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Report on the 2012 Proficiency Test on Pyrrolizidine alkaloids in honey and hay

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Executive summary

The purpose of this proficiency test (PT) was to investigate current measurement capacities of laboratories for pyrrolizidine alkaloids (PAs) in honey and hay and to provide a benchmark for their performance. PAs are part of a plant's defence mechanism and some of them are hepatotoxic. They may be present in honey and milk as a result of carry-over from bees and cows foraging on PA producing plants.

The scheme consisted of two parts: determination of pyrrolizidine alkaloids in the supplied test samples and detailed reporting on the methods used. Twenty-eight laboratories from eight different countries registered for this exercise. Two laboratories did not submit any results, and two participants submitted two independent results, using two different methodologies available in their lab.

Analysis of spiked honey showed no statistical differences between the determination of a sum parameter covering all alkaloids containing a retronecinic back bone compared to the determination of individual PAs. However, a significant difference was found for the analysis of naturally contaminated materials. Determination of individual PAs led to lower results than the sum parameter determination, indicating that not all PAs present in the sample have been identified and quantified.

Laboratories were benchmarked using z-scores. z-Scores are used to make proficiency test scores comparable and are closely related to the idea of being "fit-for-purpose". z-Scores between –2 and +2 are commonly regarded as "satisfactory". Satisfactory performance has been achieved by more than half of participants indicating that the determination of PAs using current methodologies is possible for a substantial part of testing laboratories. However, there is still a considerable need for improving the proficiency of laboratories in PA determination. One of the critical points identified was the improved supply of a wider range of individual PAs with proven identity and purity.

1 Introduction

Pyrrolizidine alkaloids (PAs) are a class of secondary plant metabolites where certain class members have a hepatotoxic activity. It is thought that they are biosynthesised to defend the plant against insect and mammalian consumption [1]. It is estimated that over 6000 plant species worldwide produce at least 600 different PAs. Exposure of mammals to these compounds can result in hepatic veno-occlusive disease, liver necrosis and ultimately death. However bees and some other insects tolerate PAs and collect pollen from PA producing plants, resulting in PA contaminated honey. There is also evidence, that PA's can enter the human food chain (f.i. milk) as non-metabolised residues [1]. Chemically all PAs toxic to mammalian species are macrocyclic or acyclic mono/diesters of a common bicyclic system called necinic base. More structural diversity is created due to oxidation forming N-oxides (**Figure 1**).

The European Food Safety Authority (EFSA) published scientific opinions for PAs in feed [2] and food [3]. Significant attention has been given to honey as one source of direct exposure of humans and lists of relevant marker PAs have been established. Furthermore, EFSA concluded that validated methods are lacking [3]. Test laboratories currently use different methodological approaches ranging from determination of a single "sum parameter" to the identification and quantification of individual PAs and their N-oxides [4].

