	-0.51	nd	
PT8608	46.7	0.21	647	1.02	nd	
PT8609	44.4	0.00	387	-1.00	nd	
PT8610	32	-1.12	364	-1.18	<10	
PT8611	56.9	1.13	515.3	0.00	nd	
PT8584#	56.62	1.10	514.47	-0.01	nd	
PT8594#	58.9	1.31	702.3	1.45	<5	

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nd = not detected.

nr = not relevant.

nt = not tested.

FN = false negative.

* results below LOQ should not be reported.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Echimidine group A: 84.7 µg/kg u: 6.46 µg/kg σ_p: 21.2 µg/kg (25%) robust σ: 24.2 µg/kg (29%)		Echimidine-N-oxide group A: 256 µg/kg u: 18.4 µg/kg σ_p: 64.0 µg/kg (25%) robust σ: 69.2 µg/kg (27%)		Intermedine group A: 325 µg/kg u: 23.4 µg/kg σ_p: 81.2 µg/kg (25%) robust σ: 87.8 µg/kg (27%)		Intermedine-N-oxide group A: 463 µg/kg u: 43.9 µg/kg σ_p:116 µg/kg (25%) robust σ: 165 µg/kg (36%)	
Lab code	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**
PT8583	9.8	**-3.38**	78.0	**-2.78**	94.7	**-2.83**	154.1	**-2.50**
PT8588	91.7	0.32	323.8	1.06	438.6	1.40	591.7	1.04
PT8589	56.2	-1.29	52.1	**-3.19**	251.5	-0.90	329.6	-1.08
PT8592	63.2	-0.97	211	-0.70	328	0.04	401	-0.50
PT8593	74.3	-0.47	261.1	0.08	306.1	-0.23	421.5	-0.33
PT8595	41.7	-1.94	33.6	**-3.47**	272	-0.65	339	-1.00
PT8596	111	1.19	207	-0.76	81.5	**-3.00**	200	**-2.12**
PT8597	82.5	-0.10	264	0.13	292	-0.40	500	0.30
PT8598	78	-0.30	283	0.42	270	-0.68	415	-0.39
PT8599	97	0.55	300	0.69	390	0.80	550	0.70
PT8600	89.6	0.22	277	0.33	369	0.54	554	0.74
PT8601	83.2	-0.07	240.5	-0.24	324.2	-0.01	489.9	0.22
PT8602	122.2	1.69	246.2	-0.15	543.7	**2.70**	671.7	1.69
PT8603	56.7	-1.27	249	-0.11	148	**-2.18**	617	1.24
PT8604	89.9	0.23	296	0.63	360	0.43	510	0.38
PT8605	375	**13.11**	360	1.63	1222	**11.05**	1103	**5.17**
PT8606	100	0.69	210	-0.72	380	0.68	330	-1.07
PT8607	64.8	-0.90	255.3	-0.01	353.3	0.35	484.1	0.17
PT8608	113	1.28	398	**2.22**	312	-0.16	198	**-2.14**
PT8609	90.1	0.24	191	-1.01	320	-0.06	374	-0.72
PT8610	74	-0.48	436	**2.81**	261	-0.79	5799	**43.11**
PT8611	103.1	0.83	266.7	0.17	427.6	1.27	472.1	0.07
PT8584#	84.98	0.01	296.62	0.64	250.65	-0.91	841.14	**3.06**
PT8594#	110.9	1.18	386.9	**2.05**	188	-1.68	1437.2	**7.87**

