Research study for pyrrolizidine alkaloids in alfalfa and herbal tea

EURLPT-MP02 (2019)

D.P.K.H. Pereboom, M. de Nijs, J.G.J. Mol, P.P.J. Mulder

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Summary

The European Commission and the Member States are preparing a harmonised regulation on the presence of 21 1,2-unsaturated pyrrolizidine alkaloids (PAs), together with 14 isomeric PAs, in certain food products, such as herbal teas, herbal infusions, food supplements, and spices, by establishing maximum limits (MLs) for the total concentration of PAs. The proposed EU legislation will not include legal limits for PAs in animal feed. However, it is recommended by European Food Safety Authority to monitor PAs in animal feed for data collection and monitoring purposes.

A research study for the quantitative analysis of PAs in alfalfa and herbal tea was organised by the European Union Reference Laboratory for mycotoxins & plant toxins between April-July 2019. This research study was conducted to obtain a comprehensive overview of the current methods in use by the NRLs, and their capabilities regarding the separation of isomeric groups of PAs. The primary goal was to assess the proficiency of National Reference Laboratories (NRLs). The research study was carried out according to ISO/IEC 17043:2010 [7], however the preparation of the test materials was not part of the WFSR accreditation (R013, Dutch Accreditation Board). During the time that the study was conducted an agreement was reached with the subcontractor for the homogenization of the proficiency test material. The subcontractor has ISO 17043 accreditation for preparation of proficiency test materials.

Twenty-seven laboratories, of which 24 National Reference Laboratories for mycotoxins & plant toxins in food and feed from 22 EU Member States, and 3 Official Laboratories participated in the research study.

Two materials, alfalfa (material A) and herbal tea (material B), were prepared. Material A was prepared by adding a small portion of alfalfa material contaminated with PAs to blank alfalfa material and material B was prepared by adding a small portion of herbal tea material contaminated with PAs to blank herbal tea. Material A contained 24 PAs in concentrations above the LOQ (10 µg/kg), of which 18 PAs will be included in the EU regulation. In material B 17 PAs were present in concentrations above the LOQ, which all will be included in the proposed EU regulation. Both materials were approved for homogeneity.

In this research study the robust mean based on the participants' results was used as consensus value. The consensus value was used as the assigned value. Results were calculated for individual PAs as well as for isomeric groups of PAs. The proficiency of the participants was assessed through z and z'-scores, calculated using the assigned values and a relative target standard deviation of 25%. Proxy z-scores were calculated from non-quantifiable results with a reported LOQ and were considered false negative (FN) when |proxy z|$\geq$2. FN scores were included in the evaluation as unsatisfactory results. False positive results (FP) were identified above a threshold value and are evaluated as unsatisfactory results.

As presented in Tables 1a and 1b, the assigned values of individual PAs in material A ranged from 12.0 to 384 µg/kg, the obtained interlaboratory reproducibility (RSD_R) ranged from 13% to 44% and 7 out of the 24 individual PAs had an RSD_R value below or equal to the target standard deviation of 25%. For the sum of 35 PAs for legislation the RSD_R was 32% and for the sum of all PAs it was 25%. 76% of the results for individual PAs were rated with satisfactory z-scores (|z|≤ 2), 6% of the results fell into the questionable range with 2<|z|<3 and 18% of the results fell into the unsatisfactory range with |z|≥ 3. A total of 31 FN and 19 FP results were reported for material A.

For material B, presented in Tables 1a and 1b, the assigned values of individual PAs ranged from 15.1 to 207 µg/kg, the obtained RSD_R ranged from 13% to 52%, while 9 out of the 17 individual PAs had an RSD_R value below or equal to the target standard deviation of 25%. For the sum of 35 PAs for legislation the RSD_R was 22% and for the sum of all PAs it was 21%. 77% of the results were rated

with satisfactory z-scores (|z|≤ 2), 5% of the results fell into the questionable range with 2<|z|<3 and 18% of the results fell into the unsatisfactory range with |z|≥ 3. A total of 20 FN and 15 FP results were reported for material B.

For all ten isomeric groups in material A, presented in Tables 1c and 1d, the RSD_R values were above the target standard deviation of 25%. For material B this was the case for one out of six isomeric groups. For the PA isomeric groups in material A, 76% of the results were rated with satisfactory z-scores (|z|≤ 2), 12% of the results fell into the questionable range with 2<|z|<3 and 11% of the results fell into the unsatisfactory range with |z|≥ 3 and for material B it was respectively, 76%, 8% and 15%. A total of 2 FN was reported for the 10 isomeric groups in material A and 8 FN were reported for the 6 isomeric groups in Material B.

From the results obtained in this research study on pyrrolizidine alkaloids in food and feed the following conclusions can be drawn. With a few exceptions, the limits of quantification reported by the participants are sufficient in view of the proposed legislative limits for PAs. The majority of participants has also included the 21 PAs together with the 14 isomeric PAs in their analytical method. Nevertheless the results indicate that there are a number of issues that need attention and that should be improved. The in many cases relatively high RSD_R values obtained for individual PAs as well as for isomeric groups indicate that there is substantial uncertainty in the assigned values. A high uncertainty indicates that there is a wide variation in the reported results. This is also substantiated by the relatively high number of questionable and unsatisfactory results reported. In this respect, it should be mentioned that the determination of PAs in animal feed and herbal tea is a relatively new area for many NRLs. Furthermore, the wide variety of analytes, including many isomeric compounds, that needs to be covered by the method, requires substantial experience by the laboratory. Efforts need to be made by NRLs to improve the robustness of the implemented methods, in order to produce reliable data. The concept of reporting of isomeric groups needs some special attention as well. It is anticipated that the formalisation of legislation for PAs will stimulate laboratories to optimise their methods and to obtain experience with the analysis of feed and food samples. The progress made will be tested in an upcoming proficiency study, projected for 2022.

Table 1a *Summary of research study materials parameters and participants' performance on individual PA – number of laboratories reporting quantitative values, <LOQ and FN.*

			Assigned value	Uncertainty	Robust RSD_R[1)]	No of labs out of 25 reporting		
	Pyrrolizidine alkaloid	Matrix	(µg/kg)	(µg/kg)	(%)	Quant. value	<LOQ	FN
Em-group	Echimidine	A	88.9	6.21	27	24		
EmNO-group	Echimidine-N-oxide	A	384	36.8	38	24	1	1
Im-group	Intermedine	A	30.0	2.65	30	18	2	1
	Lycopsamine	A	85.7	5.71	20	14	1	1
ImNO-group	Intermedine-N-oxide	A	178	18.4	32	15	1	1
	Lycopsamine-N-oxide	A	311	28.0	31	18		
Rt-group	Retrorsine	A	70.6	7.99	42	21		
		B	29.8	2.75	29	16	4	3
RtNO-group	Retrorsine-N-oxide	A	273	33.9	44	20	1	1
		B	106	7.51	25	20	1	
	Usaramine-N-oxide	A	51.0	5.62	31	12	2	2
Sn-group	Senecionine	A	79.0	4.92	23	22	1	1
		B	25.7	2.91	39	18	5	3
	Senecivernine	A	17.8	2.14	35	13	7	1
	Integerrimine	A	25.1	2.53	28	12	3	3
		B	15.1	0.95	16	10	6	
SnNO-group	Senecionine-N-oxide	A	197	25.0	43	18	1	1
		B	174	20.0	38	17	1	1
	Senecivernine-N-oxide	A	74.9	9.26	44	20	1	1
		B	28.1	4.59	52	16	4	3
	Integerrimine-N-oxide	A	68.6	3.39	14	12	2	1
		B	52.9	5.44	27	11	2	1
Sp-group	Seneciphylline	A	90.3	7.02	29	21		
		B	59.4	5.24	30	20	1	1
	Spartioidine	B	15.9	1.22	16	7	7	
SpNO-group	Seneciphylline-N-oxide	A	138	13.2	34	20	1	
		B	207	14.0	25	21		
	Spartioidine-N-oxide	B	44.0	2.81	18	12	2	2
Individual	Europine	B	26.4	1.16	16	21	3	1
	Europine-N-oxide	B	225	7.61	13	23	1	1
	Heliotrine	B	43.8	2.21	20	24	1	1
	Heliotrine-N-oxide	B	363	14.4	15	23	1	1
	Lasiocarpine	B	19.0	1.71	34	22	3	
	Lasiocarpine-N-oxide	B	145	11.5	30	23	2	2
	Senkirkine	A	12.0	0.551	17	21	4	
Other PAs	Erucifoline[4]	A	107	6.26	21	20	2	1
	Erucifoline-N-oxide[4]	A	192	16.6	29	18	3	3
	Jacobine[4]	A	46.4	3.97	27	15	6	6
	Jacobine-N-oxide[4]	A	33.1	2.39	25	18	3	3
	Jacoline[4]	A	40.7	1.92	16	18	2	2
	Jaconine[4]	A	176	11.6	22	18	1	1
Sum of 35 PAs for legislation		A	2287	185	32	25		
		B	1626	90.7	22	25		
Sum of total PAs		A	2750	175	25	25		
		B	1628	86.1	21	25		

Matrix: A= alfalfa, B= herbal tea.

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.

4) PAs not included in the list of 21 PAs + 14 isomers proposed for legislation.

Table 1b *Summary of research study materials parameters and participants' performance on individual PA – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

			z-scores[2]				Labs out of 25 with	
			Satisfact.		Quest.	Unsatisf.	Accept. z or z'-score	
	Pyrrolizidine alkaloid	Assigned Value Matrix	(µg/kg)	(% of z or z'-scores)	(% of z or z'-scores)	(% of z-or z' scores)	No[3]	%[3]
Em-group	Echimidine	A	88.9	92	4	4	22	88
EmNO-group	Echimidine-N-oxide	A	384	88	8	4	22	88
Im-group	Intermedine	A	30.0	79	11	11	15	60
	Lycopsamine	A	85.7	80		20	12	48
InNO-group	Intermedine-N-oxide	A	178	88		13	14	56
	Lycopsamine-N-oxide	A	311	83	6	11	15	60
Rt-group	Retrorsine	A	70.6	76	14	10	16	64
		B	29.8	79	5	16	15	60
RtNO-group	Retrorsine-N-oxide	A	273	76	10	14	16	64
		B	106	85	10	5	17	68
	Usaramine-N-oxide	A	51.0	64	7	29	9	36
Sn-group	Senecionine	A	79.0	83		17	19	76
		B	25.7	81	5	14	17	68
	Senecivernine	A	17.8	71	7	21	10	40
	Integerrimine	A	25.1	67	7	27	10	40
		B	15.1	80		20	8	32
SnNO-group	Senecionine-N-oxide	A	197	79	5	16	15	60
		B	174	83		17	15	60
	Senecivernine-N-oxide	A	74.9	67	19	14	14	56
		B	28.1	53	5	42	10	40
	Integerrimine-N-oxide	A	68.6	85	8	8	11	44
		B	52.9	83		17	10	40
Sp-group	Seneciphylline	A	90.3	76	10	14	16	64
		B	59.4	81	5	14	17	68
	Spartioidine	B	15.9	86	14		6	24
SpNO-group	Seneciphylline-N-oxide	A	138	85	10	5	17	68
		B	207	81	5	14	17	68
	Spartioidine-N-oxide	B	44.0	64	7	29	9	36
Individual	Europine	B	26.4	91		9	20	80
	Europine-N-oxide	B	225	83	4	13	20	80
	Heliotrine	B	43.8	88	4	8	22	88
	Heliotrine-N-oxide	B	363	79	8	13	19	76
	Lasiocarpine	B	19.0	91	5	5	20	80
	Lasiocarpine-N-oxide	B	145	80	8	12	20	80
	Senkirkine	A	12.0	90	10		19	76
Other PAs	Erucifoline[4]	A	107	86		14	18	72
	Erucifoline-N-oxide[4]	A	192	76	10	14	16	64
	Jacobine[4]	A	46.4	62		38	13	52
	Jacobine-N-oxide[4]	A	33.1	76		24	16	64
	Jacoline[4]	A	40.7	85		15	17	68
	Jaconine[4]	A	176	79	11	11	15	60
Sum of 35 PAs for legislation		A	2282	76	20	4	19	76
		B	1626	80	8	12	20	80
Sum of total PAs		A	2749	80	16	4	20	80
		B	1628	80	8	12	20	80

Matrix: A= alfalfa, B= herbal tea.

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.

4) PAs not included in the list of 21 PAs + 14 isomers proposed for legislation.

Table 1c *Summary of research study materials parameters and participants' performance on PA groups – number of laboratories reporting quantitative values, <LOQ and FN.*

Pyrrolizidine alkaloid group	Matrix	Assigned value (µg/kg)	Uncertainty (µg/kg)	Robust RSD_R[1] (%)	No of labs out of 25 reporting		
					Quant. value	<LOQ	FN
Echimidine group	A	95.1	6.56	28	25		
Echimidine-N-oxide group	A	392	37.7	38	24	1	1
Intermedine group	A	112	13.5	48	25		
Intermedine-N-oxide group	A	461	59.1	51	25		
Retrorsine group	A	81.7	9.15	44	24		
	B	33.1	2.22	24	20	3	3
Retrorsine-N-oxide group	A	325	39.6	47	23	1	1
	B	113	8.33	29	24		
Senecionine group	A	114	9.77	34	25		
	B	41.8	3.23	28	21	4	3
Senecionine-N-oxide group	A	324	31.3	39	25		
	B	233	27.0	44	23	1	1
Seneciphylline group	A	94.1	8.01	33	24		
	B	71.8	7.89	42	23	1	1
Seneciphylline-N-oxide group	A	147	15.0	40	24		
	B	245	15.8	25	24		

Matrix: A= alfalfa, B= herbal tea.

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.

Table 1d *Summary of research study materials parameters and participants' performance on PA groups – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

Pyrrolizidine alkaloid group	Matrix	Assigned Value (µg/kg)	z-scores[2] Satisfact. (% of z or z'-scores)	Quest. (% of z or z'-scores)	Unsatisf. (% of z or z'-scores)	Labs out of 25 with Accept. z or z'-score	
						No[3]	%[3]
Echimidine group	A	95.1	88	8	4	22	88
Echimidine-N-oxide group	A	392	92	4	4	23	92
Intermedine group	A	112	68	24	8	17	68
Intermedine-N-oxide group	A	461	72	12	16	18	72
Retrorsine group	A	81.7	75	8	17	18	72
	B	33.1	70	4	26	16	64
Retrorsine-N-oxide group	A	325	67	17	17	16	64
	B	113	79	13	8	19	76
Senecionine group	A	114	76	16	8	19	76
	B	41.8	71	17	13	17	68
Senecionine-N-oxide group	A	324	76	12	12	19	76
	B	233	75	4	21	18	72
Seneciphylline group	A	94.1	71	13	17	17	68
	B	71.8	75	13	13	18	72
Seneciphylline-N-oxide group	A	147	79	8	13	19	76
	B	245	88	0	13	21	84

Matrix: A= alfalfa, B= herbal tea.

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 secondary plant metabolites produced by a number of plants from the families of Asteraceae *(e.g. Senecio spp.),* Boriginaceae (e.g. *Heliotropium spp*.) and Fabeceae *(e.g. Crotalaria spp.)*. PAs occur in plants as free base or the N-oxide (NO). Isomeric forms do occur of several PAs and PA-N-oxides. Many of these PAs have been shown to be highly toxic, causing hepatic veno-occlusive disease (VOD), liver cirrhosis and ultimately death. The European Food Safety Authority (EFSA) has concluded in their opinions [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, by establishing maximum limits (MLs) for the total concentration of PAs. The underlying basis for regulation is the assumption that all 1,2-unsaturated PAs have a similar mode of action and similar toxicity (EFSA Scientific Opinion 2011:2406) [3].

Due to practical issues it is not possible to include all known PAs in legislation and it has been proposed by the EC and the MS to base legislation on the presence of 21 PAs that have been identified in the past as the most relevant with respect to occurrence in food products (primarily based on EFSA report 2016:4572 and EFSA Statement 2017:4908). These PAs are presented in Table 2. It should be noted that 4 isomeric PA pairs are included in the original EU list of PAs to be regulated: intermedine and lycopsamine, intermedine-N-oxide and lycopsamine-N-oxide, senecionine and senecivernine and senecionine-N-oxide and senecivernine-N-oxide. In addition to the isomers already in the list, for 10 PA of the 21 PAs, 14 structural isomers have been identified that can co-elute with one or more of the PAs listed 21 PAs (Table 2). If the method used can quantify each isomer, in the proposed legislation these 14 PAs need to be also analyzed and included in the sum concentration together with the 21 PAs of the main list. The proposed legislation is expected to become in effect by 1 July 2022.

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 isomeric groups. 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 the same chemical structure, were in addition assessed as isomeric groups, in which the sum of all individual isomers was considered. One aim of this study is to determine if quantitative results can be compared between methods with different levels of isomer separation.

For animal feeds no legislation is yet foreseen. Based on limited data available for animal feeds it can be expected that the list of relevant PAs may be different from the list relevant for food. For this reason, the analytical methods for animal feeds should preferably also include extra PAs that are not mentioned in the list proposed for legislation in food.

Table 2 *PAs proposed for analysis in the research study, a combination of the PAs proposed for EU legislation, their isomers and a set of other Pas.*

PA	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomeric group
Echimidine (Em)*	**Heliosupine (Hs)#**				*Echimidine group (Em group)*
Echimidine-N-oxide (EmNO)	**Heliosupine-N-oxide (HsNO)**				*Echimedine-N-oxide group (EmNO group)*
Intermedine (Im)	Lycopsamine (Ly)	**Indicine (Id)**	**Echinatine (En)**	**Rinderine (Rn)**	*Intermedine group (Im group)*
Intermedine-N-oxide (ImNO)	Lycopsamine-N-oxide (LyNO)	**Indicine-N-oxide (IdNO)**	**Echinatine-N-oxide (EnNO)**	**Rinderine-N-oxide (RnNO)**	*Intermedine-N-oxide group (ImNO group)*
Retrorsine (Rt)	**Usaramine (Us)**				*Retrorsine group (Rt group)*
Retrorsine-N-oxide (RtNO)	**Usaramine-N-oxide (UsNO)**				*Retrorsine-N-oxide group (RtNO group)*
Senecionine (Sn)	Senecivernine (Sv)	**Integerrimine (Ir)**			*Senecionine group (Sn group)*
Senecionine-N-oxide (SnNO)	Senecivernine-N-oxide (SvNO)	**Integerrimine-N-oxide (IrNO)**			*Senecionine-N-oxide group (SnNO group)*
Seneciphylline (Sp)	**Spartioidine (St)**				*Seneciphylline group (Sp group)*
Seneciphylline-N-oxide (SpNO)	**Spartioidine-N-oxide (StNO)**				*Seneciphylline-N-oxide group (SpNO group)*
Europine (Eu)					
Europine-N-oxide (EuNO)					
Heliotrine (Ht)					
Heliotrine-N-oxide (HtNO)					
Lasiocarpine (Lc)					
Lasiocarpine-N-oxide (LcNO)					
Senkirkine (Sk)					

*in brackets: abbreviation of PA

#in plain tekst original PAs proposed for legislation; in **bold** the added PA isomers

Other PAs in the research study
Erucifoline (Er)
Erucifoline-N-oxide (ErNO)
Jacobine (Jb)
Jacobine-N-oxide (JbNO)
Jacoline (Jl)
Jaconine (Jn)
Monocrotaline (Mc)
Monocrotaline-N-oxide (McNO)
Trichodesmine (Td)

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). In view of the novelty of analyte/matrix combination, this study will be conducted not as a proficiency test but as a 'research study', with the main aim to gain insight in the current capabilities of the NRLs. To facilitate NRLs in their task, official laboratories (OLs) can also participate, in consultation with their NRL.

2 Research study material

2.1 Scope of the research study

This research study focused on pyrrolizidine alkaloids in food and feed, using alfalfa (lucerne) and herbal tea as representative matrices. In view of the relative novelty of this analyte/matrix combination, this study will be conducted as a 'research study' to gain insight in the current capabilities of the participating laboratories to perform analyses on PAs. The scope includes the set of 21 PAs currently considered for legislation in food products. The scope also includes the 14 isomeric PAs that are mentioned for legislation in food products. Furthermore, 9 PAs, erucifoline, erucifoline-N-oxide, jacobine, jacobine-N-oxide, jacoline, jaconine, monocrotaline, monocrotaline-N-oxide and trichodesmine, were included because they can be relevant for feed products as well as of additional relevance for food products.

2.2 Material preparation

For preparation of the two materials A and B, alfalfa (lucerne) and herbal tea, respectively, were used. A mixture (4:1) of blank alfalfa and blank hay and a mixture of several blank herbal teas (mint tea, hibiscus cherry blossom tea, rosemary tea, herbal blend tea) were milled using a centrifugal mill (ZM 200, Retsch, Haan) to obtain a particle size of 500 µm.

Premixes for material A and B were prepared in the following way: PA containing plants (ground to <500 µm), in total 10 g for material A (*Anchusa officinalis* (6 g), *Echium vulgare* (1 g), *Jacobaea vulgaris* (2.5 g) and *Senecio inaequidens* (1 g)) and 3 g for material B (*Senecio vulgaris* (1.5 g) and *Heliotropium europaeum* (1.5 g)), were extracted with, respectively, 200 and 100 mL methanol/water/formic acid (90:10:0.2) in a rotary tumbler for 2 h at RT followed by centifugation (15 min, 3500*g*). The extraction was repeated (1 h) with, respectively, 50 and 25 mL of the same extraction solvent. The respective combined extracts were filtered through a glass fiber filter (Whatman GF1A 90 mm) and then slurried with, respectively, 200 g blank material A and 200 g blank material B. 250 mL methanol was added to each premix and mixed for 10 min using a spoon. The premixes were air dried in a fumehood and subsequently homogenized using a Stephan cutter UMC 5.

The final material A was a blend of the fortified alfalfa premix with blank alfalfa and the final material B was a blend of premix herbal tea with blank herbal tea. For both materials, 2300 g of blank material were fortified with 200 g of premix. Materials A and B were homogenised by mixing in a rotating drum during 2 hours and stored at 4 °C until use. The homogenisation of the materials was carried out at Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). WEPAL is accredited to ISO/IEC 17043 for the organisation of proficiency tests by the Dutch Accreditation Council (RvA, R002).

2.3 Sample identification

After homogenization, materials A and B were divided into sub-portions of approximately 25 grams and stored in polypropylene, airtight closed containers in the refrigerator until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was 2019/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 research 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), and shaking in an overhead shaker. 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, reconstituted in methanol/water 1/9 (v/v) (500 µL) and filtered. Analysis was done by LC-MS/MS, using reversed phase chromatography under alkaline conditions.

The isomers lycopsamine and indicine could not be separated by the method applied by the organiser of the proficiency test. The presence of indicine can, therefore, not be excluded in material A.

The homogeneity of the materials was assessed 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 $s_s < 0.3 \times \sigma_P$. 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 pyrrolizidine alkaloids (µg/kg d.w.) in material A and B obtained during homogeneity testing.*

Material A: alfalfa			Material B: herbal tea		
Compound	Conc. (µg/kg)	RSD %	Compound	Conc. (µg/kg)	RSD %
Echimidine	92.1	2.97	Europine	29.6	3.70
Echimidine-N-oxide	397	1.40	Europine N-oxide	226	2.25
Erucifoline	112	2.61	Heliotrine	47.7	2.60
Erucifoline-N-oxide	207	3.27	Heliotrine-N-oxide	397	2.42
Integerrimine	26.9	3.80	Integerrimine	14.0	7.60
Integerrimine-N-oxide	96.6	3.02	Integerrimine-N-oxide	88.8	4.45
Intermedine	41.3	5.07	Lasiocarpine	18.2	3.48
Intermedine-N-oxide	167	9.13	Lasiocarpine-N-oxide	185	2.77
Jacobine	39.8	4.48	Retrorsine	25.4	7.27
Jacobine-N-oxide	34.2	3.90	Retrorsine-N-oxide	124	3.25
Jacoline	42.3	4.19	Senecionine	39.3	4.87
Jaconine	186	2.34	Senecionine-N-oxide	176	3.45
Lycopsamine	93.6	3.27	Seneciphylline	70.9	4.59
Lycopsamine-N-oxide	273	4.94	Seneciphylline-N-oxide	224	3.66
Retrorsine	63.0	3.05	Senecivernine-N-oxide	37.0	8.29
Retrorsine-N-oxide	321	2.53	Spartioidine	14.3	8.21
Senecionine	93.9	2.27	Spartioidine-N-ox	62.6	3.48
Senecionine-N-oxide	213	2.83			
Seneciphylline	90.4	2.49			
Seneciphylline-N-oxide	153	3.83			
Senecivernine	15.4	3.54			
Senecivernine-N-oxide	89.5	4.72			
Senkirkine	12.4	3.85			
Usaramine-N-oxide	76.3	2.81			

Both materials proved to be sufficiently homogeneous for this research study. Senkirkine in material B was the only analyte for which the criteria for homogeneity testing did not comply. A high variability in

the results was obtained, causing s_s (1.72 µg/kg) to exceed the critical value of $0.3\sigma_p$ (1.30 µg/kg) and s_w (6.97 µg/kg) to exceed $0.5\sigma_p$ (2.16 µg/kg).

Senkirkine in material B was found in concentrations ranging from 11 to 38 µg/kg. Likely the high variability of senkirkine in material B is due to the fact that one of the herbal tea ingredients used to prepare the material contained traces of senkirkine as a contaminant. Senkirkine was not present in the extract prepared from the PA-containing plant materials. Despite the deviation for senkirkine, material B was considered to be homogeneous, since the other PAs fulfilled the homogeneity requirements. Senkirkine in material B was excluded from evaluation in this research study.

During the homogeneity testing, it was found that material B contained traces of echinatine, echinatine-N-oxide and rinderine-N-oxide in the range of 0 – 24 µg/kg, with RSDs ranging from 41 – 84%. The average concentration was below the LOQ of 10 µg/kg used for this study. The presence of these PAs probably resulted from a low contamination in one of the raw materials. Echinatine, echinatine-N-oxide and rinderine-N-oxide in material B were excluded from evaluation in this research study.

2.5 Stability of the materials

The stability of the PAs in the materials was assessed according to [10, 11]. On May 13th, 2019, the day of distribution of the samples, six randomly selected containers of each material A and B were stored at <-18°C. Under these conditions it is assumed that the PAs are stable in the materials. In addition, six samples of each material were stored at 4-6°C.

On July 3th, 2019, 51 days after distribution of the samples, for each of the storage conditions (<-18°C and 4-6°C) six samples of materials A and B 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 at 4-6°C. A consequential instability is observed when the average value of an analyte in the samples stored at 4-6°C is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-18°C. 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. Under the tested storage conditions no consequential difference was observed. The PAs in the materials were, therefore, considered stable for the duration of the study.

3 Organisational details

3.1 Participants

This research study focused on the determination of PAs in food and feed, using alfalfa and herbal tea. Invitations to the NRL network were sent out on April 3th, 2019 (Annex 5). Twenty-eight laboratories registered for the study (Annex 1) and 27 participants reported their results. One participant was unable to report results due to problems with their method. Of the participating laboratories, 24 were NRLs and three were OLs. Each participant was free to use their analytical method of choice reflecting their routine procedure. The participants were asked to report the results through a web application designed for proficiency tests as well as to fill out a questionnaire, where they were 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 two coded samples (Annex 2) and three vials containing a total of 45 PA standards, including all the relevant PAs and isomers proposed for legislation, were sent to the participants on May 13th, 2019. 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, the standard solutions at <-18°C, and to analyse the samples according to their routine method. As reported by participants, all parcels were received in good order within 24 hours after dispatch.

The samples were accompanied by a letter describing the requested analysis (Annex 6) and an acknowledgement of receipt form. In addition, by e-mail, each participant received instructions on how to use the web application to report the results. The questionnaire was intended to collect additional information on LOQs, 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 each PA in each sample was requested. The deadline for submitting the quantitative results was June 24th, 2019, allowing the participants six weeks for analysis of the test samples.

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 is based on assigned values and the standard deviation for proficiency assessment (σ_P). From this, z-scores are calculated to classify the participants' performance. Details on the methods used for the statistical evaluation can be found in the background document 'EURL-MP PT performance assessment' [14] on the EURL mycotoxins & plant toxins (EURL-MP) website.

4.1 Calculation of the assigned value

The robust mean was used as consensus value in this research study. The consensus value based on the participants' results (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 analytes in both materials, except for senecivernine in material B (herbal tea) since the uncertainty of the assigned value, 3.56 µg/kg, exceeds 0.7 σ_P (1.88 µg/kg).

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:

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

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 research material, is not negligible, then 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 research study, the uncertainty of the assigned value for individual pyrrolizidine alkaloids 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: echimidine-N-oxide, intermedine, intermedine-N-oxide, lycopsamine-N-oxide, retrorsine, retrorsine-N-oxide, usaramine-N-oxide, senecivernine, intergerrimine, senecionine-N-oxide, senecivernine-N-oxide, seneciphylline, seneciphylline-N-oxide and erucifoline-N-oxide and jacobine.
- material B: retrorsine, senecionine, senecionine-N-oxide, senecivernine-N-oxide, integerrimine-N-oxide, seneciphylline, spartioidine, lasiocarpine and lasiocarpine-N-oxide.

For the isomeric groups, the uncertainty of the assigned value in both materials, except for the echimidine group in material A and the retrorsine group, retrorsine-N-oxide group and seneciphylline-N-oxide 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 for legislation in material A, the uncertainty of the assigned value was taken into account for in 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]'; or 'not detected (nd)' (i.e. below their limit of quantification (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) [14], using the reported LOQ value as a result. Proxy-z-scores are for information only and indicated as a value between brackets. Values ($z < -2$) are considered as False Negatives (see 4.5). Values ($z > 2$) indicate that the LOQ is high in relation to the assigned value and high in comparison to other participants.

Other types of 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 applicable analyte/matrix.

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 research material by the organiser, and/or not detected by the majority 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.

Specific for this research study:

- Considering the fact that the proposed legislation requires a limit of quantification (LOQ) of 10 µg/kg, it was decided to use a threshold limit of 2×LOQ, in this study 20 µg/kg, to determine FP.
- The analytical method applied by the organiser of the research study did not allow to determine the presence of (trace amounts) of indicine due to co-elution of indicine with lycopsamine. Therefore, in material A, no FP were considered for indicine.
- Two participants reported for material B the sum of intermedine-N-oxide and indicine-N-oxide above the threshold value. In both cases, one of the two PAs was indicated as FP.
- The PAs echinatine, echinatine-N-oxide and rinderine-N-oxide were detected in material B in trace amounts close to the LOQ by the organiser of the research study (paragraph 2.4). It was decided that quantification of these PAs by the participants was possible. These PAs were, therefore, not considered as FP in material B.

When an PA is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', 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 (see 4.4) is <-2. False negatives are indicated as '**FN**'. False negatives are to be interpreted as unsatisfactory performance.

5 Assessment of participants' performance

5.1 Scope and LOQ

This research study was dedicated to the quantification of pyrrolizidine alkaloids in alfalfa and herbal tea samples. Annex 7 summarises the quantitative scopes of each participant, with an indication of the LOQs for each PA.

Twenty-five participants analysed the two samples, material A and material B for 44 PAs. Participant PT9566 analysed only material A and participant PT9569 analysed only material B.

Concerning the individual PAs included in the scope of the participants: 24 participants did have methods for determination of the 21 PAs first considered for legislation, two participants did not have echimidine and echimidine-N-oxide in their scope and one laboratory did not have europine, europine-N-oxide and heliotrine-N-oxide in its scope.

Participant PT9570 included only 4 PAs, retrorsine, senecionine, seneciphylline and senkirkine, in the method. The results submitted by participant PT9570 were not included in the calculation of the assigned value. For participant PT9570 z-scores were calculated separately for these four PAs using the assigned values calculated from the data submitted by the other participants.

Of the 27 participants, 18 participants also had all 14 isomeric analogues of the 21 PAs considered for legislation in their scope. Nineteen participants also included nine additional PAs in their scope, which are known to occur in food and feed products.

Several results were reported as 'detected'. In case the participant had specified an LOQ (Annex 7), for these results proxy z-scores were calculated. Also a few results were reported as 'nd' without specification of LOQ and therefore, these results were excluded from evaluation (see section 4.4).

With respect to the LOQs provided by the participants, 12 participants reported an LOQ of 10 µg/kg for all analytes, seven participants reported LOQs in the range of 2 – 5 µg/kg, four participants reported variable LOQs in the range of 0.1 – 10 µg/kg and two reported LOQs in the range of 20 – 25 µg/kg. One laboratory reported LOQs in a wider range, namely 3 - 400 µg/kg and one 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 proposed legislation.

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. One participant provided no information on their accreditation, nor any method details and the preparation conditions. An overview of the applied methods is presented in Annex 8.

Out of 26 laboratories, four had their analytical method covered by ISO 17025 accreditation, while 21 participants had not accredited their method and one participant did not provide this information.

The information provided about test material preparation showed that the most often reported intake was 2 g (88%) in combination with 40 ml extraction solvent (among which four participants did a double extraction 2×20 ml) and one laboratory used 8 ml. Two participants used 1 g of which one in combination with 20 ml of extraction solvent and one with a double extraction of 15 ml and 10 ml. One laboratory used 2.5 g with 25 ml extraction solvent. The extraction time was in most of the cases

30 min and in case of a double extraction 2×15 min. As extraction solvent participants often used an aqueous acidic solvent: 0.2% formic acid (14×), 1-2%formic acid (3×), or 0.05 M sulphuric acid (7×). One participant used a mixture of methanol and water and in one case a solution containing sodium chloride and hydrochloric acid was used.

SPE was used by 23 participants for sample extract purification. Three participants used no clean-up among which two participants only diluted the extract. The following clean-up cartridges were reported: polymeric SPE sorbent (Stata X or Oasis HLB, 14×), Bond Elut (3×), DSC (4×), SDB (1×) and Evolute express CX (1×).

All participants used LC in combination with MS for separation and identification of the PAs. Three participants used two different methods, so in total 30 methods were reported. Acetonitrile was used as organic mobile phase modifier by 18 while 12 participants used methanol. The majority of participants (67%) indicated that acidic chromatography had been used. For the preparation of the acidic mobile phase the following buffers were used: formate (formic acid with or w/o ammonium formate) (15×), acetate (acetic acid with or w/o acetate) (4×) and acetate (acetic acid with ammonium hydroxide) (1×). Ten participants employed an alkaline mobile phase with ammonium carbonate (33%).

For the acidic chromatography a wide variety of column brands were used, mostly with a C18 type stationary phase (85%). Columns from different suppliers were used: Waters Acquity BEH C18 (5×); Thermo Scientific Hypersil Gold (5×); Phenomenex Gemini (2×), Luna (2×) and Kinetex XB (1×); Agilent Portoshell SB (1×) and Zorbax SB (1×). A few methods used a different stationary phase: Phenomenex Kinetex biphenyl or phenyl-hexyl (3x). For chromatography with alkaline conditions participants also used mostly C18 type stationary phases, mostly from one supplier: Waters Acquity BEH C18 (8×), Atlantis T3 (1×) and XSelect CSH C18 (1×). There was a considerable variation in column length, between 50 and 200 mm, although typically 150 mm was used (18×). The total run time reported varied between 9 and 70 min, typically around 15 min (13×). Most participants used Very-High Pressure Liquid Chromatography (VHPLC) type columns with a small internal diameter, around 2 mm (25×), and sub-2-µm particle size (17×).

For the identification and quantification of the PAs most participants used LC-MS/MS (93%), two participants applied LC-HRMS (High resolution Mass Spectrometry) of which one participant used Quadrupole Time-of-Flight (QTOF)/MS.

On questions about the calibration approach all except two participants replied. For the quantification of the PAs 20 participants used multi-level standard addition (MLSA), among which seven used matrix-matched standard calibration, four used standard addition after extraction, six used standard addition before extraction and one participant used solvent external standard calibration. Eight participants used for quantification single point calibration, among which seven used standard addition before extraction and one after extraction.

Based on the results and method details provided by the participants no obvious effects of extraction or clean-up on the results were observed but the results indicated that subtle changes in chromatographic conditions may already result in differences in the separations obtained by the individual methods. The different stationary phase brands and types also have a significant effect on the separation of isomers and the order of elution may also differ. Because the effect of the chromatography on the separation of isomers is an important issue with respect to the adequate quantification of PAs, this was investigated in more detail. The participants were asked to analyse the three provided mixtures of standards and to report the retention times (Annex 8). First, it is important to state that to date no analytical methods have been described that can separate all the isomeric groups of Table 2 in a single run with baseline separation with the current state-of-the-art chromatographic instrumentation. It is however well established that separation of isomers can be influenced by the choice of column bonded phase material as well as the pH of the mobile phase. In Table 5 an overview is given of the main separation problems encountered by the different chromatographic methods. From the data it follows that there are frequent difficulties with the separation of isomeric pairs or groups when acidic chromatography is used. For the (smaller group of)

alkaline chromatography the problem seems to be somewhat more limited. Overlooking all methods and datasets, for almost all isomeric pairs and groups problems with (partial) co-elution can be identified. Only for the echimidine N-oxide/heliosupine N-oxide pair no problems with separation have been identified, but for all the other possible isomer combinations, at least one but often more participants reported poor separation.

Table 5 *General evaluation of problems with separation between isomeric compounds based on the information provided by the participants.*

PA isomeric group	Acidic chromatography		Alkaline chromatography	
	Frequent problem	Less frequent problem	Frequent problem	Less frequent problem
Em/Hs		Em+Hs		
EmNO/HsNO				
Im/Ly/Id/En/Rn	Ly+Id+En+Rn	Im+En	Im+Ly+Id	Id+En+Rn
ImNO/LyNO/IdNO/EnNO/RnNO	ImNO+IdNO; EnNO+RnNO	ImNO+EnNO; LyNO+RnNO	ImNO+LyNO	IdNO+EnNO+RnNO
Rt/Us	Rt+Us			
RtNO/UsNO	RtNO+UsNO			RtNO+UsNO
Sn/Sv/Ir	Ir+Sv	Sn+Sv		
SnNO/SvNO/IrNO	IrNO+SvNO	SnNO+SvNO	SnNO+SvNO	
Sp/St	Sp+St			
SpNO/StNO	SpNO+StNO			

5.3 Performance

The twenty-seven participants were asked to determine and quantify as many individual PAs as possible in the two materials. All participants received analytical standards of 44 PAs.

- Since material A contained 24 and material B 17 PAs above the LOQ (10 µg/kg), the maximum number of quantifiable **individual PAs** was 41.
- In material A, data for quantification of 10 isomeric groups could be submitted and for 6 isomeric groups in material B, amounting to a total of 16 **isomeric groups**.
- For each of the two materials, all submitted data for the 35 individual PAs and isomers proposed for legislation were summed. This sum includes, besides the compounds for which a z-score was evaluated, also quantitative results for individual PAs for which a z-score could not be calculated, trace amounts and **FP** results. The data were used to calculate the z-score for the **sum of 35 PAs for legislation** in each material.
- For each of the two materials, all submitted data for all 44 individual PAs also were summed. These data were used to calculate the **sum of total PAs** z-scores for materials A and B. Since some participants reported individual PAs belonging to the group of 'other PA' in material B, the z-score for 'total sum' differs slightly from the z-score for 'sum of 35 PAs for legislation'.
- Reporting of **FN** and **FP** results was also evaluated.

The quantitative performance was assessed through z-scores. For each participant, the results of the calculated individual z-scores for the PAs in material A (alfalfa) and B (herbal tea) are summarised in Annex 9 and Annex 10, respectively. These annexes also show graphical representations of the z-scores per individual PA (41) and the sixteen isomeric groups. Results per group were calculated based on the reported data for the individual isomers. For laboratories that had problems with separation between PA isomers, only z-scores were calculated for the total PA concentrations of each isomer group. A summary of the performance of the participants in this research study is provided in Annex 11.

A summary of the statistical evaluation of the research study results is presented in Tables 6 to 9. These tables include all relevant parameters: the assigned value (A), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σp) 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 (calculating the z'-score).

A total of 61 z-/z'-scores, 24 z-scores and 37 z'-scores were calculated from the reported data for both materials.