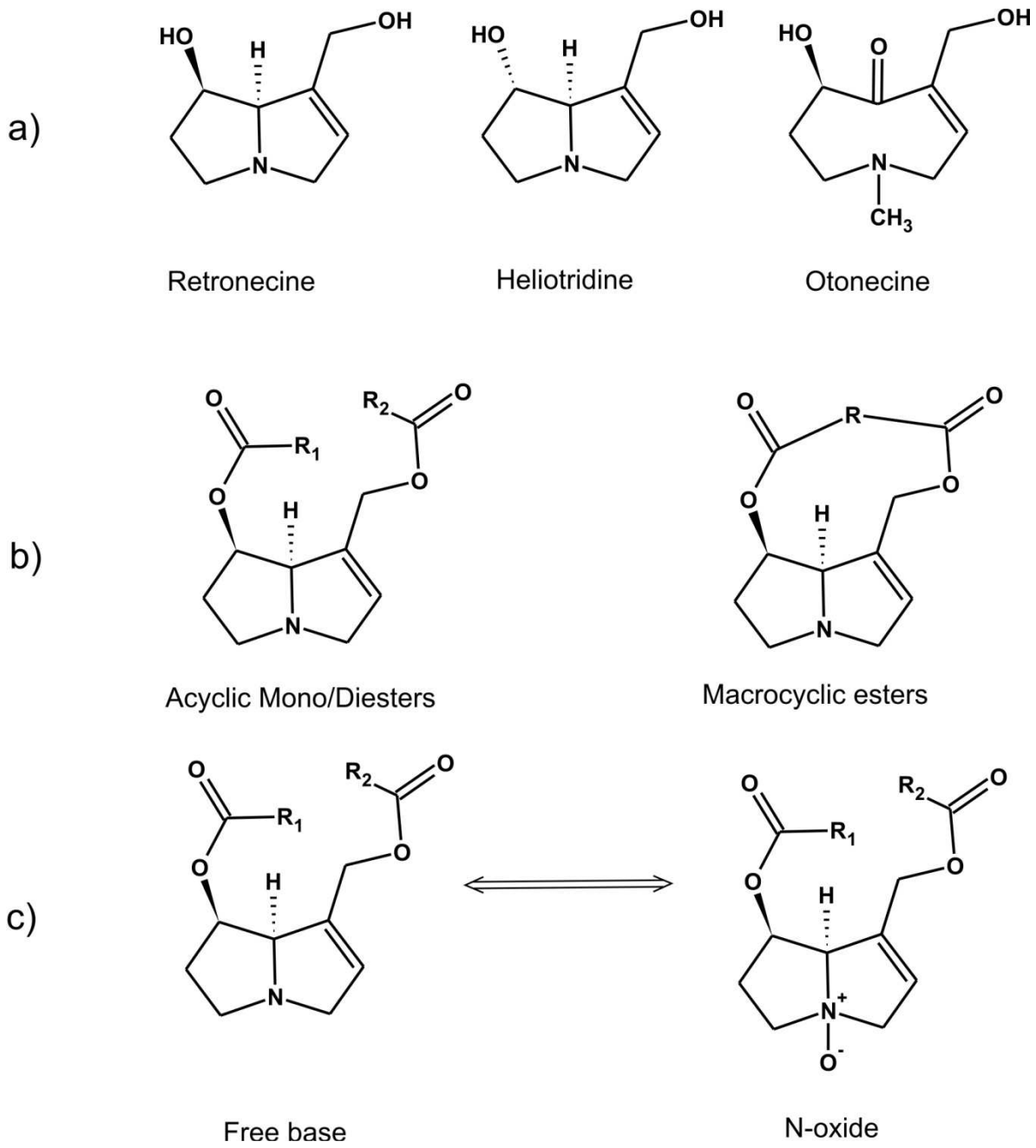

Figure 1: Structural properties of pyrrolizidine alkaloids: a) types of necinic bases, b) types of esters formed, c) oxidation of pyrrolizidine alkaloids to produce N-oxides

2 Scope

The intention of this PT was to give laboratories the possibility to obtain performance information on the method they currently use. Next to the analytical work, participants reported detailed information on their methods. This information allowed conclusions to be drawn on how currently used analytical approaches perform to determine PAs in two relevant matrices (honey and hay). The questionnaire included questions on method scopes, quantification limits and other methodological features related to the method such as reliability and simplicity. The latter two aspects are of interest identifying method features relevant for the identification of suitable methods useful for future collaborative validation studies.

The PT planning included naturally contaminated samples as well as fortified materials. The participants were requested to use the methods of analysis available in their laboratories, and report all PAs quantified. The information gathered was then used to benchmark the laboratories against the assigned values deriving from gravimetric preparation of spiked materials. Results for naturally contaminated materials were used to compare the efficiency of different analytical methods for the identification and quantification of PAs. In order to have a common basis for

comparing methods involving different ways to estimate the content of PAs (sum parameter for PAs vs. reporting individual analytes), participants were asked to calculate the total content of PAs quantified as retrorsine equivalent.

2.1 Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed by assigning random codes to participants and not disclosing their identity.

3 Time frame

The PT was announced on the JRC-IRMM website on 24 November 2011. The registration to the exercise was open until 28 February 2012. The samples have been dispatched to participants on 28 June 2012. The reporting deadline has been set to 10 August 2012, however the last results were accepted till 05 October 2012 to complete the study with the maximum number of participants possible.