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Retrorsine group A: 131 µg/kg u: 4.82 µg/kg σ_p: 32.6 µg/kg (25%) robust σ: 18.1 µg/kg (14%)		Retrorsine-N-oxide group A: 440 µg/kg u: 33.7 µg/kg σ_p: 110 µg/kg (25%) robust σ: 124 µg/kg (28%)		Senecionine group A: 216 µg/kg u: 19.4 µg/kg σ_p: 54.1 µg/kg (25%) robust σ: 72.9 µg/kg (34%)		Senecionine-N-oxide group A: 789 µg/kg u: 93.6 µg/kg σ_p: 197 µg/kg (25%) robust σ: 351 µg/kg (45%)	
Lab code	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z'-score**
PT8583	44.8	**-2.63**	232.2	-1.81	27.4	**-3.29**	290.0	**-2.29**
PT8588	140.5	0.30	582.1	1.23	243.4	0.47	1039.9	1.15
PT8589	129.4	-0.04	695.5	**2.22**	366.1	**2.61**	1529.7	**3.39**
PT8592	105	-0.78	331	-0.95	153	-1.10	315	**-2.17**
PT8593	136.3	0.17	347.4	-0.81	258.5	0.73	808.5	0.09
PT8595	105	-0.78	536	0.83	290	1.28	1282	**2.26**
PT8596	154	0.72	187	**-2.20**	70.3	**-2.54**	297	**-2.25**
PT8597	129	-0.05	417	-0.20	127	-1.55	728	-0.28
PT8598	143	0.38	498	0.50	155	-1.07	652	-0.63
PT8599	150	0.59	510	0.61	230	0.24	650	-0.64
PT8600	132	0.04	376	-0.56	175	-0.72	875	0.39
PT8601	135.6	0.15	424.6	-0.14	213.6	-0.05	743.1	-0.21
PT8602	295.6	**5.05**	959.6	**4.51**	400.4	**3.20**	1343.4	**2.54**
PT8603	122	-0.26	441	0.01	200	-0.28	811	0.10
PT8604	140	0.29	475	0.30	228	0.20	896	0.49
PT8605	113	-0.54	489	0.42	291	1.30	1328	**2.47**
PT8606	130	-0.02	nt		200	-0.28	250	**-2.47**
PT8607	104	-0.81	390.1	-0.44	165.2	-0.89	647.3	-0.65
PT8608	292	**4.94**	530	0.78	247	0.53	1134	1.58
PT8609	125	-0.17	262	-1.55	198	-0.32	641	-0.68
PT8610	116	-0.45	362	-0.68	277	1.06	560	-1.05
PT8611	138.1	0.23	501.1	0.53	239.7	0.41	972.3	0.84
PT8584#	189.43	1.80	414.65	-0.22	227.65	0.20	458.32	-1.52
PT8594#	105.3	-0.77	336.3	-0.90	260.6	0.77	759.3	-0.14

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

nt = not tested.

FN = false negative.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

	Seneciphylline group A: 212 µg/kg u: 9.04 µg/kg σ_p: 53.0 µg/kg (25%) robust σ: 33.1 µg/kg (16%)		Seneciphylline-N-oxide group A: 608 µg/kg u: 48.8 µg/kg σ_p: 152 µg/kg (25%) robust σ: 183 µg/kg (30%)		Sum of 35 PAs A: 5964 µg/kg u: 278 µg/kg σ_p: 1491 µg/kg (25%) robust σ: 1042 µg/kg (17%)	
Lab code	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z-score**
PT8583	<5	**[-3.91] FN**	244.9	**-2.27**	2550.9	**-2.29**
PT8588	216.9	0.09	704.1	0.60	7597.7	1.10
PT8589	234.4	0.42	789.7	1.14	7914.3	1.31
PT8592	179	-0.62	435	-1.08	4323	-1.10
PT8593	176.3	-0.67	804	1.23	5925	-0.03
PT8595	200	-0.23	598	-0.06	6367	0.27
PT8596	179	-0.62	236	**-2.33**	3123	-1.91
PT8597	189	-0.43	550	-0.36	5808	-0.10
PT8598	202	-0.19	662	0.34	5861	-0.07
PT8599	200	-0.23	680	0.45	5900	-0.04
PT8600	222	0.19	582	-0.16	6380	0.28
PT8601	229.6	0.33	518.7	-0.56	5708.2	-0.17
PT8602	411.9	**3.77**	1307.5	**4.39**	9962.4	**2.68**
PT8603	234	0.41	619	0.07	5850	-0.08
PT8604	212	0.00	651	0.27	6380	0.28
PT8605	258	0.87	731	0.77	9293	**2.23**
PT8606	170	-0.79	290	-1.99	2650	**-2.22**
PT8607	158.4	-1.01	573.8	-0.21	5523	-0.30
PT8608	249	0.70	896	1.81	6591	0.42
PT8609	234	0.41	479	-0.81	4465	-1.01
PT8610	208	-0.08	483	-0.78	14078	**5.44**
PT8611	234.9	0.43	726.8	0.75	5900.9	-0.04
PT8584#	218.6	0.12	925.19	1.99	6463.9	0.34
PT8594#	286.2	1.40	597.5	-0.06	7074.2	0.74