Table 6 *Parameters of the individual PAs and summary in material A.*

	Gr Em	Gr EmNO	Gr Im		Gr ImNO		Gr Rt	Gr RtNO		Gr Sn		
	Em	EmNO	Im	Ly	ImNO	LyNO	Rt	RtNO	UsNO	Sn	Sv	Ir
A (µg/kg)	88.9	384	30.0	85.7	178	311	70.6	273	51.0	79.0	17.8	25.1
u (µg/kg)	6.21	36.8	2.65	5.71	18.4	28.0	7.99	33.9	5.62	4.92	2.14	2.53
σ_p (µg/kg) (25%)	22.2	95.9	7.49	21.4	44.4	77.7	17.7	68.3	12.7	19.8	4.44	6.27
$u>0.3\sigma_p$	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
robust σ (µg/kg)	24.3	144	9.00	17.1	57.0	95.2	29.3	121	15.6	18.5	6.17	7.00
robust σ (%)	27	38	30	20	32	31	42	44	31	23	35	28
# reported	24	25	20	15	16	18	21	21	16	23	20	17
"<", nd			1	1	1				2		7	3
detected		1	1					1		1		
nd (no LOQ)									2			2
# quantitative res	24	24	18	14	15	18	21	20	12	22	13	12
$\|z\|\leq 2$	22	22	15	12	14	15	16	16	9	19	10	10
$2<\|z\|<3$	1	2	2			1	3	2	1		1	1
$\|z\|\geq 3$	1		1	2	1	2	2	2	2	3	2	1
FN		1	1	1	1			1	2	1	1	3
S z-scores (%)	92	88	79	80	88	83	76	76	64	83	71	67

S z-scores: satisfactory z-scores

	Gr SnNO			Gr Sp	Gr SpNO		Other PAs					
	SnNO	SvNO	IrNO	Sp	SpNO	Sk	Er	ErNO	Jb	JbNO	Jl	Jn
A (µg/kg)	197	74.9	68.6	90.3	138	12.0	107	192	46.4	33.1	40.7	176
u (µg/kg)	25.0	9.26	3.39	7.02	13.2	0.551	6.26	16.6	3.97	2.39	1.92	11.6
σ_p (µg/kg) (25%)	49.1	18.7	17.2	22.6	34.5	3.00	26.7	47.9	11.6	8.28	10.2	44.1
$u>0.3\sigma_p$	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	No
robust σ (µg/kg)	84.7	33.1	9.39	25.8	47.2	2.02	22.4	56.4	12.3	8.13	6.51	39.4
robust σ (%)	43	44	14	29	34	17	21	29	27	25	16	22
# reported	19	21	16	21	21	25	22	21	22	22	20	19
"<", nd		1	2		1	3	2	2	5	2	2	1
detected	1					1		1	1	1		
nd (no LOQ)			2						1	1		
# quantitative res	18	20	12	21	20	21	20	18	15	18	18	18
$\|z\|\leq 2$	15	14	11	16	17	19	18	16	13	16	17	15
$2<\|z\|<3$	1	4	1	2	2	2		2				2
$\|z\|\geq 3$	2	2		3	1		2		2	2	1	1
FN	1	1	1				1	3	6	3	2	1
S z-scores (%)	79	67	85	76	85	90	86	76	62	76	85	79

S z-scores: satisfactory z-scores

Table 7 *Parameters of 10 isomeric groups of PAs and summary in material A.*

	Group										Leg.	All
	Em	Em NO	Im	Im NO	Rt	Rt NO	Sn	Sn NO	Sp	Sp NO	Total sum	Total sum
A (µg/kg)	95.1	392	112	461	81.7	325	114	324	94.1	147	2287	2750
u (µg/kg)	6.56	37.7	13.5	59.1	9.15	39.6	9.77	31.3	8.01	15.0	185	175
σ_p (µg/kg) (25%)	23.8	97.9	27.9	115	20.4	81.2	28.4	81.1	23.5	36.7	572	687
$u>0.3\sigma_p$	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
robust σ (µg/kg)	26.2	148	53.9	236	35.8	152	39.1	125	31.4	58.7	740	699
robust σ (%)	28	38	48	51	44	47	34	39	33	40	32	25
# reported	25	25	25	25	24	24	25	25	24	24	25	25
"<", nd						1						
detected		1										
# quantitative res	25	24	25	25	24	23	25	25	24	24	25	25
$\|z\|\leq 2$	22	23	17	18	18	16	19	19	17	19	19	20
$2<\|z\|<3$	2	1	6	3	2	4	4	3	3	2	5	4
$\|z\|\geq 3$	1		2	4	4	3	2	3	4	3	1	1
FN		1				1						
S z-scores (%)	88	92	68	72	75	67	76	76	71	79	76	80

s z-scores: satisfactory z-scores

Table 8 *Parameters of the individual PAs and summary in material B.*

	Gr Rt	Gr RtNO	Gr Sn		Gr SnNO			Gr Sp		Gr SpNO	
	Rt	RtNO	Sn	Ir	SnNO	SvNO	IrNO	Sp	St	SpNO	StNO
A (µg/kg)	29.8	106	25.7	15.1	174	28.1	52.9	59.4	15.9	207	44.0
u (µg/kg)	2.75	7.51	2.91	0.95	20.0	4.59	5.44	5.24	1.22	14.0	2.81
σ_p (µg/kg) (25%)	7.45	26.6	6.42	3.77	43.5	7.02	13.2	14.8	3.98	51.8	11.0
$u>0.3\sigma_p$	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No
robust σ (µg/kg)	8.79	26.9	9.89	2.39	66.0	14.7	14.4	18.7	2.58	51.5	7.80
robust σ (%)	29	25	39	16	38	52	27	30	16	25	18
# reported	20	21	23	18	18	20	15	21	16	21	16
"<", nd	4	1	4	6	1	4	2	1	7		2
detected			1								
nd (no LOQ)				2			2		2		2
# quantitative res	16	20	18	10	17	16	11	20	7	21	12
$\|z\|\leq 2$	15	17	17	8	15	10	10	17	6	17	9
$2<\|z\|<3$	1	2	1			1		1	1	1	1
$\|z\|\geq 3$		1		2	2	5	1	2		3	2
FN	3		3		1	3	1	1			2
S z-scores (%)	79	85	81	80	83	53	83	81	86	81	64

s z-scores: satisfactory z-scores

	Other PAs					
	Eu	EuNO	Ht	HtNO	Lc	LcNO
A (µg/kg)	26.4	225	43.8	363	19.0	145
u (µg/kg)	1.16	7.61	2.21	14.4	1.71	11.5
σ_p (µg/kg) (25%)	6.61	56.4	10.9	90.8	4.75	36.3
$u>0.3\sigma_p$	No	No	No	No	Yes	Yes
robust σ (µg/kg)	4.27	29.2	8.65	55.4	6.40	44.1
robust σ (%)	16	13	20	15	34	30
# reported	25	25	25	24	25	25
"<", nd	3	1	1		2	1
detected				1	1	1
nd (no LOQ)	1	1				
# quantitative res	21	23	24	23	22	23
$\|z\|\leq 2$	20	20	22	19	20	20
$2<\|z\|<3$		1	1	2	1	2
$\|z\|\geq 3$	1	2	1	2	1	1
FN	1	1	1	1		2
S z-scores (%)	91	83	88	79	91	80

S z-scores: satisfactory z-scores

Table 9 *Parameters of 6 isomeric groups of PAs and summary in material B.*

	Group						Leg. PAs	All PAs
	Rt	RtNO	Sn	SnNO	Sp	SpNO	Total sum	Total sum
A (µg/kg)	33.1	113	41.8	233	71.8	245	1626	1628
u (µg/kg)	2.22	8.33	3.23	27.0	7.89	15.8	90.7	86.1
σ_p (µg/kg) (25%)	8.27	28.2	10.5	58.3	18.0	61.1	407	407
$u>0.3\sigma_p$	No	No	Yes	Yes	Yes	No	No	No
robust σ (µg/kg)	7.93	32.6	11.8	104	30.3	61.9	363	344
robust σ (%)	24	29	28	44	42	25	22	21
# reported	23	24	25	24	24	24	25	25
"<", nd	3		4	1	1			
# quantitative res	20	24	21	23	23	24	25	25
$\|z\| \leq 2$	16	19	17	18	18	21	20	20
$2<\|z\|<3$	1	3	4	1	3		2	2
$\|z\| \geq 3$	3	2		4	2	3	3	3
FN	3		3	1	1			
S z-scores (%)	70	79	71	75	75	88	80	80

S z-scores: satisfactory z-scores

For the individual PAs in material A, 76% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 6% of the results fell into the questionable range with $2<|z|<3$ and 18% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). For the isomeric groups in material A was this respectively 76%, 12% and 11% (Table 7).

For the individual PAs in material B, 77% 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 18% of the results fell into the unsatisfactory range with $|z| \geq 3$. For the isomeric groups in material B was this respectively 76%, 8% and 15%.

In case of the total sum of the 35 PAs considered for legislation, for material A, 76% of submitted results were satisfactory and for material B 80%. In case of the total sum of the PAs, for both materials, 80% of submitted results were satisfactory.

In Annex 11 an overview of the overall performance for each participant in this research study is summarized. For the two materials combined, a maximum of 40 satisfactory z-scores for the individual PAs could be obtained, and '41 out of 41' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. Out of 27 participants, none of the participants showed optimal performance by detecting all individual PAs with correct quantification, the absence of false positive and false negative results. In case of the isomeric groups, a maximum of 16 satisfactory z-scores for the individual PAs could be obtained, and '16 out of 16' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. Five of 27, showed satisfactory performance. With respect to the total sum of Pas considered for legislation, 19 participants, and in case of the total sum, 17 participants showed satisfactory performance.

A total of 19 FP results was reported by participants for material A. Six FP results concerned heliosupine N-oxide, in the range of 21.3 to 35 µg/kg. Heliosupine was reported twice (64 and 70.9 µg/kg). Indicine-N-oxide was reported two times (100 and 161 µg/kg) and one participant reported the presence of echinatine-N-oxide and rinderine-N-oxide (565 and 656 µg/kg). Usaramine was reported three times (73.4, 120 and 164.3 µg/kg). One participant reported spartioidine (63 µg/kg) and two partipants spartioidine-N-oxide (90 and 405 µg/kg). Europine-N-oxide (243 µg/kg) was reported by one participant.

For material B 15 FP results were reported. One participant reported the presence of lycopsamine (139.4 µg/kg), one participant reported indicine (22.2 µg/kg) and one reported rinderine (25.5 µg/kg). Two participants reported intermedine-N-oxide (35.1 and 38.8 µg/kg) and one reported indicine-N-oxide (30 µg/kg). Usaramine was reported three times (53.7, 54.6 and 120 µg/kg) and

usaramine-N-oxide two times (85.5 and 150 µg/kg). Senecivernine was reported two times (28.1 and 31.6 µg/kg as well as jacobine (26 and 50 µg/kg).

The reported FPs can in part be attributed to the fact that participants experienced difficulties with the correct identification of individual compounds of isomeric groups (e.g. heliosupine, heliosupine-N-oxide, indicine-N-oxide, usaramine, spartioidine and spartioidine-N-oxide in material A and usaramine, usaramine-N-oxide and senecivernine in material B). A number of participants indicated that they had very limited experience with the inclusion of the 14 PA isomers in their method.

A total of 31 false negative (FN) results was reported for material A. With respect to the regulated PAs, three FNs were reported for integerrimine and two for usaramine-N-oxide. Single FN results were reported for echimidine-N-oxide, intermedine, lycopsamine, intermedine-N-oxide, retrorsine-N-oxide, usaramine-N-oxide, senecionine, senecivernine, senecionine-N-oxide, senecivernine-N-oxide and integerrimine. For the additional, non-regulated, PAs, six FNs were reported for jacobine, three for jacobine-N-oxide and erucifoline-N-oxide, two for jacoline and one FN result for erucifoline and jaconine.

For material B 19 FN results were reported: three FNs for retrorsine, senecionine and senecivernine-N-oxide, two FNs for spartioidine-N-oxide and lasiocarpine-N-oxide and one FN for senecionine-N-oxide, integerrimine-N-oxide, seneciphylline, europine, europine-N-oxide, heliotrine and heliotrine-N-oxide.

With respect to the isomeric groups, for material A in two cases, the echimidine-N-oxide group and the retrorsine-N-oxide group, one participant reported a FN. For material B eight FNs were reported for the following isomeric groups: three FNs for the retrorsine group and the senecionine group and one FN for the senecionine-N-oxide and the seneciphylline group.

To some extent the reported FNs for the additional PAs in material A can be due to inexperience of participants to monitor for these PAs. The concentrations of the six PAs (erucifoline, erucifoline-N-oxide, jacobine, jacobine-N-oxide, jacoline and jaconine) in material A ranged from 33 to 192 µg/kg and were well above the LOQs reported by the majority of participants. Only in three instances the reported FN can be correlated to a reported LOQ > 10 µg/kg.

The (possible) coelution and reporting of isomeric PAs showed to be a complex issue for a number of participants. Many different combinations of co-eluting isomers were reported (Table 5, Annex 8, 9, 10). A number of participants correctly indicated which isomers were reported as a sum result. However, some participants reported contradictionary results. Double reporting of isomers may have occurred in a number of these cases, resulting in a in a too high isomeric group total concentration. In cases were double reporting was evident (exact the same concentration for two co-eluting isomers), only one of the results was used for the calculation of the isomeric group total concentration. However, double reporting could not always be verified, e.g. in case a participant reported two slightly different concentrations for two coeluting isomers. Unfortunately, the participants had not been asked to report isomeric group concentrations. The calculation of the isomeric group totals was now done by the study organiser based on the provided results. Asking the participants to report isomeric group totals together with the results for individual isomers, could possibly have prevented or reduced the risk of including double reporting in the group totals.

The option of reporting isomeric groups instead of reporting all compounds individually was evaluated. The concept of reporting isomeric groups is introduced to address the differences between methods with respect to the separation of isomers. The idea is that in case isomers are not (fully) separated the sum of the isomers can still be determined. A representative standard is used to quantify those isomers that have similar properties with respect to fragmentation and sensitivity. E.g. lycopsamine has very similar mass spectrometric properties as intermedine and can therefore be quantified by using intermedine (or vice versa). Care should be taken with isomers that have different fragmentation properties such as echimidine and heliosupine. In these cases it is highly preferred when the isomers are chromatographically separated and quantified individually. In the two materials in total 16 isomeric groups and 28 different PAs were present for which z-scores could be assigned (Tables 6-9). The percentage satisfactory z-scores for the isomeric groups varied between 67 and

92%, with an average of 76%. This is comparable with the satisfactory z-scores for the individual PAs present in the materials (range 53-92%, average 78%). The results indicate that there is a relatively strong link between the results for the individual PAs in an isomeric group and the sum of the isomers. This is not really surprising because for many isomeric groups only one isomer was present, or one isomer was dominating. For the more complex groups (intermedine and senecionine groups and corresponding N-oxides), this correlation was somewhat weaker, likely due to the increased number of possibilities that could result in a satisfactory or unsatisfactory result. Furthermore, in the total group concentration, theoretically an unsatisfactory low concentration for one isomer can be compensated by an unsatisfactory high concentration of another isomer, resulting in an overall higher satisfactory rate. This compensatory effect is not evident from the data. Rather the opposite is found: for the four groups mentioned above the satisfactory rate for the 14 individual compounds is 77% (range 53-88%), while for the four groups it is only 73% (range 68-75%). The reporting as isomeric groups may therefore need some more attention.

5.4 Robust relative standard deviation

The robust standard deviation (RSD_R) was calculated according to ISO13528:2015 [12] for informative purposes only. 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 the Tables 6 to 9 and also in Table 1 (Summary section).

For material A, the robust standard deviations (RSD_R) of the reported results (ranging between 14-44%), were below the target standard deviation (25%) for eight out of 24 individual PAs. For the isomeric groups in material A, none of the RSD_R (ranging between 28-51%) were below the target standard deviation. For material B, for the individual PAs, nine out of 17 RSD_R (ranging between 13-52%) were below the target standard deviation (25%). For the isomeric groups in material B, two out of six RSD_R (ranging between 24-44%) were below the target standard deviation.

The RSD_R values for the total sum of 35 PAs considered for legislation was for material A 32%, which is above the target standard deviation (25%) and for material B it was 25%. The RSD_R values for the total sum of PAs was below the target standard deviation (25%) for material A (22%) as well as for material B (21%).

6 Conclusions

Twenty-seven laboratories, of which 24 NRLs for mycotoxins and plant toxins in food and feed from 22 EU Member States, and 3 OLs participated in the research study EURL-PT-MP02 on the quantitative determination of PAs in alfalfa and herbal tea.

Two materials, alfalfa (material A) and herbal tea (material B), were prepared. Material A was prepared by adding blank alfalfa to alfalfa material contaminated with PAs and material B was prepared by adding blank herbal tea to herbal tea material contaminated with PAs. Material B contained only PAs from the list that will be regulated, and material A contained PAs that will be regulated as well as PAs that are not included in the list of regulated PAs.

Fifteen out of 27 participants determined all 44 PAs and 24 participants determined the 35 PAs considered for legislation. LOQs varied widely from 0.1 µg/kg to 250 µg/kg. Twenty-two participants reported LOQs equal or lower than 10 µg/kg, which is the minimally required LOQ specified in the upcoming legislation.

Most of the participants used methods based on LC-MS/MS (93%) with SPE clean-up (85%).

In this research study the robust mean was used as consensus value. The consensus value based on the participants' results was used as the assigned value. Results were calculated for individual PAs as well as for isomeric groups of PAs. The assigned values of individual PAs in material A ranged from 12.0 to 384 µg/kg and in material B from 15.1 to 207 µg/kg. Obtained interlaboratory reproducibility (RSD_R) ranged from 13% to 52%. For material A, RSD_R values were below the target standard deviation (25%) for seven out of 24 individual PAs and for material B this was the case for nine out of 17 individual PAs.

For the isomeric groups in material A, RSD_R values were below the target standard deviation (25%) for none of ten isomeric PA groups and for material B this was the case for three out of six groups of PA isomers. For the sum of 35 PAs considered for legislation the RSD_R was 32% and 25% for material A and B, respectively and for the sum of total PAs 25% and 21% for material A and B, respectively.

The proficiency of the participants was assessed through z-scores, calculated using the assigned values and a relative target standard deviation of 25%. For material A, 76% of the results for individual PAs were rated with satisfactory z-scores ($|z| \leq 2$), 6% of the results fell into the questionable range with $2<|z|<3$ and 18% of the results fell into the unsatisfactory range with $|z| \geq 3$. For material B 77% 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 18% of the results fell into the unsatisfactory range with $|z| \geq 3$. For the PA isomeric groups in material A, 76% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 12% of the results fell into the questionable range with $2<|z|<3$ and 11% of the results fell into the unsatisfactory range with $|z| \geq 3$ and for material B it was respectively, 76%, 8% and 15%.

A total of 19 FP results was reported by participants for material A and 15 FP were reported for material B. The reported false positives can in part be attributed to the fact that participants experienced difficulties with the correct identification of individual compounds of isomeric groups (e.g. heliosupine, heliosupine-N-oxide, indicine-N-oxide, usaramine, spartioidine and spartioidine-N-oxide in material A and usaramine, usaramine-N-oxide and senecivernine in material B).

A total of 31 FN results was reported for material A, while for material B 19 FN results were reported. With respect to the isomeric groups, for material A two FNs were reported and for material B there were eight FNs. Sixteen FNs in material A concerned the non-regulated, additional PAs of interest for analysis of feed. Only three FN results could be correlated to a reported LOQ > 10 µg/kg.

The (possible) coelution and reporting of isomeric PAs showed to be a complex issue for a number of participants. Many different combinations of co-eluting isomers were reported. A number of participants correctly indicated which isomers were reported as a sum result. However, some participants reported contradictionary results. Double reporting of isomers may have occurred in a number of cases, resulting in a in a too high isomeric group total concentration. Unfortunately, the participants had not been asked to report isomeric group concentrations. The calculation of the isomeric group totals was now done by the study organiser based on the provided results. Asking the participants to report isomeric group totals together with the results for individual isomers, could possibly have prevented or reduced the risk of including double reporting in the group totals.

From the results obtained in this research study on PAs in food and feed the following conclusions can be drawn. With a few exceptions, the limits of quantification reported by the participants are sufficient in view of the proposed legislative limits for PAs. The majority of participants has also included the 21 PAs together with the 14 isomeric PAs in their analytical method. Nevertheless the results indicate that there are a number of issues that need attention and that should be improved. The in many cases relatively high RSD_R values obtained for individual PAs as well as for isomeric groups indicate that there is substantial uncertainty in the assigned values. A high uncertainty indicates that there is a wide variation in the reported results. This is also substantiated by the relatively high number of questionable and unsatisfactory results reported. In this respect, it should be mentioned that the determination of PAs in animal feed and herbal tea is a relatively new area for many NRLs. Furthermore, the wide variety of analytes, including many isomeric compounds, that needs to be covered by the method, requires substantial experience by the laboratory. Efforts need to be made by NRLs to improve the robustness of the implemented methods, in order to produce reliable data. The concept of reporting of isomeric groups needs some special attention as well. It is anticipated that the formalisation of legislation for PAs will stimulate laboratories to optimise their methods and to obtain experience with the analysis of feed and food samples. The progress made will be tested in an upcoming proficiency study, projected for 2022.

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Annex 1 List of participants

Country	Organisation	NRL
AUSTRIA*	Austrian Agency for Health and Food Safety	NRL
BELGIUM*	Sciensano	NRL
CROATIA*	A. Stampar Teaching Institute of Public Health	NRL in formation
CYPRUS*	STATE GENERAL LABORATORY	NRL
CZECH REPUBLIC*	UKZUZ (Central Institute for Supervising and Testing in Agriculture	NRL
DENMARK*	National Food Institute	NRL
FINLAND*	Finnish Food Safety Authority Evira	NRL
FRANCE*	Laboratoire SCL de Strasbourg	NRL
GERMANY*	Federal Institute fur Risk Assessment (BfR)	NRL
ICELAND*	Represented by Eurofins WEJ Contaminants GmbH	NRL
GREECE*	General Chemical State Laboratory (GCSL)	NRL
HUNGARY*	National Food Chain Safety Office	NRL
IRELAND*	Public Analyst's Laboratory, Dublin	NRL
IRELAND*	The State Laboratory	NRL
ITALY	IZSLER	OL
ITALY*	Istituto superiore di sanita	NRL information
LITHUANIA*	National Food and Veterinary Risk Assessment Institute	NRL
LUXEMBOURG*	Laboratoire national de Sante	NRL
NETHERLANDS*	WFSR	NRL
POLAND*	National Institute of Public Health - National Institute of Hygiene	NRL
ROMANIA*	Institute for Hygiene and Veterinary Public Health	NRL
SLOVENIA*	University of Ljubljana, Veterinary Faculty, National Veterinary Institute	NRL
SLOVENIA*	National laboratory of health, environment and food	NRL
SWEDEN*	National Veterinary Institute, SVA	NRL
SWITZERLAND	Kantonales Laboratorium Thurgau	OL
SWITZERLAND	Service de la consommation et des affaires veterinaires (SCAV)	OL
UNITED KINGDOM*	FERA Science Ltd	NRL

* National Reference Laboratory of EU Member State at the time of the study.

Annex 2 Codification of the samples

Participants code	Material A*	Material B*
PT9547	187	376
PT9548	794	292
PT9549	402	947
PT9550	378	918
PT9551	832	940
PT9552	301	163
PT9553	900	953
PT9554	355	943
PT9555	279	578
PT9556	999	106
PT9557	594	893
PT9558	159	960
PT9559	877	226
PT9560	347	989
PT9561	411	123
PT9562	317	413
PT9563	898	763
PT9564	614	460
PT9565	783	859
PT9566	984	912
PT9567	275	771
PT9568	117	490
PT9569	277	501
PT9570	392	109
PT9571	225	371
PT9572	298	529
PT9573	620	905
PT9574	693	231

* All sample codes start with 2019/EURL PT MP/PAs/.

Annex 3 Statistical evaluation of homogeneity data

Sample No.	Echimidine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	90.5	92.9
Hom/A002	90.8	92.4
Hom/A003	89.9	90.6
Hom/A004	98.9	94.6
Hom/A005	91.3	93.5
Hom/A006	88.8	94.9
Hom/A007	90.2	92.8
Hom/A008	95.5	87.2
Hom/A009	89.4	94.7
Hom/A010	90.5	91.9
Grand mean	92.1	
Cochran's test		
C	0.393	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	23.0	
s_x	1.75	
s_w	2.95	
s_s	0.000	
Critical= 0.3 σ_P	6.90	
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.

Sample No.	Echimidine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001		
Hom/A002	398	397
Hom/A003	403	391
Hom/A004	410	392
Hom/A005	402	400
Hom/A006	389	392
Hom/A007	391.	402
Hom/A008	403	398
Hom/A009	394	393
Hom/A010	398	395
Grand mean	397	
Cochran's test		
C	0.541	
Ccrit	0.638	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	99.3	
s_x	3.61	
s_w	5.90	
s_s	0.000	
Critical= 0.3 σ_P	29.8	
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.	Erucifoline in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	108	109
Hom/A002	112	113
Hom/A003	115	109
Hom/A004	112	108
Hom/A005	111	112
Hom/A006	108	115
Hom/A007	112	116
Hom/A008	113	117
Hom/A009	106	114
Hom/A010	111	113
Grand mean	112	
Cochran's test		
C	0.350	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	27.9	
s_x	1.93	
s_w	3.07	
s_s	0.000	
Critical= 0.3 σ_P	8.38	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Erucifoline-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	215	215
Hom/A002	213	208
Hom/A003	206	194
Hom/A004	216	210
Hom/A005	195	208
Hom/A006	197	207
Hom/A007	203	201
Hom/A008	203	208
Hom/A009	212	215
Hom/A010	215	210
Grand mean	207	
Cochran's test		
C	0.318	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	51.9	
s_x	5.80	
s_w	5.19	
s_s	4.49	
Critical= 0.3 σ_P	15.6	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	25.8	26.8
Hom/A002	27.1	25.9
Hom/A003	28.1	27.5
Hom/A004	26.1	27.3
Hom/A005	27.0	26.6
Hom/A006	26.0	28.4
Hom/A007	27.1	27.6
Hom/A008	29.3	25.2
Hom/A009	27.2	26.0
Hom/A010	25.6	27.0
Grand mean	26.9	
Cochran's test		
C	0.548	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.72	
s_x	0.488	
s_w	1.25	
s_s	0.000	
Critical= 0.3 σ_P	2.02	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	91.5	94.4
Hom/A002	93.9	99.6
Hom/A003	95.0	96.6
Hom/A004	101	95.7
Hom/A005	91.4	96.8
Hom/A006	99.0	95.9
Hom/A007	99.9	95.6
Hom/A008	93.4	95.1
Hom/A009	97.5	101
Hom/A010	98.5	99.8
Grand mean	96.6	
Cochran's test		
C	0.222	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	24.1	
s_x	2.23	
s_w	2.69	
s_s	1.16	
Critical= 0.3 σ_P	7.24	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	39.8	40.3
Hom/A002	36.5	45.2
Hom/A003	43.2	42.4
Hom/A004	44.6	39.4
Hom/A005	42.5	39.9
Hom/A006	41.9	38.5
Hom/A007	40.1	39.8
Hom/A008	42.0	43.6
Hom/A009	42.0	41.1
Hom/A010	42.3	41.1
Grand mean	41.3	
Cochran's test		
C	0.600	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.3	
s_x	1.05	
s_w	2.52	
s_s	0.000	
Critical= 0.3 σ_P	3.10	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Intermedine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	183	155
Hom/A002	157	194
Hom/A003	174	151
Hom/A004	193	157
Hom/A005	153	150
Hom/A006	179	173
Hom/A007	158	148
Hom/A008	169	166
Hom/A009	190	171
Hom/A010	176	147
Grand mean	167	
Cochran's test		
C	0.258	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	41.8	
s_x	9.86	
s_w	16.4	
s_s	0.000	
Critical= 0.3 σ_P	12.5	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Jacobine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	40.5	38.4
Hom/A002	37.0	40.9
Hom/A003	40.5	41.0
Hom/A004	43.6	37.6
Hom/A005	40.2	38.9
Hom/A006	38.0	41.4
Hom/A007	37.5	41.4
Hom/A008	40.5	39.0
Hom/A009	38.5	42.5
Hom/A010	41.0	38.5
Grand mean	39.8	
Cochran's test		
C	0.335	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	9.96	
s_x	0.577	
s_w	2.34	
s_s	0.000	
Critical= 0.3 σ_P	2.99	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Jacobine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	31.9	34.0
Hom/A002	35.4	36.0
Hom/A003	32.0	34.3
Hom/A004	36.2	36.2
Hom/A005	31.4	33.7
Hom/A006	33.9	34.2
Hom/A007	33.5	32.8
Hom/A008	34.5	33.8
Hom/A009	35.5	35.1
Hom/A010	34.0	34.0
Grand mean	34.2	
Cochran's test		
C	0.365	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.54	
s_x	1.21	
s_w	0.842	
s_s	1.06	
Critical= 0.3 σ_P	2.56	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Jacoline in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	39.9	41.0
Hom/A002	40.2	44.9
Hom/A003	42.3	39.2
Hom/A004	42.3	40.0
Hom/A005	42.3	42.2
Hom/A006	44.1	41.9
Hom/A007	42.5	42.9
Hom/A008	42.9	45.7
Hom/A009	41.6	44.5
Hom/A010	44.3	41.0
Grand mean	42.3	
Cochran's test		
C	0.312	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.6	
s_x	1.17	
s_w	1.87	
s_s	0.000	
Critical= 0.3 σ_P	3.17	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Jaconine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	177	179
Hom/A002	185	189
Hom/A003	186	182
Hom/A004	194	180
Hom/A005	183	189
Hom/A006	183	190
Hom/A007	187	185
Hom/A008	184	192
Hom/A009	185	189
Hom/A010	184	191
Grand mean	186	
Cochran's test		
C	0.417	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	46.4	
s_x	2.80	
s_w	4.65	
s_s	0.000	
Critical= 0.3 σ_P	13.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	87.9	99.0
Hom/A002	91.9	91.7
Hom/A003	94.4	91.5
Hom/A004	96.0	97.0
Hom/A005	92.4	93.7
Hom/A006	87.4	97.6
Hom/A007	93.2	97.8
Hom/A008	96.5	93.4
Hom/A009	92.2	92.9
Hom/A010	93.8	92.3
Grand mean	93.6	
Cochran's test		
C	0.449	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	23.4	
s_x	1.50	
s_w	3.70	
s_s	0.000	
Critical= 0.3 σ_P	7.02	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Lycopsamine-N-oxide in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	238	281
Hom/A002	269	260
Hom/A003	267	278
Hom/A004	266	272
Hom/A005	284	283
Hom/A006	255	273
Hom/A007	275	295
Hom/A008	280	281
Hom/A009	253	281
Hom/A010	273	288
Grand mean	273	
Cochran's test		
C	0.478	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	68.2	
s_x	9.21	
s_w	13.9	
s_s	0.000	
Critical= 0.3 σ_P	20.5	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	63.7	62.7
Hom/A002	61.2	64.3
Hom/A003	63.6	59.4
Hom/A004	64.7	58.9
Hom/A005	63.7	62.2
Hom/A006	60.3	65.0
Hom/A007	64.6	63.1
Hom/A008	64.4	66.3
Hom/A009	61.6	63.5
Hom/A010	64.5	62.8
Grand mean	63.0	
Cochran's test		
C	0.341	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	15.6	
s_x	1.10	
s_w	2.21	
s_s	0.000	
Critical= 0.3 σ_P	4.73	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	310	329
Hom/A002	320	329
Hom/A003	318	307
Hom/A004	333	329
Hom/A005	308	313
Hom/A006	321	321
Hom/A007	317	314
Hom/A008	312	325
Hom/A009	327	323
Hom/A010	328	332
Grand mean	321	
Cochran's test		
C	0.436	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	80.2	
s_x	6.86	
s_w	6.37	
s_s	5.18	
Critical= 0.3 σ_P	24.1	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	91.7	93.1
Hom/A002	95.8	93.8
Hom/A003	98.5	96.1
Hom/A004	95.4	92.7
Hom/A005	91.3	92.0
Hom/A006	90.3	93.4
Hom/A007	91.7	93.0
Hom/A008	97.1	93.3
Hom/A009	95.0	93.2
Hom/A010	94.5	96.2
Grand mean	93.9	
Cochran's test		
C	0.283	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	23.5	
s_x	1.83	
s_w	1.61	
s_s	1.44	
Critical= 0.3 σ_P	7.04	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	209	212
Hom/A002	208	225
Hom/A003	212	217
Hom/A004	224	211
Hom/A005	206	217
Hom/A006	214	210
Hom/A007	215	203
Hom/A008	211	211
Hom/A009	200	217
Hom/A010	213	217
Grand mean	213	
Cochran's test		
C	0.276	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	53.1	
s_x	3.04	
s_w	7.22	
s_s	0.000	
Critical= 0.3 σ_P	15.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	86.9	95.0
Hom/A002	90.7	90.7
Hom/A003	94.0	90.2
Hom/A004	94.0	90.9
Hom/A005	89.5	89.3
Hom/A006	88.3	92.8
Hom/A007	88.9	88.8
Hom/A008	91.0	86.7
Hom/A009	88.9	88.8
Hom/A010	91.2	91.4
Grand mean	90.4	
Cochran's test		
C	0.514	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	22.6	
s_x	1.35	
s_w	2.51	
s_s	0.000	
Critical= 0.3 σ_P	6.78	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	144	156
Hom/A002	147	160
Hom/A003	157	158
Hom/A004	159	152
Hom/A005	145	148
Hom/A006	149	153
Hom/A007	150	147
Hom/A008	152	150
Hom/A009	148	164
Hom/A010	151	164
Grand mean	153	
Cochran's test		
C	0.299	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	38.2	
s_x	3.70	
s_w	6.35	
s_s	0.000	
Critical= 0.3 σ_P	11.5	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	15.6	15.0
Hom/A002	15.9	15.8
Hom/A003	16.1	14.8
Hom/A004	14.9	15.9
Hom/A005	15.8	15.5
Hom/A006	14.3	14.9
Hom/A007	15.4	16.4
Hom/A008	15.0	14.9
Hom/A009	15.7	14.7
Hom/A010	15.5	15.6
Grand mean	15.4	
Cochran's test		
C	0.321	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.84	
s_x	0.396	
s_w	0.529	
s_s	0.131	
Critical= 0.3 σ_P	1.15	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	86.6	88.9
Hom/A002	86.3	92.8
Hom/A003	93.0	90.4
Hom/A004	87.6	87.1
Hom/A005	82.4	83.2
Hom/A006	86.6	87.9
Hom/A007	88.2	91.5
Hom/A008	90.9	91.3
Hom/A009	88.2	98.1
Hom/A010	88.9	99.3
Grand mean	89.5	
Cochran's test		
C	0.395	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	22.4	
s_x	3.33	
s_w	3.72	
s_s	2.05	
Critical= 0.3 σ_P	6.71	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Senkirkine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	12.0	12.0
Hom/A002	12.5	12.2
Hom/A003	13.0	12.0
Hom/A004	12.7	11.7
Hom/A005	12.5	12.5
Hom/A006	13.1	13.0
Hom/A007	12.4	12.5
Hom/A008	12.5	12.5
Hom/A009	11.1	12.4
Hom/A010	12.8	12.5
Grand mean	12.4	
Cochran's test		
C	0.444	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.10	
s_x	0.348	
s_w	0.462	
s_s	0.120	
Critical= 0.3 σ_P	0.930	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	72.6	78.6
Hom/A002	77.4	76.5
Hom/A003	75.1	76.0
Hom/A004	81.0	75.7
Hom/A005	72.7	75.1
Hom/A006	76.6	78.0
Hom/A007	78.1	76.1
Hom/A008	77.1	78.1
Hom/A009	78.9	74.1
Hom/A010	74.2	74.6
Grand mean	76.3	
Cochran's test		
C	0.359	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	19.1	
s_x	1.42	
s_w	2.26	
s_s	0.000	
Critical= 0.3 σ_P	5.72	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Europine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	29.2	29.4
Hom/B002	29.4	30.0
Hom/B003	29.8	30.4
Hom/B004	30.0	28.1
Hom/B005	29.9	31.0
Hom/B006	30.4	28.5
Hom/B007	28.3	30.7
Hom/B008	30.2	31.6
Hom/B009	31.0	28.4
Hom/B010	28.9	27.5
Grand mean	29.6	
Cochran's test		
C	0.262	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.041	
s_x	0.751	
s_w	1.13	
s_s	0.000	
Critical= 0.3 σ_P	2.22	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Europine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	220	219
Hom/B002	218	226
Hom/B003	222	229
Hom/B004	220	233
Hom/B005	226	225
Hom/B006	225	231
Hom/B007	218	235
Hom/B008	232	231
Hom/B009	224	224
Hom/B010	229	226
Grand mean	226	
Cochran's test		
C	0.456	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	56.4	
s_x	3.20	
s_w	5.53	
s_s	0.000	
Critical= 0.3 σ_P	16.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Heliotrine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	46.8	46.9
Hom/B002	48.2	48.1
Hom/B003	46.2	49.4
Hom/B004	46.7	48.3
Hom/B005	48.0	49.3
Hom/B006	46.4	46.7
Hom/B007	46.0	47.9
Hom/B008	48.8	49.5
Hom/B009	50.2	47.6
Hom/B010	47.2	46.2
Grand mean	47.7	
Cochran's test		
C	0.386	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.9	
s_x	0.954	
s_w	1.14	
s_s	0.515	
Critical= 0.3 σ_P	3.58	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Heliotrine-N-oxide in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	395	381
Hom/B002	386	397
Hom/B003	392	396
Hom/B004	389	411
Hom/B005	400	397
Hom/B006	390	401
Hom/B007	392	413
Hom/B008	419	405
Hom/B009	393	385
Hom/B010	396	401
Grand mean	397	
Cochran's test		
C	0.316	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	99.2	
s_x	7.15	
s_w	9.09	
s_s	3.12	
Critical= 0.3 σ_P	29.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	13.9	14.2
Hom/B002	13.4	13.8
Hom/B003	14.4	15.6
Hom/B004	15.6	13.4
Hom/B005	14.5	13.7
Hom/B006	11.9	13.9
Hom/B007	14.1	13.2
Hom/B008	15.8	12.5
Hom/B009	14.5	14.2
Hom/B010	14.8	12.2
Grand mean	14.0	
Cochran's test		
C	0.362	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.49	
s_x	0.604	
s_w	1.22	
s_s	0.000	
Critical= 0.3 σ_P	1.05	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	85.7	83.9
Hom/B002	87.9	99.7
Hom/B003	88.4	92.3
Hom/B004	92.8	86.6
Hom/B005	88.6	89.0
Hom/B006	81.7	88.4
Hom/B007	87.8	89.5
Hom/B008	88.5	92.4
Hom/B009	91.9	83.30
Hom/B010	90.2	87.4
Grand mean	88.8	
Cochran's test		
C	0.405	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	22.2	
s_x	2.63	
s_w	4.14	
s_s	0.000	
Critical= 0.3 σ_P	6.66	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	18.2	17.9
Hom/B002	17.8	18.5
Hom/B003	17.7	18.3
Hom/B004	18.1	18.1
Hom/B005	18.9	19.2
Hom/B006	16.9	18.3
Hom/B007	17.8	18.4
Hom/B008	17.3	18.6
Hom/B009	19.9	18.3
Hom/B010	18.3	18.3
Grand mean	18.2	
Cochran's test		
C	0.331	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.56	
s_x	0.47	
s_w	0.605	
s_s	0.195	
Critical= 0.3 σ_P	1.37	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	181	182
Hom/B002	179	192
Hom/B003	186	181
Hom/B004	181	193
Hom/B005	178	196
Hom/B006	183	184
Hom/B007	182	191
Hom/B008	187	187
Hom/B009	178	184
Hom/B010	187	185
Grand mean	185	
Cochran's test		
C	0.430	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	46.2	
s_x	2.38	
s_w	6.28	
s_s	0.000	
Critical= 0.3 σ_P	13.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	25.7	28.1
Hom/B002	27.7	24.0
Hom/B003	25.9	25.8
Hom/B004	19.	25.8
Hom/B005	24.8	24.2
Hom/B006	25.2	28.1
Hom/B007	24.7	26.0
Hom/B008	24.9	26.4
Hom/B009	25.5	25.4
Hom/B010	24.0	26.7
Grand mean	25.4	
Cochran's test		
C	0.505	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.35	
s_x	1.18	
s_w	1.99	
s_s	0.000	
Critical= 0.3 σ_P	1.91	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	129	125
Hom/B002	119	125
Hom/B003	118	126
Hom/B004	118	126
Hom/B005	128	127
Hom/B006	123	127
Hom/B007	121	130
Hom/B008	125	122
Hom/B009	119	131
Hom/B010	126	123
Grand mean	124	
Cochran's test		
C	0.305	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	31.1	
s_x	2.10	
s_w	4.80	
s_s	0.000	
Critical= 0.3 σ_P	9.32	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	38.8	38.4
Hom/B002	36.1	40.1
Hom/B003	39.4	42.8
Hom/B004	40.1	37.9
Hom/B005	41.2	42.0
Hom/B006	38.7	40.1
Hom/B007	38.2	37.8
Hom/B008	42.1	36.3
Hom/B009	38.3	39.1
Hom/B010	41.6	37.3
Grand mean	39.3	
Cochran's test		
C	0.377	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	9.83	
s_x	1.18	
s_w	2.11	
s_s	0.000	
Critical= 0.3 σ_P	2.95	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	175	175
Hom/B002	176	184
Hom/B003	178	186
Hom/B004	186	177
Hom/B005	172	175
Hom/B006	178	166
Hom/B007	164	186
Hom/B008	181	174
Hom/B009	174	174
Hom/B010	176	169
Grand mean	176	
Cochran's test		
C	0.493	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	44.1	
s_x	3.62	
s_w	6.83	
s_s	0.000	
Critical= 0.3 σ_P	13.2	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	73.4	67.6
Hom/B002	69.1	73.2
Hom/B003	71.3	71.2
Hom/B004	72.8	71.0
Hom/B005	75.4	72.2
Hom/B006	66.3	76.3
Hom/B007	68.7	72.4
Hom/B008	75.4	64.1
Hom/B009	72.8	68.3
Hom/B010	68.8	67.8
Grand mean	70.9	
Cochran's test		
C	0.389	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	17.7	
s_x	1.42	
s_w	4.06	
s_s	0.000	
Critical= 0.3 σ_P	5.32	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	225	220
Hom/B002	219	242
Hom/B003	234	232
Hom/B004	233	228
Hom/B005	228	221
Hom/B006	216	215
Hom/B007	209	225
Hom/B008	226	222
Hom/B009	234	215
Hom/B010	220	216
Grand mean	224	
Cochran's test		
C	0.404	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	56.0	
s_x	6.06	
s_w	7.86	
s_s	2.40	
Critical= 0.3 σ_P	16.8	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	6.87	6.49
Hom/B002	4.58	5.57
Hom/B003	5.74	5.56
Hom/B004	5.67	6.53
Hom/B005	5.62	5.49
Hom/B006	5.40	6.12
Hom/B007	6.65	5.45
Hom/B008	6.06	5.12
Hom/B009	5.88	5.54
Hom/B010	6.14	6.50
Grand mean	5.85	
Cochran's test		
C	0.287	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.46	
s_x	0.452	
s_w	0.500	
s_s	0.281	
Critical= 0.3 σ_P	0.439	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	33.8	35.6
Hom/B002	32.8	41.0
Hom/B003	38.0	36.9
Hom/B004	38.0	36.7
Hom/B005	39.6	37.0
Hom/B006	32.9	42.1
Hom/B007	33.0	36.5
Hom/B008	34.7	40.7
Hom/B009	41.4	33.6
Hom/B010	35.7	40.6
Grand mean	37.0	
Cochran's test		
C	0.286	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	9.26	
s_x	1.28	
s_w	3.86	
s_s	0.000	
Critical= 0.3 σ_P	2.78	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