4 Materials

Table 4-1: Coding of materials and abbreviations used further in the text

Label as dispatched to participants	Abbreviated in the text
PA/PT/2012/STD (standard solution)	STD
PA/PT/2012/SNH (spiked natural honey)	SNH
PA/PT/2012/SAH (spiked invert sugar)	SAH
PA/PT/2012/NCH (naturally contaminated honey)	NCH
PA/PT/2012/CPM (naturally contaminated hay)	CPM

4.1 Preparation

4.1.1 Standard solution

Senecionine (Phytolab), seneciphylline (Phytolab) and retrorsine (Sigma) have been used as reference materials. Stock solutions in methanol-D4, containing benzoic acid) as internal standard for quantitative nuclear magnetic resonance spectrometry (qNMR) have been prepared by substitution weighing [5]. The purity of senecionine, seneciphylline and retrorsine was determined by qNMR by RIKILT Institute for Food Safety, Wageningen, NL.

A standard solution (**STD**) was prepared gravimetrically in methanol (total volume 500 ml) from the stock solutions described above. The final concentrations of analytes including total PA content in the solution and expanded uncertainties are indicated in **Tables 6-2**, **6-4**, **6-6** and **6-8**.

4.1.2 Spiked honey materials

About 4 kilograms of monofloral *Acacia* honey (**SNH**) and invert sugar syrup, which mimics some on honey properties (**SAH**), were used for the preparation of spiked samples by gravimetry. An intermediate solution containing the three PAs, which was prepared from the stock solutions described above, was added. Masses were recorded using a laboratory balance with a resolution of 0.1 g for honey. Spiking solutions were added using substitution weighing with an analytical balance having a resolution of 0.01 mg.

The invert sugar syrup was made from (60/40/0.1 Sugar/Water/Citric acid) which was stirred for 3 hours at 80 ^{o}C. Then it was cooled down and spiked with the intermediate spiking solution. After spiking, materials were mixed in a rotating drum for 48 h prior to packaging.

4.1.3 Naturally contaminated honey material (NCH)

Six different honey samples which have been found to have a similar content of PAs were blended in a rotating drum for 48 hours prior packaging to obtain sufficient bulk material for the study. The concentrations found in the starting materials were:

Retrorsine 40 – 50 µg/kg
Seneciphylline 23 - 50 µg/kg
Senecionine 20 - 35 µg/kg
Lycopsamine <LOQ (3.9 µg/kg) – 10 µg/kg.

4.1.4 Naturally contaminated hay (CPM)

Hay was obtained from within the institute as surplus material for the production of a certified reference material and tested for the presence of PAs. The content of PAs was below the respective limits of detection (monocrotaline, LOD=28 µg/kg; intermedine, LOD=24 µg/kg; lycopsamine, LOD=33 µg/kg; trichodesmine, LOD=8 µg/kg; retrorsine, LOD=5 µg/kg; seneciphylline LOD=5 µg/kg; senecionine, LOD=9 µg/kg; senkirkine, LOD=8 µg/kg and echimidine, LOD=12 µg/kg).

Tansy Ragwort (*Jacobea vulgaris*) was collected locally and dried. Both materials were milled (Retsch ZM 100 using a 0.5 mm sieve) and then sieved through a 100 µm sieve to obtain a fine powder. Prior to blending particle size distribution analysis was carried out. **Annex 10.1** shows that the materials coded as 17043 (hay) and 17044 (Tansy Ragwort) had similar particle distributions and were considered compatible for blending. The final composition of the sample was 30 g Tansy Ragwort and 4970 g hay. The level of contamination with Tansy Ragwort has been chosen to be approximately twice the regulated level laid down in 2002/32/EC [6]. The materials have been blended together step-by-step diluting Tansy Ragwort with hay. The final material was mixed in a rotating drum for 48 hours.

4.2 Homogeneity

To verify homogeneity 10 units per each material were selected at random. Two independent determinations per unit were performed using an LC-MS/MS based method, which has been single-laboratory validated at IRMM showing a fit-for-purpose repeatability. The measurement batch order was randomised. Sufficient homogeneity was assumed if the between-sample variance (s_s) was smaller than a critical factor ($0.3\sigma_p$) [7].