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

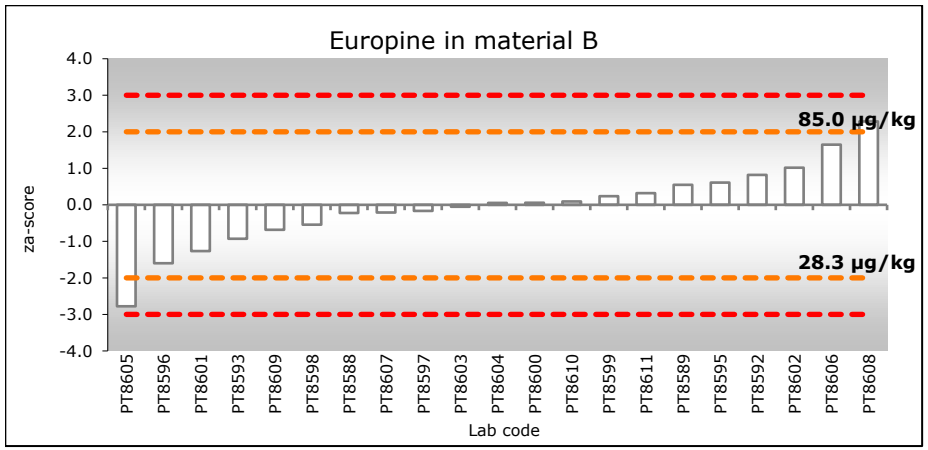

Figure 13 *Graphical representation of the z-scores for europine in material B. in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

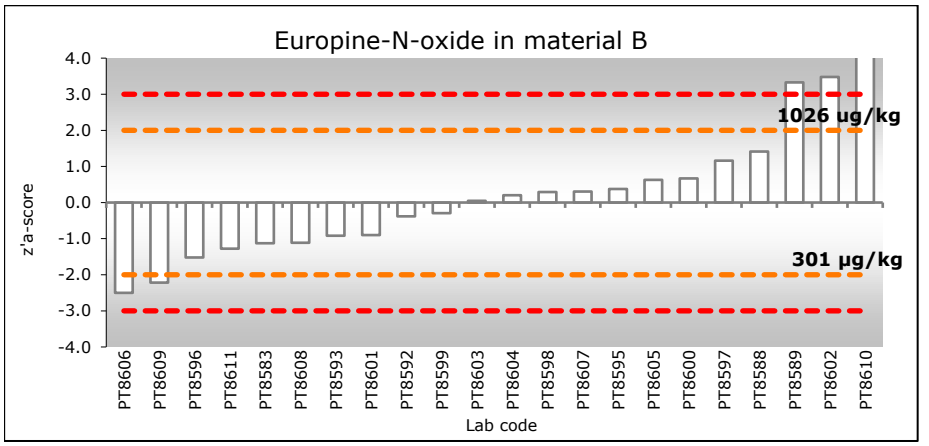

Figure 14 *Graphical representation of the z'-scores for europine-N-oxide in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

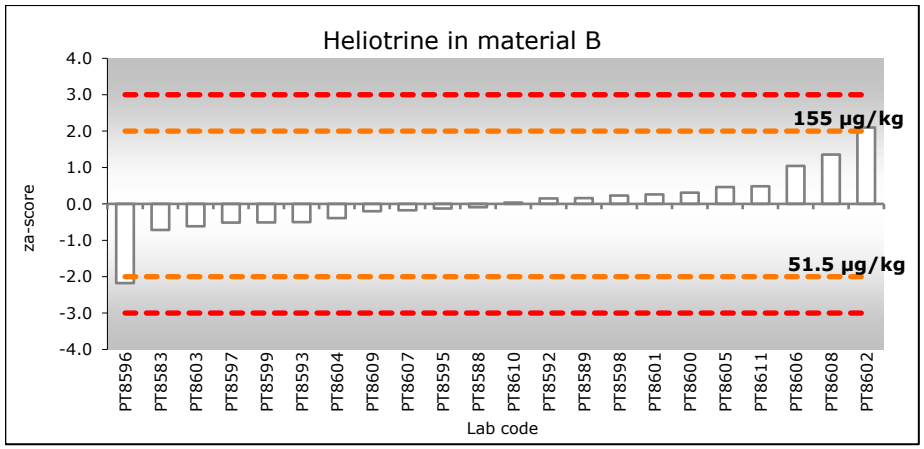

Figure 15 *Graphical representation of the z-scores for heliotrine in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