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

Sample No.	Senkirkine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	38.1	16.3
Hom/B002	13.0	18.4
Hom/B003	14.2	13.2
Hom/B004	11.2	13.8
Hom/B005	13.0	16.1
Hom/B006	18.1	17.3
Hom/B007	19.4	16.6
Hom/B008	36.3	15.6
Hom/B009	13.6	12.5
Hom/B010	16.0	12.7
Grand mean	17.3	
Cochran's test		
C	0.490	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.32	
s_x	5.22	
s_w	6.97	
s_s	1.72	
Critical= 0.3 σ_P	1.30	
s_s < critical?	NOT ACCEPTED	
s_w < 0.5 σ_H?	NOT 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	13.1	14.1
Hom/B002	14.3	14.9
Hom/B003	13.4	14.7
Hom/B004	12.5	16.3
Hom/B005	14.0	15.5
Hom/B006	12.3	15.9
Hom/B007	13.7	14.4
Hom/B008	15.2	14.6
Hom/B009	16.2	14.9
Hom/B010	13.3	13.0
Grand mean	14.3	
Cochran's test		
C	0.406	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.58	
s_x	0.684	
s_w	1.34	
s_s	0.000	
Critical= 0.3 σ_P	1.07	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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	63.2	63.7
Hom/B002	60.1	64.1
Hom/B003	63.2	67.6
Hom/B004	63.6	60.9
Hom/B005	62.8	61.7
Hom/B006	60.4	63.0
Hom/B007	57.7	64.7
Hom/B008	64.4	62.0
Hom/B009	64.0	59.9
Hom/B010	63.2	60.8
Grand mean	62.6	
Cochran's test		
C	0.385	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	15.6	
s_x	1.20	
s_w	2.53	
s_s	0.000	
Critical= 0.3 σ_P	4.69	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 echimidine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	84.2	88.5
	88.6	89.8
	90.1	86.7
	87.2	86.2
	80.8	91.1
	83.5	89.5
Average amount (µg/kg)	85.7	88.7
n	6	6
st. dev (µg/kg)	3.49	1.88
Difference		-2.91
$0.3*\sigma_P$		6.43
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	333	358
	328	356
	359	351
	350	343
	336	340
	343	341
Average amount (µg/kg)	342	349
n	6	6
st. dev (µg/kg)	11.6	7.79
Difference		-6.96
$0.3*\sigma_P$		25.6
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for erucifoline in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	97.1	102
	101	99.4
	103	100
	97.3	100
	98.0	102
	97.3	103
Average amount (µg/kg)	98.9	101
n	6	6
st. dev (µg/kg)	2.41	1.33
Difference		-2.18
$0.3*\sigma_P$		7.42
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	176	179
	186	181
	188	182
	183	185
	177	186
	172	185
Average amount (µg/kg)	181	183
n	6	6
st. dev (µg/kg)	6.16	2.65
Difference		-2.56
$0.3*\sigma_P$		13.5
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for integerrimine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	21.7	20.8
	22.6	21.9
	21.4	21.2
	23.1	24.4
	21.3	21.0
	20.4	23.5
Average amount (µg/kg)	21.7	22.1
n	6	6
st. dev (µg/kg)	0.956	1.48
Difference		-0.410
$0.3*\sigma_P$		1.63
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	83.8	87.8
	89.0	88.3
	85.1	86.0
	83.5	88.3
	84.6	87.2
	84.8	90.6
Average amount (µg/kg)	85.1	88.0
n	6	6
st. dev (µg/kg)	1.97	1.51
Difference		-2.86
$0.3*\sigma_P$		6.39
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for intermedine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	41.7	42.2
	45.6	42.0
	41.1	35.1
	41.2	38.0
	34.9	41.9
	40.0	45.3
Average amount (µg/kg)	40.8	40.7
n	6	6
st. dev (µg/kg)	3.45	3.61
Difference		0.01
$0.3*\sigma_P$		3.06
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	138	144
	142	140
	140	138
	138	139
	137	139
	133	143
Average amount (µg/kg)	138	140
n	6	6
st. dev (µg/kg)	3.09	2.29
Difference		-2.43
$0.3*\sigma_P$		10.3
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for jacobine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	31.1	33.7
	32.6	32.0
	32.8	33.0
	30.8	30.3
	31.1	33.4
	32.1	31.1
Average amount (µg/kg)	31.7	32.2
n	6	6
st. dev (µg/kg)	0.847	1.37
Difference		-0.492
$0.3*\sigma_P$		2.38
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	26.2	28.1
	28.7	26.6
	28.2	28.1
	28.0	28.2
	27.6	27.1
	25.9	27.1
Average amount (µg/kg)	27.4	27.5
n	6	6
st. dev (µg/kg)	1.12	0.685
Difference		-0.109
$0.3*\sigma_P$		2.06
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for jacoline in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	36.6	37.5
	38.0	37.2
	38.5	38.1
	36.4	36.9
	36.4	36.6
	36.3	37.7
Average amount (µg/kg)	37.0	37.3
n	6	6
st. dev (µg/kg)	0.959	0.574
Difference		-0.294
$0.3*\sigma_P$		2.78
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for jaconine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	171	174
	175	178
	175	174
	170	178
	172	177
	171	176
Average amount (µg/kg)	172	176
n	6	6
st. dev (µg/kg)	2.15	1.76
Difference		-3.85
$0.3*\sigma_P$		12.9
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lycopsamine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	83.1	82.2
	85.3	80.9
	82.0	84.9
	80.0	85.6
	80.5	81.7
	78.0	88.3
Average amount (µg/kg)	81.5	83.9
n	6	6
st. dev (µg/kg)	2.57	2.83
Difference		-2.45
$0.3*\sigma_P$		6.11
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	263	282
	267	267
	282	274
	271	266
	262	266
	270	279
Average amount (µg/kg)	269	272
n	6	6
st. dev (µg/kg)	7.35	6.92
Difference		-3.31
$0.3*\sigma_P$		20.2
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	55.0	56.4
	56.1	57.9
	57.9	57.1
	54.9	55.5
	54.4	56.4
	55.1	56.9
Average amount (µg/kg)	55.6	56.7
n	6	6
st. dev (µg/kg)	1.26	0.786
Difference		-1.13
$0.3*\sigma_P$		4.17
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	277	285
	297	284
	287	292
	292	295
	281	291
	276	292
Average amount (µg/kg)	285	290
n	6	6
st. dev (µg/kg)	8.61	4.56
Difference		-4.66
$0.3*\sigma_P$		21.4
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecionine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	81.2	81.8
	88.3	83.9
	84.7	83.8
	84.5	89.5
	81.4	80.1
	81.1	86.1
Average amount (µg/kg)	83.5	84.2
n	6	6
st. dev (µg/kg)	2.86	3.32
Difference		-0.670
$0.3*\sigma_P$		6.26
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	205	214
	214	212
	208	211
	201	217
	203	206
	206	206
Average amount (µg/kg)	206	211
n	6	6
st. dev (µg/kg)	4.72	4.43
Difference		-4.81
$0.3*\sigma_P$		15.5
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	76.7	77.7
	84.9	80.9
	79.8	79.2
	80.7	85.4
	76.7	77.6
	76.5	82.8
Average amount (µg/kg)	79.2	80.6
n	6	6
st. dev (µg/kg)	3.30	3.07
Difference		-1.38
$0.3*\sigma_P$		5.94
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	141	146
	150	145
	146	145
	140	150
	141	139
	141	149
Average amount (µg/kg)	143	146
n	6	6
st. dev (µg/kg)	3.87	3.92
Difference		-2.60
$0.3*\sigma_P$		10.7
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecivernine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	14.5	14.2
	15.3	14.5
	15.0	14.6
	15.2	15.2
	14.3	14.7
	14.3	15.5
Average amount (µg/kg)	14.8	14.8
n	6	6
st. dev (µg/kg)	0.458	0.479
Difference		-0.02
$0.3*\sigma_P$		1.11
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	62.4	64.8
	71.0	68.7
	66.9	65.0
	64.5	67.9
	66.6	66.1
	64.5	72.6
Average amount (µg/kg)	66.0	67.5
n	6	6
st. dev (µg/kg)	2.96	2.92
Difference		-1.54
$0.3*\sigma_P$		4.95
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senkirkine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	11.5	11.7
	11.6	11.5
	11.6	11.9
	11.2	12.0
	11.2	11.8
	11.1	11.4
Average amount (µg/kg)	11.4	11.7
n	6	6
st. dev (µg/kg)	0.213	0.240
Difference		-0.332
$0.3*\sigma_P$		0.853
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for usaramine in material A.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	9.86	10.9
	10.2	10.5
	10.5	10.3
	10.4	10.5
	9.87	10.3
	9.77	10.5
Average amount (µg/kg)	10.1	10.5
n	6	6
st. dev (µg/kg)	0.297	0.214
Difference		-0.408
$0.3*\sigma_P$		0.756
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	65.4	64.2
	68.5	67.2
	67.2	66.6
	65.9	68.3
	64.7	67.4
	61.3	69.7
Average amount (µg/kg)	65.5	67.2
n	6	6
st. dev (µg/kg)	2.46	1.83
Difference		-1.73
$0.3*\sigma_P$		4.91
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for europine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	27.4	27.0
	27.8	21.3
	26.0	27.2
	27.2	26.3
	28.3	28.8
	27.5	26.5
Average amount (µg/kg)	27.3	26.2
n	6	6
st. dev (µg/kg)	0.777	2.57
Difference		1.17
$0.3*\sigma_P$		2.05
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	200	195
	202	206
	221	231
	201	205
	200	206
	208	209
Average amount (µg/kg)	205	209
n	6	6
st. dev (µg/kg)	8.06	11.9
Difference		-3.23
$0.3*\sigma_P$		15.4
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for heliotrine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	45.1	43.2
	45.3	42.5
	45.0	45.9
	44.5	42.5
	44.6	46.6
	45.3	42.9
Average amount (µg/kg)	45.0	43.9
n	6	6
st. dev (µg/kg)	0.333	1.82
Difference		1.03
$0.3*\sigma_P$		3.37
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	322	318
	334	334
	359	377
	324	332
	308	340
	336	339
Average amount (µg/kg)	331	340
n	6	6
st. dev (µg/kg)	17.2	19.9
Difference		-9.30
$0.3*\sigma_P$		24.8
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for integerrimine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	14.3	13.1
	14.2	14.1
	13.2	14.1
	14.2	13.7
	14.2	12.6
	14.0	13.7
Average amount (µg/kg)	14.0	13.5
n	6	6
st. dev (µg/kg)	0.432	0.591
Difference		0.469
$0.3*\sigma_P$		1.05
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	79.9	75.4
	78.8	88.4
	76.3	80.9
	76.3	76.4
	78.8	78.5
	78.8	78.5
Average amount (µg/kg)	78.1	79.7
n	6	6
st. dev (µg/kg)	1.49	4.67
Difference		-1.55
$0.3*\sigma_P$		5.86
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for lasiocarpine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	17.3	17.4
	16.8	15.0
	17.3	17.4
	17.0	16.8
	17.9	17.6
	17.0	16.9
Average amount (µg/kg)	17.2	16.8
n	6	6
st. dev (µg/kg)	0.376	0.966
Difference		0.356
$0.3*\sigma_P$		1.29
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	162	149
	159	182
	180	178
	170	159
	150	170
	168	167
Average amount (µg/kg)	165	167
n	6	6
st. dev (µg/kg)	10.2	12.3
Difference		-2.76
$0.3*\sigma_P$		12.3
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for retrorsine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	25.5	22.2
	27.4	27.4
	25.5	27.9
	27.9	24.5
	26.0	27.7
	25.0	25.9
Average amount (µg/kg)	26.2	25.9
n	6	6
st. dev (µg/kg)	1.16	2.25
Difference		0.299
$0.3*\sigma_P$		1.97
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	119	111
	120	127
	120	132
	119	116
	117	120
	117	115
Average amount (µg/kg)	119	120
n	6	6
st. dev (µg/kg)	1.19	7.75
Difference		-1.59
$0.3*\sigma_P$		8.89
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecionine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	35.3	34.6
	34.4	36.5
	36.3	37.0
	34.9	36.1
	35.0	36.2
	36.1	35.7
Average amount (µg/kg)	35.3	36.0
n	6	6
st. dev (µg/kg)	0.721	0.822
Difference		-0.702
$0.3*\sigma_P$		2.65
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	v
Time (days)	0	51
Calculated amounts (µg/kg)	161	155
	166	181
	160	170
	160	159
	164	168
	162	160
Average amount (µg/kg)	162	165
n	6	6
st. dev (µg/kg)	2.28	9.56
Difference		-3.17
$0.3*\sigma_P$		12.2
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for seneciphylline in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	63.3	59.9
	63.5	54.2
	63.0	63.7
	62.2	63.0
	63.8	63.0
	62.8	61.1
Average amount (µg/kg)	63.1	60.8
n	6	6
st. dev (µg/kg)	0.554	3.53
Difference		2.29
$0.3*\sigma_P$		4.73
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	206	202
	215	205
	207	214
	207	199
	214	214
	211	209
Average amount (µg/kg)	210	207
n	6	6
st. dev (µg/kg)	3.93	6.41
Difference		2.77
$0.3*\sigma_P$		15.7
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for senecivernine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	5.85	6.74
	6.13	5.26
	6.08	6.13
	5.97	6.57
	5.95	5.69
	5.56	5.83
Average amount (µg/kg)	5.93	6.04
n	6	6
st. dev (µg/kg)	0.207	0.559
Difference		-0.111
$0.3*\sigma_P$		0.444
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	26.1	25.9
	27.5	28.6
	26.7	27.8
	29.2	25.6
	29.8	25.5
	27.0	28.7
Average amount (µg/kg)	27.7	27.0
n	6	6
st. dev (µg/kg)	1.45	1.51
Difference		0.677
$0.3*\sigma_P$		2.08
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for spartioidine in material B.

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	13.5	12.8
	14.0	12.8
	14.5	14.7
	14.4	14.7
	14.2	14.3
	14.1	14.2
Average amount (µg/kg)	14.1	13.9
n	6	6
st. dev (µg/kg)	0.366	0.884
Difference		0.195
$0.3*\sigma_P$		1.06
Consequential difference? Diff < $0.3*\sigma_P$		No

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

Storage temperature	<-18 °C	4-6 °C
Time (days)	0	51
Calculated amounts (µg/kg)	52.8	50.7
	54.1	60.6
	51.4	54.7
	52.6	53.2
	54.2	55.5
	52.8	54.2
Average amount (µg/kg)	53.0	54.8
n	6	6
st. dev (µg/kg)	1.05	3.26
Difference		-1.83
$0.3*\sigma_P$		3.97
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

RIKILT

DATE
April 3, 2019

SUBJECT
Research study for pyrrolizidine alkaloids in food and feed matrices

POSTAL ADDRESS
P.O. Box 230
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The Netherlands

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CoC NUMBER
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HANDLED BY
Diana Pereboom

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EMAIL
pt.rikilt@wur.nl

Dear colleague,

The EURL mycotoxins & plant toxins will organize a Research Study regarding pyrrolizidine alkaloids in food and feed matrices. This study will be organised according to ISO 17043 (General requirements for proficiency testing - R013), except the preparation of the test materials. The study will focus on the quantification of pyrrolizidine alkaloids. Harmonised EU regulation for pyrrolizidine alkaloids in these matrices is being prepared and their inclusion in national monitoring is recommended by EFSA. In view of the relative novelty of this analyte/matrix combination, this study will be conducted as a 'Research Study' to gain insight in the current capabilities of the NRLs. The scope includes the set of 21 PAs currently considered for legislation in food products. The scope also includes 14 PAs that are isomeric analogues of the PAs considered for legislation as well as 13 PAs that can be of additional relevance for food and feed. The EURL mycotoxins & plant toxins will evaluate the results of the research study in the same way as a proficiency test, ensuring an appropriate follow-up and informing the Commission and the Member States of the results. The primary goal of this research study is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of these compounds in food and feed matrices. A secondary goal of this study is to evaluate the capabilities of laboratories to discriminate between PAs considered for legislation and PAs not considered for legislation.

According to Regulation (EU) 2017/625 all EU National Reference Laboratories (NRLs) plant toxins in food and/or feed are strongly encouraged to participate. I would like to invite you to participate in this test.

1. Test materials
- One herbal tea and one alfalfa (lucerne) material will be supplied.
- The test amount sent will be approximately 25 g.
- Test materials will be sent in April/May 2019. 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.

2. Scope of analysis
- The materials may contain one or more of the following analytes, included in the list of 21 PAs considered for legislation:
  - Echimidine and echimidine-N-oxide
  - Europine and europine-N-oxide
  - Heliotrine and heliotrine-N-oxide

Wageningen Research Foundation/RIKILT is part of Wageningen University & Research. RIKILT carries out research into the safety and reliability of food and feed. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

- Intermedine and intermedine-N-oxide
- Lasiocarpine and lasiocarpine-N-oxide
- Lycopsamine and lycopsamine-N-oxide
- Retrorsine and retrorsine-N-oxide
- Senecionine and senecionine-N-oxide
- Seneciphylline and seneciphylline-N-oxide
- Senecivernine and senecivernine-N-oxide
- Senkirkine

- The materials may also contain one or more of the following analytes, which are isomeric analogues of the PAs included in the list of 21 PAs considered for legislation:
  - Echinatine and echinatine-N-oxide
  - Heliosupine and heliosupine-N-oxide
  - Indicine and indicine-N-oxide
  - Integerrimine and integerrimine-N-oxide
  - Rinderine and rinderine-N-oxide
  - Spartioidine and spartioidine-N-oxide
  - Usaramine and usaramine-N-oxide

- The materials may also contain one or more of the following analytes not included in the list of 21 PAs, nor being isomeric analogues, but known to occur in food or feed products:
  - Erucifoline and erucifoline-N-oxide
  - Jacobine and jacobine-N-oxide
  - Jacoline
  - Jaconine
  - Monocrotaline and monocrotaline-N-oxide
  - Trichodesmine

3. Mixed standard solutions

Mixed standard solutions with different combinations of the substances indicated above will be provided. These mixed standard solutions can be used by the participant for optimisation of the analytical method and for quantification of the test materials. They will also be used to evaluate the possibility to discriminate between PAs considered for legislation and PAs not considered for legislation.

4. Questionnaire

A questionnaire will be send electronically. In this questionnaire the particants will be asked to provide information about the laboratory method used. This information is necessary to conduct a more in depth analysis of the results obtained in this research study.

5. Report

- A report of the research study will be dispatched in December 2019.
- Results of the research study will be presented anonymously.
- The follow-up protocol on Research studies from DG Santé by the EURL will be applied.

6. Additional information

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

7. Costs

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

Note: The participant should arrange the necessary import permits for the sample materials (if necessary).

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it to me before 22 April 2019 to: pt.rikilt@wur.nl.

Hoping to welcome you for this test,

D. Pereboom

Diana Pereboom-de Fauw
Proficiency tests

EURL mycotoxins & plant toxins
RIKILT Wageningen University & Research
Wageningen
Netherlands

Annex 6 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

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

Dear Madam/Sir,

Thank you very much for your interest in the Research Study regarding pyrrolizidine alkaloids in food and feed.

The parcel shipped to you should contain:

- One herbal tea and one alfalfa (lucerne) sample. Each sample contains approximately 25 grams of test material.
- Three mixtures of PA standards. Each vial contains 1.25 mL of the standard mixture dissolved in methanol at a concentration of 5 µg/mL. PA standard mix 1 contains the 21 PAs currently considered for legislation, PA standard mix 2 contains 14 PAs that are isomers of the PAs considered for legislation. PA standard mix 3 contains 9 PAs that are of additional interest for analysis of food and feed samples. The PA standard mixtures can be used to optimise your analytical method and they can also be used for quantification of PAs in the test materials.

- PA standard mixture 1 contains:
  - Echimidine and echimidine-N-oxide
  - Europine and europine-N-oxide
  - Heliotrine and heliotrine-N-oxide
  - Intermedine and intermedine-N-oxide
  - Lasiocarpine and lasiocarpine-N-oxide
  - Lycopsamine and lycopsamine-N-oxide
  - Retrorsine and retrorsine-N-oxide
  - Senecionine and senecionine-N-oxide
  - Seneciphylline and seneciphylline-N-oxide
  - Senecivernine and senecivernine-N-oxide
  - Senkirkine

- PA standard mixture 2 contains:
  - Echinatine and echinatine-N-oxide
  - Heliosupine and heliosupine-N-oxide
  - Indicine and indicine-N-oxide
  - Integerrimine and integerrimine-N-oxide
  - Rinderine and rinderine-N-oxide
  - Spartioidine and spartioidine-N-oxide
  - Usaramine and usaramine-N-oxide

RIKILT

DATE
May 13, 2019

SUBJECT
Instruction research study pyrrolizidine alkaloids in food and feed matrices

OUR REFERENCE
1926393/RIK

POSTAL ADDRESS
P.O. Box 230
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Wageningen Research Foundation/RIKILT is part of Wageningen University & Research. RIKILT carries out research into the safety and reliability of food and feed. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

100years
1918 — 2018

- PA standard mixture 3 contains:
- Erucifoline and erucifoline-N-oxide
- Jacobine and jacobine-N-oxide
- Jacoline
- Jaconine
- Monocrotaline and monocrotaline-N-oxide
- Trichodesmine

Instructions:

- After arrival the samples should be stored at +4°C. The standard solutions should be stored at -20°C.
- Please fill out the accompanying acknowledgement of receipt form and return it upon receipt of the samples, preferably by e-mail (pt.rikilt@wur.nl).
- You are requested to analyse all PAs in standard mix 1 and mix 2 and report the retention times obtained with your analytical method for all individual PAs, even in case one or more PAs are not within the scope of your method. These data will be used to evaluate the chromatographic separation of PAs considered for legislation from their isomeric analogues that are not considered for legislation. Provide detailed information on the analysis of the PA mixes and the analytical method used in the questionnaire and send it by e-mail (pt.rikilt@wur.nl).
- You can download the EURL method for analysis of PAs using LC-MS/MS v1, used for the EURLMP trainings in November 2018, from the EURL mycotoxins & plant toxins website (www/rikilt.nl/eurl) under 'Library'. Please note that the EURL-MP-method-002-v2, for analysis of PAs with specific instructions for the proficiency test, will be placed on the website soon. EURL-MP-method-002-v2 will contain detailed information on the analysis of all PAs included in mixtures 1, 2, and 3.
- Before analysis, homogenize the samples according to your laboratory's procedure.
- Treat the test material as if it was a sample for routine analysis. Report one result and not an average of multiple measurements.
- Report for the test materials all results in µg/kg for the product as received. When a toxin is not within your scope, please report 'nt (not tested)' in the web application. Do not use the option 'detected' from the web application. When a toxin is 'not detected' or the result is below your LOQ, report the result as <LOQ-value and specify the value (e.g. <10 µg/kg).
- Please use the web application for entering your results for the test samples (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.
- The deadline for submitting test-results for this test is **June 24th 2019.**

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,

D. Pereboom

Diana Pereboom

Proficiency tests
EURL mycotoxins & plant toxins
RIKILT Wageningen University & Research, The Netherlands

Annex 7 Scope and LOQ

	LOQ (µg/kg)																					
	Group Im					Group ImNO					Group Sp		Group SpNO		Group Sn			Group SnNO			Group RtNO	
Lab code	Im	Ly	Id	En	Rn	ImNO	LyNO	IdNO	EnNO	RnNO	Sp	St	SpNO	StNO	Sn	Sv	Ir	SnNO	SvNO	IrNO	Rt	Us
PT9547	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
PT9548	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9549	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PT9550	0.2	0.2				0.3	0.5				0.5	1.8	1.1	0.4	1.0	1.0	2.0	1.1	1.0	1.0	1.4	1.6
PT9551	0.2	0.2				0.3	0.5				0.5	1.8	1.1	0.4	1.0	1.0	2.0	1.1	1.0	1.0	1.4	1.6
PT9552	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9553	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9554	2	5	5	/	/	2	10	2	/	/	5	/	2	/	2	5	/	2	2	/	2	/
PT9555	25	25				25	25				25		25		25	25		25	25		25	
PT9556	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9557	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9558	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	10
PT9559	10	10		10	10	10	10				10		10		10	10	10	10	10		10	
PT9560	2.5	2.5				2.5	2.5				2.5		2.5		2.5	2.5		2.5	2.5		2.5	
PT9561	10	10	10	10	10	10	10	10	10	10	10	10	25	10	10	10	10	10	10	25	10	10
PT9562[1]	24 (F)	21 (F)	7.1 (F)	19 (F)	7.5 (F)	13 (F)	12 (F)	5.6 (F)	7.2 (F)	5.9 (F)	16 (F)	3.0 (F)	250(F)	11 (F)	23 (F)	8.5 (F)	8.6 (F)	13 (F)	11 (F)	250(F)	12 (F)	49 (F)
	28 (T)	29 (T)	8.4 (T)	5.6 (T)	8 (T)	18 (T)	15 (T)	9.9 (T)	13 (T)	9.9 (T)	28 (T)	14 (T)	42 (T)	14 (T)	0.7 (T)	8.2(T)	8.4(T)	7.2 (T)	18 (T)	60 (T)	19 (T)	29 (T)
PT9563	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9564																						
PT9565	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	
PT9566	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT9568	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9569	0.5	0.6	1.0	1.0	1.5	1.5	1.5	1.0	2.0	3.0	3.0	10	6.0	6.0	2.0	3.0	8.0	7.0	6.0	5.0	5.0	10
PT9570											0.75				0.75						0.75	
PT9571	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9572	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9573	10	10		10		10	10		10		10		10		10	10	10	10	10	10	10	
PT9574	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

1. Participant reported separate LOQs for feed (F) and tea (T).

	LOQ (µg/kg)																					
	Group RtNO		Group Em		Group EmNO																	
Lab code	RtNO	UsNO	Em	Hs	EmNO	HsNO	Sk	Mc	McNO	Td	Ht	HtNO	Eu	EuNO	Lc	LcNO	Er	ErNO	Jb	JbNO	Jl	Jn
PT9547	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9548	10	10	10	10	10	10	10	/	/	/	10	10	10	10	10	10	/	/	/	/	/	/
PT9549	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PT9550	1.0	1.2	0.5	0.5	0.2	0.5	0.1	0.4	0.5	1.0	0.1	0.2	0.2	0.1	0.1	0.3	0.4	0.6	1.0	0.6	0.2	0.3
PT9551	1.0	1.2	0.5	0.5	0.2	0.5	0.1	0.4	0.5	1.0	0.1	0.2	0.2	0.1	0.1	0.3	0.4	0.6	1.0	0.6	0.2	0.3
PT9552	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9553	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9554	2	/		/		/	1	2	2	2	1	2	1	1	1	2	5	2	5	2	/	/
PT9555	25		25		25		25	25	25	25	25	25	25	25	25	25	25	25				
PT9556	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9557	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9558	5	5	5	5	5	10	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5
PT9559	10		10		10		10	10	10		10	10	10	10	10	10	10	10	10	10		
PT9560	2.5		2.5		2.5		2.5				2.5				2.5	2.5						
PT9561	10	-	10	10	10	10	10	10	10	10	10	10	25	10	10	10	10	10	10	25	10	25
PT9562[1]	82 (F)	14 (F)	9 (F)	11 (F)	4 (F)	7.4 (F)	4.9 (F)	250(F)	250(F)	8.0 (F)	84 (F)	22 (F)	32 (F)	4.6 (F)	13 (F)	20 (F)	250(F)	250(F)	7.0 (F)	7.4 (F)	94 (F)	10 (F)
	110(T)	31 (T)	18 (T)	11 (T)	10 (T)	12 (T)	14 (T)	40 (T)	82 (T)	11 (T)	224(T)	43 (T)	19 (T)	13.5(T)	17 (T)	14 (T)	31 (T)	25 (T)	13 (T)	4.0(T)	141(T)	9.7(T)
PT9563	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9564																						
PT9565	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	2.5	
PT9566	2	2	2	2	2	2	2		2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT9568	10	10		10		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9569	6.0	4.0	0.6	0.3	1.0	1.5	3.0	10	10	3.0	0.6	3.0	5.0	0.7	0.5	2.0	10	5.0	7.0	4.0	8.0	5.0
PT9570							0.75															
PT9571	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9572	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9573	10		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PT9574	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

1. Participant reported separate LOQs for feed (F) and tea (T).

Annex 8 Analytical method details

Lab code	Method	Colum	Column length (mm)	Total run time (min)	Retention time (min)									
					Group Im					Group ImNO				
					Im	Ly	Id	En	Rn	ImNO	LyNO	IdNO	EnNO	RnNO
PT9570	Acid	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100	9										
PT9566	Acid	Waters Acquity BEH C18, 50 x 2.1, 1.7 um	50	14	2.00	2.03	2.05	2.03	2.12	2.51	2.49	2.22	2.23	2.21
PT9550	Acid	Waters Acquity UPLC BEH C18, 2.1×100 mm, 1.7 µm,	100	13.5	3.66	3.75				4.12	4.21			
PT9548	Acid	Waters Acquity BEH C18, 2.1 ×150 mm, 1.7 µm	150	14.2	3.95	4.10	4.13	4.25	4.25	5.00	5.20	5.10	4.66	4.76
PT9557	Acid	Waters Acquity UPLC BEH C18, 150 x 2.1 mm, 1.7 µm,	150	15.2	3.38	3.49	3.53	3.58	3.58	4.27	4.40	4.27	3.93	3.97
PT9555	Acid	Waters BEH C18, 2.1x150 mm, 1.7µm	150	19	5.40	5.62	5.34	5.34	5.33	6.81	7.09	6.04	6.04	5.91
PT9554	Acid	Thermo Scientific Hypersil Gold aQ, 150x2.1 mm, 3 µm	150	15	3.64	3.88	3.88	3.88	3.88	4.63	4.92	4.63	4.92	4.33
PT9574	Acid	Thermo Hypersil gold, 150 x 2.1 mm, 1.9 µm	150	15	4.83	4.94	4.94	4.94	4.93	5.34	5.45	5.19-5.37	5.19-5.37	5.19-5.37
PT9551	Acid	C18, 150x2.1 mm; 1.3 µm	150	16	5.37	5.52	5.52	5.50	5.50	5.99	6.14	5.99	5.89	5.81
PT9560	Acid	Thermoscientific Hypersil GOLD 150 x 2.1 mm; 1.9 µm	150	25	7.33	7.66	7.92?	7.65?	7.65?	7.85	8.15	8.48?	8.29?	8.29?
PT9572	Acid	Hypersil Gold, C17, 200*2.1 mm, 1.9 µm	200	26	7.84	8.03	8.01	7.94	7.89	8.75	8.94	8.76	8.55	8.44
PT9568	Acid	Agilent Poroshell SB-18, 100x2.1 mm	100	20	5.3	5.5	5.45	5.45	5.45	6.2	6.5	6.2	5.9	6.0
PT9547	Acid	Agilent Zorbax SB-C18, 2.1x50 mm, 1.8 µm	50	22	6.08	6.45	6.45	6.75	6.75	8.44	8.99	8.44	7.70	8.00
PT9553	Acid	Phenomenex Gemini C18, 3.0x150 mm, 3µm	150	21	4.76	5.08	4.85	5.09	5.20	7.08	7.66	6.40	6.67	6.79
PT9565	Acid	Phenomenex Luna, C18, 150x2 mm	150	25	8.00	8.28	8.25-8.32	8.25-8.32	8.25-8.32	9.33	9.59	9.37	9.25	9.09
PT9556	Acid	Phenomenex Luna C8, 150 x 2 mm, 3µm	150	30	10.9	11.4	11.4	11.2	11.2	13.8	14.3	13.8	13.3	13.3
PT9564	Acid	Phenomenex Kinetex XB-C18, 100 x 4.6 mm, 2.6 µm	100	16	4.9	5.1	5.1	5.01/5.17	5.01/5.17	5.4	5.5	5.5	5.41/5.50	5.41/5.50
PT9572	Acid	Phenomenex Kinetex, Biphenyl, 150*2.1 mm, 1.7 µm	150	26	6.66	6.83	6.95	7.43	7.42	7.89	8.04	7.98	7.78	7.82
PT9549	Acid	Phenomenex kinetex biphenyl and kinetex phenyl hexyl	150	70	18.2	19.1	20.9	18.1	21.2	23.5	24.2	22.8	22.2	23.3
PT9559	Acid	Phenomenex Kinetex Phenyl-Hexyl, 2.1x150 mm, 2.6 µm	150	70	8.20	8.97		11.16	10.84	14.48	15.92			
PT9561	Alkaline	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.2	4.85	4.85	4.87	5.84	5.98	2.64	2.64	2.76	3.05	2.98
PT9573	Alkaline	Waters Acquity UPLC BEH, 100x2.0 mm, 1.7 µm	100	15	4.71	4.73		5.62		2.89	2.88		3.15	
PT9552	Alkaline	Waters Acquity C18 BEH, 2.1x150 mm	150	15	5.56	5.61	5.61	6.51	6.65	3.53	3.57	3.70	3.89	3.99
PT9569	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	5.80	5.80	5.84	6.70	6.87	3.37	3.42	3.55	3.74	3.84
PT9547	Alkaline	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100		9.46	9.46	9.46	10.55	10.73	7.01	7.01	7.14	7.39	7.48
PT9571	Alkaline	Waters Acquity UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	100	21	9.20	9.34	9.27	11.71	11.92	4.50	4.61	4.92	5.44	5.64
PT9562	Alkaline	Waters X-Select CSH C18, 2.1 x 150 mm, 3.5 µm	150	14.2	4.9	4.9	5.2	5.2	5.2	3.0	3.0	3.2	3.2	3.2
PT9558	Alkaline	Waters Atlantis T3, 100Å, 3 mm X 150 mm, 3 µm	150	17	8.33	8.33	8.43	9.11	9.31	6.37	6.37	6.45	6.57	6.67
PT9563	Alkaline	Phenomenex Gemini-NX C18, 150* 2.00 mm, 5 µm	150	24.2			9.32	10.65	10.65	5.68	5.84	5.97	6.54	6.54

Lab code	Method	Column	Column length (mm)	Total run time (min)	Retention time (min)									
					Group Sp			Group SpNO		Group Sn			Group SnNO	
					Sp	St	SpNO	StNO	Sn	Sv	Ir	SnNO	SvNO	IrNO
PT9570	Acid	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100	9	4.5				5.0					
PT9566	Acid	Waters Acquity BEH C18, 50 x 2.1, 1.7 um	50	14	3.74	3.52	4.01	3.83	4.72	4.68	4.49	4.73	4.68	4.77
PT9550	Acid	Waters Acquity UPLC BEH C18, 2.1×100 mm, 1.7 µm,	100	13.5	4.72	4.69	4.91	4.88	5.36	5.45	5.37	5.59	5.47	5.56
PT9548	Acid	Waters Acquity BEH C18, 2.1 ×150 mm, 1.7 µm	150	14.2	8.22	7.95	8.70	8.47	9.48	9.44	9.27	9.74	9.58	9.57
PT9557	Acid	Waters Acquity UPLC BEH C18, 150 x 2.1 mm, 1.7 µm,	150	15.2	7.11	6.82	7.81	7.45	8.87	8.78	8.62	9.14	8.96	8.94
PT9555	Acid	Waters BEH C18, 2.1x150 mm, 1.7µm	150	19	10.88	10.09	11.52	10.96	12.48	12.42	12.03	12.82	12.64	12.43
PT9554	Acid	Thermo Scientific Hypersil Gold aQ, 150x2.1 mm, 3 µm	150	15	5.88	/	6.41	/	7.42	7.08	7.34	7.89	7.24	7.88
PT9574	Acid	Thermo Hypersil gold, 150 x 2.1 mm, 1.9 µm	150	15	5.98	5.97	6.21	6.20	6.72	6.64	6.65	6.92	6.80	6.89
PT9551	Acid	C18, 150x2.1 mm; 1.3 µm	150	16	6.78	6.76	7.10	7.09	7.68	7.60	7.60	7.98	7.86	7.96
PT9560	Acid	Thermoscientific Hypersil GOLD 150 x 2.1 mm; 1.9 µm	150	25	15.10	14.42	12.92	12.79	18.22	18.54	17.45	15.74	15.44	15.45
PT9572	Acid	Hypersil Gold, C17, 200*2.1 mm, 1.9 µm	200	26	9.87	9.80	10.34	10.27	11.23	11.08	11.05	11.63	11.43	11.54
PT9568	Acid	Agilent Poroshell SB-18, 100x2.1 mm	100	20	7.3	7.3	8.0	8.0	8.3	8.2	8.2	8.9	8.8	9.1
PT9547	Acid	Agilent Zorbax SB-C18, 2.1x50 mm, 1.8 µm	50	22	11.36	11.36	12.80	12.80	14.12	13.96	13.52	15.08	14.24	14.24
PT9553	Acid	Phenomenex Gemini C18, 3.0x150 mm, 3 µm	150	21	11.39	10.80	13.11	12.82	13.56	13.28	13.28	14.28	14.03	14.11
PT9565	Acid	Phenomenex Luna, C18, 150x2 mm	150	25	10.59	10.52	11.63	11.54	12.38	12.07	12.21	13.57	13.15	13.48
PT9556	Acid	Phenomenex Luna C8, 150 x 2 mm, 3µm	150	30	18.4	18.1	20.1	19.7	22.4	21.9	21.9	23.8	23.0	23.0
PT9564	Acid	Phenomenex Kinetex XB-C18, 100 x 4.6 mm, 2.6 µm	100	16	7.3	7.08	7.8	7.59	8.4	8.4	8.14	8.8	8.9	8.65
PT9572	Acid	Phenomenex Kinetex, Biphenyl, 150*2.1 mm, 1.7 µm	150	26	10.38	10.39	11.59	11.59	11.56	11.56	11.56	12.53	12.52	12.66
PT9549	Acid	Phenomenex kinetex biphenyl and kinetex phenyl hexyl	150	70	31.5	31.2	36.2	35.5	38.1	38.5	38.4	42.8	44.9	45.0
PT9559	Acid	Phenomenex Kinetex Phenyl-Hexyl, 2.1x150 mm, 2.6 µm	150	70	27.72		29.78		30.94	30.73	30.28	33.56	32.79	
PT9561	Alkaline	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.2	8.51	8.24	5.12	5.00	9.48	9.69	9.20	7.86	7.86	5.82
PT9573	Alkaline	Waters Acquity UPLC BEH, 100x2.0 mm, 1.7 µm	100	15	8.33		4.98		9.47	9.67	9.11	5.85	5.87	5.66
PT9552	Alkaline	Waters Acquity C18 BEH, 2.1x150 mm	150	15	9.41	9.14	5.93	5.83	10.40	10.56	10.13	6.79	6.85	6.61
PT9569	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7µm	150	14.2	9.43	9.17	5.82	5.71	10.40	10.54	10.18	6.69	6.74	6.51
PT9547	Alkaline	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100		13.95	13.59	9.64	9.48	15.15	15.35	14.89	10.67	10.67	10.43
PT9571	Alkaline	Waters Acquity UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	100	21	14.74	14.51	9.85	9.61	15.50	15.62	15.33	11.91	11.90	11.59
PT9562	Alkaline	Waters X-Select CSH C18, 2.1 x 150 mm, 3.5 µm	150	14.2	8.5	7.9	4.9	4.7	10.0	10.1	9.4	5.2	5.2	5.0
PT9558	Alkaline	Waters Atlantis T3, 100Å, 3 mm X 150 mm, 3 µm	150	17	13.05	12.84	8.50	8.50	13.63	13.75	13.45	9.47	9.59	9.21
PT9563	Alkaline	Phenomenex Gemini-NX C18, 150* 2.00 mm, 5 µm	150	24.2	14.38	14.00	9.31	9.15	15.86	16.19	15.49			10.25

Lab code	Method	Colum	Column Length (mm)	Total run Time (min)	Retention time (min)											
					Group Rt		Group RtNO		Group Em		Group EmNO					
					Rt	Us	RtNO	UsNO	Em	Hs	EmNO	HsNO	Sk	Mc	McNO	Td
PT9570	Acid	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100	9	4.3								5.5			
PT9566	Acid	Waters Acquity BEH C18, 50 x 2.1, 1.7 um	50	14	3.29	4.72	3.32	3.22	5.62	5.47	5.55	6.07	5.45			3.22
PT9550	Acid	Waters Acquity UPLC BEH C18, 2.1×100 mm, 1.7 µm,	100	13.5	4.47	4.44	4.56	4.59	6.14	6.24	6.10	6.75	6.30	2.63	3.32	4.53
PT9548	Acid	Waters Acquity BEH C18, 2.1 ×150 mm, 1.7 µm	150	14.2	7.18	6.93	7.44	7.20	10.15	10.05	10.05	10.54	10.10	/	/	/
PT9557	Acid	Waters Acquity UPLC BEH C18, 150 x 2.1 mm, 1.7 µm,	150	15.2	6.07	5.80	6.33	6.13	9.67	9.57	9.59	10.07	9.55	2.27	3.09	6.22
PT9555	Acid	Waters BEH C18, 2.1x150 mm, 1.7µm	150	19	9.53	8.76	9.95	9.27	13.34	13.08	13.24	13.73	13.27	3.75	5.17	9.68
PT9554	Acid	Thermo Scientific Hypersil Gold aQ, 150x2.1 mm, 3 µm	150	15	5.55	5.56	5.79	5.93		9.35		10.13	9.77	2.40	3.61	5.32
PT9574	Acid	Thermo Hypersil gold, 150 x 2.1 mm, 1.9 µm	150	15	5.73	5.72	5.87	5.90	7.41	7.49	7.38	8.02	7.55	3.67	4.48	5.77
PT9551	Acid	C18, 150x2.1 mm; 1.3 µm	150	16	6.47	6.45	6.66	6.72	8.44	8.55	8.46	9.11	8.64	4.04	4.95	6.56
PT9560	Acid	Thermoscientific Hypersil GOLD 150 x 2.1 mm; 1.9 µm	150	25	14.51	13.71	12.02	11.85	20.72	20.08	18.44	18.76	16.87	5.87	5.17	13.37
PT9572	Acid	Hypersil Gold, C17, 200*2.1 mm, 1.9 µm	200	26	9.35	9.43	9.71	9.68	12.46	12.60	12.49	13.61	12.74	5.78	7.17	9.47
PT9568	Acid	Agilent Poroshell SB-18, 100x2.1 mm	100	20	6.9	6.9	7.3	7.4		9.4		10.2	9.4	3.7	5.2	6.7
PT9547	Acid	Agilent Zorbax SB-C18, 2.1x50 mm, 1.8 µm	50	22	10.51	10.51	11.51	11.15	17.46	17.46	17.06	18.41	17.4	3.4	6.35	9.48
PT9553	Acid	Phenomenex Gemini C18, 3.0x150 mm, 3µm	150	21	10.42	9.96	12.17	12.07	14.65	14.50	14.91	15.39	14.63	3.20	5.39	9.01
PT9565	Acid	Phenomenex Luna, C18, 150x2 mm	150	25	10.06	10.04	10.75	10.85	14.80	15.09	15.42	16.86	15.33	4.59	7.97	10.10
PT9556	Acid	Phenomenex Luna C8, 150 x 2 mm, 3µm	150	30	16.5	16.1	18.1	18.1	26.5	26.0	25.9	27.1	25.9	3.8	11.2	16.4
PT9564	Acid	Phenomenex Kinetex XB-C18, 100 x 4.6 mm, 2.6 µm	100	16	6.7	6.56	7.1	6.96	9.1	8.98	9.4	9.28	9.2	3.9	4.6	6.8
PT9572	Acid	Phenomenex Kinetex, Biphenyl, 150*2.1 mm, 1.7 µm	150	26	9.80	9.99	10.74	10.99	12.21	12.39	12.47	13.41	12.79	5.52	7.56	8.95
PT9549	Acid	Phenomenex kinetex biphenyl and kinetex phenyl hexyl	150	70	29.3	29.4	31.7	32.0	46.3	46.3	46.5	47.7	46.7	14.4	22.1	27.7
PT9559	Acid	Phenomenex Kinetex Phenyl-Hexyl 2.1x150 mm, 2.6 µm	150	70	26.23		27.63		34.47		34.74		34.72	5.96	13.09	
PT9561	Alkaline	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.2	6.03	7.59	4.69	-	9.98	9.73	6.80	6.50	6.36	4.88	1.74	7.91
PT9573	Alkaline	Waters Acquity UPLC BEH, 100x2.0 mm, 1.7 µm	100	15	7.67		4.54			9.94	6.68	6.39	6.43	4.85	2.13	7.79
PT9552	Alkaline	Waters Acquity C18 BEH, 2.1x150 mm	150	15	8.66	8.32	5.49	5.32	10.69	10.52	7.54	7.24	7.21	5.75	2.61	8.79
PT9569	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	8.65	8.38	5.37	5.21	10.66	10.54	7.45	7.15	7.13	5.75	2.45	8.82
PT9547	Alkaline	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100		13.05	12.66	9.14	8.98	15.60	15.39	11.61	11.25	11.14	9.43	5.39	13.18
PT9571	Alkaline	Waters Acquity UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	100	21	14.11	11.59	8.96	8.61	15.71	15.62	13.04	12.72	12.45	9.23	2.45	14.17
PT9562	Alkaline	Waters X-Select CSH C18, 2.1 x 150 mm, 3.5 µm	150	14.2	5.2	5.1	4.8	4.8	10.2	9.9	5.5	5.3	5.5	4.9	2.3	7.5
PT9558	Alkaline	Waters Atlantis T3, 100Å, 3 mm X 150 mm, 3 µm	150	17	12.11	11.71	7.77	7.58	13.51	13.49	10.19	9.78	10.36	8.73	5.74	12.61
PT9563	Alkaline	Phenomenex Gemini-NX C18, 150* 2.00 mm, 5 µm	150	24.2	13.18	12.91	8.80	8.07	16.53	16.22	11.55	11.22	10.95	9.52	4.14	13.48