The between-sample variance (s_s) and the within-sample variance (s_w) were obtained from one-way analysis of variance (ANOVA). The allowable variance was calculated as $0.3\sigma_p$ (target standard deviation) from the Horwitz equation modified by Thompson [8].

Annex 10.2 lists the details of the homogeneity tests for all the materials. For all materials the between-sample variance (s_s) was smaller than the critical factor ($0.3\sigma_p$) and, therefore, sufficient homogeneity was concluded.

4.3 Stability

The stability of PAs in some matrices has been demonstrated in literature [9]. However, an isochronous stability testing was started upon sample dispatch. The samples were kept at three different temperatures (-20°C; +4°C and room temperature) for a period of 12 weeks. Every fourth week a sample was moved to reference conditions (-70°C). At the end of the stability testing period samples were analysed at once, making two independent determinations per sample. The concentrations found were plotted as a function of storage time. Samples were considered sufficiently stable if the slope of the regression function was not significantly different

from zero. Most samples were found to be stable with the exception of seneciphyline and retrorsine.

Seneciphyline showed some signs of instability in standard solution at room temperature and at +4°C, the drop in concentration observed was 5.6 % over 12 weeks and 2.0% within 8 weeks.

Retrorsine was apparently instable in naturally contaminated hay at +4 °C over a period of 12 weeks, while it was found to be stable for that period at -20 °C and at room temperature (RT), see **Table 10-9**. The decay for 12 weeks was 4.7%. The instability at +4 °C seems to be inconclusive as the material was stable at RT. The material was however found to be stable for a period of 8 weeks, which was the period in which results were requested. The details of the stability test can be found in **Annex 10.3**.

4.4 Distribution and instructions to participants

All samples were packed in cardboard boxes and sent to the participant via DHL express mail. One set of material was sent to every participant. The test materials were dispatched to the participants by IRMM on 28 June 2012. The samples were mostly received within 24 hours after dispatch.

Each participant received:

a) four packages containing approximately 50 g of test materials, and an ampoule containing 4 ml of test solution containing senecionine, seneciphylline and retrorsine in methanol
b) a accompanying letter with laboratory code, instructions on sample handling, and reporting **Annex 10.5**
c) a sample receipt form **Annex 10.6**
d) a questionnaire with results reporting form **Annex 10.7**

The materials were shipped refrigerated (+4°C); storage upon arrival was required to be at +4°C until the analysis was performed.

5 Reference values and their uncertainties

The assigned reference values and uncertainties have been calculated from the preparation data taking into account the uncertainty coming from the purity of standard materials and uncertainties related to preparation processes. Purity has been estimated using proton qNMR, analysing three replicates for each standard material. Benzoic acid purchased from Sigma (product Nr. 06185, Lot: BCBC1484V, traceable to NIST SRM 350b) has been used as internal standard.

Combined and expanded uncertainties were calculated as summarised in Figure 2 and equations 1 and 2.