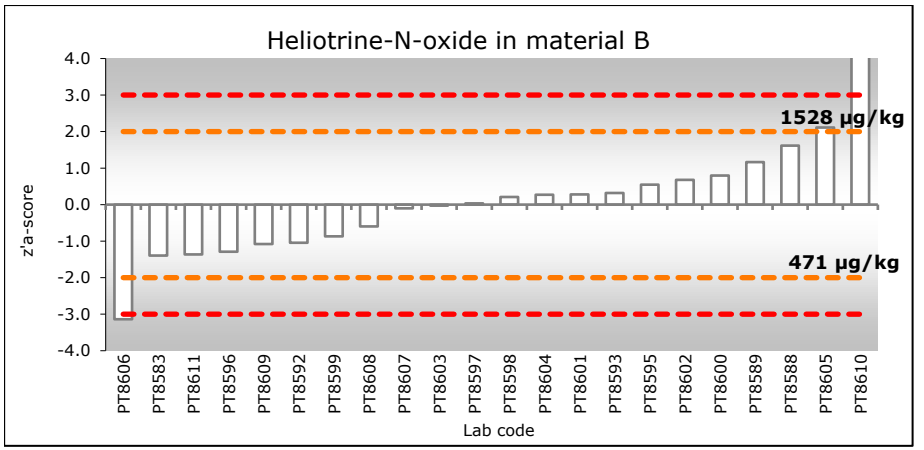

Figure 16 *Graphical representation of the z'-scores for heliotrine-N-oxide in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

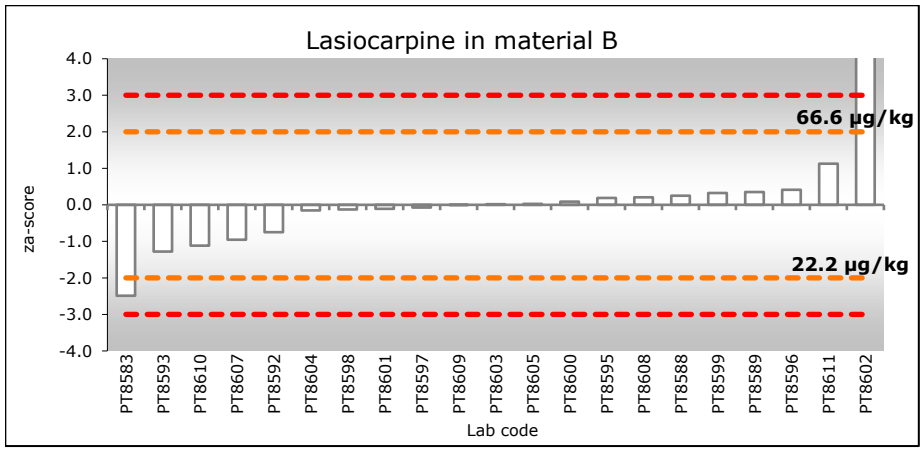

Figure 17 *Graphical representation of the z-scores for lasiocarpine in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

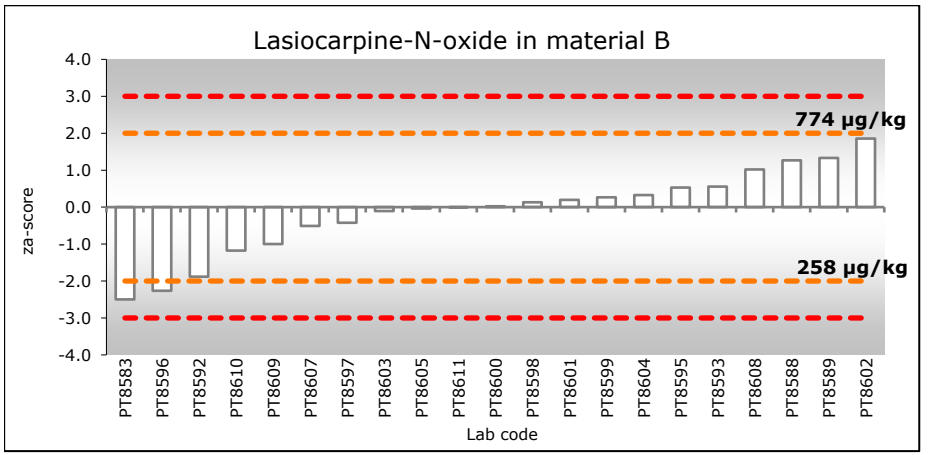

Figure 18 *Graphical representation of the z-scores for lasiocarpine-N-oxide in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