Lab code	Method	Colum	Column length (mm)	Total run time (min)	Retention time (min)											
					Ht	HtNO	Eu	EuNO	Lc	LcNO	Er	ErNO	Jb	JbNO	Jl	Jn
PT9570	Acid	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100	9	4.6											
PT9566	Acid	Waters Acquity BEH C18, 50 x 2.1, 1.7 um	50	14	3.61	3.91	2.09	2.19	6.85	7.29	1.73	2.04	2.23	2.54	1.47	2.8
PT9550	Acid	Waters Acquity UPLC BEH C18, 2.1×100 mm, 1.7 µm,	100	13.5	4.86	5.16	3.62	3.87	7.13	7.62	3.03	3.43	3.52	3.73	2.63	3.99
PT9548	Acid	Waters Acquity BEH C18, 2.1 ×150 mm, 1.7 µm	150	14.2	7.80	8.32	4.00	4.40	11.14	11.42	/	/	/	/	/	/
PT9557	Acid	Waters Acquity UPLC BEH C18, 150 x 2.1 mm, 1.7 µm,	150	15.2	6.72	7.36	3.44	3.79	10.66	11.10	2.91	3.63	4.26	4.82	2.28	5.21
PT9555	Acid	Waters BEH C18, 2.1x150 mm, 1.7µm	150	19	10.27	11.06	5.49	6.05	14.56	15.00	4.83	6.04	6.32	7.21	3.24	7.48
PT9554	Acid	Thermo Fisher Scientific, Hypersil Gold aQ, 150x2.1 mm, 3 µm	150	15	5.89	6.59	3.77	4.27	10.34	10.56	3.29	4.03	3.70	4.23	2.24	4.03
PT9574	Acid	Thermo Hypersil gold, 150 x 2.1 mm, 1.9 µm	150	15	6.12	6.43	4.84	5.09	8.35	8.83	4.25	4.70	4.71	4.99	3.64	5.16
PT9551	Acid	C18, 150x2.1 mm; 1.3 µm	150	16	6.97	7.35	5.31	5.68	9.37	9.60	4.67	5.23	5.21	5.60	3.98	5.72
PT9560	Acid	Thermoscientific Hypersil GOLD 150 x 2.1 mm; 1.9 µm	150	25	11.85	12.49	7.62	8.00	23.11	22.23	11.09	7.45	11.66	8.32	6.45	14.18
PT9572	Acid	Hypersil Gold, C17, 200*2.1 mm, 1.9 µm	200	26	10.12	10.70	7.74	8.26	14.22	15.20	6.66	7.50	7.56	8.09	5.76	8.41
PT9568	Acid	Agilent Poroshell SB-18, 100x2.1 mm	100	20	7.3	7.8	5.6	6.1	10.8	11.6	5.2	6.5	5.4	6.3	3.6	5.8
PT9547	Acid	Agilent Zorbax SB-C18, 2.1x50 mm, 1.8 µm	50	22	11.76	13.03	7.06	7.98	18.84	19.28	5.54	9.71	6.23	7.77	3.71	7.96
PT9553	Acid	Phenomenex Gemini C18, 3.0x150 mm, 3µm	150	21	10.78	12.46	5.38	6.64	15.82	16.68	4.65	7.06	5.67	8.30	3.24	6.88
PT9565	Acid	Phenomenex Luna, C18, 150x2 mm	150	25	10.70	11.73	8.22	8.98	16.95	17.20	7.11	8.50	8.09	9.12	4.63	
PT9556	Acid	Phenomenex Luna C8, 150 x 2 mm, 3µm	150	30	17.9	18.8	11.9	13.2	30.6	29.8	9.3	13.5	12.1	15.1	3.6	14.4
PT9564	Acid	Phenomenex Kinetex XB-C18, 100 x 4.6 mm, 2.6 µm	100	16	6.9	7.2	5.1	5.4	10.6	10.8	5	5.7	5.8	6.3	3.8	6.2
PT9572	Acid	Phenomenex Kinetex, Biphenyl, 150*2.1 mm, 1.7 µm	150	26	9.94	10.38	7.52	7.85	14.22	14.9	7.61	9.06	8.25	9.28	5.51	8.36
PT9549	Acid	Phenomenex kinetex biphenyl and kinetex phenyl hexyl	150	70	29.1	31.1	21.4	22.6	48.2	48.8	21.3	25.8	23.7	27.0	14.2	25.2
PT9559	Acid	Phenomenex Kinetex Phenyl-Hexyl 100A, 2.1x150 mm, 2.6 µm	150	70	25.33	26.53	10.30	12.29	42.48	43.60	14.09	23.1	21.22	25.40		
PT9561	Alkaline	Waters Acquity UPLC BEH C18, 2.1x100 mm, 1.7 µm	100	14.2	7.48	4.83	5.58	2.80	10.82	7.63	6.59	2.46	6.94	3.53	4.64	8.01
PT9573	Alkaline	Waters Acquity UPLC BEH, 100x2.0 mm, 1.7 µm	100	15	7.31	4.81	5.45	3.06	12.30	7.57	6.40	2.67	6.79	3.54	4.71	7.97
PT9552	Alkaline	Waters Acquity C18 BEH, 2.1x150 mm	150	15	8.21	5.67	6.28	3.72	11.44	8.40	7.41	3.37	7.81	4.51	5.53	8.95
PT9569	Alkaline	Waters Acquity UPLC BEH C18, 2.1x150 mm, 1.7 µm	150	14.2	8.45	5.54	6.44	3.57	11.38	8.32	7.38	3.23	7.74	4.36	5.45	8.91
PT9547	Alkaline	Waters Acquity BEH C18, 2.1x100 mm, 1.7 µm	100		12.61	9.30	10.24	7.23	16.54	12.71	11.41	6.85	11.86	7.94	9.15	13.29
PT9571	Alkaline	Waters Acquity UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	100	21	13.74	9.26	11.22	5.15	16.38	13.90	12.74	4.15	13.10	6.38	8.90	14.28
PT9562	Alkaline	Waters X-Select CSH C18, 2.1 x 150 mm, 3.5 µm	150	14.2	6.6	4.8	5.0	3.1	10.7	6.3	4.9	2.9	6.2	4.1	4.8	7.7
PT9558	Alkaline	Waters Atlantis T3, 100Å, 3 mm X 150 mm, 3 µm	150	17	11.81	8.16	8.86	6.42	14.62	11.52	10.68	6.28	11.48	7.18	8.18	12.79
PT9563	Alkaline	Phenomenex Gemini-NX C18, 150* 2.00 mm, 5 µm	150	24.2	12.60	8.84	10.10	6.28	18.62	12.71	12.05	6.04	12.13	7.20	9.12	12.60

Lab. code	Method	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
PT9547	EURL-MP-method_002	2	0.2% formic acid	40	mechanical shaking	30	SPE	Strata-X	5	0.25	acidic/alkaline
PT9548	EURL-MP-method_002	2	0.2% formic acid	40	mechanical shaking	30	SPE	Strata X-33µm Reversed Phase 200 mg/6mL	6	0.33	acidic
PT9549	modified EURL method 002	1	0.2%formic acid	20	ultrasonic	30	SPE	strata X	5	0.5	acidic
PT9550	BfR method	2	0.05M H_2SO_4	40	ultrasonic	30	SPE	Discovery DSC-18, 500mg/6mL	10	0.5	acidic
PT9551	BfR method	2	0.28% V/V solution of H_2SO_4	2x20	ultrasonic	2x15	dilution			0.0025	acidic
PT9552	in-house method	2	0.2%FA	40	mechanical shaking	30	SPE	Strata X, 200 mg/6ml	5		alkaline
PT9553	EURL-MP-method_002	2	0.2% formic acid solution	40	mechanical shaking	30	SPE	Oasis HLB 6cc/200mg	5	0.5	acidic
PT9554	in-house method	2	MeOH/H_2O	20	ultrasonic	2	dilution	/	/	0.05	acidic
PT9555	EURL-MP-method_002	2	water + 0.2 HCOOH	40	mechanical shaking	30	SPE	Strata-X	5	0.5	acidic
PT9556	EURL-MP-method_002	2	formic acid 0.2%	40	mechanical shaking	30	SPE	OASIS HLB C18 150 mg	5	0.5	acidic
PT9557											acidic/alkaline
PT9558	EURL-MP-method_002	2	2% formic acid	40	mechanical shaking	30	SPE	Waters, Oasis HLB 3cc (60 mg)	5	0.05	alkaline
PT9559	BfR method	2	sulfuric acid 0.05 mol/L	2x20	ultrasonic	2x15	SPE	Supelco DSC-C18, 500 mg, 6 ml	10	0.125	acidic
PT9560	BfR method	1	H_2SO_4 50mM in Water	1x15; 1x10	ultrasonic	2x15	SPE	Bond Elut Agilent (Mega BE- C18; 1gm; 6ml)"	10	0.4	acidic
PT9561	EURL-MP-method_002	2	H_2O 0.2% HCOOH	40	mechanical shaking	30	SPE	Strata XL SPE	5	0.5	alkaline
PT9562	EURL-MP-method_002	2	0.2% Formic acid	40	shaking (hand/vortex)	30	SPE	Strata X 200 mg/6 ml	5	0.5	alkaline
PT9563	EURL-MP-method_002	2	Water-formic acid 0.2%	40	mechanical shaking	30	SPE	Strata X (Phenomenex)	5	0.5	alkaline
PT9564	in-house method	2.5	20 g NaCl in 800 mL H_2O and make up till 1L with HCl 37%	25	mechanical shaking	60	none				acidic
PT9565	BfR method	2	dilute sulfuric acid	20	ultrasonic	15	SPE	C18, Supelco	5	0.5	acidic
PT9566	EURL-MP-method_002	2	water /formic acid	40	shaking (hand/vortex)	30	SPE	Strata-X	5	0.5	acidic
PT9568	in-house method	2	0.2% formic acid	40	mechanical shaking	30	SPE	biotage evolute express cx, 150 mg/6 ml	2	0.4	acidic
PT9569	EURL-MP-method_002	2	0.2% formic acid	40	mechanical shaking	30	SPE	Bond Elut C18, 500mg, 6ml	5	0.5	alkaline
PT9570	in-house method	2	1% formic acid in water	8	mechanical shaking	45-60	SPE	Baker SDB 1, 200 mg/3 ml	8	10	acidic
PT9571	EURL-MP-method_002	2	1% Formic Acid	40	mechanical shaking	30	SPE	Strata-X 3um 200mg/6ml	5	0.5	alkaline
PT9572	BfR method	2	H_2SO_4 0.05M	2x20	ultrasonic	2x15	SPE	DSC-C18 (Supelco), 500 mg, 6ml	10	0.5	acidic
PT9573	EURL-MP-method_002	2	0.2% formic acid in water	40	mechanical shaking	30	SPE	Strata-X 200 mg/6 mL	5	0.5	alkaline
PT9574	BfR method	2	0.05M sulfuric acid	2x20	ultrasonic	2x15	SPE	Discovery DSC 18 500mg/6ml	10	0.5	acidic

ACN = acetonitrile; MeOH = methanol; FA = HCOOH = formic acid; H_2SO_4 = sulfuric acid; HCl = hydrochloric acid; NaCl = sodium chloride.

Annex 9 Results material A (alfalfa)

Material A, echimidine group					
	Echimidine (Em) A: 88.9 µg/kg u: 6.21 µg/kg σ_p: 22.2 µg/kg (25%) robust σ: 24.3 µg/kg (27%)		Heliosupine (Hs)	Sum echimidine group (Em-group) A: 95.1 µg/kg u: 6.56 µg/kg σ_p: 23.8 µg/kg (25%) robust σ: 26.2 µg/kg (28%)	
Lab code	Result (µg/kg)	z-score	Results (µg/kg)	Result (µg/kg)	z-score
PT9547	35	**-2.43**	12	47	**-2.02**
PT9548	91.8	0.13	<10	91.8	-0.14
PT9549	86	-0.13	<20	86	-0.38
PT9550	62.747	-1.18	<0.5	62.747	-1.36
PT9551	91.7 (sum Em/Hs)		91.7 (sum Em/Hs)	91.7	-0.14
PT9552	86	-0.13	nd	86	-0.38
PT9553	85.98	-0.13	70.85 **FP**	156.83	**2.60**
PT9554	67	-0.99	nd	67	-1.18
PT9555	65.1	-1.07	nt	65.1	-1.26
PT9556	332.6	**10.96**	nd	332.6	**9.99**
PT9557	81.1	-0.35	<10	81.1	-0.59
PT9558	58	-1.39	64 **FP**	122	1.13
PT9559	124.8	1.61	nt	124.8	1.25
PT9560	87	-0.09	nd	87	-0.34
PT9561	111	0.99	<10	111	0.67
PT9562	120	1.40	<11	120	1.05
PT9563	100.6	0.53	11.1	111.7	0.70
PT9564	111	0.99	nt	111	0.67
PT9565	90.5	0.07	<2.5	90.5	-0.19
PT9566	51.1	-1.70	nd	51.1	-1.85
PT9568	87	-0.09	nd	87	-0.34
PT9570	Nt		nt	nt	
PT9571	92.74	0.17	11.58	104.32	0.39
PT9572	109.8	0.94	<5	109.8	0.62
PT9573	87	-0.09	nd	87	-0.34
PT9574	100	0.50	nd	100	0.21

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

Material A, echimidine-N-oxide group					
	Echimidine-N-oxide (EmNO) A: 384 µg/kg u: 36.8 µg/kg σ_p: 95.9 µg/kg (25%) robust σ: 144 µg/kg (38%)		Heliosupine-N-oxide (HsNO)	Sum echimidine-N-oxide group (EmNO-group) A: 392 µg/kg u: 37.7 µg/kg σ_p: 97.9 µg/kg (25%) robust σ: 148 µg/kg (38%)	
Lab code	Result (µg/kg)	z'-score	Results (µg/kg)	Result (µg/kg)	z'-score
PT9547	510	1.23	35 **FP**	545	1.46
PT9548	496	1.09	22.6 **FP**	518.6	1.21
PT9549	413	0.29	<20	413	0.20
PT9550	276.28	-1.05	<0.5	276.28	-1.10
PT9551	373.3	-0.10	19.5	392.8	0.01
PT9552	373.4	-0.10	nd	373.4	-0.17
PT9553	404.2	0.20	<10	404.2	0.12
PT9554	395	0.11	nd	395	0.03
PT9555	199.1	-1.80	nt	199.1	-1.84
PT9556	detected, <10	**(-3.64) FN**	nd	detected, <10	**(-3.64) FN**
PT9557	293.3	-0.88	<10	293.3	-0.94
PT9558	199	-1.80	24 **FP**	223	-1.61
PT9559	595.5	**2.06**	nt	595.5	1.94
PT9560	415	0.30	nd	415	0.22
PT9561	439	0.54	28 **FP**	467	0.72
PT9562	200	-1.79	<7.4	200	-1.83
PT9563	388.2	0.04	26.9 **FP**	415.1	0.22
PT9564	303	-0.79	nt	303	-0.84
PT9565	538	1.50	<2.5	538	1.40
PT9566	184.4	-1.94	nd	184.4	-1.98
PT9568	247	-1.33	nd	247	-1.38
PT9570	nt		nt	nt	
PT9571	388.48	0.05	21.29 **FP**	409.77	0.17
PT9572	669.2	**2.78**	<5	669.2	**2.65**
PT9573	423.7	0.39	nd	423.7	0.31
PT9574	558	1.70	nd	558	1.59

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material A, intermedine group								
	Intermedine (Im) A: 30.0 µg/kg u: 2.65 µg/kg σ_p: 7.49 µg/kg (25%) robust σ: 9.00 µg/kg (30%)		Lycopsamine (Ly) A: 85.7 µg/kg u: 5.71 µg/kg σ_p: 21.4 µg/kg (25%) robust σ: 17.1 µg/kg (20%)		Indicine (Id)	Echinatine (En)	Rinderine (Rn)	Sum intermedine group (Im-group) A: 112 µg/kg u: 13.5 µg/kg σ_p: 27.9 µg/kg (25%) robust σ: 53.9 µg/kg (48%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z-score	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	z'-score
PT9547	43 (sum Im/Ly/Id)		43 (sum Im/Ly/Id)		43 (sum Im/Ly/Id)	<10	<10	43	**-2.22**
PT9548	20.9	-1.14	273	**8.74**	116	<10	<10	409.9	**9.61**
PT9549	33	0.38	101	0.71	<20	<20	<20	134	0.72
PT9550	18.828	-1.40	nt		nt	nt	nt	18.828	**-3.00**
PT9551	37.7 (sum Im/Rn)		68.7 (sum Ly/Id/En)		68.7 (sum Ly/Id/En)	68.7 (sum Ly/Id/En)	37.7 (sum Im/Rn)	106.4	-0.17
PT9552	28.8	-0.14	79 (sum Ly/Id)		79 (sum Ly/Id)	nd	nd	107.8	-0.13
PT9553	28.55	-0.18	78.02	-0.36	66.73	<10	<10	173.3	1.98
PT9554	37.7	0.98	66.4 (sum Ly/Id)		66.4 (sum Ly/Id)	nd	nd	104.1	-0.25
PT9555	detected, <25	(-0.62)	63.6	-1.03	nt	nt	nt	63.6	-1.55
PT9556	22.6	-0.93	117.8 (sum Ly/Id)		117.8 (sum Ly/Id)	nd	nd	140.4	0.92
PT9557	23.5	-0.81	79.9	-0.27	nt	nt	nt	103.4	-0.27
PT9558	<10	**(-2.51) FN**	<10	**(-3.41) FN**	19	<10	<10	19	**-2.99**
PT9559	47.4	**2.20**	95.7	0.47	nt	nd	nd	143.1	1.01
PT9560	34	0.51	76	-0.45	nd	nd	nd	110	-0.06
PT9561	50 (sum Im/Ly)		50 (sum Im/Ly)		114	20	16	200	**2.85**
PT9562	60	**3.78**	80	-0.27	<7.1	<19	<7.5	140	0.91
PT9563	33.3	0.42	99.5	0.64	nd	nd	nd	132.8	0.68
PT9564	31	0.13	44 (sum Ly/Id)		44 (sum Ly/Id)	nt	nt	75	-1.18
PT9565	36.7	0.85	76.8	-0.42	nt	<2.5	<2.5	113.5	0.06
PT9566	10.1	**-2.50**	14.4	**-3.33**	nd	nd	nd	24.5	**-2.81**
PT9568	21	-1.13	83	-0.13				104	-0.25
PT9570	nt		nt		nt	nt	nt	nt	
PT9571	31.03	0.14	92	0.29	73	nd; <10	nd	196.03	**2.72**
PT9572	34.3	0.55	105.8	0.94	<5	<5	<5	140.1	0.91
PT9573	49.2 (sum Im/Ly)		49.2 (sum Im/Ly)		nd	nd	nd	49.2	**-2.02**
PT9574	150 (sum Im/Ly/Id/En/Rn)		150 (sum Im/Ly/Id/En/Rn		150 (sum Im/Ly/Id/En/Rn	150 (sum Im/Ly/Id/En/Rn	150 (sum Im/Ly/Id/En/Rn	150	1.23

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material A, intermedine-N-oxide group									
	Intermedine-N-oxide (ImNO) A: 178 µg/kg u: 18.4 µg/kg σ_p: 44.4 µg/kg (25%) robust σ: 57.0 µg/kg (32%)		Lycopsamine-N-oxide (LyNO) A: 311 µg/kg u: 28.0 µg/kg σ_p: 77.7 µg/kg (25%) robust σ: 95.2 µg/kg (31%)		Indicine-N-oxide (IdNO)	Echinatine-N-oxide (EnNO)	Rinderine-N-oxide (RnNO)	Sum intermedine-N-oxide group (ImNO-group) A: 461 µg/kg u: 59.1 µg/kg σ_p: 115 µg/kg (25%) robust σ: 236 µg/kg (51%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	z'-score
PT9547	<10	**(-3.49) FN**	300	-0.13	100 **FP**	<10	<10	400	-0.47
PT9548	158	-0.41	379	0.82	161 **FP**	<10	<10	698	1.83
PT9549	211	0.69	278	-0.40	<20	<20	<20	489	0.22
PT9550	nt		190.597 (sum ImNO/LyNO/IdNO/EnNO/RnNO)		nt	nt	nt	190.597	**-2.09**
PT9551	159.3 (sum ImNO/IdNO)		247.5	-0.77	159.3 (sum ImNO/IdNO)	nd	nd	406.8	-0.42
PT9552	152.4	-0.52	259.6	-0.62	nd	nd	nd	412	-0.38
PT9553	261.04	1.74	455.49	1.75	<10	<10	<10	716.53	1.98
PT9554	208 (sum ImNO/IdNO)		349	0.46	208 (sum ImNO/IdNO)	nd	nd	557	0.74
PT9555	105.3	-1.50	134.8	**-2.13**	nt	nt	nt	240.1	-1.70
PT9556	356.6 (sum ImNO/IdNO)		1516.2	**14.59**	356.6 (sum ImNO/IdNO)	nd	nd	1872.8	**10.91**
PT9557	147.5	-0.63	247.1	-0.77	nt	<10	<10	394.6	-0.51
PT9558	32 (sum ImNo/LyNO)		32 (sum ImNO/LyNO)		<10	<10	<10	32	**-3.31**
PT9559	227.7	1.04	381	0.85	nt	nt	nt	608.7	1.14
PT9560	219	0.86	392	0.98	nd	nd	nd	611	1.16
PT9561	396* (sum ImNO/LyNO/IdNO)		375* (sum ImNO/LyNO/IdNO)		789* (sum ImNO/LyNO/IdNO)	565* (sum EnNO/RnNO) **FP**	656* (sum EnNO/RnNO) **(FP**	1445	**7.60**
PT9562	130 (sum ImNO/LyNO)		130 (sum ImNO/LyNO)		<5.6	<7.2	<5.9	130	**-2.55**
PT9563	194.2	0.34	350	0.47	nd	nd	nd	544.2	0.64
PT9564	399	**4.61**	1344 (sum LyNO/IdNO)		1344 (sum LyNO/IdNO)	nt	nt	1743	**9.91**
PT9565	165	-0.26	320	0.11	nt	<2.5	<2.5	485	0.19
PT9566	95.4	-1.71	56.8	**-3.08**	nd	nd	nd	152.2	**-2.38**
PT9568	110	-1.41	213	-1.18	nd	nd	nd	323	-1.06
PT9570	nt		nt		nt	nt	nt	nt	
PT9571	176.08	-0.03	282.31	-0.35	nd; <10	nd; <10	nd; <10	458.39	-0.02
PT9572	195	0.36	357	0.56	<5	<5	<5	552	0.71
PT9573	239.3 (sum ImNO/LyNO)		239.3 (sum ImNO/LyNO)		nd	nd	nd	239.3	-1.71
PT9574	420 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)		420 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)		420 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	420 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	420 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	420	-0.31

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

*participant PT9561: mentions co-elution of ImNO/LyNO/IdNO as well as individual concentrations for each isomer, the concentration of 789 µg/kg was included in the calculations of the sum totals; mentions coelution of EnNo/RnNO as well as individual concentrations for each isomer; the concentration of 656 µg/kg was included in the calculations of the sum totals.

nd = not detected; nt = not tested.

Material A, retrorsine group					
	Retrorsine (Rt) A: 70.6 µg/kg u: 7.99 µg/kg σ_p: 17.7 µg/kg (25%) robust σ: 29.3 µg/kg (42%)		Usaramine (Us)	Sum retrorsine group (Rt-group) A: 81.7 µg/kg u: 9.15 µg/kg σ_p: 20.4 µg/kg (25%) robust σ: 35.8 µg/kg (44%)	
Lab code	Result (µg/kg)	z'-score	Results (µg/kg)	Result (µg/kg)	z'-score
PT9547	90	1.00	<10	90	0.37
PT9548	79	0.43	<10	79	-0.12
PT9549	109	1.98	<20	109	1.22
PT9550	nt		nt	nt	
PT9551	83.6 (sum Rt/Us)		83.6 (sum Rt/Us)	83.6	0.09
PT9552	62.9	-0.40	11.5	74.4	-0.33
PT9553	49.75	-1.08	164.32 **FP**	214.07	**5.92**
PT9554	50 (sum Rt/Us)		50 (sum Rt/Us)	50	-1.42
PT9555	42.6	-1.45	nt	42.6	-1.75
PT9556	243.5	**8.92**	nd	243.5	**7.23**
PT9557	52.3	-0.95	<10	52.3	-1.31
PT9558	24	**-2.41**	18	42	-1.77
PT9559	85.9	0.79	nt	85.9	0.19
PT9560	77	0.33	nd	77	-0.21
PT9561	138	**3.48**	14	152	**3.14**
PT9562	110	**2.03**	120 **FP**	230	**6.63**
PT9563	62.8	-0.40	10.9	73.7	-0.36
PT9564	97	1.36	nt	97	0.68
PT9565	71.9	0.07	nt	71.9	-0.44
PT9566	15	**-2.87**	nd	15	**-2.98**
PT9568	45	-1.32	nd	45	-1.64
PT9570#	*30.32*	***(-2.08#)***	*nt*	*30.32*	***(-2.30#)***
PT9571	56.2	-0.74	73.43 **FP**	129.63	**2.14**
PT9572	67.9	-0.14	16.5	84.4	0.12
PT9573	56.4	-0.73	nd	56.4	-1.13
PT9574	70 (sum Rt/Us)		70 (sum Rt/Us)	70	-0.52

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

participant PT9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material A, retrorsine-N-oxide group					
	Retrorsine-N-oxide (RtNO) A: 273 µg/kg u: 33.9 µg/kg σ_p: 68.3 µg/kg (25%) robust σ: 121 µg/kg (44%)		Usaramine-N-oxide (UsNO) A: 51.0 µg/kg u: 5.62 µg/kg σ_p: 12.7 µg/kg (25%) robust σ: 15.6µg/kg (31%)		Sum retrorsine-N-oxide group (RtNO-group) A: 325 µg/kg u: 39.6 µg/kg σ_p: 81.2 µg/kg (25%) robust σ: 152 µg/kg (47%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score
PT9547	410	1.80	56	0.36	466	1.57
PT9548	183	-1.18	31.6	-1.39	214.6	-1.22
PT9549	296	0.30	47	-0.28	343	0.20
PT9550	nt		nt		nt	
PT9551	432.4 (sum RtNO/UsNO)		432.4 (sum RtNO/UsNO)		432.4	1.19
PT9552	285.7	0.17	53.9	0.21	339.6	0.17
PT9553	424.86	1.99	240.44	**13.61**	665.3	**3.77**
PT9554	225 (sum RtNO/UsNO)		225 (sum RtNO/UsNO)		225	-1.10
PT9555	141	-1.73	nt		141	**-2.03**
PT9556	detected, <10	**(-3.45) FN**	nd, <10	**(-2.94) FN**	nd, <10	**(-3.48) FN**
PT9557	246.6	-0.35	42.1	-0.64	288.7	-0.40
PT9558	39	**-3.07**	15	**-2.58**	54	**-3.00**
PT9559	370.7	1.28	nt		370.7	0.51
PT9560	356	1.09	nd (no loq)		356	0.35
PT9561	320	0.62	nt		320	-0.05
PT9562	230	-0.56	350	**21.48**	580	**2.83**
PT9563	279.2	0.08	43.2	-0.56	322.4	-0.02
PT9564	769	**6.51**	nt		769	**4.92**
PT9565	276	0.04	nt		276	-0.54
PT9566	116	**-2.06**	nd, <2	**(-3.52) FN**	116	**-2.31**
PT9568	207	-0.87	65	1.01	272	-0.58
PT9570	nt		nt		nt	
PT9571	282.48	0.12	42.12	-0.63	324.6	0.00
PT9572	369.1	1.26	60.1	0.66	429.2	1.16
PT9573	112.7	**-2.10**	nd (no loq)		112.7	**-2.35**
PT9574	385 (sum RtNO/UsNO)		385 (sum RtNO/UsNO)		385	0.67

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material A, senecionine group							
	Senecionine (Sn) A: 79.0 µg/kg u: 4.92 µg/kg σ_p: 19.8 µg/kg (25%) robust σ: 18.5 µg/kg (23%)		Senecivernine (Sv) A: 17.8 µg/kg u: 2.14 µg/kg σ_p: 4.44 µg/kg (25%) robust σ: 6.17 µg/kg (35%)		Integerrimine (Ir) A: 25.1 µg/kg u: 2.53 µg/kg σ_p: 6.27 µg/kg (25%) robust σ: 7.00 µg/kg (28%)		Sum senecionine group (Sn-group) A: 114 µg/kg u: 9.77 µg/kg σ_p: 28.4 µg/kg (25%) robust σ: 39.1 µg/kg (34%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score
PT9547	63	-0.81	16	-0.36	25	-0.01	104	-0.32
PT9548	73.4	-0.28	<10	(-1.57)	29.5	0.66	102.9	-0.36
PT9549	67	-0.61	<20	(0.46)	22	-0.45	89	-0.82
PT9550	nt		44.449	**5.42**	nt		44.449	**-2.30**
PT9551	98.4	0.98	31.4 (sum Sv/Ir)		31.4 (sum Sv/Ir)		129.8	0.54
PT9552	73.2	-0.29	16.1	-0.34	27.2	0.32	116.5	0.09
PT9553	62.3	-0.85	11.78	-1.21	44.42	**2.86**	118.5	0.16
PT9554	72.4	-0.33	12.1	-1.15	nd (no loq)		84.5	-0.97
PT9555	detected, <250	**(-2.73) FN**	40.6	**4.64**	nt		40.6	**-2.43**
PT9556	335.9	**13.01**	441.7 (sum Sv/Ir))		441.7 (sum Sv/Ir)		777.6	**22.09**
PT9557	62.2	-0.85	14.2	-0.72	15.5	-1.42	91.9	-0.72
PT9558	42	-1.87	<10	(-1.57)	<10	**(-2.23) FN**	42	**-2.39**
PT9559	112	1.67	26.8	1.84	29	0.58	167.8	1.80
PT9560	100	1.06	nd, <2.5	**(-3.10) FN**	nd (no loq)		100	-0.46
PT9561	95	0.81	28	**2.08**	16	-1.34	139	0.84
PT9562	150	**3.59**	<8.5	(-1.88)	<8.6	**(-2.44) FN**	150	1.21
PT9563	86.3	0.37	nd, <10	(-1.57)	25.2	0.02	111.5	-0.07
PT9564	169 (sum Sn/Sv)		169 (sum Sn/Sv)		nt		169	1.84
PT9565	96.7	0.90	15.6	-0.44	nt		112.3	-0.05
PT9566	6.9	**-3.65**	12	-1.17	nd, <2	**(-3.41) FN**	18.9	**-3.15**
PT9568	73	-0.30	nd, <10	(-1.57)	89	**9.46**	162	1.61
PT9570#	*41.38*	*(-1.91#)*	*nt*		*nt*		*41.38*	***(-2.41#)***
PT9571	72.1	-0.35	21.67	0.79	18.59	-0.96	112.36	-0.04
PT9572	82.2	0.16	54 (identification)*		42.3 (identification)*		178.5	**2.16**
PT9573	88.4	0.48	13.2	-0.92	23.9	-0.17	125.5	0.39
PT9574	71	-0.41	23 (sum Sv/Ir)		23 (sum Sv/Ir)		94	-0.66

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

*participant PT9572 had problems with identification, these values are not included in the evaluation of results.

participant PT9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material A, senecionine-N-oxide group							
	Senecionine-N-oxide (SnNO) A: 197 µg/kg u: 25.0 µg/kg σ_p: 49.1 µg/kg (25%) robust σ: 84.7 µg/kg (43%)		Senecivernine-N-oxide (SvNO) A: 74.9 µg/kg u: 9.26 µg/kg σ_p: 18.7 µg/kg (25%) robust σ: 33.1 µg/kg (44%)		Integerrimine-N-oxide (IrNO) A: 68.6 µg/kg u: 3.39 µg/kg σ_p: 17.2 µg/kg (25%) robust σ: 9.39 µg/kg (14%)		Sum senecionine-N-oxide group (SnNO-group) A: 324 µg/kg u: 31.3 µg/kg σ_p: 81.1 µg/kg (25%) robust σ: 125 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
PT9547	140	-1.03	70	-0.24	65	-0.21	275	-0.57
PT9548	138	-1.06	81	0.29	91.1	1.31	310.1	-0.16
PT9549	193	-0.06	134	**2.83**	68	-0.04	395	0.81
PT9550	nt		43.041	-1.53	nt		43.041	**-3.24**
PT9551	324.4 (sum SnNO/IrNO)		62.6	-0.59	324.4 (sum SnNO/IrNO)		387	0.72
PT9552	242.1	0.83	78.9	0.19	81	0.72	402	0.89
PT9553	244.57	0.87	68.24	-0.32	44.72	-1.39	357.53	0.38
PT9554	427	**4.18**	66.8	-0.39	nd (no loq)		493.8	1.95
PT9555	171.6	-0.45	116	1.97	nt		287.6	-0.42
PT9556	detected, <10	**(-3.38) FN**	455.8 (sum SvNO/IrNO)		455.8 (sum SvNO/IrNO)		455.8	1.51
PT9557	223.8	0.50	137	**2.97**	Nt		360.8	0.42
PT9558	89	-1.95	21	**-2.58**	18	**-2.95**	128	**-2.26**
PT9559	284	1.59	182.4	**5.14**	Nt		466.4	1.63
PT9560	259	1.13	75	0.00	nd (no loq)		334	0.11
PT9561	65 (sum SvNO/SvNO)		65 (sum SvNO/SvNO)		68	-0.04	133	**-2.20**
PT9562	110	-1.57	<11	**(-3.06) FN**	<250	**(10.6)**	110	**-2.47**
PT9563	256	1.08	82.7	0.37	69.5	0.05	408.2	0.96
PT9564	316 (sum SnNO/SvNO)		316 (sum SnNO/SvNO)		nt		316	-0.10
PT9565	151	-0.83	42.1	-1.57	nt		193.1	-1.51
PT9566	36	**-2.91**	15.7	**-2.83**	nd, <2	**(-3.88) FN**	51.7	**-3.14**
PT9568	162	-0.63	43	-1.53	70	0.08	275	-0.57
PT9570	nt		Nt		nt		nt	
PT9571	173.52	-0.42	163.43	**4.24**	68.68	0.00	405.63	0.93
PT9572	445.7	**4.52**	78.7	0.18	83.3	0.86	607.7	**3.26**
PT9573	148.9 (sum SnNO/SvNO)		148.9 (sum SnNO/SvNO)		60.9	-0.45	209.8	-1.32
PT9574	350 (sum SnNO/IrNO)		70	-0.24	350 (sum SnNO/IrNO)		420	1.10

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

Material A, seneciphylline group					
	Seneciphylline (Sp) A: 90.3 µg/kg u: 7.02 µg/kg σ_p: 22.6 µg/kg (25%) robust σ: 25.8 µg/kg (29%)		Spartioidine (St)	Sum seneciphylline group (Sp-group) A: 94.1 µg/kg u: 8.01 µg/kg σ_p: 23.5 µg/kg (25%) robust σ: 31.4 µg/kg (33%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	Result (µg/kg)	z'-score
PT9547	105	0.62	10	115	0.84
PT9548	112	0.92	<10	112	0.72
PT9549	100	0.41	63 **FP**	163	**2.77**
PT9550	nt		Nt	nt	
PT9551	94.4 (sum Sp/St)		94.4 (sum Sp/St)	94.4	0.01
PT9552	78	-0.52	nd	78	-0.65
PT9553	70.24	-0.85	<10	70.24	-0.96
PT9554	77.9	-0.52	nt	77.9	-0.65
PT9555	229.6	**5.90**	nt	229.6	**5.45**
PT9556	483.2	**16.63**	nd	483.2	**15.65**
PT9557	69.9	-0.86	<10	69.9	-0.97
PT9558	45	-1.92	<10	45	-1.98
PT9559	107.9	0.75	nt	107.9	0.55
PT9560	95	0.20	nd	95	0.03
PT9561	95	0.20	<10	95	0.03
PT9562	160	**2.95**	<3.0	160	**2.65**
PT9563	94.6	0.18	nd	94.6	0.02
PT9564	80	-0.43	nt	80	-0.57
PT9565	95.2	0.21	nt	95.2	0.04
PT9566	12.2	**-3.30**	nd	12.2	**-3.30**
PT9568	40	**-2.13**	nd	40	**-2.18**
PT9570#	*19.42*	***(-3.00#)***	*nt*	*19.42*	***(-3.01#)***
PT9571	81.26	-0.38	nd, <10	81.26	-0.52
PT9572	104.2 (identification)*		87.7 (identification)*	191.9	**3.93**
PT9573	74	-0.69	nd	74	-0.81
PT9574	80 (sum Sp/St)		80 (sum Sp/St)	80	-0.57

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

*participant PT9572 had problems with identification, these values are not included in the evaluation of results.

participant PT9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

Material A, seneciphylline-N-oxide group					
	Seneciphylline-N-oxide (SpNO) A: 138 µg/kg u: 13.2 µg/kg σ_p: 34.5 µg/kg (25%) robust σ: 47.2 µg/kg (34%)		Spartioidine-N-oxide (StNO)	Sum seneciphylline-N-oxide group (SpNO-group) A:147 µg/kg u: 15.0 µg/kg σ_p: 36.7 µg/kg (25%) robust σ: 58.7 µg/kg (40%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	Result (µg/kg)	z'-score
PT9547	135	-0.09	<10	135	-0.30
PT9548	81.3	-1.54	<10	81.3	-1.65
PT9549	172	0.91	<20	172	0.64
PT9550	nt		nt	nt	
PT9551	174.8 (sum SpNO/StNO)		174.8 (sum SpNO/StNO)	174.8	0.71
PT9552	134.6	-0.10	5.3	139.9	-0.17
PT9553	152.9	0.40	<10	152.9	0.16
PT9554	154	0.43	nt	154	0.18
PT9555	109.2	-0.78	nt	109.2	-0.95
PT9556	416.9	**7.54**	nd	416.9	**6.82**
PT9557	126.2	-0.32	<10	126.2	-0.52
PT9558	44	**-2.55**	<10	44	**-2.59**
PT9559	205.3	1.82	nt	205.3	1.48
PT9560	193	1.48	nd	193	1.17
PT9561	149	0.29	405 **FP**	554	**10.28**
PT9562	<250	(3.02)	90 **FP**	90	-1.43
PT9563	150.2	0.33	nd	150.2	0.09
PT9564	204	1.78	nt	204	1.44
PT9565	129	-0.25	nt	129	-0.45
PT9566	63.3	**-2.02**	nd	63.3	**-2.11**
PT9568	91	-1.28	nd	91	-1.41
PT9570	nt		nt	nt	
PT9571	137.31	-0.02	4.71	142.02	-0.12
PT9572	257.1 (identification)*		120.8 (identification)*	377.9	**5.83**
PT9573	104.8	-0.90	nd	104.8	-1.06
PT9574	150 (sum SpNO/StNO)		150 (sum SpNO/StNO)	150	0.08

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

*participant PT9572 had problems with identification, these values are not included in the evaluation of results.

nd = not detected; nt = not tested.