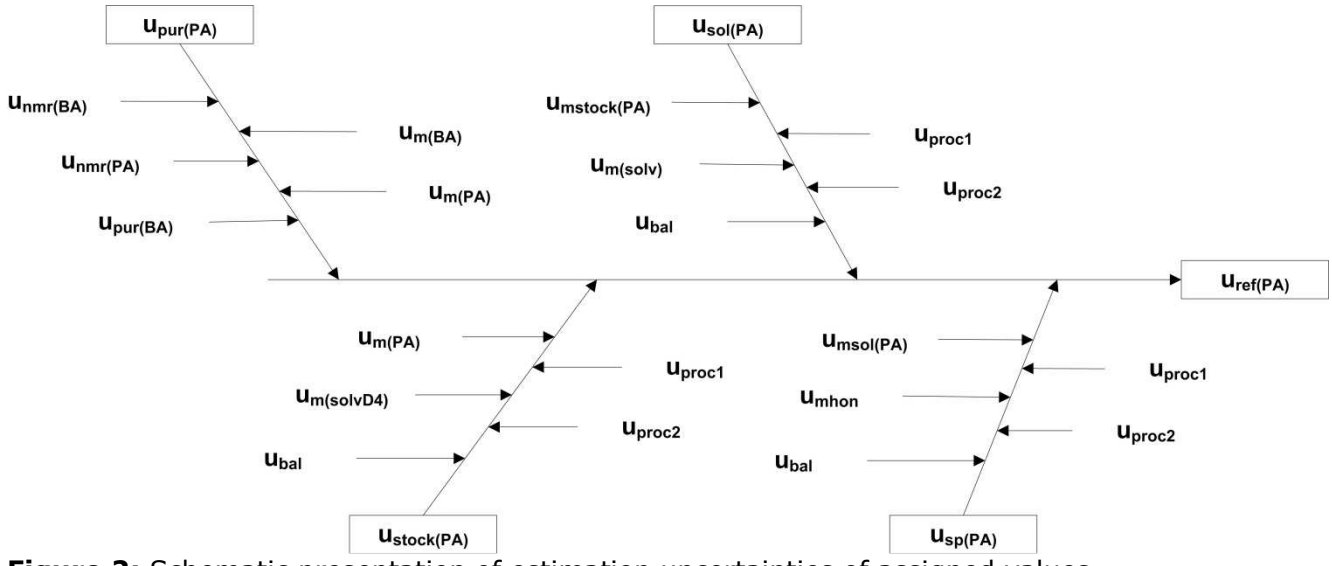

Figure 2: Schematic presentation of estimation uncertainties of assigned values

- Combined uncertainties for sum parameter:

$$u_{\sum ref(PA)} = \sqrt{\sum_{n=1}^{i} u_{ref(PA)i}^2} \qquad \textbf{Equation 1}$$

- Expanded uncertainties:

$$U_{(PA)} = 2 \times u_{ref(PA)} \qquad \textbf{Equation 2}$$

Explanations to the formulas and Figure 2:

$u_{nmr(BA)}$ - standard uncertainty of qNMR measurement of internal standard (benzoic acid)

$u_{nmr(PA)}$ - standard uncertainty of qNMR measurement of pyrrolizidine alkaloid

$u_{m(PA)}$ - standard uncertainty of substitution weighing of pyrrolizidine alkaloid

$u_{m(BA)}$ - standard uncertainty of substitution weighing of internal standard (benzoic acid)

$u_{m(solvD4)}$ - standard uncertainty of mass of deuterated solvent (methanol D4)

u_{msolv} - standard uncertainty of mass of solvent for dilution

$u_{pur(BA)}$ - standard uncertainty of internal standard purity

$u_{pur(PA)}$ - combined uncertainty of pyrrolizidine alkaloid purity

$u_{stock(PA)}$ - combined uncertainty of pyrrolizidine alkaloid stock solution concentration

$u_{mstock(PA)}$ - standard uncertainty of mass of pyrrolizidine alkaloid stock solution used for dilution

u_{bal} - combined uncertainty of calibration of different balances used

$u_{proc1;2}$ - standard uncertainties of weighing initial material (alkaloid/stock/spiking solution, proc1) and final material solvent/matrix (proc2)

$u_{sol(PA)}$ - combined uncertainty of pyrrolizidine alkaloid spiking/standard solution concentration

$u_{msol(PA)}$ - standard uncertainty for mass of pyrrolizidine alkaloid solution used for spiking

u_{mhon} - standard uncertainty for mass of matrix used for spiking

$u_{sp(PA)}$ - combined uncertainty of spiking procedure

$u_{ref(PA)}$ - combined uncertainty of assigned value for pyrrolizidine alkaloid

$u_{\sum ref(PA)}$ - combined uncertainty of concentration of total pyrrolizidine alkaloids in spiked material

As major uncertainty contributors the qNMR measurement and the uncertainty of the purity of internal standard were identified. As a result the relative uncertainties for the purities of retrorsine, senecionine and seneciphylline were 1.76 %, 1.33 % and 0.58 % respectively.

The other sources of uncertainties illustrated in **Figure 2**, for gravimetric preparation contributed with less than 0.05 % to the uncertainty budget. Reference values and related expanded uncertainties are presented in **Tables 6-2, 6-4, 6-6 and 6-8**.

For naturally contaminated honey, robust means and standard deviations were calculated with algorithm A of ISO 13528 [7] using a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [10].