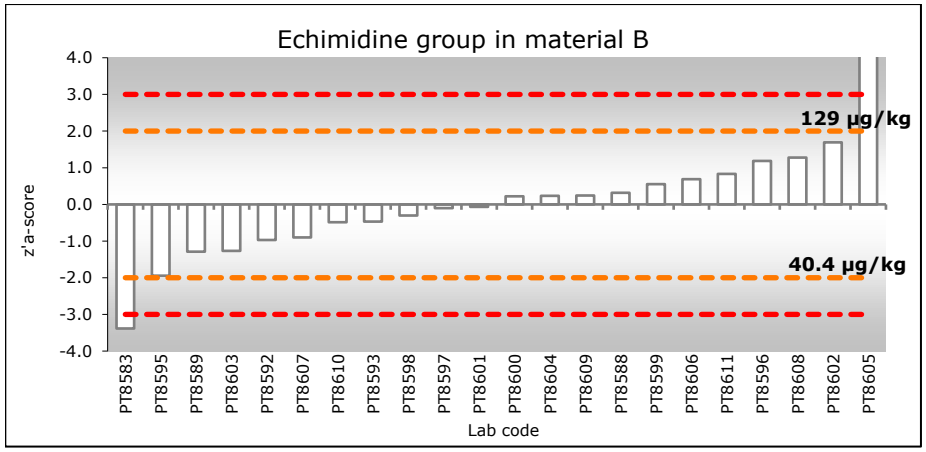

Figure 19 *Graphical representation of the z-scores for the echimidine group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

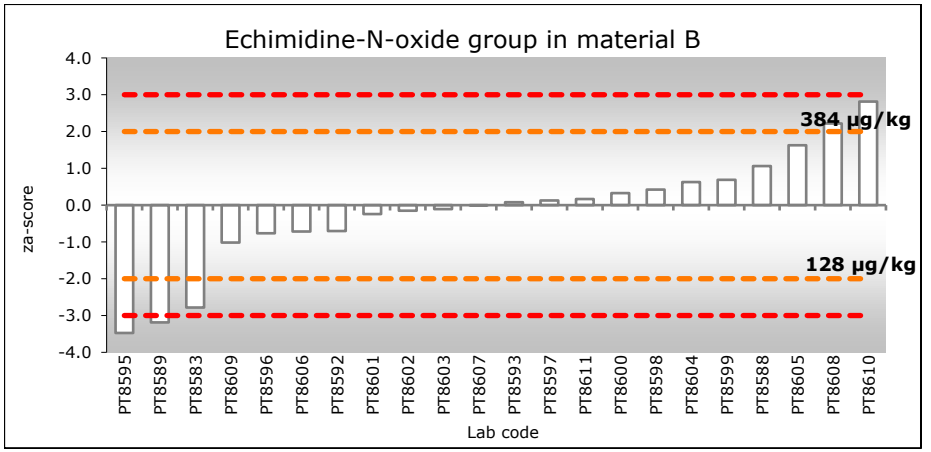

Figure 20 *Graphical representation of the z-scores for the echimidine-N-oxide group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

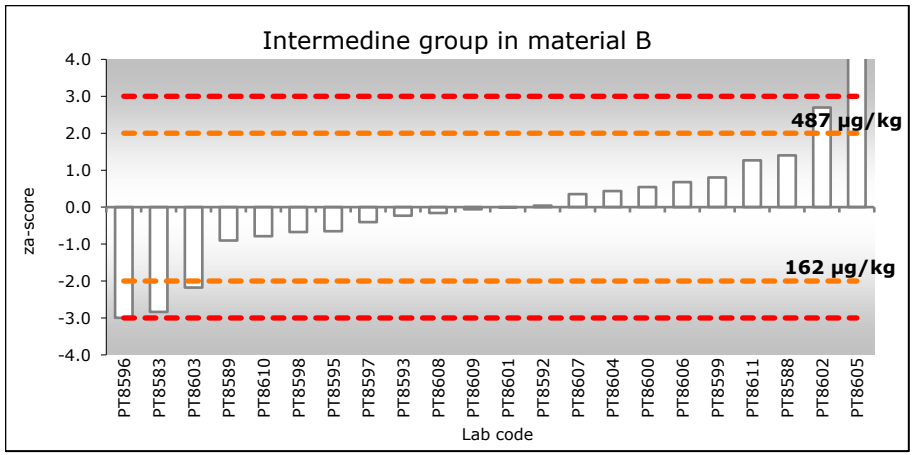

Figure 21 *Graphical representation of the z-scores for the intermedine group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