	Material A, individual PAs proposed for regulation							
	Europine (Eu)	Europine-N-oxide (EuNO)	Heliotrine (Ht)	Heliotrine-N-oxide (HtNO)	Lasiocarpine (lc)	Lasiocarpine-N-oxide (LcNO)	Senkirkine (Sk) A: 12.0 µg/kg u: 0.551 µg/kg σ_p: 3.00 µg/kg (25%) robust σ: 2.02 µg/kg (17%)	
Lab code	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	z-score
PT9547	<10	<10	<10	<10	<10	<10	12	0.00
PT9548	<10	<10	<10	<10	<10	<10	11.7	-0.10
PT9549	<20	<20	<20	<20	<20	<20	<20	**(2.66)**
PT9550	<0.2	<0.1	<0.1	<0.2	<0.1	<0.3	9.38	-0.87
PT9551	nd	nd	nd	nd	nd	12.2	11.7	-0.10
PT9552	nd	nd	nd	nd	nd	nd	13.2	0.40
PT9553	<10	<10	<10	<10	<10	<10	10.79	-0.40
PT9554	<1	<1	<1	<2	<1	<2	9.7	-0.77
PT9555	nd	nd	nd	nd	detected	nd	detected, <25	**(4.33)**
PT9556	nd	nd	nd	nd	nd	nd	18.2	**2.06**
PT9557	<10	<10	<10	<10	<10	<10	11.8	-0.07
PT9558	<10	<10	<10	<10	<10	<10	<10	(-0.67)
PT9559	nd	nd	nd	nd	nd	nd	14.8	0.93
PT9560	nd	nd	nd	nd	nd	nd	17	1.66
PT9561	<25	**243 FP**	<10	<10	<10	<10	13	0.33
PT9562	<32	<4.6	<84	<23	<13	<20	13	0.33
PT9563	nd	nd	nd	nd	nd	nd	12.4	0.13
PT9564	nd;< Loq=1	nd; <loq=2.5	nd; <loq=1	nd; <loq=2.5	nd;<loq=0.5	nd;<loq=0.5	12	0.00
PT9565	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	8.59	-1.14
PT9566	<2	<2	nd	nd	nd	nd	3.9	**-2.70**
PT9568	nd	nd	nd	nd	nd	nd	nd, <10	(-0.67)
PT9570#	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*	*3.84*	***(-2.72#)***
PT9571	nd; <10	nd; <10	nd; <10	nd; <10	4.65; <10	nd; <10	17.12	1.70
PT9572	<5	<5	<5	<5	<5	<5	12.8	0.27
PT9573	nd	nd	nd	nd	nd	nd	10.4	-0.53
PT9574	nd	nd	nd	nd	nd	nd	11	-0.33

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

participant 9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material A, other PA											
	Erucifoline (Er) A: 107 µg/kg u: 6.26 µg/kg σ_p: 26.7 µg/kg (25%) robust σ: 22.4 µg/kg (21%)		Erucifoline-N-oxide (ErNO) A: 192 µg/kg u: 16.6 µg/kg σ_p: 47.9 µg/kg (25%) robust σ: 56.4 µg/kg (29%)		Jacobine (Jb) A: 46.4 µg/kg u: 3.97 µg/kg σ_p: 11.6 µg/kg (25%) robust σ: 12.3 µg/kg (27%)		Jacobine-N-oxide (JbNO) A: 33.1 µg/kg u: 2.39 µg/kg σ_p: 8.28 µg/kg (25%) robust σ: 8.13 µg/kg (25%)		Jacoline (Jl) A: 40.7 µg/kg u: 1.92 µg/kg σ_p: 10.2 µg/kg (25%) robust σ: 6.51 µg/kg (16%)		Jaconine (Jn) A: 176 µg/kg u: 11.6 µg/kg σ_p: 44.1 µg/kg (25%) robust σ: 39.4 µg/kg (22%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9547	135	1.05	300	**2.14**	<10	**(-2.97) FN**	60	**3.24**	60	1.89	200	0.53
PT9548	nt		nt		nt		nt		nt		nt	
PT9549	84	-0.86	198	0.12	48	0.13	39	0.71	42	0.12	258	1.85
PT9550	96.842	-0.38	165.526	-0.52	43.831	-0.21	30.123	-0.36	26.063	-1.44	155.853	-0.47
PT9551	109.2	0.09	208.1	0.32	30.2	-1.32	24.6	-1.03	25	-1.55	86.5	**-2.04**
PT9552	107.7	0.03	179.8	-0.23	42.3	-0.34	28.3	-0.58	44.7	0.39	186.2	0.22
PT9553	112.89	0.22	191.04	-0.01	106.89	**4.93**	30.46	-0.32	41.09	0.03	145.29	-0.71
PT9554	99	-0.30	130	-1.22	32.9	-1.10	24.2	-1.08	24.2	-1.62	123	-1.21
PT9555	66.6	-1.51	nt		nt		nt		nt		nt	
PT9556	348.5	**9.04**	detected, <10	**(-3.58) FN**	nd, <10	**(-2.97) FN**	44	1.31	28.7	-1.18	6159.2	**135.59**
PT9557	86.7	-0.76	153.5	-0.75	35.8	-0.87	22.6	-1.27	36	-0.47	159.9	-0.38
PT9558	25	**-3.06**	57	**-2.66**	<10	**(-2.97) FN**	<10	**(-2.79) FN**	<10	**(-3.02) FN**	57	**-2.71**
PT9559	128.8	0.82	250.1	1.15	39.9	-0.53	31.3	-0.22	nt		nt	
PT9560	nt		nt		nd (no loq)		nd (no loq)		nt		nt	
PT9561	118	0.41	255	1.25	49	0.21	30	-0.38	54	1.30	198	0.49
PT9562	<250	**(5.35)**	90	-2.00	<7.0	**(-3.21) FN**	32	-0.14	230	**18.58**	210	0.76
PT9563	119.7	0.48	194	0.05	52.6	0.50	40.6	0.90	44.9	0.41	183.4	0.16
PT9564	nt		nt		nt		nt		nt		nt	
PT9565	83.5	-0.88	126	-1.30	detected, <2.5	**(-3.58) FN**	detected, <2.5	**(-3.70) FN**	41	0.03	nt	
PT9566	nd, <2	**(-3.93) FN**	nd, <2	**(-3.74) FN**	nd, <2	**(-3.62) FN**	nd, <2	**(-3.76) FN**	nd, <2	**(-3.80) FN**	nd, <2	**(-3.95) FN**
PT9568	92	-0.56	195	0.07	43	-0.28	48	1.80	41	0.03	158	-0.42
PT9570	nt		nt		nt		nt		nt		nt	
PT9571	107.61	0.03	nd, <10	**(-3.58) FN**	36.03	-0.85	34.95	0.22	41	0.03	168.37	-0.18
PT9572	119.8	0.48	217.5	0.51	59.4	1.06	30.4	-0.33	44.3	0.35	197.5	0.48
PT9573	153.2	1.73	217.1	0.50	114.8	**5.58**	26	-0.86	37.6	-0.31	228.3	1.17
PT9574	107	0.00	262	1.39	70	1.92	67	**4.09**	41	0.03	159	-0.40

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material A, other PA		
	Monocrotaline (Mc)	Monocrotaline-N-oxide (McNO)	Trichodesmine (Td)
Lab code	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)
PT9547	10	<10	<10
PT9548	nt	nt	nt
PT9549	<20	<20	<20
PT9550	<0.4	<0.5	<1.0
PT9551	nd	nd	nd
PT9552	nd	nd	nd
PT9553	<10	<10	<10
PT9554	<2	<2	<2
PT9555	detected	nt	nd
PT9556	nd	nd	nd
PT9557	<10	<10	<10
PT9558	<10	<10	<10
PT9559	nd	nd	nt
PT9560	nd	nd	nd
PT9561	<10	<10	<10
PT9562	<250	3.9	<8.0
PT9563	nd	nd	nd
PT9564	nd; <log=4	nd; <log=2.5	nt
PT9565	<2.5	<2.5	<2.5
PT9566	nt	nt	nd
PT9568	nd	nd	nd
PT9570	nt	nt	nt
PT9571	nd; <10	nd; <10	nd;<10
PT9572	<5	<5	<5
PT9573	nd	nd	nd
PT9574	nd	nd	nd

nd = not detected; nt = not tested.

Material A, sum of 35 PAs for legislation and sum of total PAs				
	Total sum of 35 PAs for legislation A: 2287 µg/kg u: 185 µg/kg σ_p: 572 µg/kg (25%) robust σ: 740 µg/kg (32%)		Sum of total PAs A: 2750 µg/kg u: 175 µg/kg σ_p: 687µg/kg (25%) robust σ: 699 µg/kg (25%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z-score
PT9547	2232	-0.09	2997	0.36
PT9548	2630	0.57	2630	-0.17
PT9549	2393	0.18	3062	0.45
PT9550	645	**-2.73**	1164	**-2.31**
PT9551	2324	0.06	2807	0.08
PT9552	2143	-0.24	2732	-0.03
PT9553	3040	1.25	3668	1.34
PT9554	2218	-0.11	2651	-0.14
PT9555	1419	-1.45	1485	-1.84
PT9556	4741	**4.08**	11321	**12.47**
PT9557	1874	-0.69	2369	-0.55
PT9558	751	**-2.56**	890	**-2.71**
PT9559	2891	1.01	3341	0.86
PT9560	2395	0.18	2395	-0.52
PT9561	3872	**2.64**	4576	**2.66**
PT9562	1923	-0.61	2489	-0.38
PT9563	2377	0.15	3012	0.38
PT9564	3879	**2.65**	3879	1.64
PT9565	2113	-0.29	2364	-0.56
PT9566	693	**-2.65**	693	**-2.99**
PT9568	1646	-1.07	2223	-0.77
PT9570#	*95*	***(-3.65#)***	*95*	***(-3.86#)***
PT9571	2381	0.17	2769	0.03
PT9572	3354	1.79	4022	1.85
PT9573	1493	-1.32	2270	-0.70
PT9574	2438	0.26	3144	0.57

A = assigned value (robust mean).

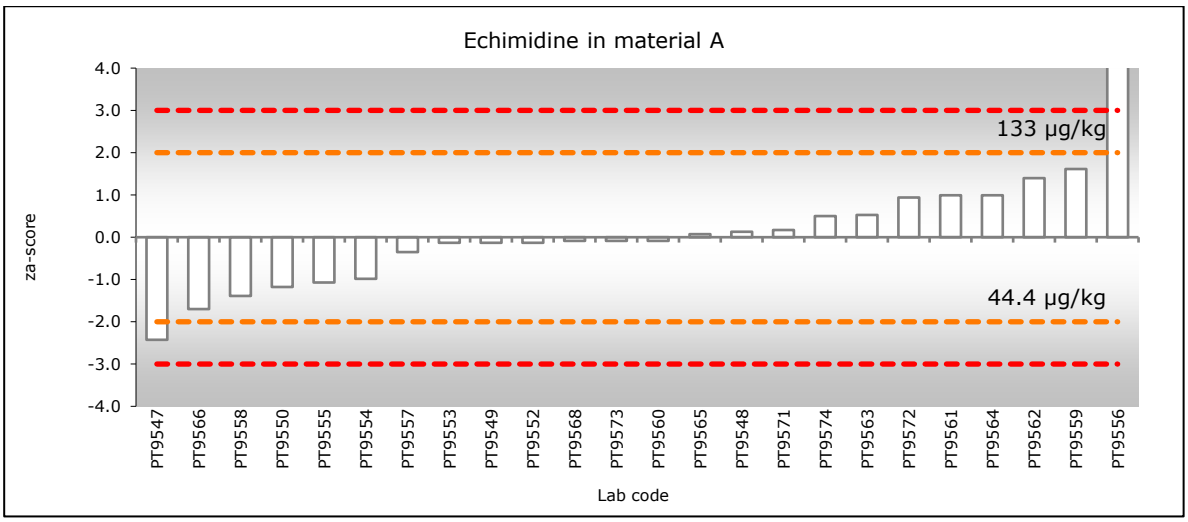
u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

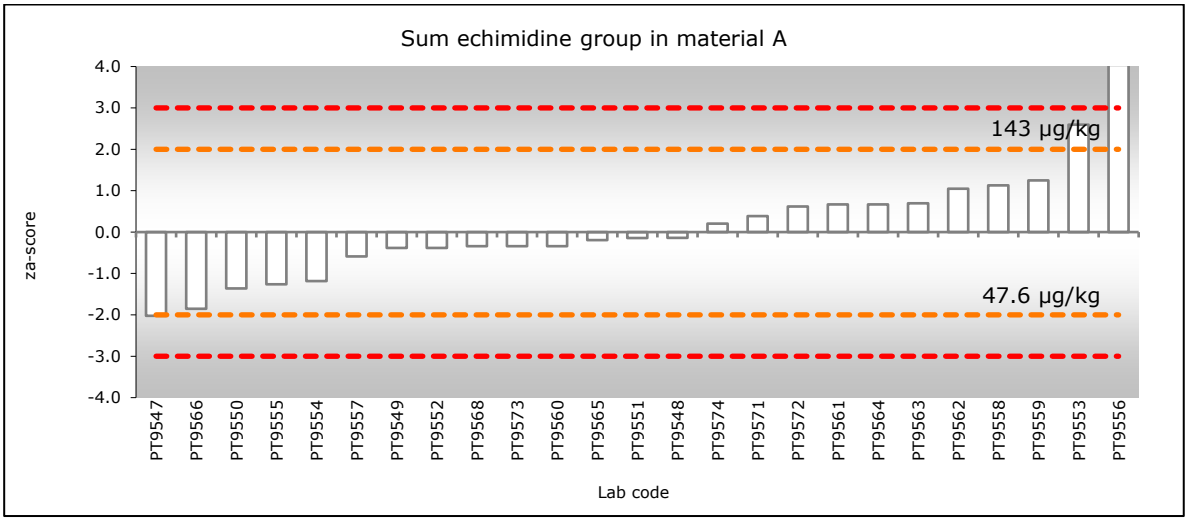
robust σ = robust (relative) standard deviation based on participants' result.

participant PT9570 reported 4 components in method. Not included in overall calculations.

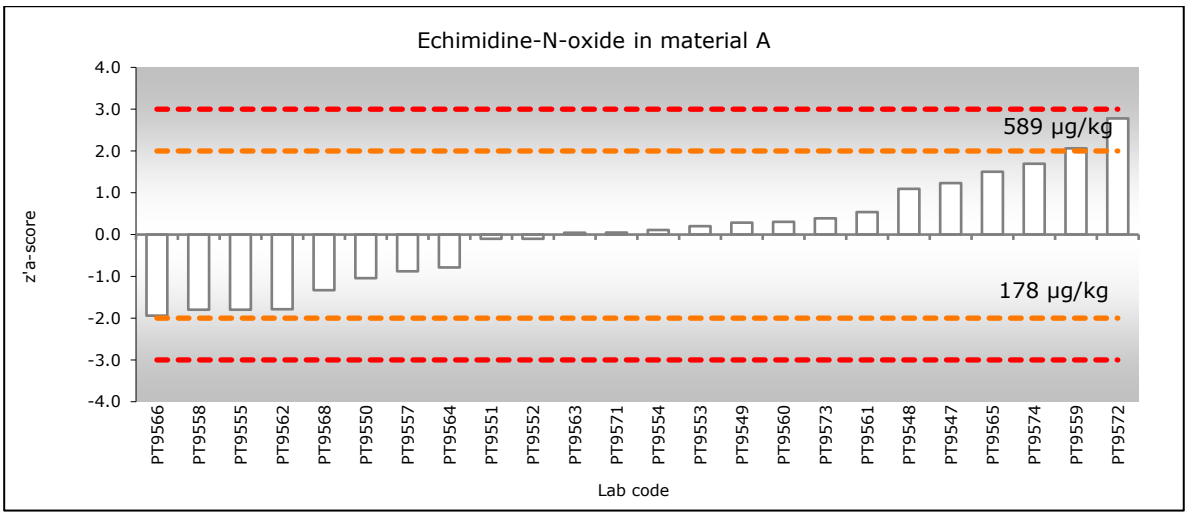
nd = not detected; nt = not tested.



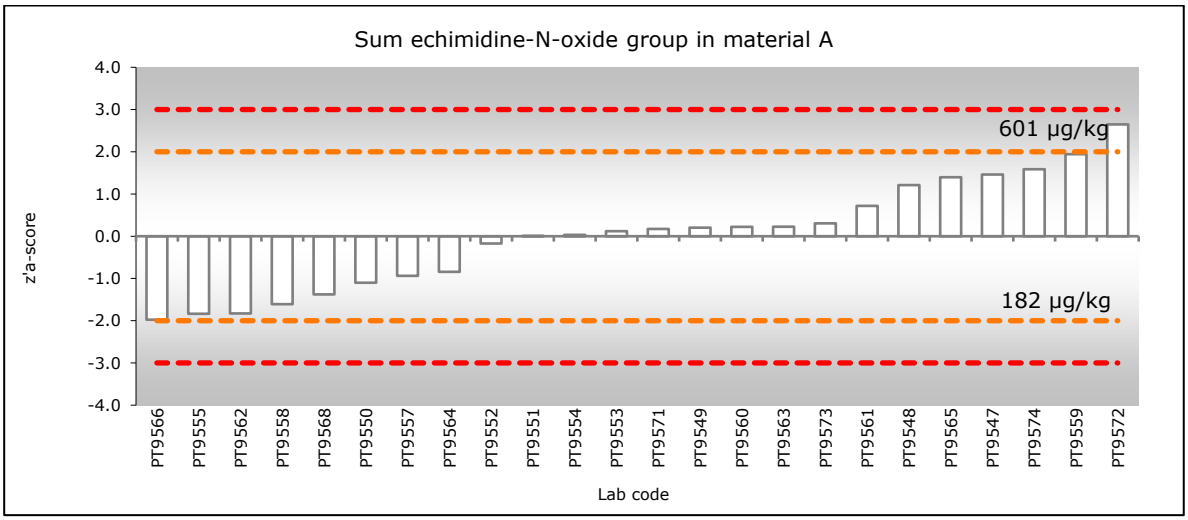
Graphical representation of the z-scores for echimidine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



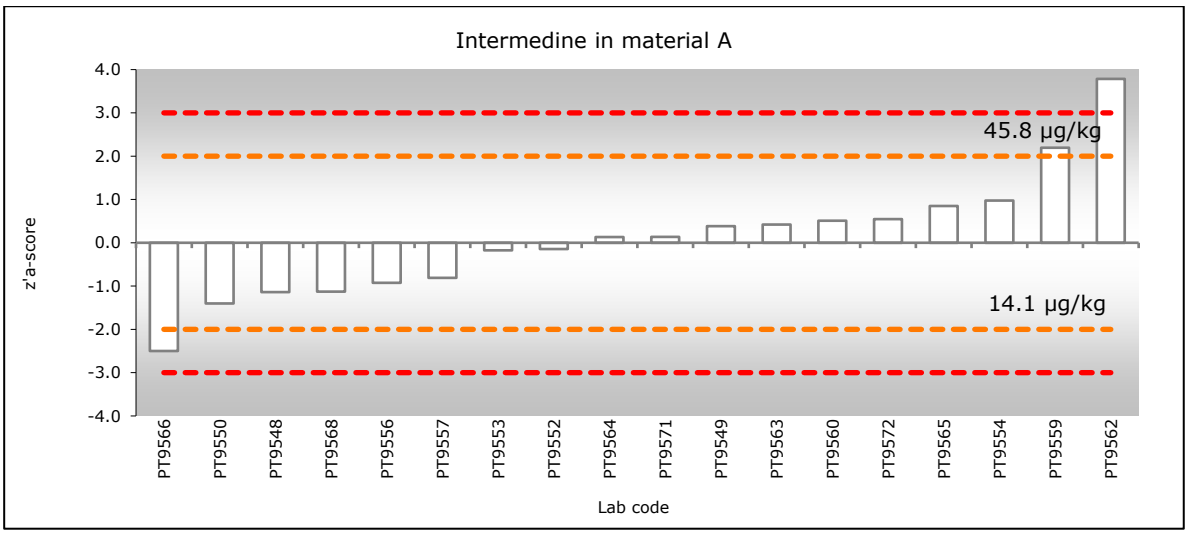
Graphical representation of the z-scores for sum echimidine group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



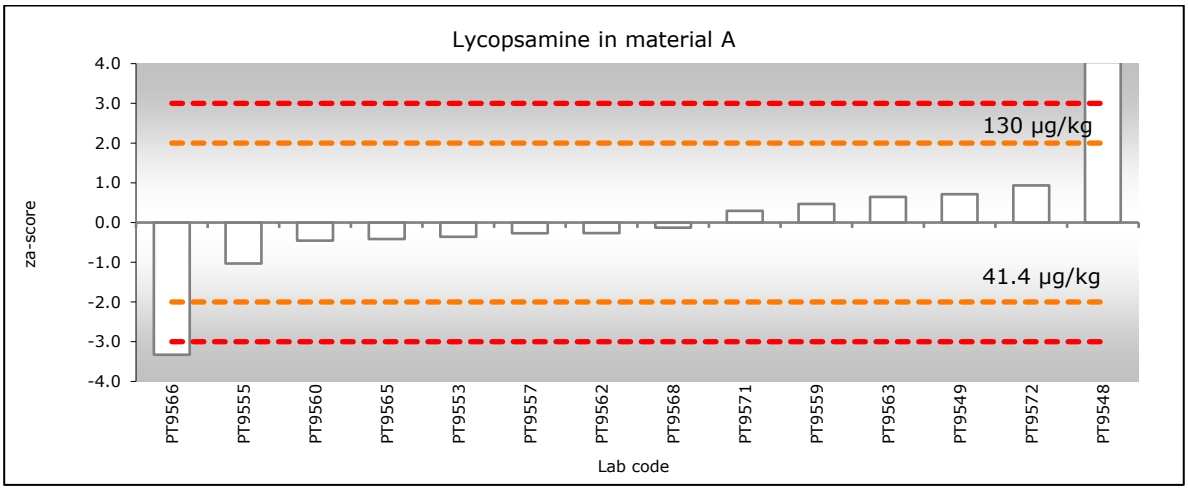
Graphical representation of the z'-scores for echimidine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



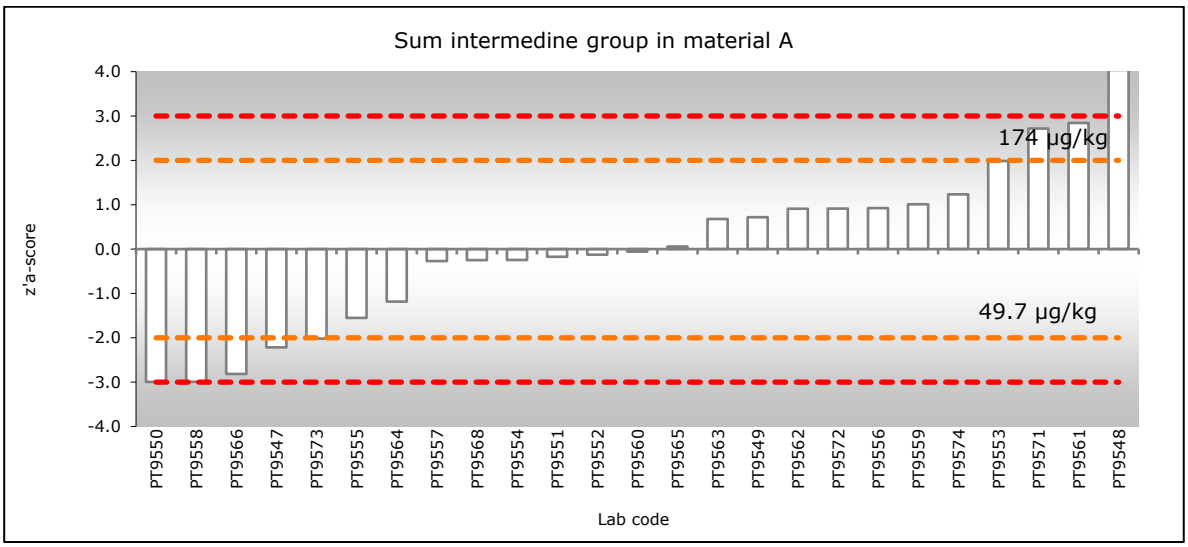
Graphical representation of the z'-scores for sum echimidine-N-oxide group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



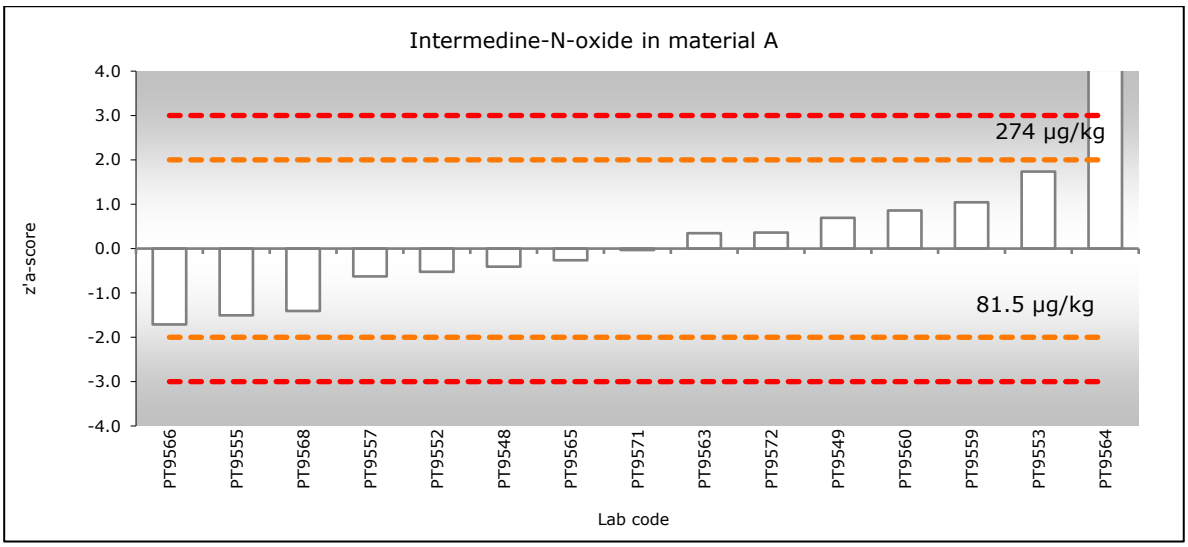
Graphical representation of the z'-scores for Intermedine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



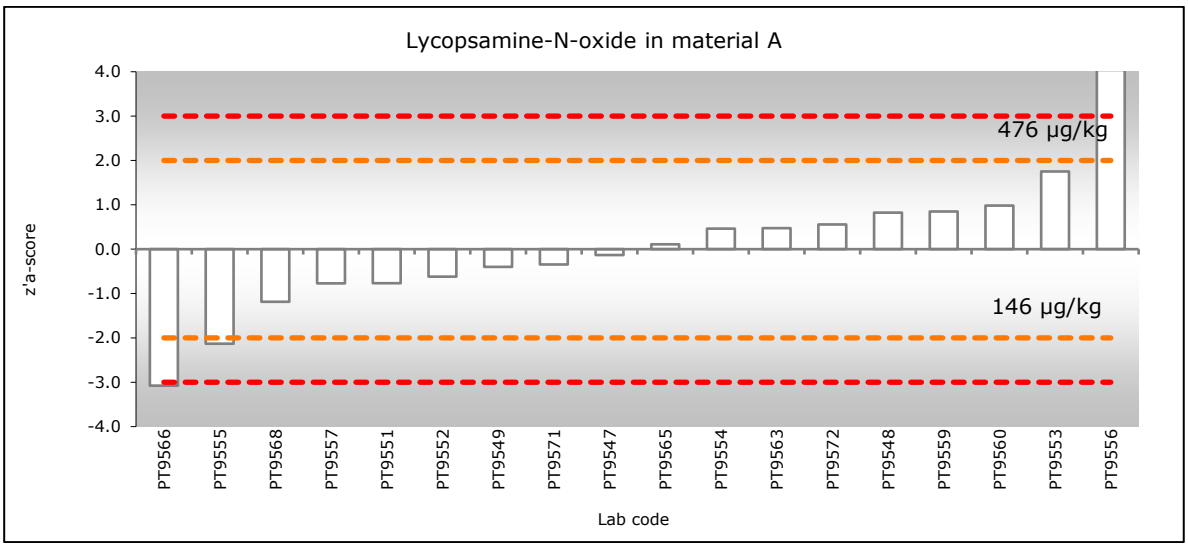
Graphical representation of the z-scores for lycopsamine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



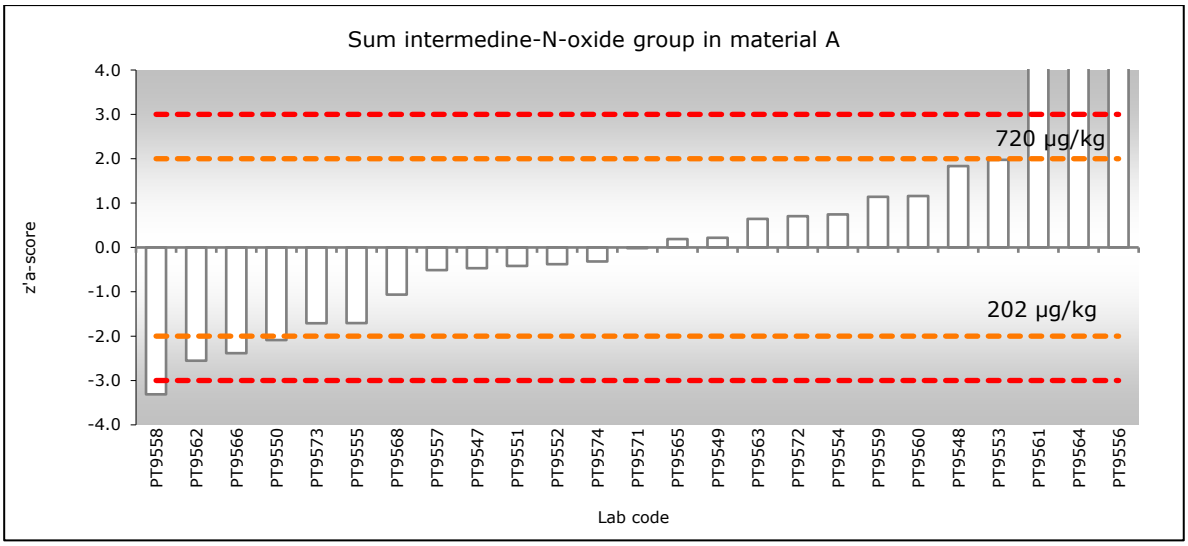
Graphical representation of the z'-scores for sum intermedine group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



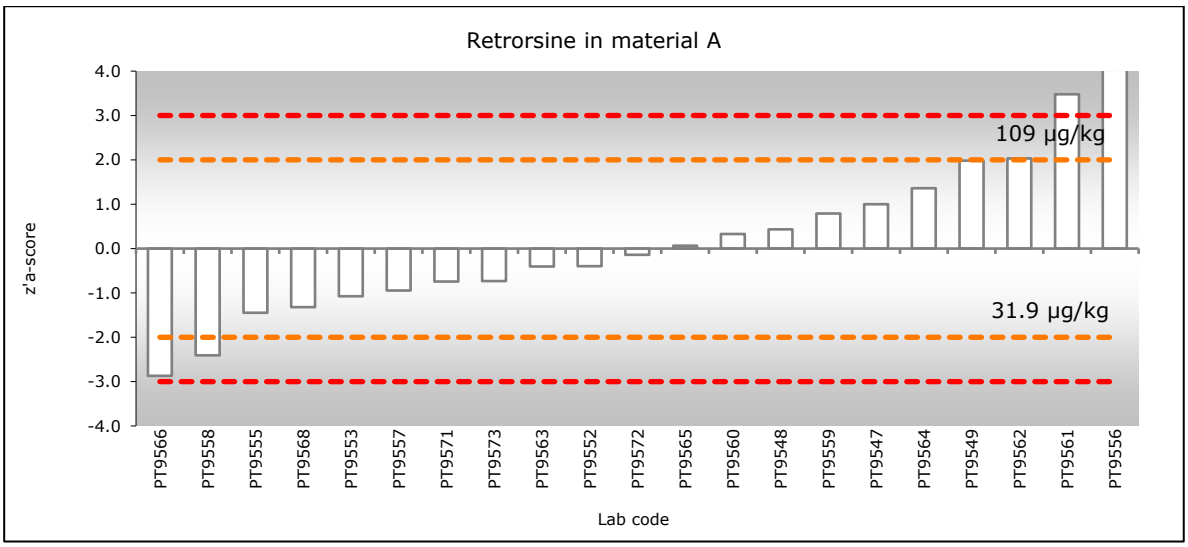
Graphical representation of the z'-scores for intermedine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



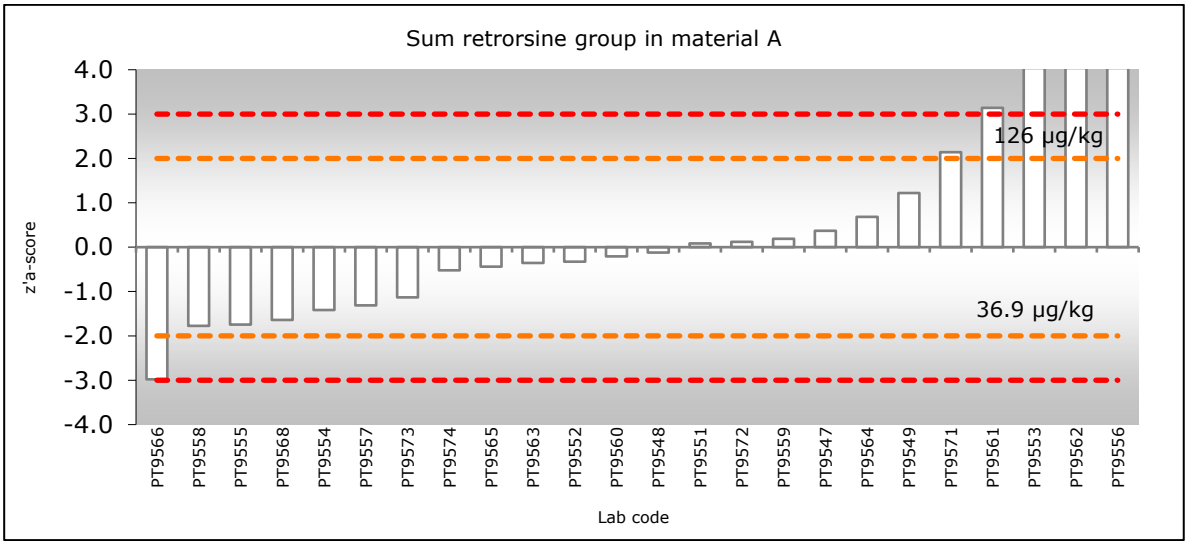
Graphical representation of the z'-scores for lycopsamine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



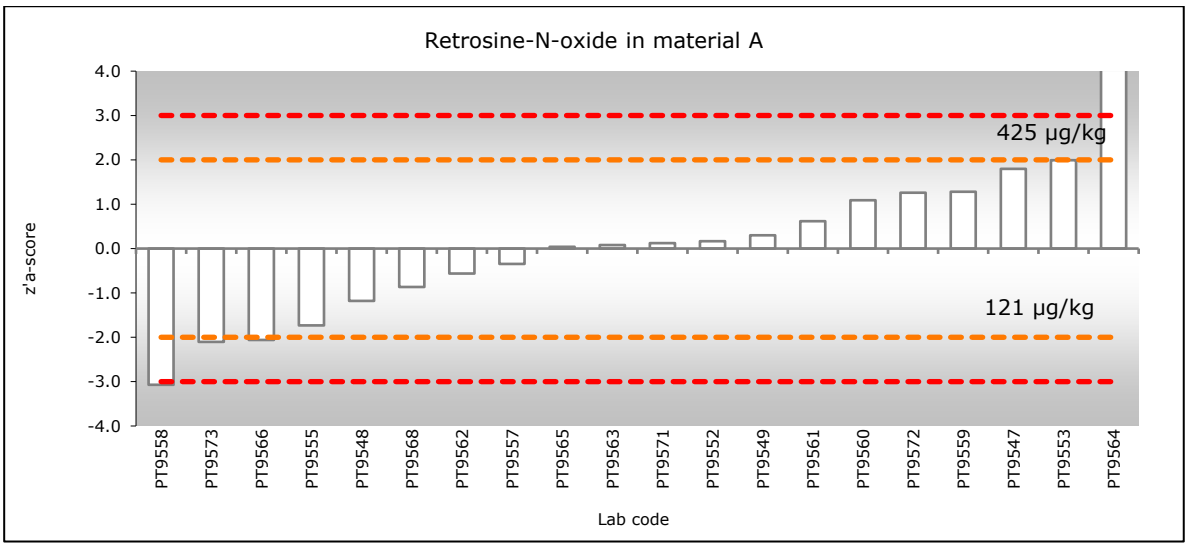
Graphical representation of the z'-scores for sum Intermedine-N-oxide group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



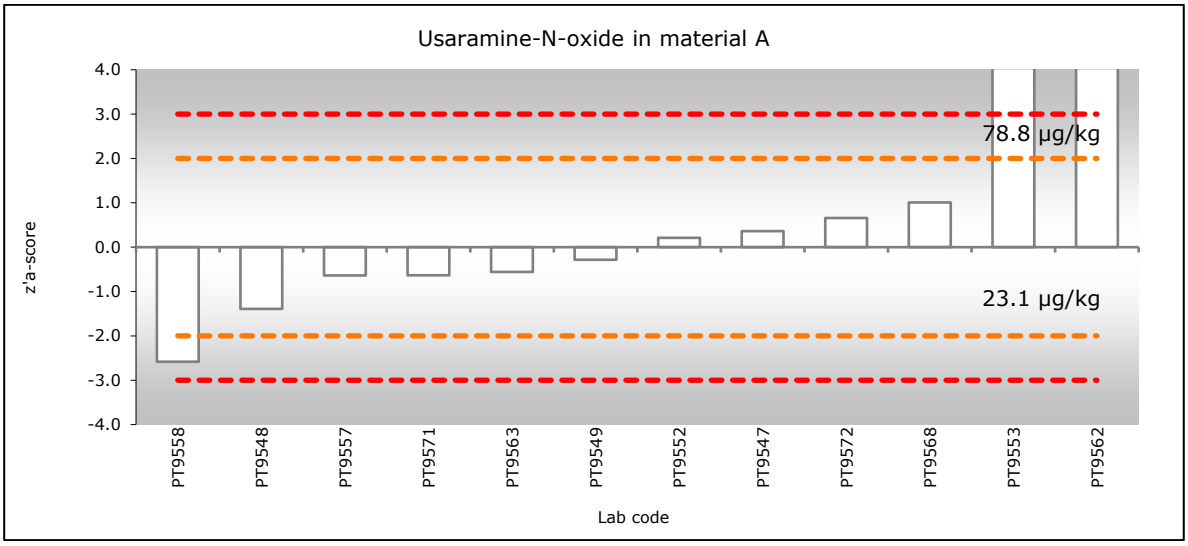
Graphical representation of the z'-scores for retrorsine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



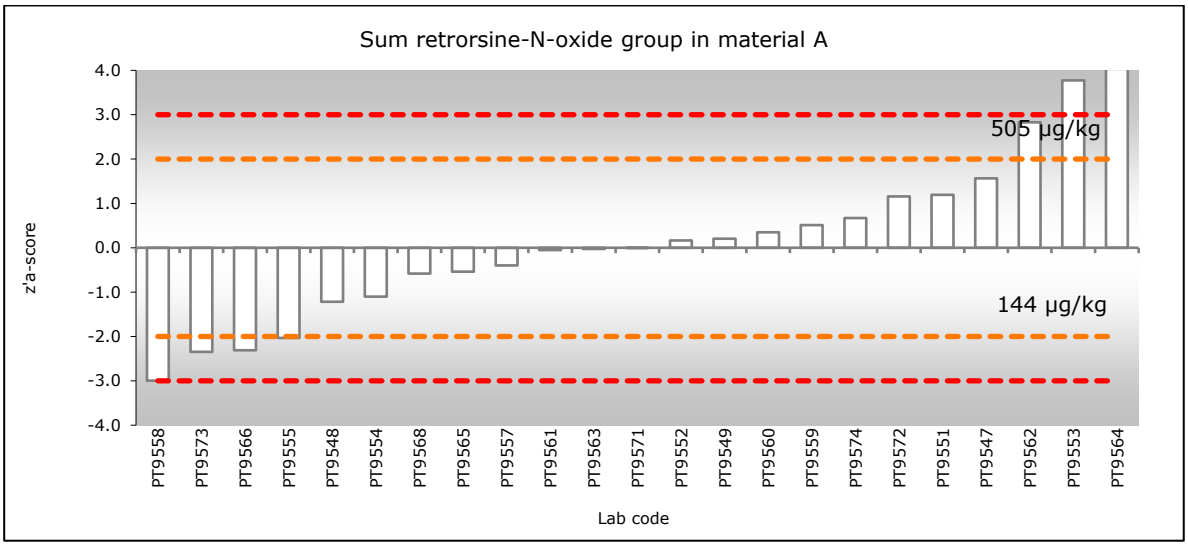
Graphical representation of the z'-scores for sum retrorsine group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



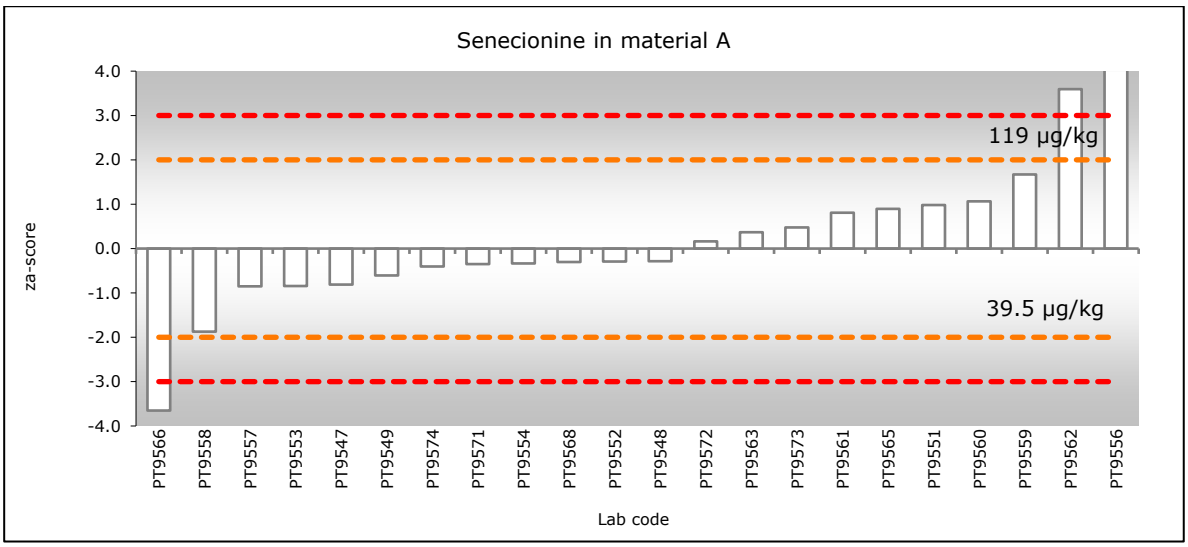
Graphical representation of the z'-scores for retrorsine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