6 Evaluation of results

6.1 General observations

Twenty-eight participants from different EU Member States, Switzerland and Singapore registered to participate at this exercise. Laboratories 110 and 115 did not report results. Two laboratories 106 and 118 reported results using two different analytical approaches. One was estimating a sum parameter, the other was reporting individual analytes. To make a clear

distinction further, 106a, 118a lab codes are used to indicate results reported for the "individual" analyte determination approach, and 106b, 118b to indicate the "sum parameter" determination approach. Laboratory 106a, in order to detect individual analytes, used a reduction step with Zn in the process of sample preparation. This leads to a combined parameter for each PA and its respective N-oxide. Results of participants indicate that PA N-oxides were not an issue in honey samples and standard solutions; however these compounds were present in substantial amounts in hay. To be able to compare results of the different analytical approaches, the sum of PA-base and PA-N-oxide was calculated. The results were expressed as PA-base compound (retrorsine, senecionine, or seneciphylline). The results are presented in **Tables 6-5, 6-7, 6-9**, and **Figures 14, 21, 28**.

z-Scores for individual and sum parameters were only assigned for gravimetrically spiked samples.

For the naturally contaminated samples **NCH** and **CPM** 28 results were available. The robust standard deviations for these materials were rather high. This influences in an undesirable manner the uncertainties of the consensus values (f.i. robust standard deviations 45 µg/kg for **NCH** and 3947 µg/kg for **CPM**, vs. robust means 140 µg/kg for **NCH** and 4352 µg/kg for **CPM** when analysing total PA's in the samples), thereby reducing the information content of the generated z-scores. Consequently, z-scoring for naturally contaminated materials was not found appropriate.

The success rate of laboratories to estimate PAs in spiked natural honey and spiked invert sugar syrup was comparable, suggesting that laboratories analysing PAs could use the latter as a quality control material if no honey blanks are available. It must be noted, that one laboratory (111) stated having difficulties with linearity of detector response in this matrix.

All participants were asked to report results in µg/kg for all samples analysed including the standard solution (**STD**). Nevertheless, some participants submitted their results in µg/volume for **STD;** those were recalculated taking into account the density of the methanolic solution d=0.7873 g/cm^3 (preparation temperature: 24^{o}C). The recalculated values are presented in **Table 6-1**. The raw data reported by participants are presented in **Tables 10-51** to **10-71.**

Despite converting the incorrectly reported measurement unit for the standard solution **STD** into µg/kg (requested reporting unit), the robust mean was nonetheless statistically different from the assigned value derived from the gravimetric preparation (1090 µg/kg vs. 1345 µg/kg for total PAs in the sample). It is strongly suggested that laboratories which reported values significantly different from the assigned value should perform a root cause analysis keeping in mind that the requested reporting was µg/kg Some laboratories might have reported values as mass fraction (µg/kg), assuming that the density of the standard solution is unity. The reason for this assumption is that the robust mean, if corrected by the density of the solution, comes very close to the assigned value.

Table 6-1: Conversion of results for sample **STD**. Abbreviations mean: Total – total content of PA's in the sample, RETRO – retrorsine, SNCP – seneciphylline, SNC - senecionine

Lab. Code	Units Reported	Values Reported				Units Converted	Values converted			
		Total	RETRO	SNCP	SNC		Total	RETRO	SNCP	SNC
101	µg/kg	1457	313	737	351	µg/kg	1457.00	313.00	737.00	351.00
102	µg/kg	987.37	263.57	422.9	256.8	µg/kg	987.37	263.57	422.90	256.80
103	µg/kg	850.54	186.56	418.84	211.37	µg/kg	850.54	186.56	418.84	211.37
104	µg/kg	907.9	239.4	372.5	262.7	µg/kg	907.90	239.40	372.50	262.70
105	µg/kg	44	20	15	9	µg/kg	44.00	20.00	15.00	9.00
106a	µg/kg	945	245	456	210	µg/kg	945.00	245.00	456.00	210.00
106b	µg/kg	938	SUM	SUM	SUM	µg/kg	938.00	SUM	SUM	SUM
107	µg/kg	1635.64	446.75	875	254.64	µg/kg	1635.64	446.75	875.00	254.64
108	µg/l	891	210	480	167	µg/kg	1131.72	266.73	609.68	212.12
109	µg/kg	880	229.6	391.9	226.4	µg/kg	880.00	229.60	391.90	226.40
111	µg/kg	974.1	229.6	497.6	210	µg/kg	974.10	229.60	497.60	210.00
112	µg/kg	389.89	205.53	97.52	58.38	µg/kg	389.89	205.53	97.52	58.38
113	µg/ml	1.14	SUM	SUM	SUM	µg/kg	1447.99	SUM	SUM	SUM
114	µg/l	956.09	248.38	441.37	231.45	µg/kg	1214.39	315.48	522.51	293.98
116	µg/kg	1113.08	263.33	510.83	297.17	µg/kg	1113.08	263.33	510.83	297.17
117	µg/kg	27.57	3.95	13.45	9.02	µg/kg	27.57	3.95	13.45	9.02
118a	µg/ml	1.032	0.228	0.535	0.229	µg/kg	1310.81	289.60	679.54	290.87