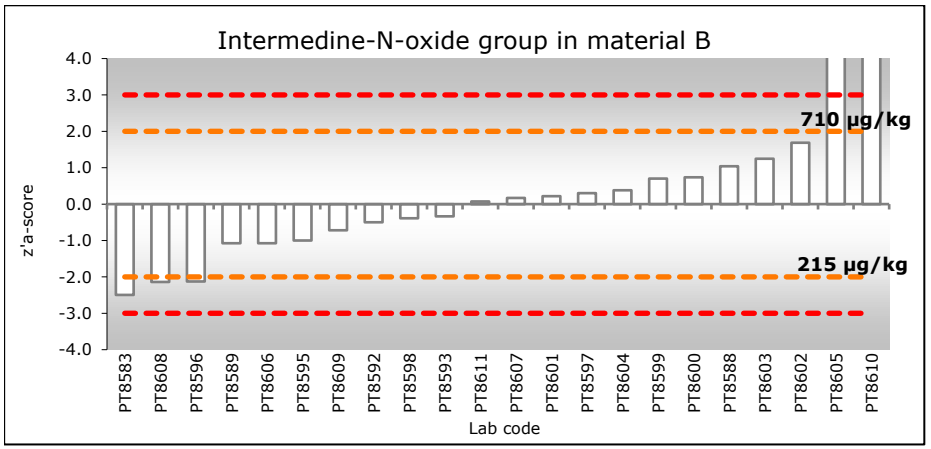

Figure 22 *Graphical representation of the z-scores for the intermedine-N-oxide group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

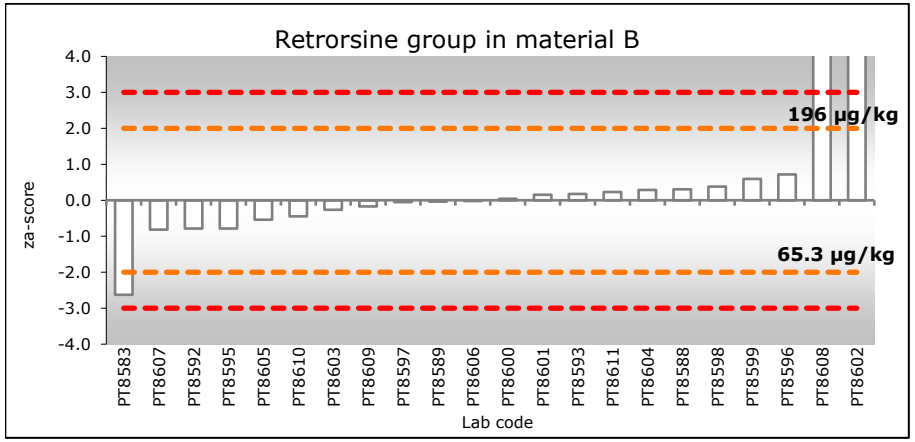

Figure 23 *Graphical representation of the z-scores for the retrorsine group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

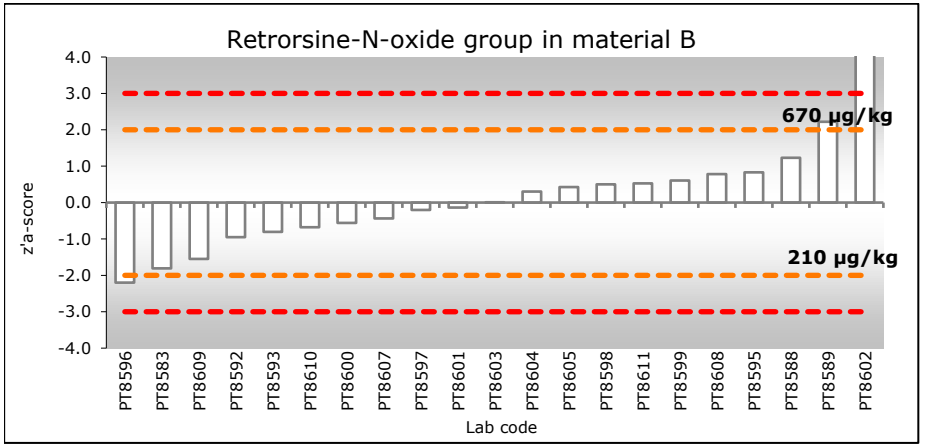

Figure 24 *Graphical representation of the z-scores for the retrorsine-N-oxide group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