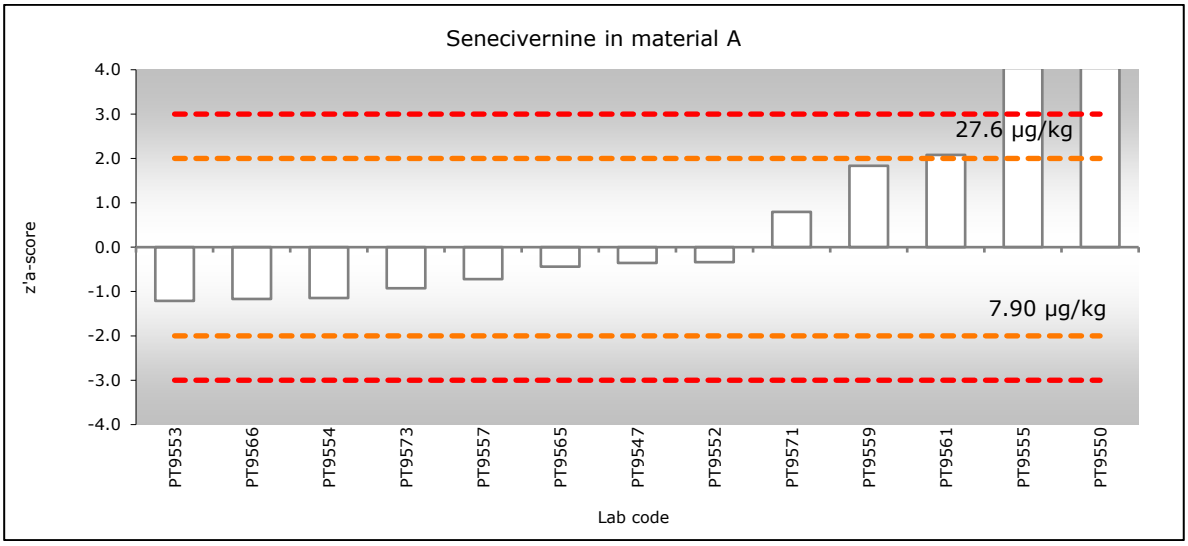
Graphical representation of the z'-scores for usaramarine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



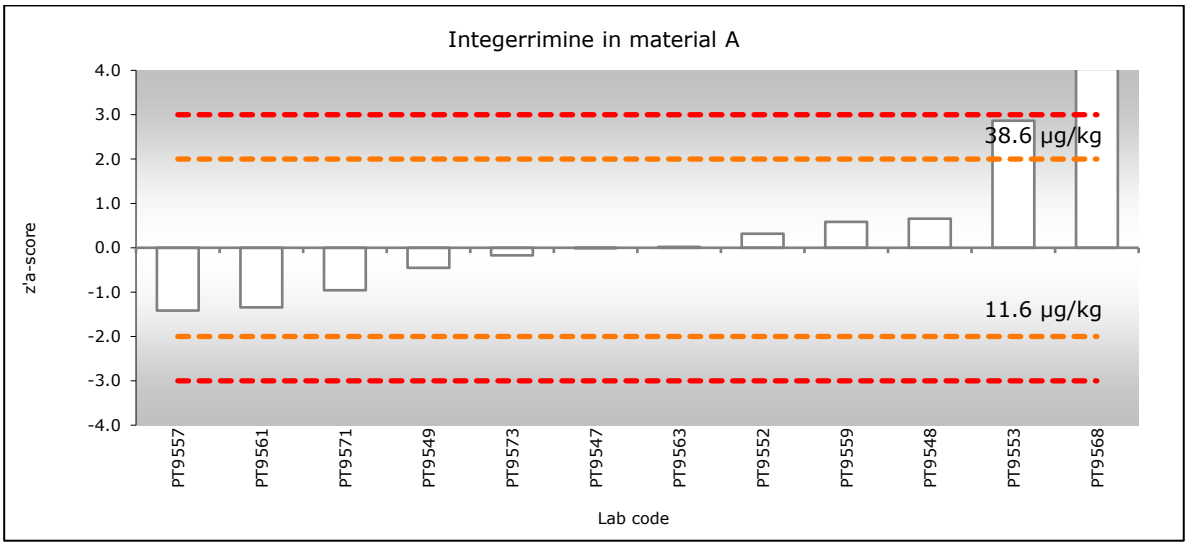
Graphical representation of the z'-scores for sum retrorsine-N-oxide group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



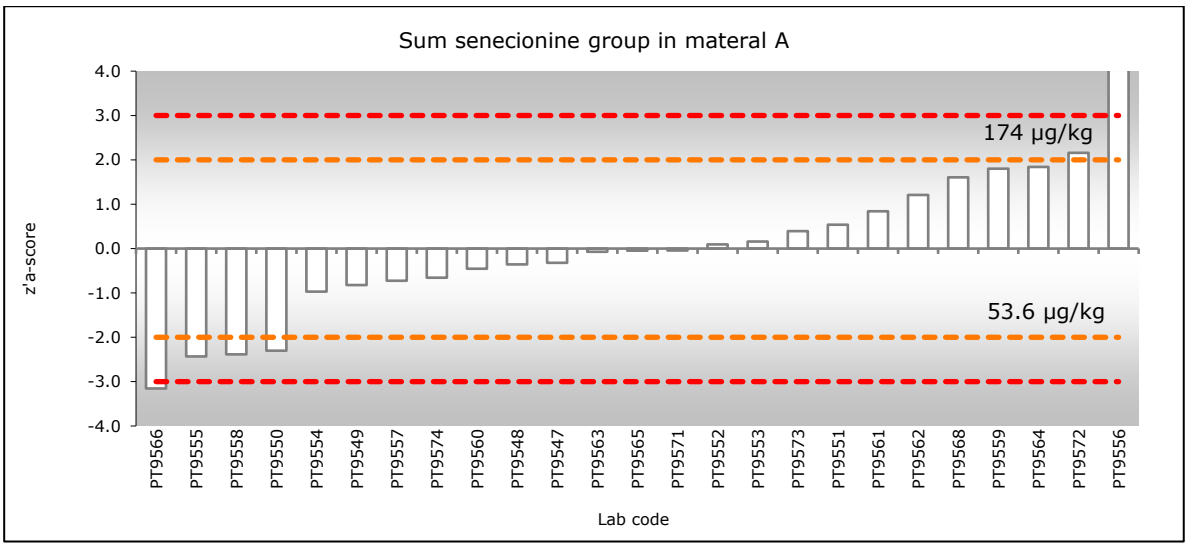
Graphical representation of the z-scores for senecionine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



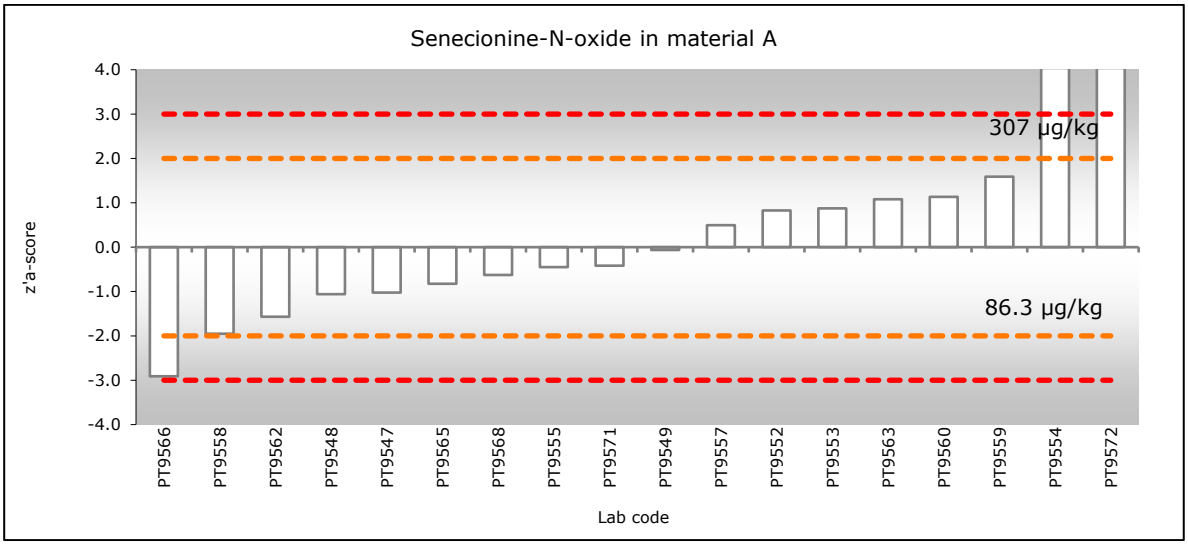
Graphical representation of the z'-scores for senecivernine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



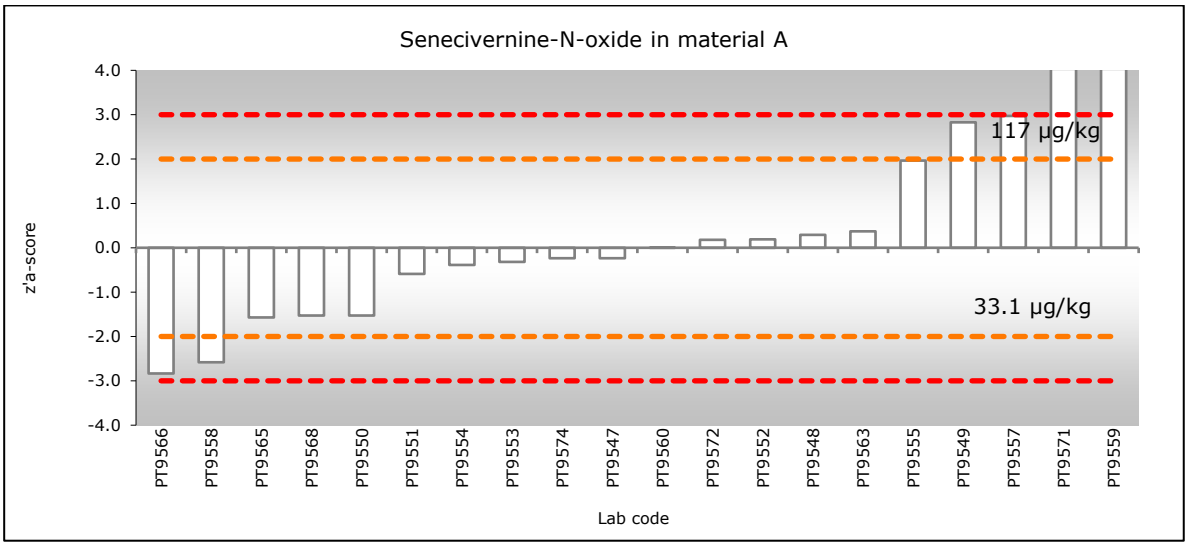
Graphical representation of the z'-scores for integerrimine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



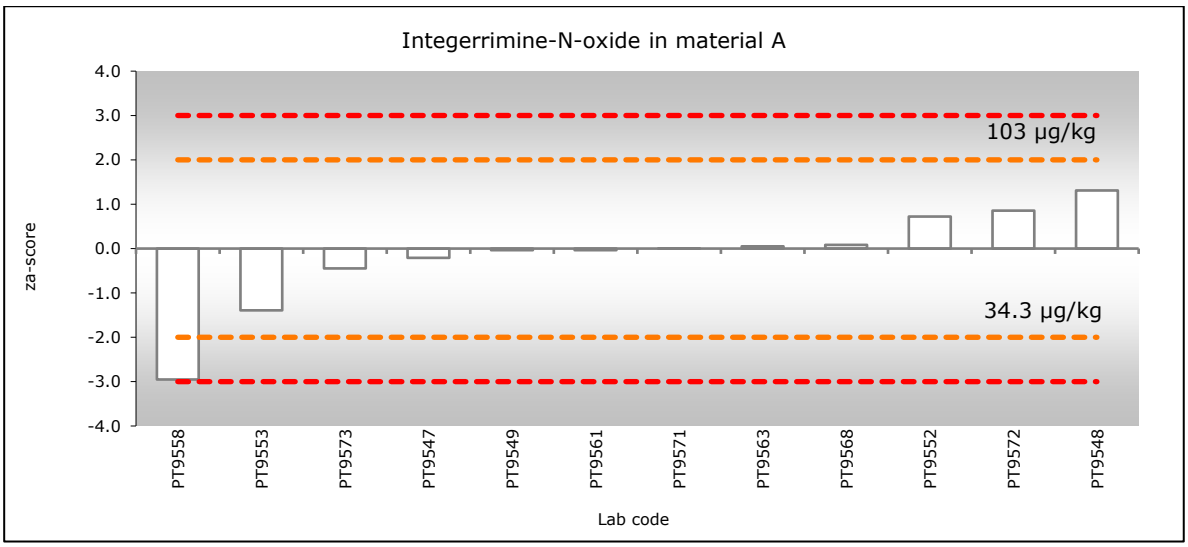
Graphical representation of the z'-scores for sum senecionine group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



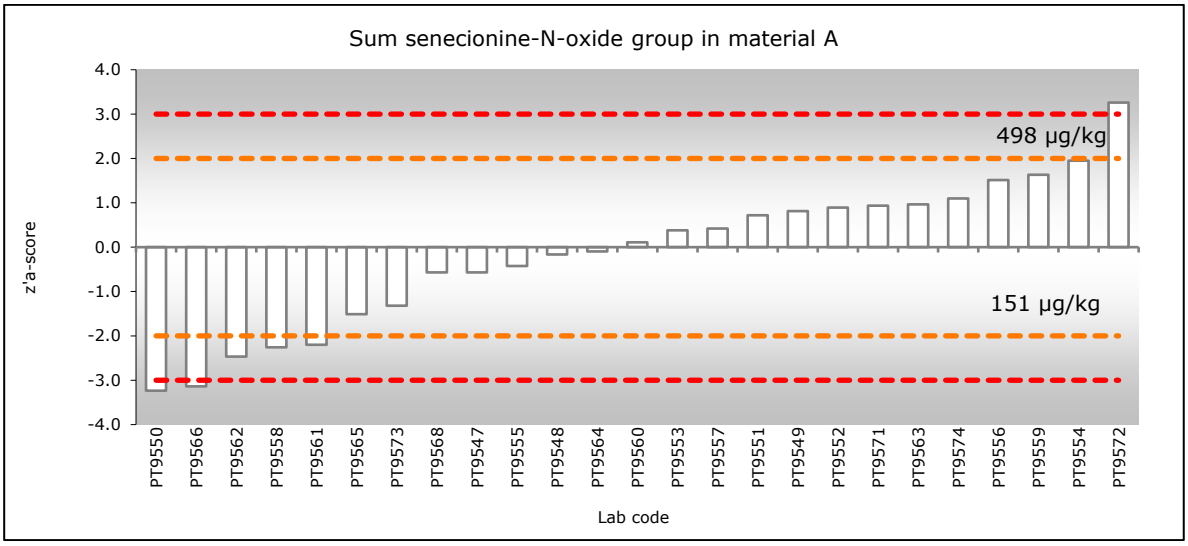
Graphical representation of the z'-scores for senecionine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



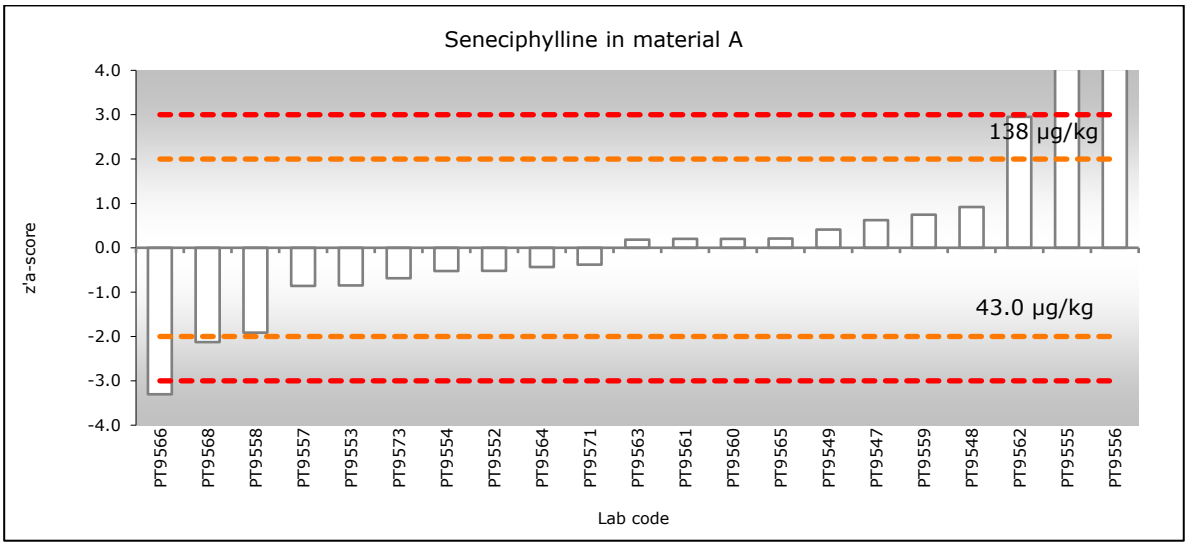
Graphical representation of the z'-scores for senecivernine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



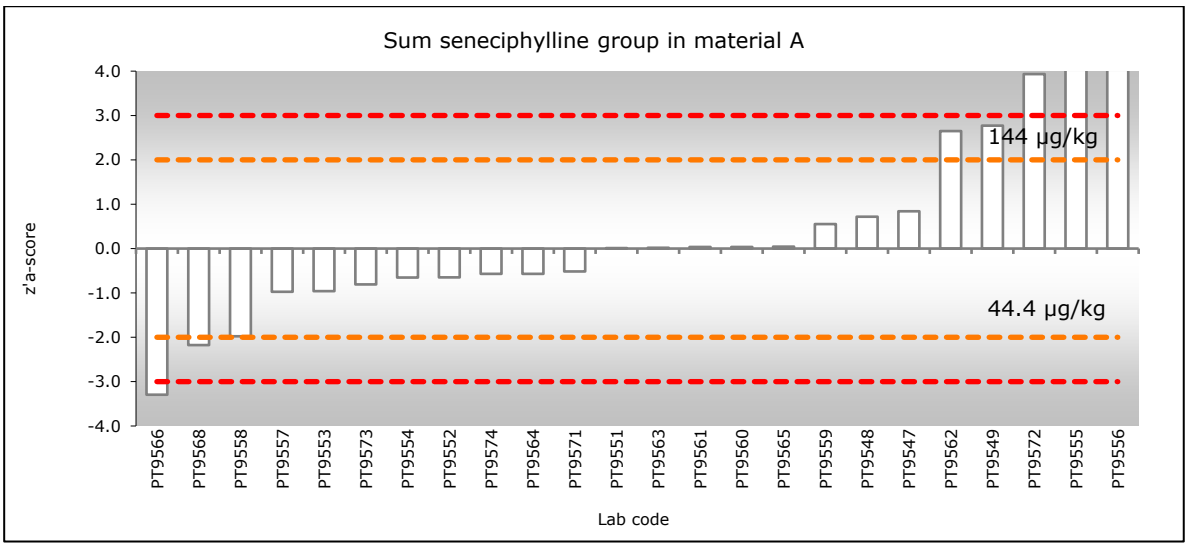
Graphical representation of the z-scores for integerrimine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



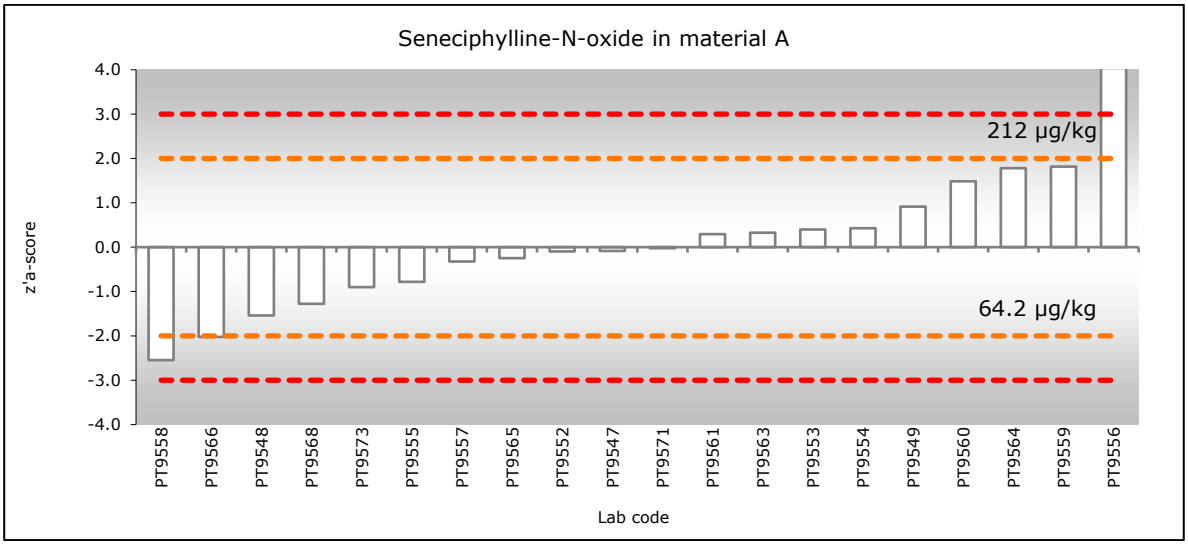
Graphical representation of the z'-scores for sum senecionine-N-oxide group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



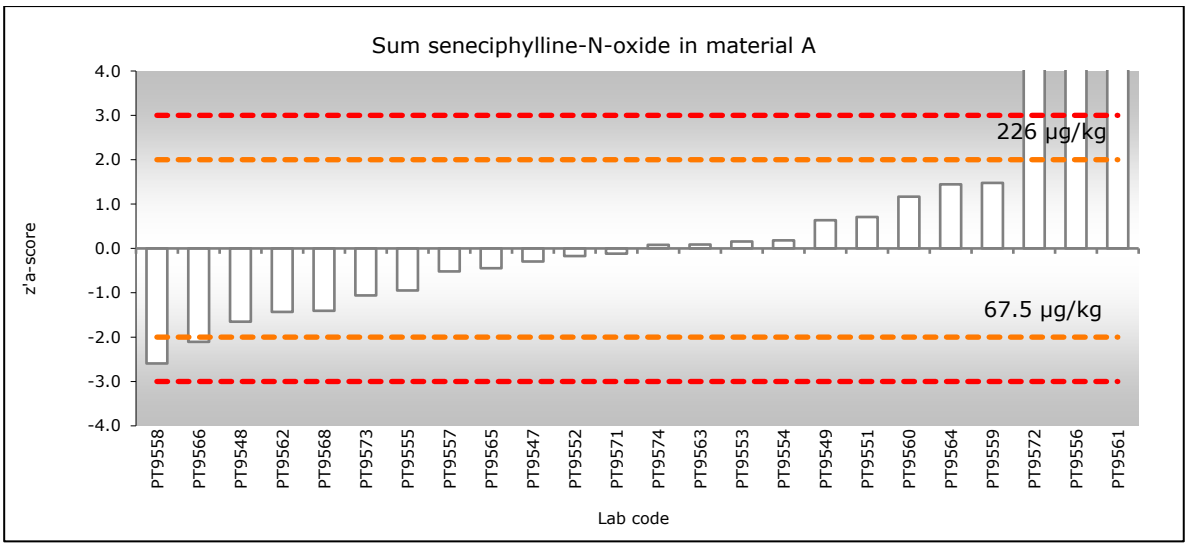
Graphical representation of the z'-scores for seneciphylline in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



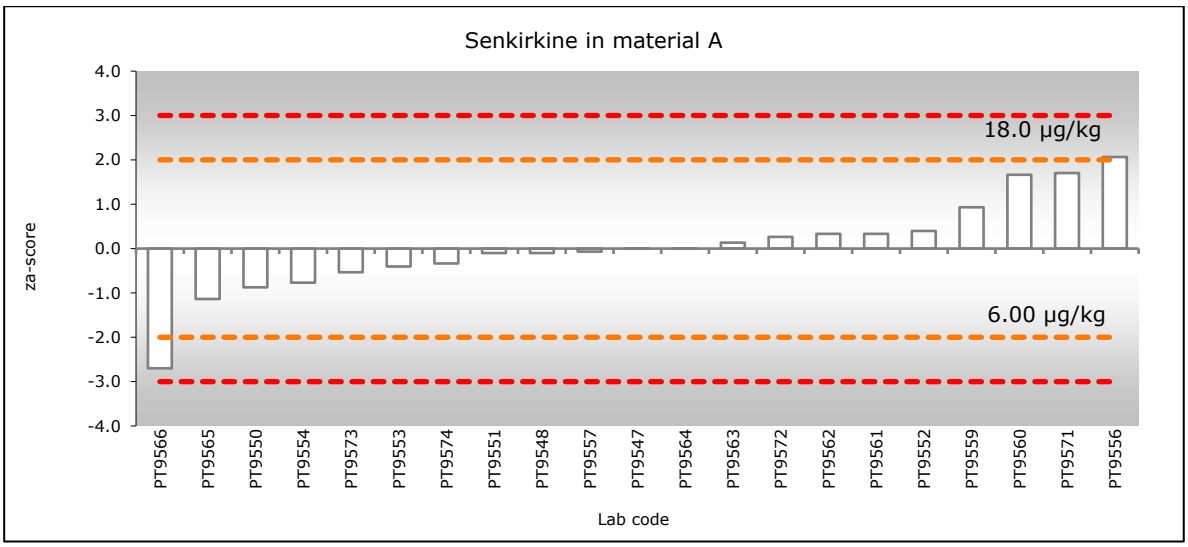
Graphical representation of the z'-scores for sum seneciphylline group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



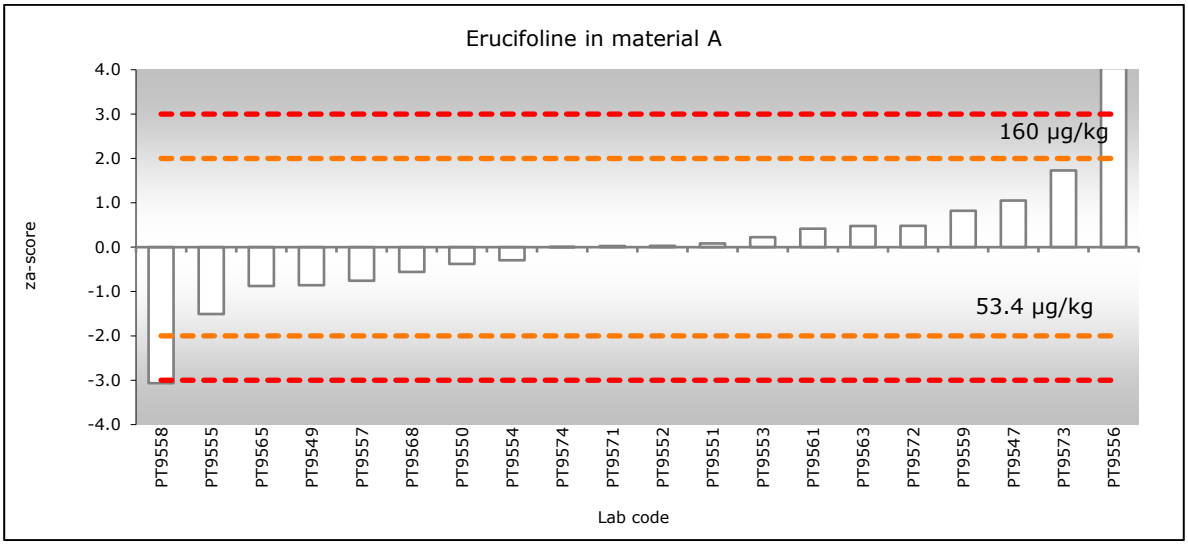
Graphical representation of the z'-scores for seneciphylline-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



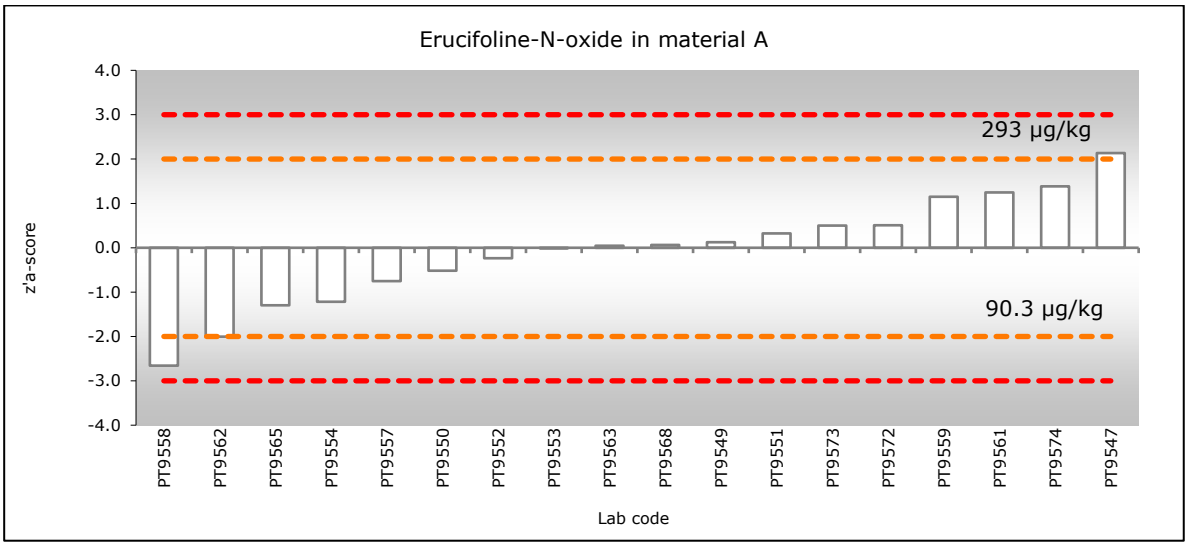
Sum seneciphylline-N-oxide group - Graphical representation of the z'-scores for sum seneciphylline-N-oxide group in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



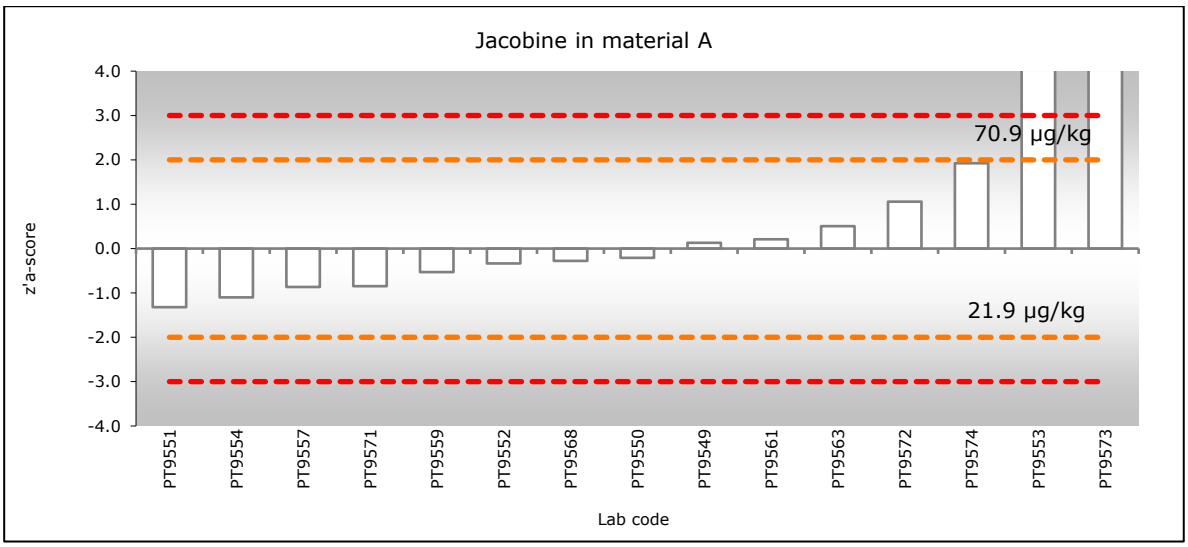
Graphical representation of the z-scores for senkirkine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



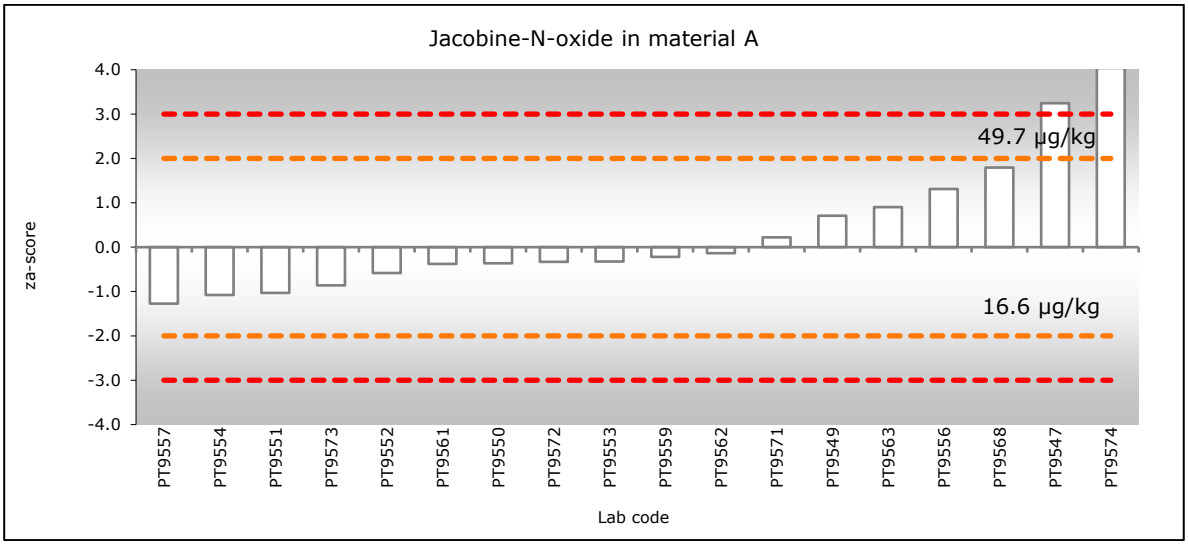
Graphical representation of the z-scores for erucifoline in material A. Dotted lines show research study performance boundaries± 2 (also in µg/kg) and ± 3.



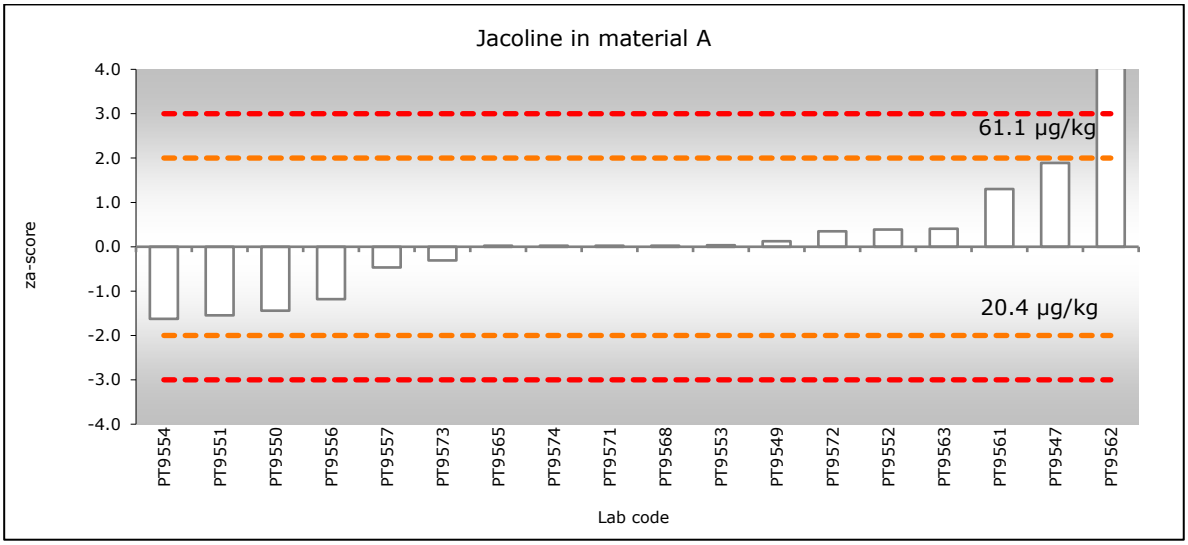
Graphical representation of the z'-scores for erucifoline-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



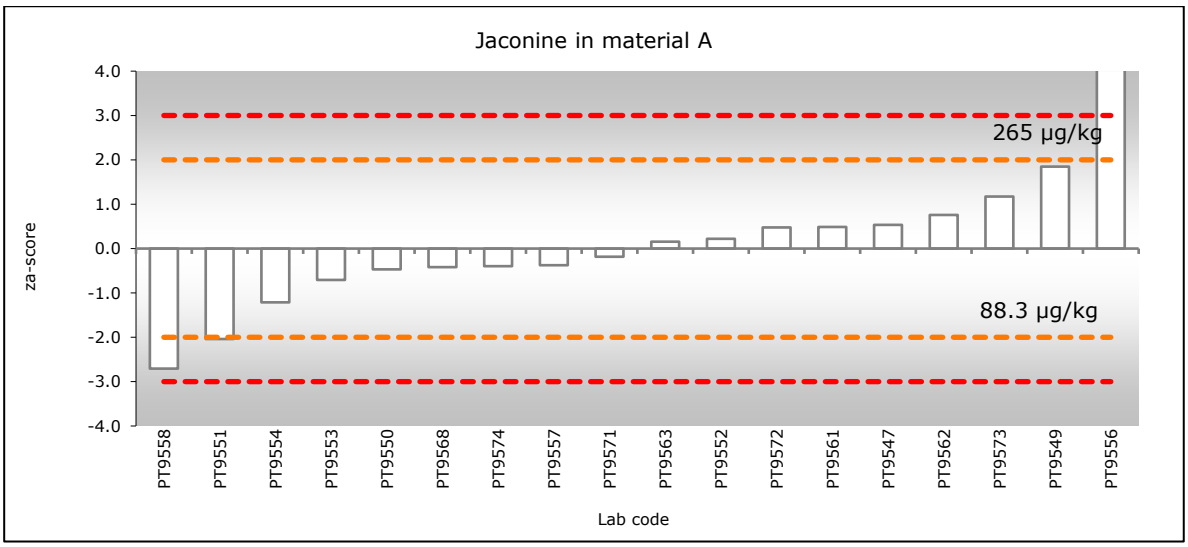
Graphical representation of the z'-scores for jacobine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



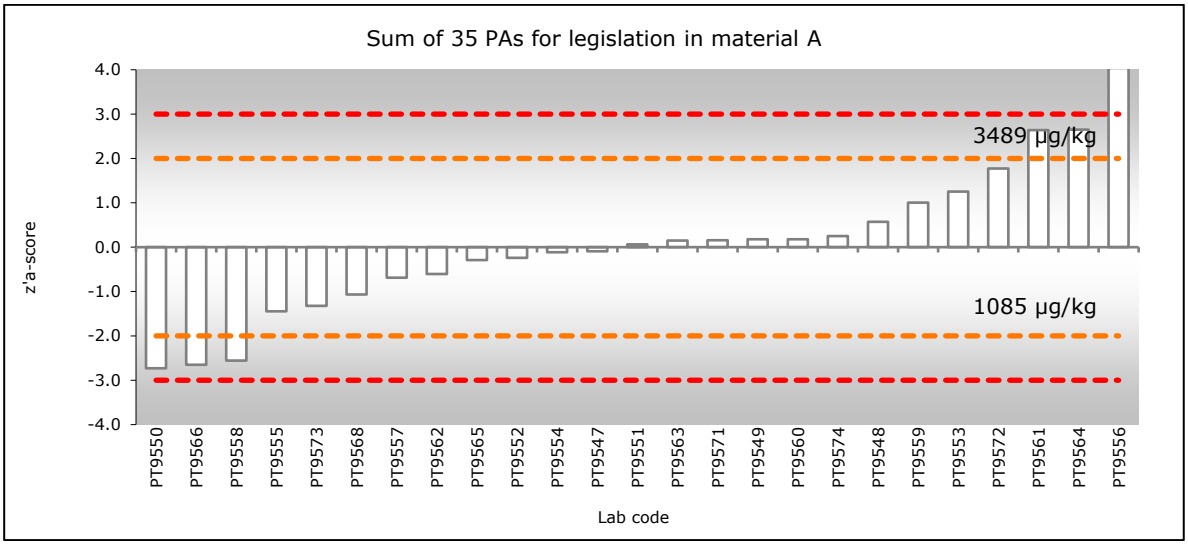
Graphical representation of the z-scores for jacobine-N-oxide in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



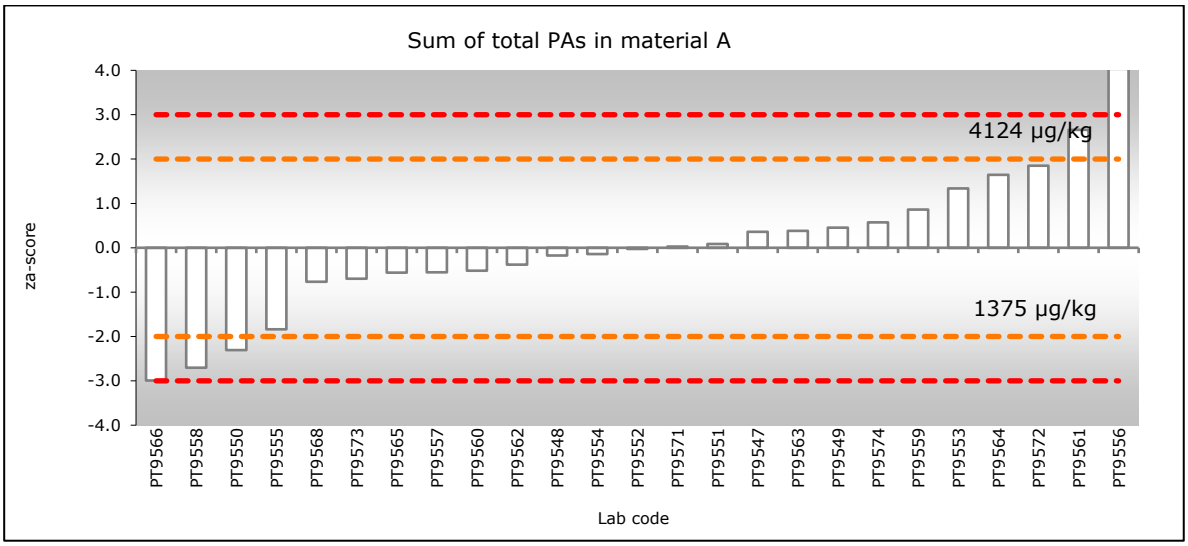
Graphical representation of the z-scores for jacoline in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



Graphical representation of the z-scores for jaconine in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



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



Graphical representation of the z-scores for sum of total PAs in material A. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.

Annex 10 Results material B (herbal tea)

	Material B, echimidine group	
	Echimidine (Em)	Heliosupine (Hs)
Lab code	Result (µg/kg)	Result (µg/kg)
PT9547	<10	<10
PT9548	<10	<10
PT9549	<20	<20
PT9550	<0.5	<0.5
PT9551	nd	nd
PT9552	nd	nd
PT9553	<10	<10
PT9554	<2	nd
PT9555	detected	nt
PT9556	nd	nd
PT9557	<10	<10
PT9558	<10	<10
PT9559	nd	nt
PT9560	nd	nd
PT9561	<10	<10
PT9562	<9.4	<11
PT9563	nd	nd
PT9564	1.5	nt
PT9565	<2.5	<2.5
PT9568	nd	nd
PT9569	<0.6	0.4
PT9570	nt	nt
PT9571	<10	<10
PT9572	<5	<5
PT9573	nd	nd
PT9574	nd	nd

nd = not detected; nt = not tested.

	Material B, echimidine-N-oxide group	
	Echimidine-N-oxide (EmNO)	Heliosupine-N-oxide (HsNO)
Lab code	Result (µg/kg)	Result (µg/kg)
PT9547	<10	<10
PT9548	<10	<10
PT9549	<20	<20
PT9550	2.848	<0.5
PT9551	nd	nd
PT9552	nd	nd
PT9553	<10	<10
PT9554	<2	nd
PT9555	detected	nt
PT9556	nd	nd
PT9557	<10	<10
PT9558	<10	<10
PT9559	<10	nt
PT9560	nd	nd
PT9561	<10	<10
PT9562	<9.6	<12
PT9563	nd	nd
PT9564	4.9	nt
PT9565	<2.5	<2.5
PT9568	nd	nd
PT9569	3.7	2.6
PT9570	nt	nt
PT9571	<10	<10
PT9572	<5	<5
PT9573	nd	nd
PT9574	nd	nd

nd = not detected; nt = not tested.

	Material B, intermedine group				
	Intermedine (Im)	Lycopsamine (ly)	Indicine (Id)	Echinatine (En)	Rinderine (Rn)
Lab code	Results (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)
PT9547	<10	<10	<10	<10	<10
PT9548	<10	<10	22.2 FP	22.1	25.5 FP
PT9549	<20	<20	<20	<20	<20
PT9550	<0.2	nt	nt	nt	nt
PT9551	Nd	nd	nd	nd	nd
PT9552	Nd	nd	nd	nd	nd
PT9553	<10	<10	<10	<10	<10
PT9554	<2	<2	<2	<15	<15
PT9555	Nd	139.4 FP	nt	nt	nt
PT9556	Nd	nd	nd	nd	nd
PT9557	<10	<10	<10	<10	<10
PT9558	<10	<10	<10	<10	<10
PT9559	Nd	detected	nt	detected	detected
PT9560	Nd	nd	nd	nd	nd
PT9561	<10	<10	<10	<10	<10
PT9562	<28	<29	<8.4	<5.6	<8.0
PT9563	Nd	nd	nd	nd	nd
PT9564	Nd	nd	nt	nt	nt
PT9565	<2.5	<2.5	nt	<2.5	<2.5
PT9568	Nd	nd	nd	nd	nd
PT9569	0.6	0.7	2.3	3.7	2.4
PT9570	Nt	nt	nt	nt	nt
PT9571	Nd	nd	nd	nd	2.78; <10
PT9572	<5	<5	<5	<5	<5
PT9573	Nd	nd	nd	nd	nd
PT9574	Nd	nd	nd	nd	nd

nd = not detected; nt = not tested.

	Material B, intermedine-N-oxide group				
	Intermedine-N-oxide (ImNO)	Lycopsamine-N-oxide (LyNO)	Indicine-N-oxide (IdNO)	Echinatine-N-oxide (EnNO)	Rinderine-N-oxide (Rn)
Lab code	Results (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)
PT9547	<10	<10	<10	<10	17
PT9548	<10	<10	15.1	37.8	31.5
PT9549	<20	<20	<20	<20	<20
PT9550	Nt	<0.5	nt	nt	nt
PT9551	38.8 **FP** (sum ImNO/IdNO)	13.5	38.8 (sum ImNO/IdNO)	nd	nd
PT9552	Nd	nd	nd	14.4	14.6
PT9553	<10	<10	16.89	23.89	<10
PT9554	<2	<10	<2	<20	<20
PT9555	Detected	detected	nt	nt	nt
PT9556	35.1 **FP** (sum ImNO/IdNO)	nd	35.1 (sum ImNO/IdNO)	nd	nd
PT9557	<10	<10	<10	nt	nt
PT9558	<10	<10	<10	<10	<10
PT9559	Detected	detected	nt	nt	nt
PT9560	Nd	nd	nd	nd	nd
PT9561	<10	<10	<10	<10	<10
PT9562	<18	<15	30 **FP**	24	26
PT9563	Nd	nd	nd	nt	nt
PT9564	Nd	detected	nt	nt	nt
PT9565	<2.5	<2.5	nt	13.9	11.5
PT9568	Nd	nd	nd	12	nd
PT9569	<1.5	<1.5	<1	19.1	13.5
PT9570	Nt	nt	nt	nt	nt
PT9571	Nd	nd	nd	10.48	15.53
PT9572	<5	<5	<5	11.4	11.9
PT9573	Nd	nd	nd	18.6	nd
PT9574	40 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	40 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	40 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	40 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)	40 (sum ImNO/LyNO/ IdNO/EnNO/RnNO)

nd = not detected; nt = not tested.

	Material B, retrorsine group				
	Retrorsine (Rt) A: 29.8 µg/kg u: 2.75 µg/kg σ_p: 7.45 µg/kg (25%) robust σ: 8.79 µg/kg (29%)		Usaramine (Us)	Sum retrorsine group (Rt-group) A: 33.1 µg/kg u: 2.22 µg/kg σ_p: 8.27 µg/kg (25%) robust σ: 7.93 µg/kg (24%)	
Lab code	Result (µg/kg)	z'-score	Results (µg/kg)	Result (µg/kg)	z-score
PT9547	32	0.28	<10	32	-0.13
PT9548	36.7	0.87	<10	36.7	0.44
PT9549	Nt		nt	nt	
PT9550	Nt		nt	nt	
PT9551	37.2 (sum Rt/Us)		37.2 (sum Rt/Us)	37.2	0.50
PT9552	37.9	1.02	nd	37.9	0.58
PT9553	15.07	-1.86	54.62 **FP**	69.69	**4.43**
PT9554	28 (sum Rt/Us)		28 (sum Rt/Us)	28	-0.61
PT9555	44.2	1.81	nt	44.2	1.34
PT9556	nd, <10	**(-2.49) FN**	nd	nd, <10	**(-2.70) FN**
PT9557	33.3	0.44	<10	33.3	0.03
PT9558	<10	**(-2.49) FN**	<10	<10	**(-2.70) FN**
PT9559	24	-0.73	nt	24	-1.10
PT9560	nd, <2.5	**(-3.44) FN**	nd	nd, <2.5	**(-3.57) FN**
PT9561	37	0.90	<10	37	0.47
PT9562	<19	(-1.36)	120 **FP**	120	**10.51**
PT9563	31	0.15	nd	31	-0.25
PT9564	36	0.78	nt	36	0.35
PT9565	29.9	0.01	nt	29.9	-0.38
PT9568	12	**-2.24**	nd	12	**-2.55**
PT9569	31.3	0.19	<10	31.3	-0.22
PT9570#	*0.96*	***(-3.63#)***	*nt*	*0.96*	***(-3.75#)***
PT9571	17.79	-1.51	53.66 **FP**	71.45	**4.64**
PT9572	19.9	-1.25	<5	19.9	-1.59
PT9573	28.8	-0.13	nd	28.8	-0.52
PT9574	20 (sum Rt/Us)		20 (sum Rt/Us)	20	-1.58

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

participant 9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material B, retrorsine-N-oxide group				
	Retrorsine-N-oxide (RtNO) A: 106 µg/kg u: 7.51 µg/kg σ_p: 26.6 µg/kg (25%) robust σ: 26.9 µg/kg (25%)		Usaramine-N-oxide (UsNO)	Sum retrorsine-N-oxide group (RtNO-group) A: 113 µg/kg u: 8.33 µg/kg σ_p: 28.2 µg/kg (25%) robust σ: 32.6 µg/kg (29%)	
Lab code	**Result (µg/kg)**	**z-score**	**Results (µg/kg)**	**Result (µg/kg)**	**z-score**
PT9547	54	-1.97	<10	54	**-2.08**
PT9548	92	-0.54	<10	92	-0.74
PT9549	107	0.02	<20	107	-0.20
PT9550	Nt		nt	nt	
PT9551	133 (sum RtNO/UsNO)		133 (sum RtNO/UsNO)	133	0.72
PT9552	112.4	0.23	nd	112.4	-0.01
PT9553	114.32	0.30	85.8 **FP**	200.12	**3.10**
PT9554	147 (sum RtNO/UsNO)		147 (sum RtNO/UsNO)	147	1.21
PT9555	137.1	1.15	nt	137.1	0.86
PT9556	183	**2.88**	nd	183	**2.49**
PT9557	116.4	0.38	<10	116.4	0.13
PT9558	19	**-3.29**	<10	19	**-3.33**
PT9559	134.93	1.07	nt	134.93	0.79
PT9560	95	-0.43	nd	95	-0.63
PT9561	30	**-2.87**	nt	30	**-2.94**
PT9562	<110	(0.14)	150 **FP**	150	1.32
PT9563	128.9	0.85	nd	128.9	0.57
PT9564	138	1.19	nt	138	0.89
PT9565	100	-0.24	nt	100	-0.45
PT9568	95	-0.43	nd	95	-0.63
PT9569	116	0.36	2.2	118.2	0.19
PT9570	Nt		nt	nt	
PT9571	112.53	0.23	nd	112.53	-0.01
PT9572	89.9	-0.62	<5	89.9	-0.81
PT9573	83.8	-0.85	nd	83.8	-1.03
PT9574	100 (sum RtNO/UsNO)		100 (sum RtNO/UsNO)	100	-0.45

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material B, senecionine group						
	Senecionine (Sn) A: 25.7 µg/kg u: 2.91 µg/kg σ_p: 6.42 µg/kg (25%) robust σ: 9.89 µg/kg (39%)		Senecivernine (Sv)	Integerrimine (Ir) A: 15.1 µg/kg u: 0.947 µg/kg σ_p: 3.77 µg/kg (25%) robust σ: 2.39 µg/kg (16%)		Sum senecionine group (Sn-group) A: 41.8 µg/kg u: 3.23 µg/kg σ_p: 10.5 µg/kg (25%) robust σ: 11.8 µg/kg (28%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	Result (µg/kg)	z-score	Result (µg/kg)	z'-score
PT9547	13	-1.80	<10	<10	(-1.35)	13	**-2.63**
PT9548	19.6	-0.86	31.6 **FP**	<10	(-1.35)	51.2	0.86
PT9549	<20	(-0.80)	<20	<20	(-1.35)	<20	(-1.99)
PT9550	nt		17.113	nt		17.113	**-2.26**
PT9551	32.8	1.01	11.4 (sum Sv/Ir)	11.4 (sum Sv/Ir)		44.2	0.22
PT9552	31.9	0.88	7.3	15.7	0.17	54.9	1.20
PT9553	24.74	-0.13	<10	45.75	**8.14**	70.49	**2.62**
PT9554	42.6	**2.40**	5.5	nd (no loq)		48.1	0.58
PT9555	detected, <25	(-0.10)	28.1 **FP**	nt		28.1	-1.25
PT9556	36.4	1.52	nd	nd, <10	(-1.35)	36.4	-0.49
PT9557	28.2	0.36	10.2	14.4	-0.18	52.8	1.01
PT9558	12	-1.94	<10	<10	(-1.35)	12	**-2.72**
PT9559	28.1	0.34	detected	11.3	-1.00	39.4	-0.22
PT9560	nd, <2.5	**(-3.29) FN**	nd	nd (no loq)		nd, <2.5	**(-3.59) FN**
PT9561	15	-1.51	<10	10	-1.35	25	-1.54
PT9562	<0.7	**(-3.54) FN**	<8.2	<8.4	(-1.77)	<8.4	**(-3.05) FN**
PT9563	36.6	1.55	nd	14.8	-0.07	51.4	0.88
PT9564	49 (sum Sn/Sv)		49 (sum Sn/Sv)	nt		49	0.66
PT9565	22.2	-0.49	3.81	nt		26.01	-1.44
PT9568	17	-1.23	nd	27	**3.17**	44	0.20
PT9569	24	-0.24	3.4	16	0.25	43.4	0.15
PT9570#	*1.043*	***(-3.49#)***	*nt*	*nt*		*1.043*	***(-3.73#)***
PT9571	24.76	-0.13	nd	15.63	0.15	40.39	-0.13
PT9572	20.1	-0.79	14.5	12.2 (identification)*		46.8	0.46
PT9573	35.6	1.41	nd	13.3	-0.47	48.9	0.65
PT9574	nd, <10	**(-2.22) FN**	nd (sum Sv/Ir)	nd (sum Sv/Ir)		nd, <10	**(-2.91) FN**

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

* participant PT9572 had problems with identification, these values are not included in the evaluation of results.

participant 9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material B, senecionine-N-oxide group							
	Senecionine-N-oxide (SnNO) A: 174 µg/kg u: 20.0 µg/kg σ_p: 43.5 µg/kg (25%) robust σ: 66.0 µg/kg (38%)		Senecivernine-N-oxide (SvNO) A: 28.1 µg/kg u: 4.59 µg/kg σ_p: 7.02 µg/kg (25%) robust σ: 14.7 µg/kg (52%)		Integerrimine-N-oxide (IrNO) A: 52.9 µg/kg u: 5.44 µg/kg σ_p: 13.2 µg/kg (25%) robust σ: 14.4 µg/kg (27%)		Sum senecionine-N-oxide group (SnNO-group) A: 233 µg/kg u: 27.0 µg/kg σ_p: 58.3 µg/kg (25%) robust σ: 104 µg/kg (44%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score
PT9547	120	-1.13	10	**-2.15**	38	-1.04	168	-1.02
PT9548	187	0.27	56.5	**3.39**	56.1	0.22	299.6	1.03
PT9549	nt		Nt		nt		nt	
PT9550	nt		16.311	-1.40	nt		16.311	**-3.38**
PT9551	207.6 (sum SnNO/IrNO)		18.7	-1.12	207.6 (sum SnNO/IrNO)		226.3	-0.11
PT9552	197.7	0.49	34.5	0.77	63.8	0.76	296	0.98
PT9553	170.72	-0.07	29.13	0.13	41.66	-0.79	241.51	0.13
PT9554	193	0.39	18.4	-1.15	nd (no loq)		211.4	-0.34
PT9555	262.6	1.85	89.7	**7.35**	nt		352.3	1.85
PT9556	411.8	**4.96**	85.5 (sum SvNO/IrNO)		85.5 (sum SvNO/IrNO		497.3	**4.11**
PT9557	190.5	0.34	223.9	**23.36**	nt		414.4	**2.82**
PT9558	26	**-3.09**	<10	**(-2.15) FN**	<10	**(-3.00) FN**	26	**-3.22**
PT9559	172.9	-0.03	77.9	**5.95**	nt		250.8	0.27
PT9560	nd, <2.5	**(-3.58) FN**	nd, <2.5	**(-3.05) FN**	nd (no loq)		nd, <2.5	**(-3.59) FN**
PT9561	36 (sum SnNO/SvNO)		36 (sum SnNO/SvNO)		<25	(-1.95)	36	**-3.07**
PT9562	140	-0.71	<18	(-1.20)	150	**6.79**	290	0.88
PT9563	261.9	1.83	18.2	-1.18	67.8	1.04	347.9	1.78
PT9564	207 (sum SnNO/SvNO)		207 (sum SnNO/SvNO)		nt		207	-0.41
PT9565	128	-0.96	20	-0.96	nt		148	-1.33
PT9568	125	-1.03	14	-1.68	40	-0.90	179	-0.84
PT9569	222	1.00	31	0.35	59.5	0.46	312.5	1.23
PT9570	nt		Nt		nt		nt	
PT9571	98.13	-1.59	112.97	**10.13**	49.39	-0.24	260.49	0.42
PT9572	143.6	-0.64	18	-1.20	38.5	-1.01	200.1	-0.52
PT9573	101.9 (sum SnNO/SvNO)		101.9 (sum SnNO/SvNO)		53.4	0.04	155.3	-1.21
PT9574	221 (sum SnNO/IrNO)		nd, <10	**(-2.15) FN**	221 (sum SnNO/IrNO)		221	-0.19

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material B, seneciphylline group					
	Seneciphylline (Sp) A: 59.4 µg/kg u: 5.24 µg/kg σ_p: 14.8 µg/kg (25%) robust σ: 18.7 µg/kg (30%)		Spartioidine (St) A: 15.9 µg/kg u: 1.22 µg/kg σ_p: 3.98 µg/kg (25%) robust σ: 2.58 µg/kg (16%)		Sum senciphylline group (Sp-group) A: 71.8 µg/kg u: 7.89 µg/kg σ_p: 18.0 µg/kg (25%) robust σ: 30.3 µg/kg (42%)	
Lab code	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score
PT9547	30	-1.87	12	-0.94	42	-1.52
PT9548	62.5	0.20	26.6	**2.57**	89.1	0.88
PT9549	48	-0.72	<20	(0.98)	48	-1.21
PT9550	Nt		nt		nt	
PT9551	67.1 (sum Sp/St)		67.1 (sum Sp/St)		67.1	-0.24
PT9552	70.3	0.69	16.8	0.21	87.1	0.78
PT9553	59.14	-0.01	22.36	1.55	81.5	0.49
PT9554	43.5	-1.01	nt		43.5	-1.44
PT9555	61.3	0.12	nt		61.3	-0.54
PT9556	157.7	**6.25**	nd, <10	(-1.42)	157.7	**4.38**
PT9557	63	0.23	15.2	-0.17	78.2	0.33
PT9558	16	**-2.76**	<10	(-1.42)	16	**-2.85**
PT9559	58.2	-0.07	nt		58.2	-0.69
PT9560	nd, <2.5	**(-3.61) FN**	nd (no loq)		nd, <2.5	**(-3.53) FN**
PT9561	36	-1.49	<10	(-1.42)	36	-1.83
PT9562	90	1.95	<14	(-0.46)	90	0.93
PT9563	77.8	1.17	15.1	-0.20	92.9	1.08
PT9564	49	-0.66	nt		49	-1.16
PT9565	112	**3.34**	nt		112	**2.05**
PT9568	47	-0.79	nd, <10	(-1.42)	47	-1.27
PT9569	53.7	-0.36	15	-0.22	68.7	-0.16
PT9570#	*0.956*	***(-3.71#)***	*nt*		*0.956*	***(-3.61#)***
PT9571	68.83	0.60	nd, <10	(-1.42)	68.83	-0.15
PT9572	62.7 (identification)*		56 (identification)*		118.7	**2.39**
PT9573	62.7	0.21	nd (no loq)		62.7	-0.46
PT9574	140 (sum Sp/Sv)		140 (sum Sp/St)		140	**3.48**

A = assigned value (robust mean).

U = uncertainty of consensus value.

Σ_p = target standard deviation for proficiency.

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

*participant PT9572 had problems with identification, these values are not included in the evaluation of results.

participant 9570 reported 4 components in method. Not included in overall calculations.

nd = not detected; nt = not tested.

	Material B, senciphylline-N-oxide group					
	Seneciphylline-N-oxide (SpNO) A: 207 µg/kg u: 14.0 µg/kg σ_p: 51.8 µg/kg (25%) robust σ: 51.5 µg/kg (25%)		Spartioidine-N-oxide (StNO) A: 44.0 µg/kg u: 2.81 µg/kg σ_p: 11.0 µg/kg (25%) robust σ: 7.80 µg/kg (18%)		Sum seneciphylline-N-oxide group (SpNO-group) A: 245 µg/kg u: 15.8 µg/kg σ_p: 61.1 µg/kg (25%) robust σ: 61.9 µg/kg (25%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9547	160	-0.91	21	**-2.09**	181	-1.04
PT9548	158	-0.95	43.5	-0.05	201.5	-0.70
PT9549	246	0.75	41	-0.27	287	0.69
PT9550	Nt		nt		nt	
PT9551	260.8 (sum SpNO/StNO)		260.8 (sum SpNO/StNO)		260.8	0.27
PT9552	202.9	-0.08	45.8	0.16	248.7	0.07
PT9553	224.73	0.34	37.08	-0.63	261.81	0.28
PT9554	216	0.17	nt		216	-0.47
PT9555	214.9	0.15	nt		214.9	-0.48
PT9556	1061.9	**16.49**	57.9	1.26	1119.8	**14.32**
PT9557	207.9	0.01	43.4	-0.06	251.3	0.11
PT9558	39	**-3.25**	<10	**(-3.09) FN**	39	**-3.36**
PT9559	208.3	0.02	nt		208.3	-0.59
PT9560	330	**2.37**	nd (no loq)		330	1.40
PT9561	111	-1.86	255	**19.17**	366	1.99
PT9562	480	**5.26**	580	**48.70**	1060	**13.34**
PT9563	241.6	0.66	36.3	-0.70	277.9	0.55
PT9564	253	0.88	nt		253	0.14
PT9565	178	-0.57	nt		178	-1.09
PT9568	179	-0.55	nd, <10	**(-3.09) FN**	179	-1.07
PT9569	195	-0.24	41.3	-0.25	236.3	-0.13
PT9570	Nt		nt		nt	
PT9571	186.57	-0.40	46.36	0.21	232.93	-0.19
PT9572	201.6 (identification)*		122 (identification)*		323.6	1.29
PT9573	168.6	-0.75	nd (no loq)		168.6	-1.24
PT9574	226 (sum SpNO/StNO)		226 (sum SpNO/StNO)		226	-0.30

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

*participant PT9572 had problems with identification, these values are not included in the evaluation of results.

nd = not detected; nt = not tested.

	Material B, individual PAs proposed for regulation											
	Europine (Eu) A: 26.4 µg/kg u: 1.16 µg/kg σ_p: 6.61 µg/kg (25%) robust σ: 4.27 µg/kg (16%)		Europine-N-oxide (EuNO) A: 225 µg/kg u: 7.61 µg/kg σ_p: 56.4 µg/kg (25%) robust σ: 29.2 µg/kg (13%)		Heliotrine (Ht) A: 43.8 µg/kg u: 2.21 µg/kg σ_p: 10.9 µg/kg (25%) robust σ: 8.65 µg/kg (20%)		Heliotrine-N-oxide (HtNO) A: 363 µg/kg u: 14.4 µg/kg σ_p: 90.8 µg/kg (25%) robust σ: 55.4 µg/kg (15%)		Lasiocarpine (Lc) A: 19.0 µg/kg u: 1.71 µg/kg σ_p: 4.75 µg/kg (25%) robust σ: 6.40 µg/kg (34%)		Lasiocarpine-N-oxide (LcNO) A: 145 µg/kg u: 11.5 µg/kg σ_p: 36.3 µg/kg (25%) robust σ: 44.1 µg/kg (30%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z'-score	Result (µg/kg)	z'-score
PT9547	20	-0.97	245	0.35	33	-0.98	480	1.29	10	-1.78	90	-1.45
PT9548	49.7	**3.52**	236	0.19	50.4	0.61	357	-0.07	11.8	-1.43	120	-0.66
PT9549	27	0.08	240	0.26	34	-0.89	344	-0.21	<20	(0.20)	129	-0.43
PT9550	26.78	0.05	157.514	-1.20	35.356	-0.77	288.312	-0.83	13.242	-1.14	129.791	-0.41
PT9551	25.5	-0.14	212.6	-0.23	47.9	0.38	336.8	-0.29	16.4	-0.51	157.9	0.33
PT9552	27.9	0.22	211.3	-0.25	44.7	0.09	362.8	-0.01	24.1	1.01	156.9	0.30
PT9553	29.72	0.50	238.84	0.24	37.58	-0.56	339.18	-0.27	19.8	0.16	220.63	1.98
PT9554	22.5	-0.60	300	1.32	37.8	-0.54	354	-0.10	20.2	0.24	156	0.28
PT9555	32.6	0.93	167.3	-1.03	45	0.11	423.3	0.66	detected, <25	(1.19)	detected, <25	**(-3.16) FN**
PT9556	23.9	-0.38	1335.2	**19.69**	52.1	0.76	detected, <10	**(-3.89) FN**	39.4	**4.04**	393.4	**6.51**
PT9557	27.5	0.16	223.4	-0.04	46.3	0.23	372.6	0.10	23.1	0.81	175.4	0.79
PT9558	<10	**(-2.49) FN**	<10	**(-3.82) FN**	15	**-2.63**	89	**-3.02**	11	-1.58	40	**-2.76**
PT9559	30.6	0.63	237.5	0.21	53.6	0.90	418.6	0.61	21.1	0.42	138.7	-0.17
PT9560	nd (no loq)		nd (no loq)		nd, <2.5	**(-3.77) FN**	nt		19	0.00	175	0.78
PT9561	<25	(-0.22)	216	-0.17	39	-0.43	2230	**20.55**	16	-0.59	<10	**(-3.55) FN**
PT9562	<19	(-1.13)	100	**-2.23**	190	**13.37**	170	**-2.13**	25	1.19	100	-1.19
PT9563	30.6	0.63	217.1	-0.15	50.1	0.58	371.3	0.09	29.7	**2.12**	244.8	**2.61**
PT9564	23	-0.52	472	**4.38**	53	0.85	560	**2.17**	17	-0.40	147	0.04
PT9565	21.4	-0.76	206	-0.34	43.5	-0.02	335	-0.31	13.9	-1.01	133	-0.32
PT9568	22	-0.67	192	-0.59	35	-0.80	326	-0.41	nd, <10	(-1.78)	75	-1.85
PT9569	23.3	-0.48	205	-0.36	37.9	-0.54	354	-0.10	19.1	0.02	154	0.23
PT9570	nt		nt		nt		nt		nt		nt	
PT9571	30.88	0.67	255.1	0.53	50.26	0.59	436.91	0.81	28.26	1.84	206.64	1.61
PT9572	26.5	0.01	219.9	-0.10	43.1	-0.06	380.1	0.19	16.9	-0.42	125.1	-0.53
PT9573	24.7	-0.26	241.4	0.28	53	0.85	289.6	-0.81	21.2	0.44	97	-1.27
PT9574	26	-0.07	247	0.38	40	-0.34	500	1.51	14	-0.99	158	0.33

A = assigned value (robust mean).

U = uncertainty of consensus value.

Σ_p = target standard deviation for proficiency.

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

nd = not detected; nt = not tested.

	Material B, other PA								
	Erucifoline (Er)	Erucifoline-N-oxide (ErNO)	Jacobine (Jb)	Jacobine-N-oxide (JbNO)	Jacoline (Jl)	Jaconine (Jn)	Monocrataline (Mc)	Monocrotaline-N-oxide (McNO)	Trichodesmine (Td)
Lab code	Result µg/kg	Result µg/kg	Result µg/kg	Result µg/kg	Result µg/kg	Result µg/kg	Results µg/kg	Results µg/kg	Result µg/kg
PT9547	<10	<10	50 **FP**	<10	<10	<10	<10	<10	<10
PT9548	nt	nt	nt	nt	nt	nt	nt	nt	nt
PT9549	<20	<20	nt	<20	<20	<20	<20	<20	<20
PT9550	<0.4	<0.6	<1.0	<0.6	<0.2	<0.3	<0.4	<0.5	<1.0
PT9551	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9552	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9553	<10	<10	<10	<10	<10	<10	<10	<10	<10
PT9554	<5	<2	<5	<2	nd	nd	<2	<2	<2
PT9555	nd	nt	nt	nt	nt	nt	nd	nt	nd
PT9556	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9557	<10	<10	<10	<10	<10	<10	<10	<10	<10
PT9558	<10	<10	<10	<10	<10	<10	<10	<10	<10
PT9559	nd	nd	nd	nd	nt	nt	nd	nd	nt
PT9560	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9561	<10	<10	26 **FP**	<25	<10	<25	<10	<10	<10
PT9562	<32	<25	<13	<4.0	<140	<9.7	<400	<80	<11
PT9563	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9564	nt	nt	nt	nt	nt	nt	loq=4	loq=2.5	nt
PT9565	<2.5	<2.5	<2.5	<2.5	<2.5	nt	<2.5	<2.5	<2.5
PT9568	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9569	<10	<5	<7	<4	<8	<5	<10	<10	<3
PT9570	nt	nt	nt	nt	nt	nt	nt	nt	nt
PT9571	<10	<10	<10	<10	<10	<10	nd	<10	<10
PT9572	<5	<5	<5	<5	<5	<5	<5	<5	<5
PT9573	nd	nd	nd	nd	nd	nd	nd	nd	nd
PT9574	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not detected; nt = not tested.

	Material B Sum of 35 PAs for regulation and sum of all PA			
	Sum of 35 PAs for legislation A: 1626 µg/kg u: 90.7 µg/kg σ_p: 407 µg/kg (25%) robust σ: 363 µg/kg (22%)		Sum of total PAs A: 1628 µg/kg u: 86.1 µg/kg σ_p: 407 µg/kg (25%) robust σ: 344 µg/kg (21%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9547	1385	-0.59	1435	-0.47
PT9548	1749	0.30	1749	0.30
PT9549	1216	-1.01	1216	-1.01
PT9550	687	**-2.31**	687	**-2.31**
PT9551	1618	-0.02	1618	-0.02
PT9552	1694	0.17	1694	0.16
PT9553	1852	0.55	1852	0.55
PT9554	1585	-0.10	1585	-0.11
PT9555	1646	0.05	1646	0.04
PT9556	3873	**5.53**	3873	**5.52**
PT9557	1815	0.46	1815	0.46
PT9558	267	**-3.34**	267	**-3.34**
PT9559	1616	-0.03	1616	-0.03
PT9560	619	**-2.48**	619	**-2.48**
PT9561	3031	**3.46**	3057	**3.51**
PT9562	2375	1.84	2375	1.83
PT9563	1874	0.61	1874	0.60
PT9564	2010	0.95	2010	0.94
PT9565	1372	-0.62	1372	-0.63
PT9568	1218	-1.00	1218	-1.01
PT9569	1652	0.06	1652	0.06
PT9570 #	*3*	***(-3.99#)***	*3*	***(-3.99#)***
PT9571	1821	0.48	1821	0.47
PT9572	1634	0.02	1634	0.01
PT9573	1294	-0.82	1294	-0.82
PT9574	1732	0.26	1732	0.26

A = assigned value (robust mean).

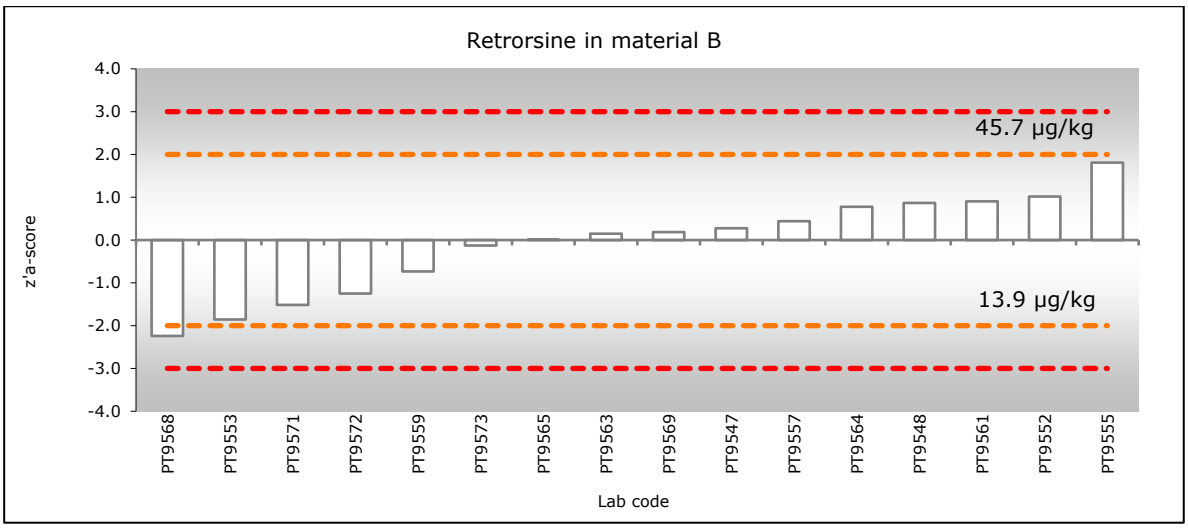
u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

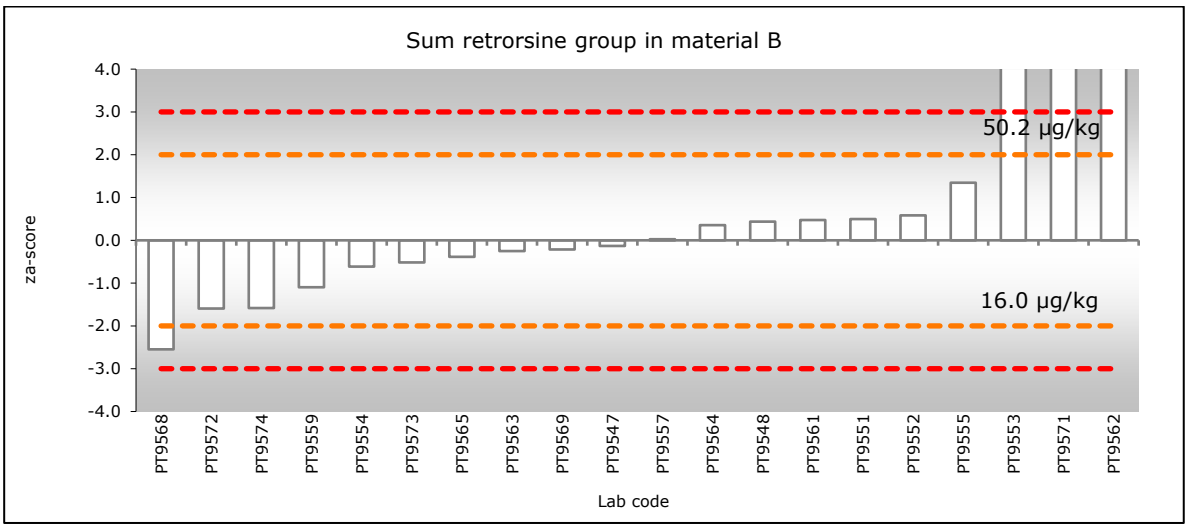
robust σ = robust (relative) standard deviation based on participants' results.

participant 9570 reported 4 components in method. Not included in overall calculations.

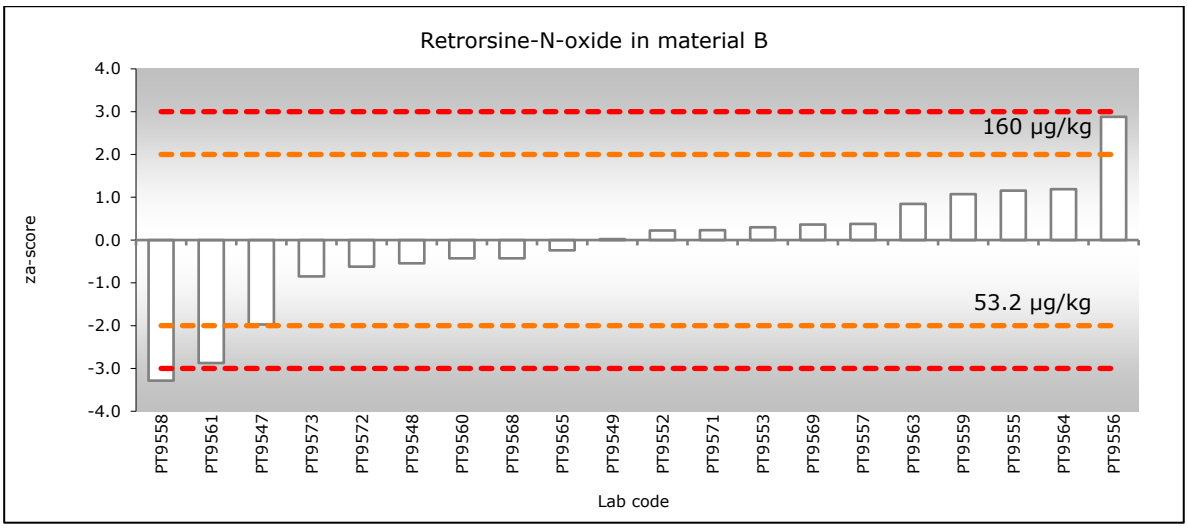
nd = not detected; nt = not tested.