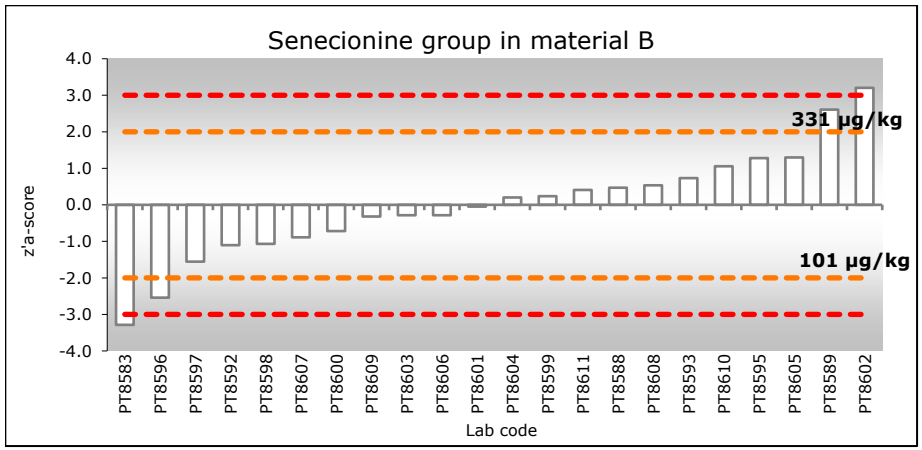

Figure 25 *Graphical representation of the z-scores for the senecionine group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

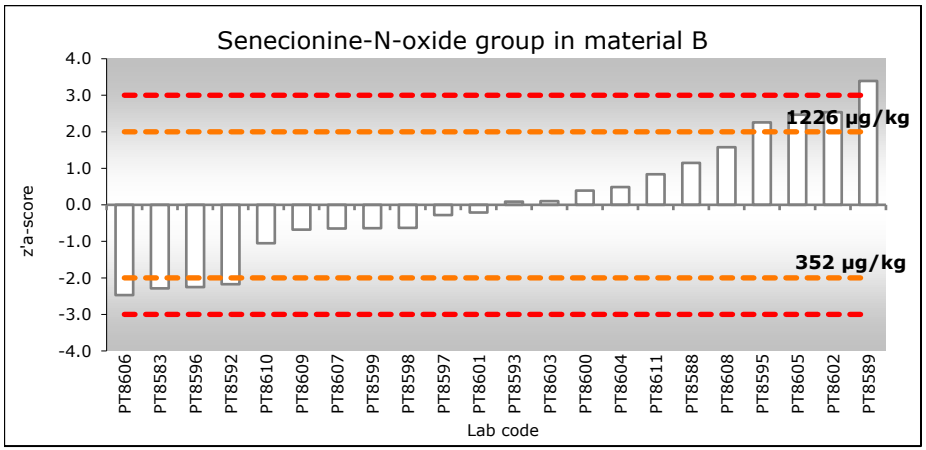

Figure 26 *Graphical representation of the z-scores for the senecionine-N-oxide group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

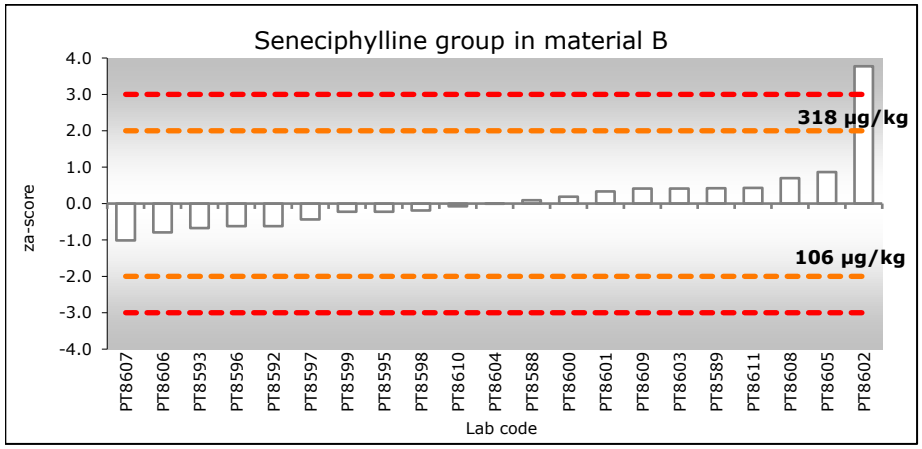

Figure 27 *Graphical representation of the z-scores for the seneciphylline group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