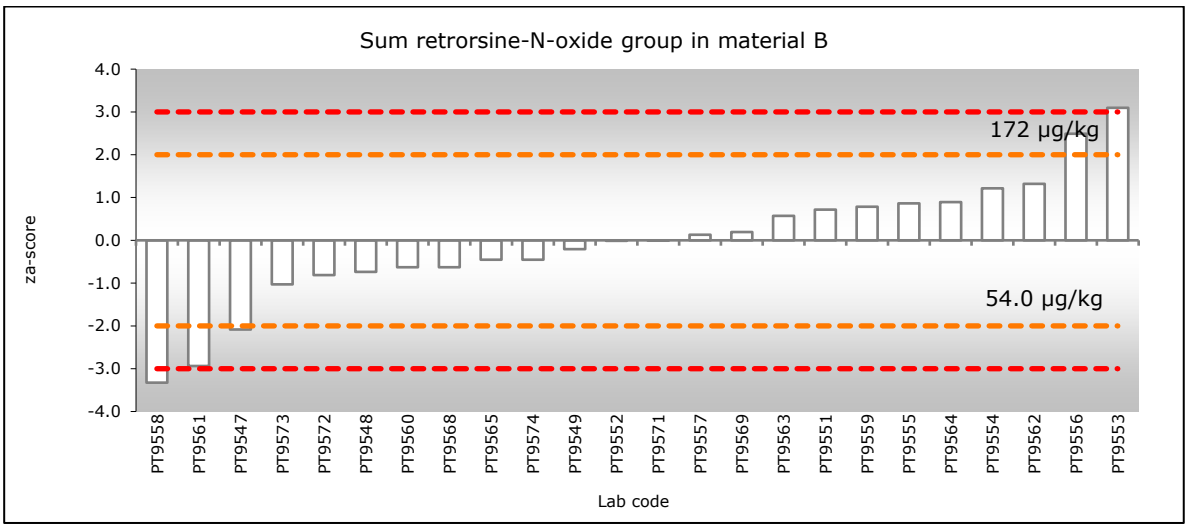
Graphical representation of the z'-scores for retrorsine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



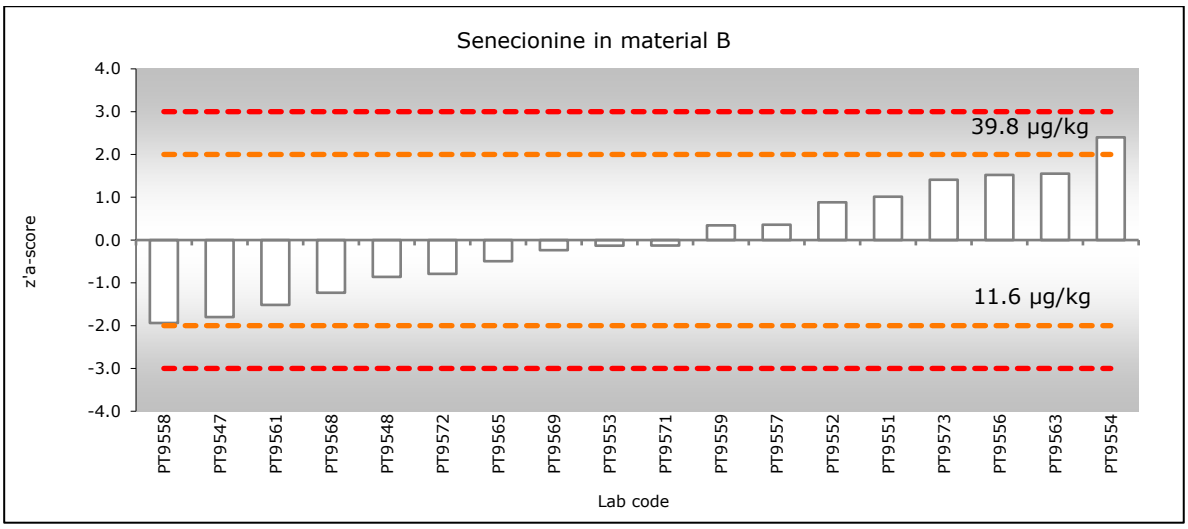
Graphical representation of the z-scores for sum retrorsine group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



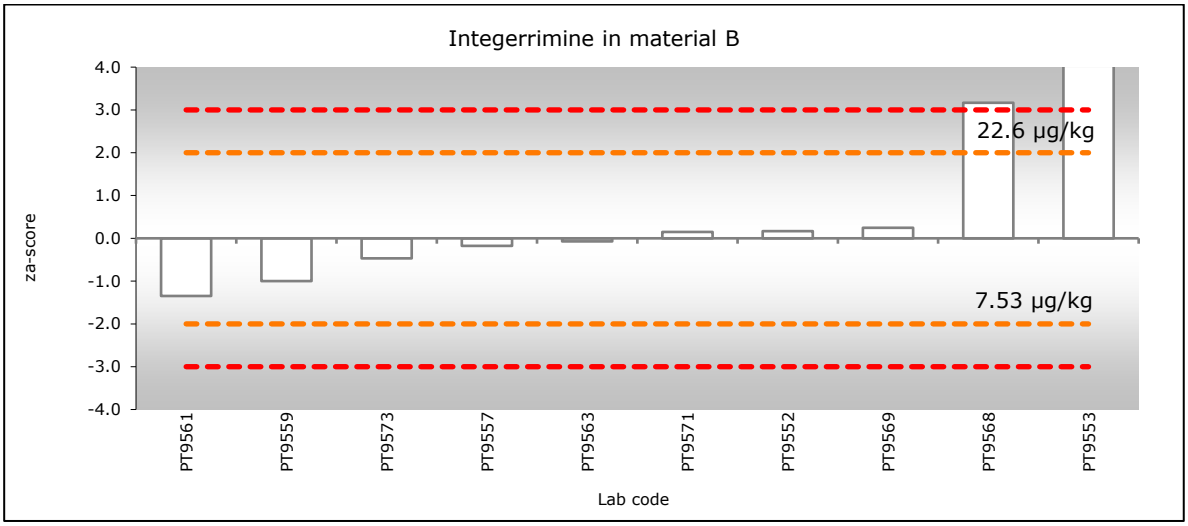
Graphical representation of the z-scores for retrorsine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



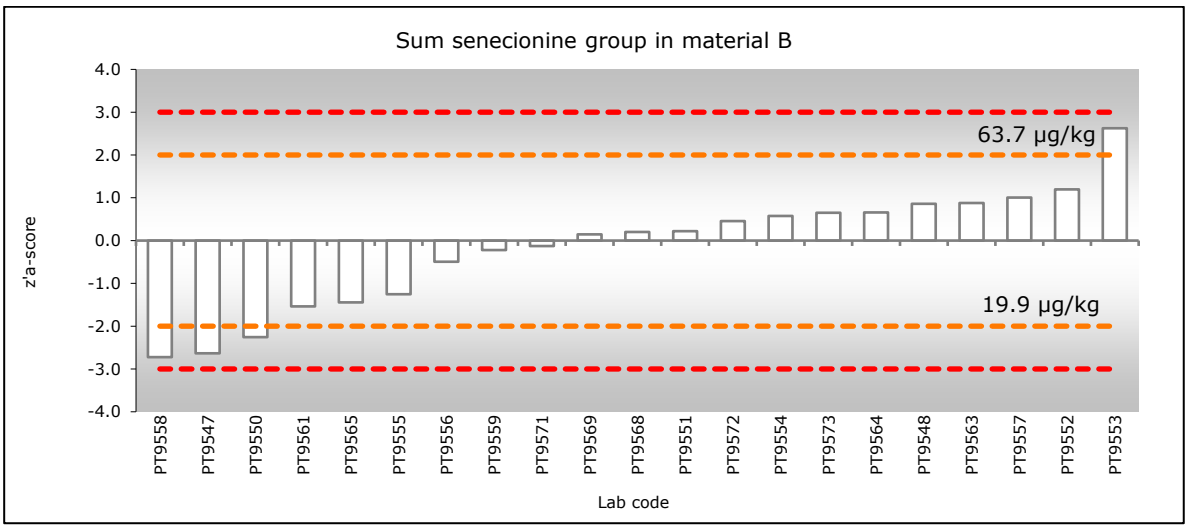
Graphical representation of the z-scores for sum retrorsine-N-oxide group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



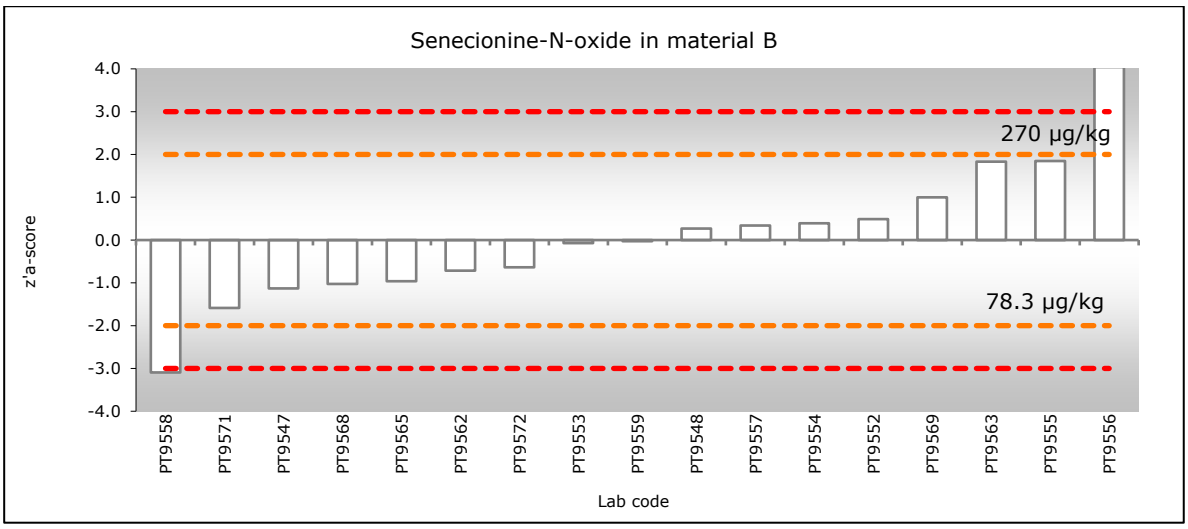
Graphical representation of the z'-scores for senecionine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



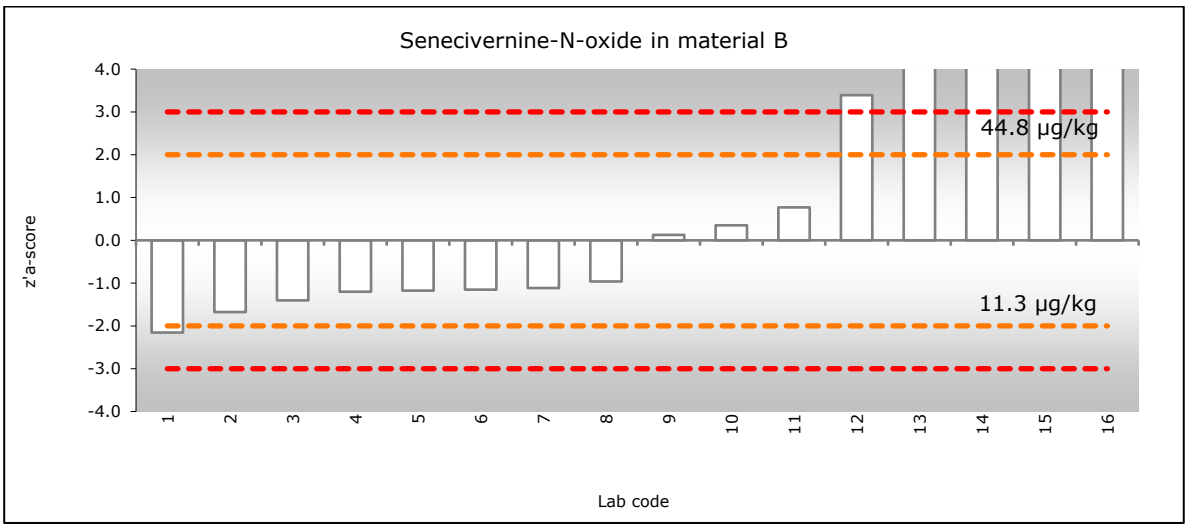
Graphical representation of the z-scores for intergerrimine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



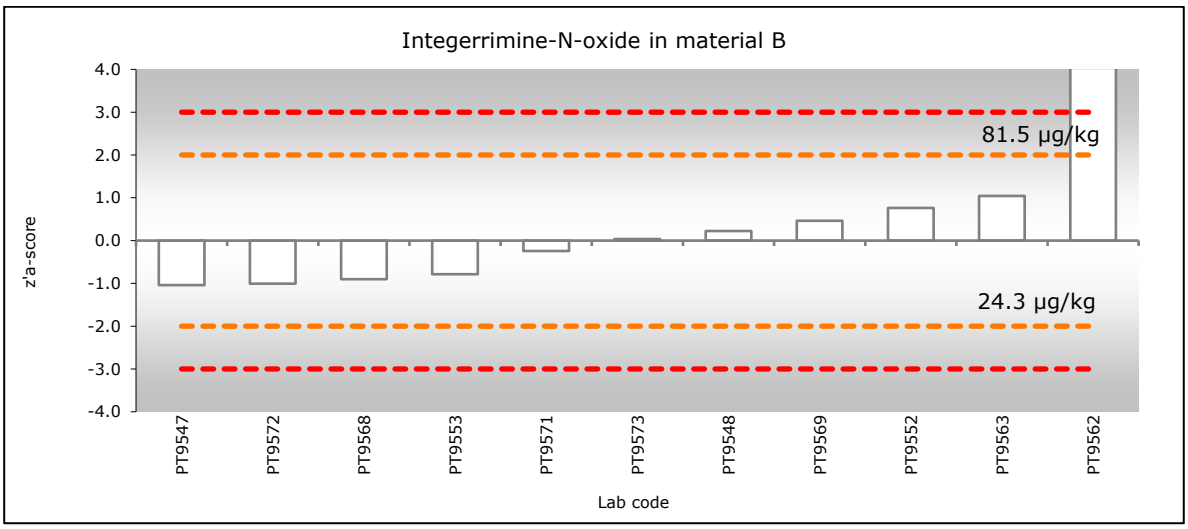
Graphical representation of the z'-scores for sum senecionine group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



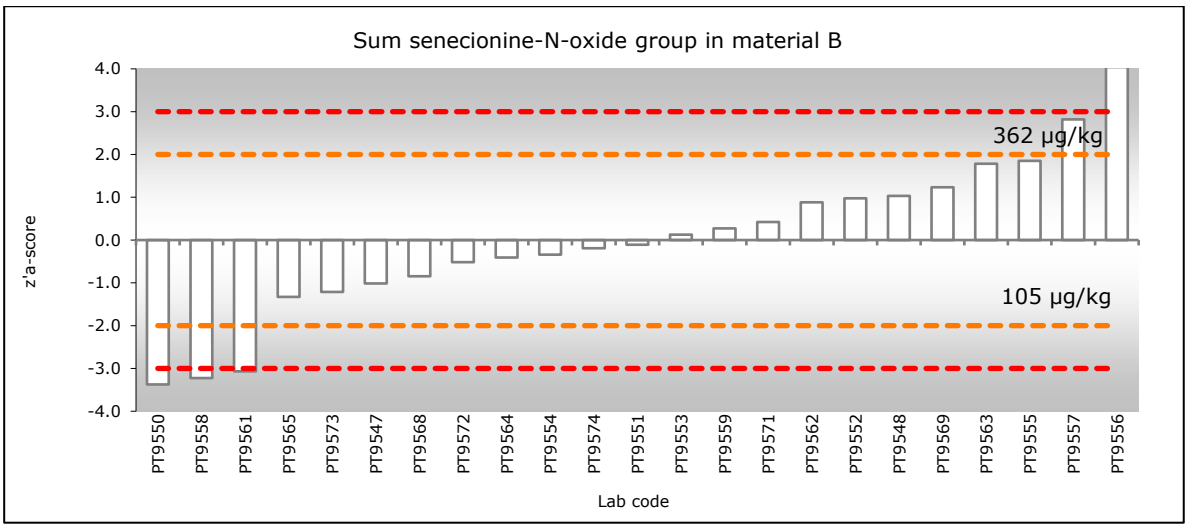
Graphical representation of the z'-scores for senecionine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



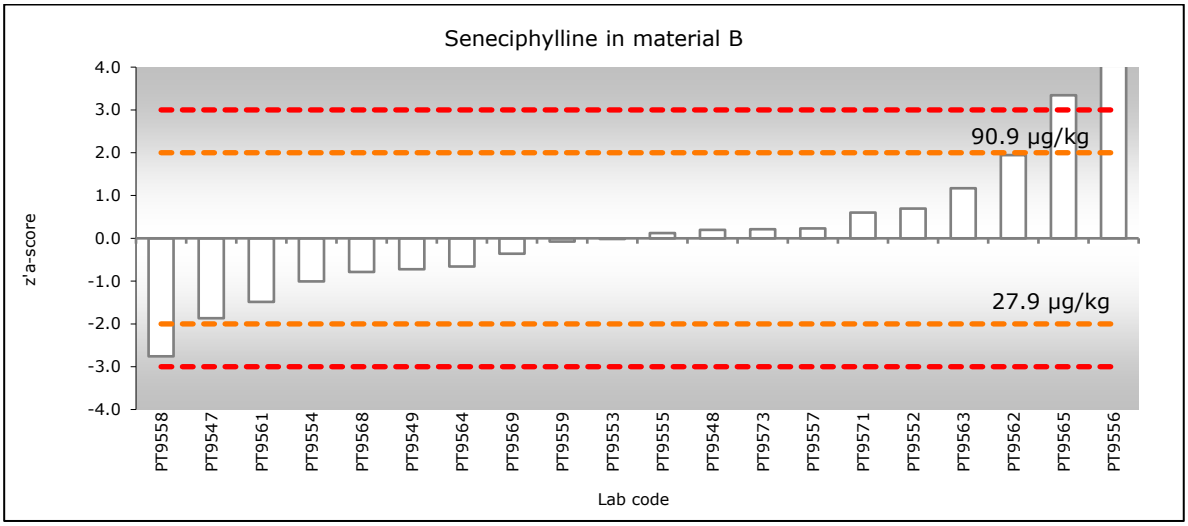
Graphical representation of the z'-scores for senecivernine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



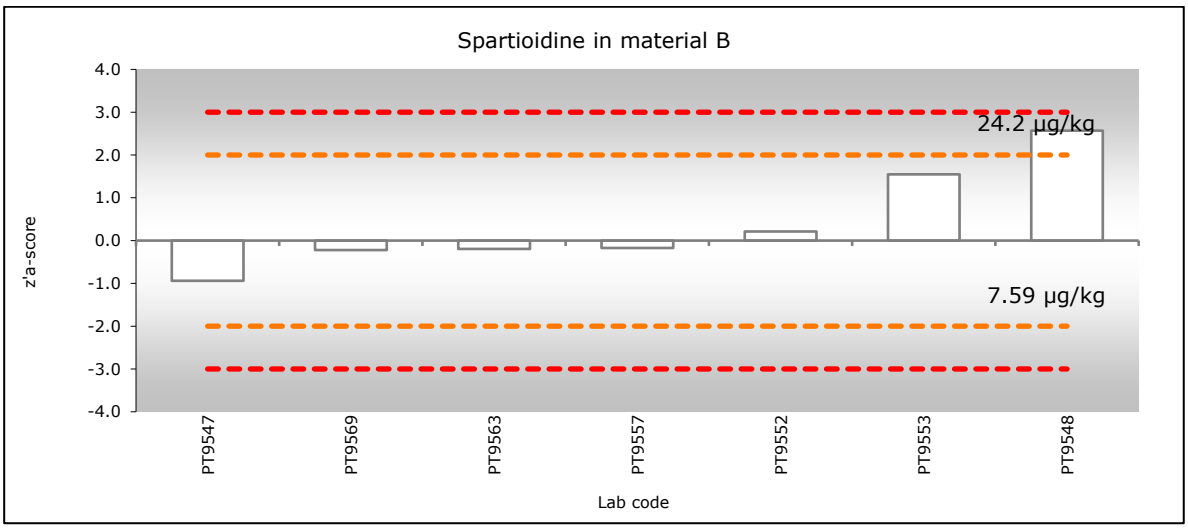
Graphical representation of the z'-scores for integerrimine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



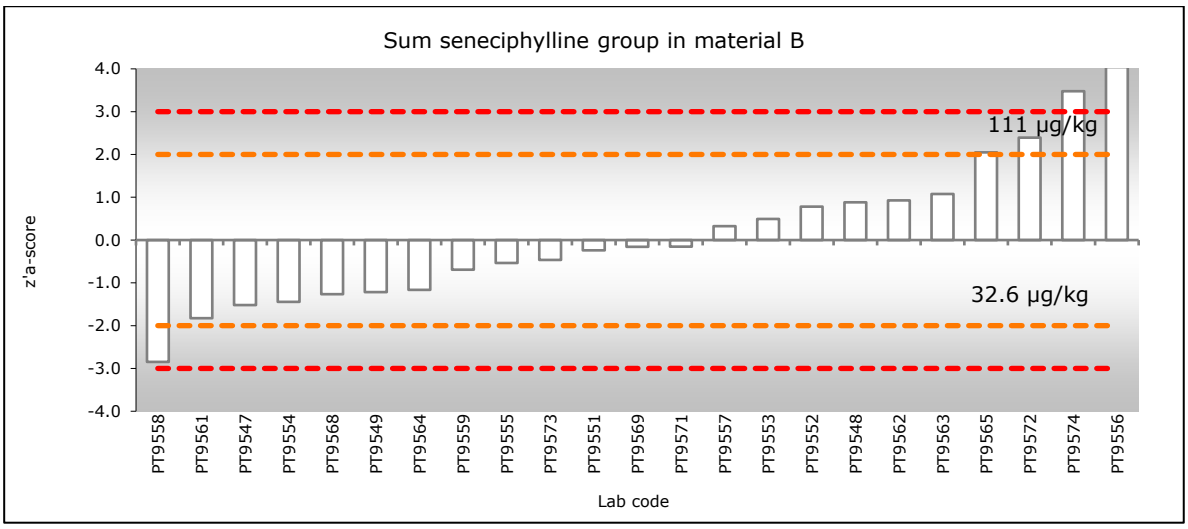
Graphical representation of the z'-scores for sum senecionine-N-oxide group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



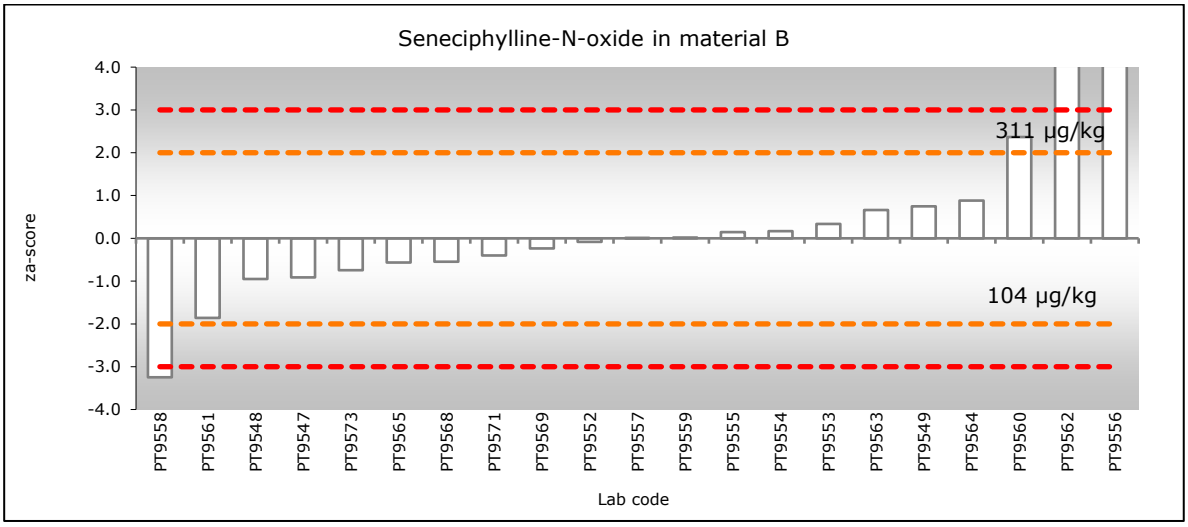
Graphical representation of the z'-scores for seneciphylline in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



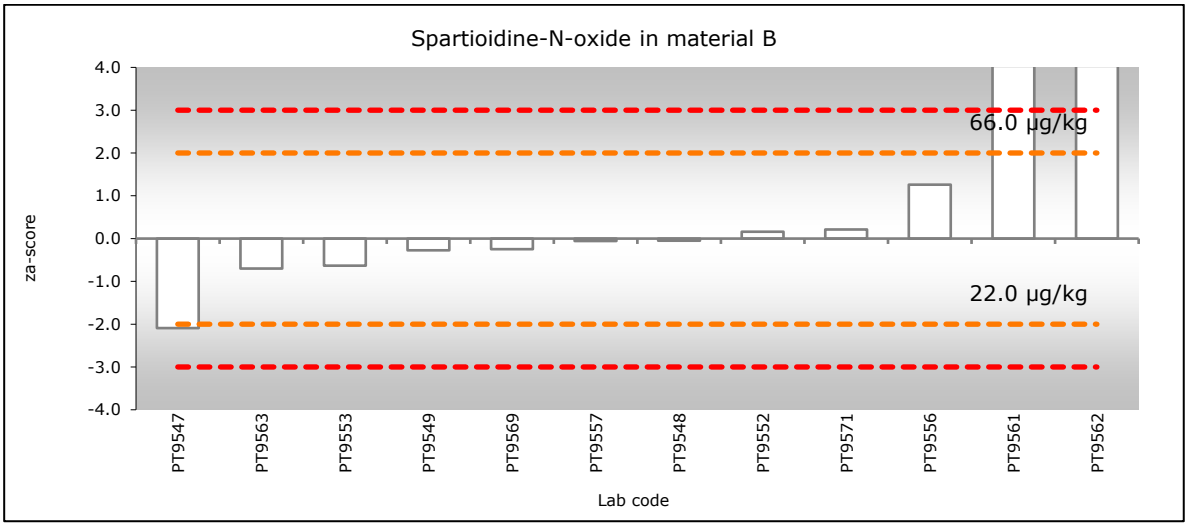
Graphical representation of the z'-scores for spartioidine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



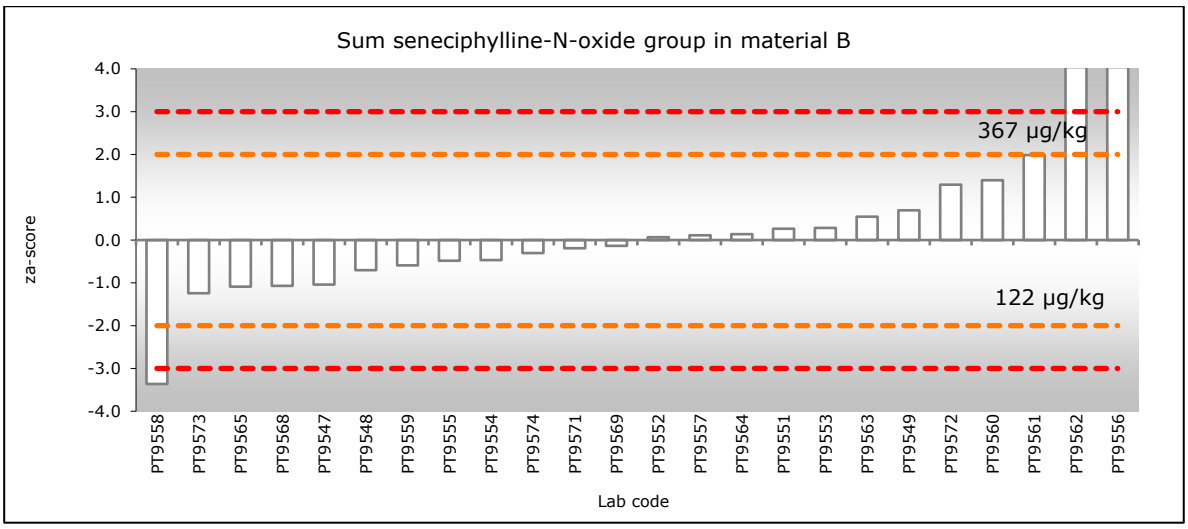
Sum seneciphylline group - Graphical representation of the z'-scores for sum seneciphylline group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



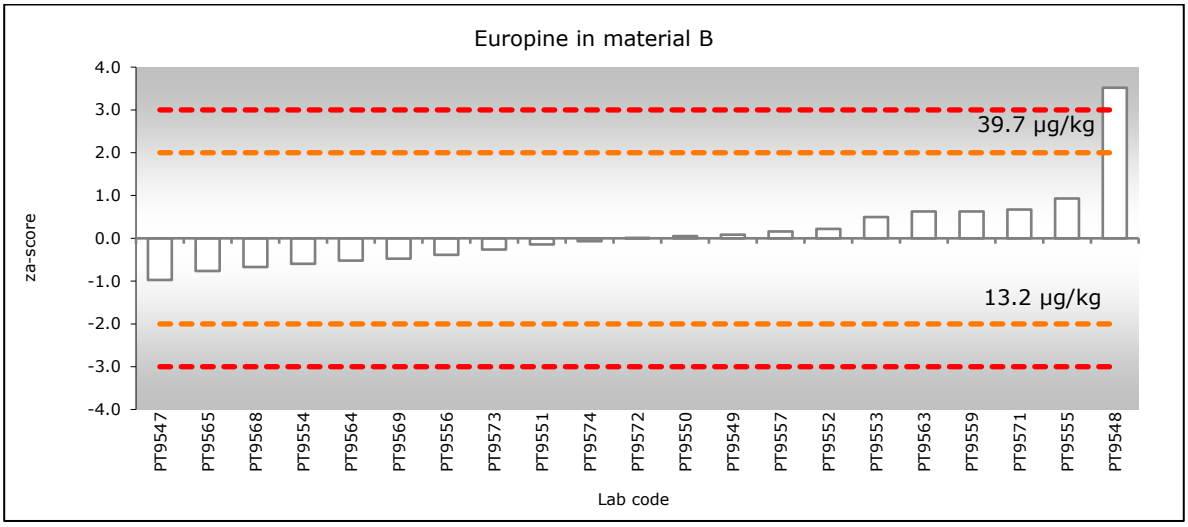
Graphical representation of the z-scores for seneciphylline-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



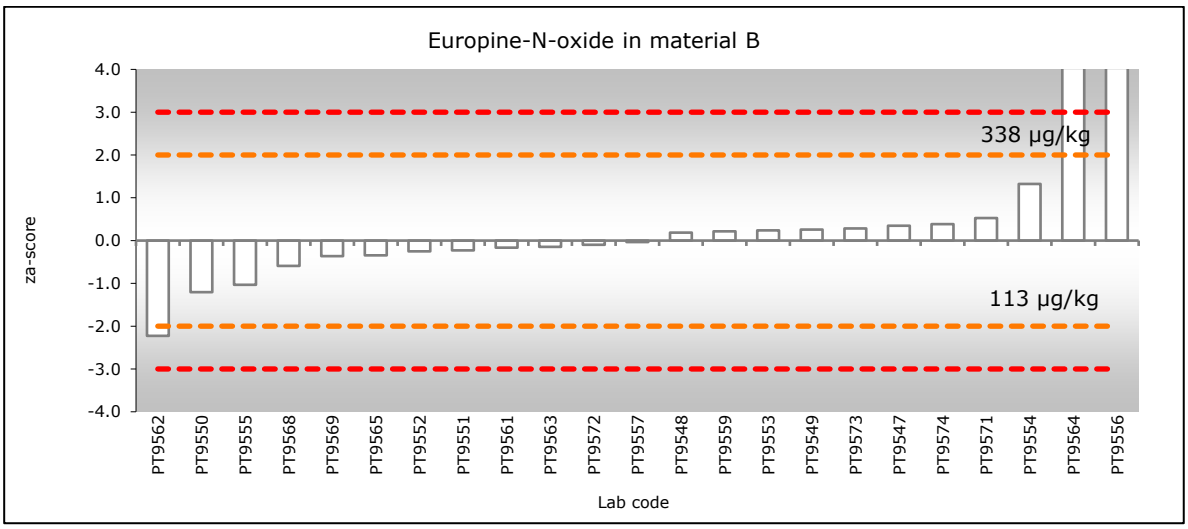
Graphical representation of the z-scores for spartioidine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



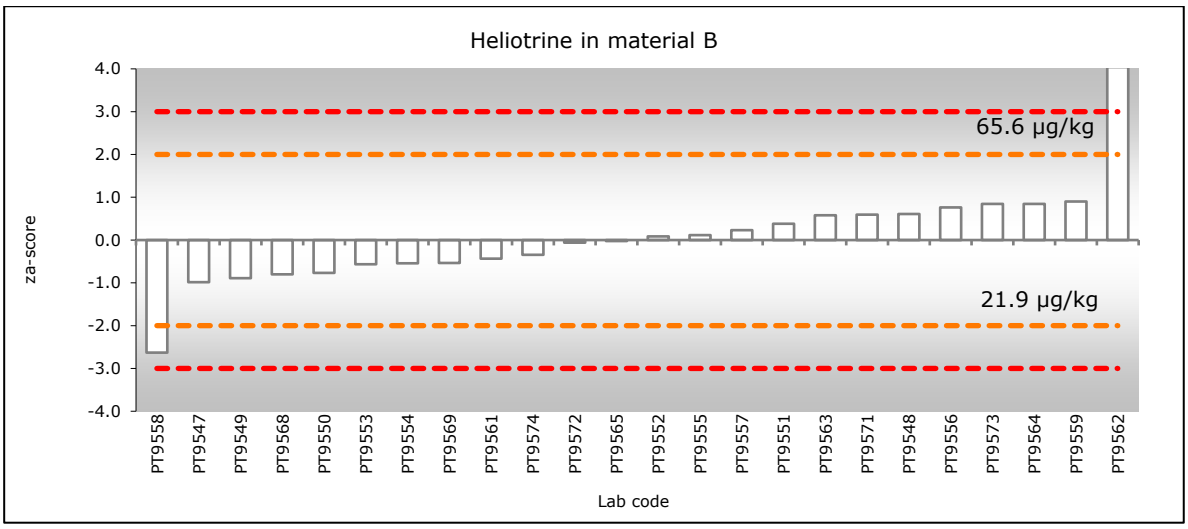
Graphical representation of the z-scores for sum seneciphylline-N-oxide group in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



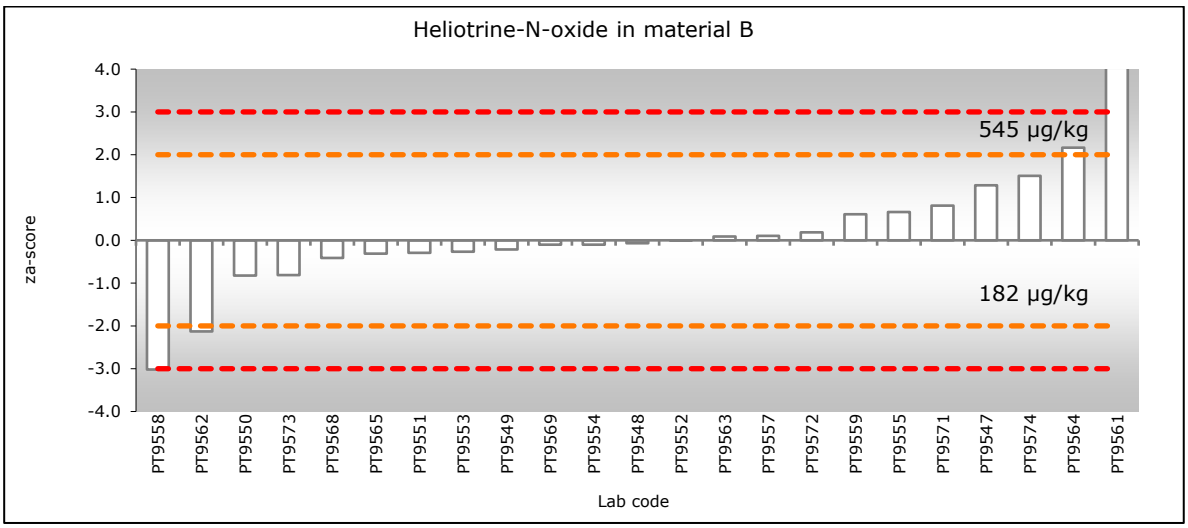
Graphical representation of the z-scores for europine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



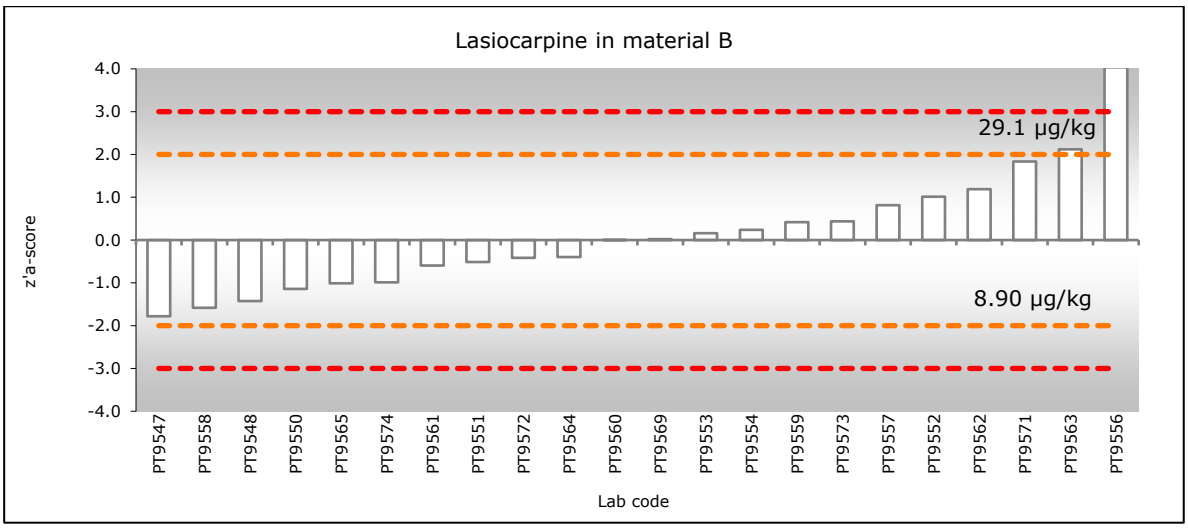
Graphical representation of the z-scores for europine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



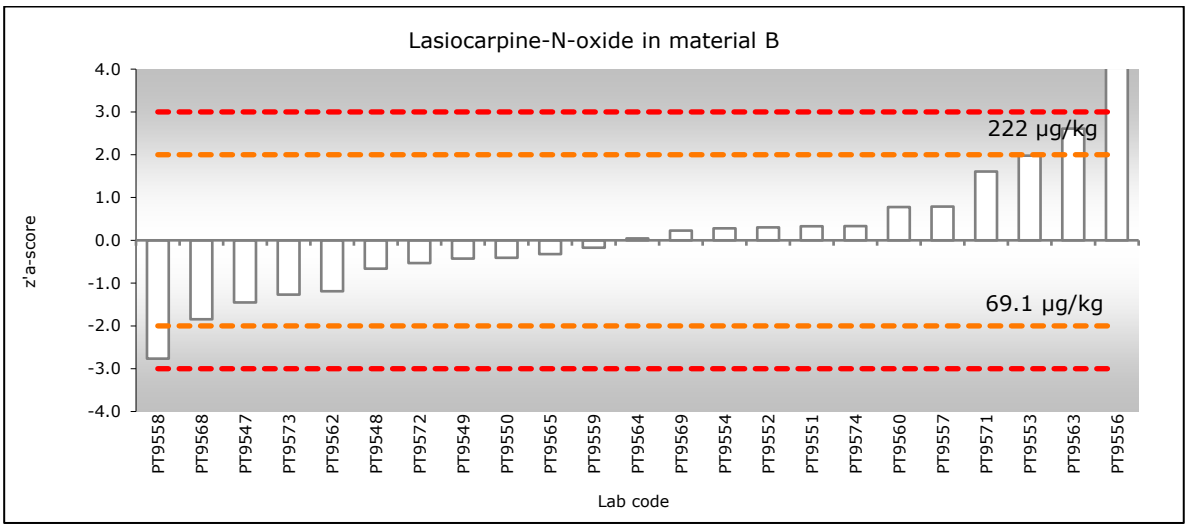
Graphical representation of the z-scores for heliotrine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



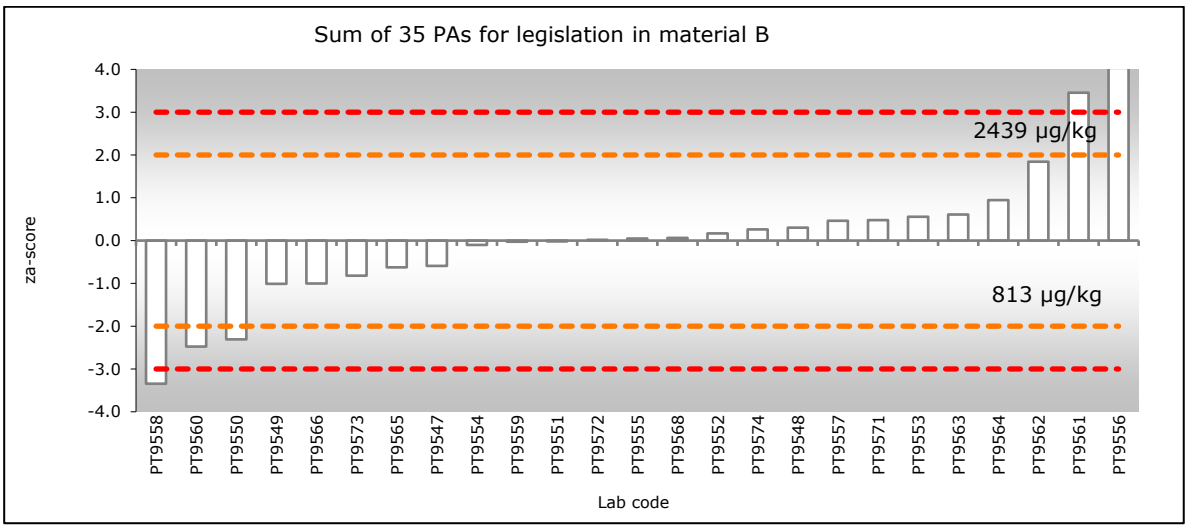
Graphical representation of the z-scores for heliotrine-N-oxide in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



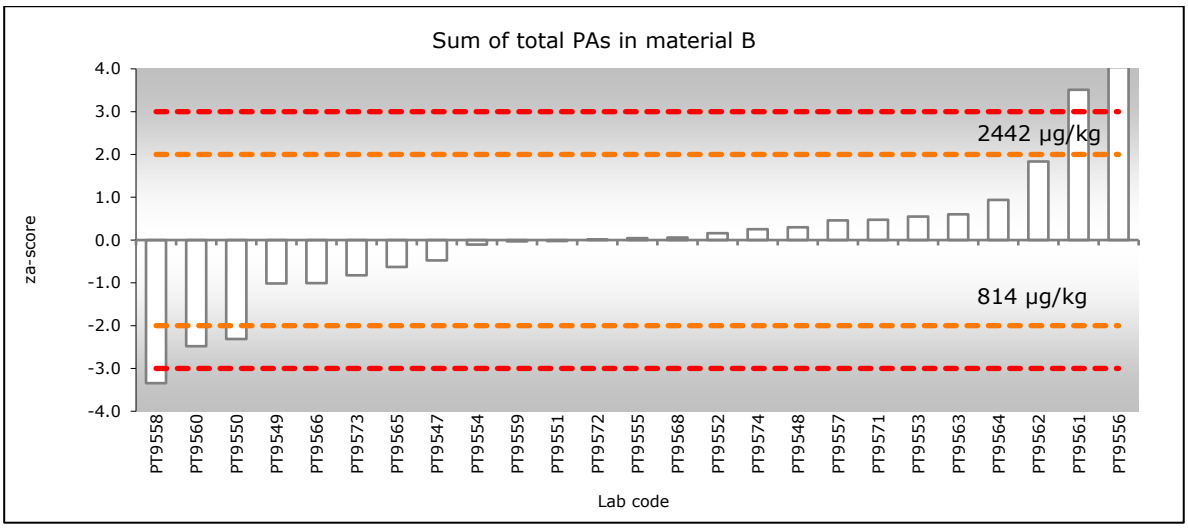
Graphical representation of the z'-scores for lasiocarpine in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



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



Graphical representation of the z-scores for sum of 35 PAs for legislation in material B. Dotted lines show research study performance boundaries ± 2 (also in µg/kg) and ± 3.



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

Annex 11 Overview performance per laboratory

Lab code	Individual PAs Satisfactory performance *	Isomeric groups Satisfactory performance *	Total sum legislation Satisfactory performance *	Total sum Satisfactory performance *	FN	FP
PT9547	31 of 41	12 of 16	**2 of 2**	**2 of 2**	2	3
PT9548	29 of 41	15 of 16	**2 of 2**	**2 of 2**		5
PT9549	30 of 41	12 of 16	**2 of 2**	**2 of 2**		1
PT9550	18 of 41	2 of16	0 of 2	0 of 2		
PT9551	18 of 41	**16 of 16**	**2 of 2**	**2 of 2**		1
PT9552	40 of 41	**16 of 16**	**2 of 2**	**2 of 2**		
PT9553	37 of 41	10 of 16	**2 of 2**	**2 of 2**		4
PT9554	26 of 41	**16 of 16**	**2 of 2**	**2 of 2**		
PT9555	19 of 41	13 of 16	**2 of 2**	**2 of 2**	2	2
PT9556	7 of 41	3 of 16	0 of 2	0 of 2	11	1
PT9557	37 of 41	15 of 16	**2 of 2**	**2 of 2**		
PT9558	7 of 41	4 of 16	0 of 2	0 of 2	13	2
PT9559	30 of 41	**16 of 16**	**2 of 2**	**2 of 2**		
PT9560	17 of 41	12 of 16	1 of 2	1 of 2	11	
PT9561	23 of 41	9 of 16	0 of 2	0 of 2	1	6
PT9562	13 of 41	8 of 16	**2 of 2**	**2 of 2**	5	5
PT9563	38 of 41	**16 of 16**	**2 of 2**	**2 of 2**		1
PT9564	15 of 41	14 of 16	1 of 2	**2 of 2**		
PT9565	30 of 41	15 of 16	**2 of 2**	**2 of 2**	2	
PT9566	4 of 24**	2 of 10**	0 of 1**	0 of 1**	9	
PT9568	32 of 41	14 of 16	**2 of 2**	**2 of 2**	1	
PT9569	**17 of 17****	**6 of 6****	**1 of 1****	**1 of 1****		
PT9570	1 of 41	0 of 16	0 of 2	0 of 2		
PT9571	37 of 41	13 of 16	**2 of 2**	**2 of 2**	1	3
PT9572	30 of 41	10 of 16	**2 of 2**	**2 of 2**		
PT9573	28 of 41	14 of 16	**2 of 2**	**2 of 2**		
PT9574	16 of 41	14 of 16	**2 of 2**	**2 of 2**	3	

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

** PT9566 analysed only material A and PT9569 analysed only material B.

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WFSR report 2021.006

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, 6,500 employees (5,500 fte) and 12,500 students, 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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