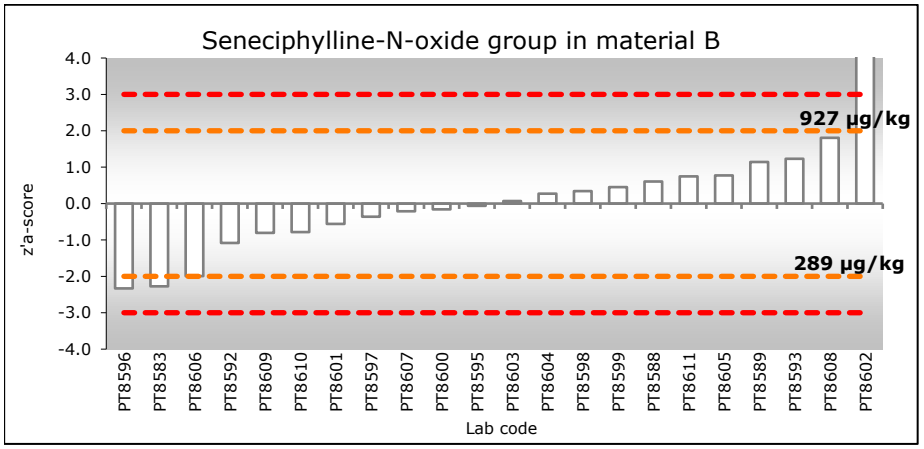

Figure 28 *Graphical representation of the z-scores for the seneciphylline-N-oxide group in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

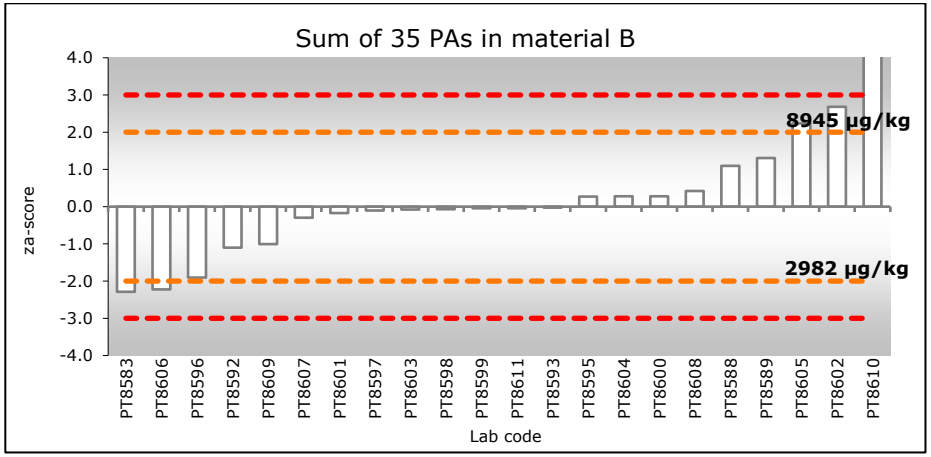

Figure 29 *Graphical representation of the z-scores for the sum of PAs in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 11 Overview performance per laboratory

Lab code	Individual PAs and isomer groups Satisfactory performance *	Total sum Satisfactory performance *	FN	FP
PT8583	4 of 27	0 of 2	7	2
PT8588	**27 of 27**	**2 of 2**		
PT8589	21 of 27	**2 of 2**		
PT8592	19 of 27	1 of 2	2	
PT8593	24 of 27	**2 of 2**	1	
PT8595	24 of 27	**2 of 2**		
PT8596	16 of 27	**2 of 2**		
PT8597	24 of 27	**2 of 2**		
PT8598	26 of 27	**2 of 2**		
PT8599	**27 of 27**	**2 of 2**		
PT8600	**27 of 27**	**2 of 2**		
PT8601	**27 of 27**	**2 of 2**		
PT8602	13 of 27	1 of 2		
PT8603	26 of 27	**2 of 2**		
PT8604	26 of 27	**2 of 2**		
PT8605	15 of 27	1 of 2		
PT8606	16 of 27	1 of 2	2	
PT8607	26 of 27	**2 of 2**		
PT8608	20 of 27	**2 of 2**		
PT8609	26 of 27	**2 of 2**		1
PT8610	22 of 27	1 of 2		
PT8611	25 of 27	**2 of 2**		
PT8584#	24 of 27	**2 of 2**		
PT8594#	23 of 27	**2 of 2**		

* Satisfactory performance means a satisfactory z-score was obtained for the pyrrolizidine alkaloids present in material A and B.

participant submitted the results after closure date; z-scores, NP and FN are calculated using the results submitted by the other participants.

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The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,200 employees (6,400 fte) and 13,200 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

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