118b	µg/ml	0.909	SUM	SUM	SUM	µg/kg	1154.58	SUM	SUM	SUM
119	µg/kg	384.8	76.6	192.3	100.8	µg/kg	384.80	76.60	192.30	100.80
120	µg/kg	996	226	487	245	µg/kg	996.00	226.00	487.00	245.00
121	ng/ml	927	253	361	195	µg/kg	1177.44	321.35	458.53	247.68
122	µg/kg	832.2	195.1	383.1	222.6	µg/kg	832.20	195.10	383.10	222.60
123	µg/kg	2180	SUM	SUM	SUM	µg/kg	2180.00	SUM	SUM	SUM
124	µg/kg	1101.95	278.25	536.25	269.25	µg/kg	1101.95	278.25	536.25	269.25
125	µg/ml	1.6	n/a	1.41	0.08	µg/kg	2032.26	n/a	1790.93	101.61
126	µg/kg	14052.47	3626	7438.1	2460	µg/kg	14052.47	3626.00	7438.10	2460.00
127	µg/ml	0.71	<LOD	0.47	0.21	µg/kg	901.82	<LOD	596.98	266.73
128	µg/kg	1058.01	270	516.35	232.7	µg/kg	1058.01	270.00	516.35	232.70

6.2 Scores and evaluation criteria

Individual laboratory performance was expressed in terms of z-scores in accordance with ISO 13528 [7] and the International Harmonised Protocol [11].

$$z = \frac{x_{lab} - X_{ref}}{\sigma_p}$$ **Equation 3**

where:

x_{lab} - result submitted by participant

X_{ref} - assigned value

σ_p - target standard deviation.

σ_p was calculated by the Horwitz equation (equations 5,6) :

- for analyte concentrations < 120 µg/kg

$$\sigma_p = 0.22 \cdot c$$ **Equation 4**

where:

c - concentration of the assigned value, expressed as a mass fraction,
e.g. 1 µg/kg = 10^{-9}, 1 mg/kg = 10^{-6}

The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, σ_p. The z-score is interpreted as:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

6.3 Data evaluation

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528 [7] by application of a MS Excel® macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC). The representative figures are tabulated for each test sample in the following sections of the report.

All results have been evaluated for individual z-Scores and for the total PA content in the samples.

6.3.1 z-Score evaluation

Table 6-2: Summary statistics for total PA determination

Sample		STD	SNH	SAH	NCH	CPM
No. Results		28	28	28	28	25
Min	µg/kg	27.57	56.89	46.74	82.25	188.00
Max	µg/kg	14052.47	819.21	758.15	10100.00	40985.02
x_m	µg/kg	1027.01	85.66	87.27	133.45	2909.5
x_r	µg/kg	1090.04	88.70	86.13	139.84	4352.2
X_{ref}	µg/kg	1345.39	85.23	85.18	n/a	n/a
U_{ref}	µg/kg	61.43	3.89	3.88	n/a	n/a
s_{rob}	µg/kg	453.7	29.5	28.8	44.8	3947.7
σ_p	µg/kg	205.86	18.75	18.73	n/a	n/a
NR /z/>2		n/a	5	4	n/a	n/a

x_m – median of results
x_r – robust mean of results
X_{ref} – assigned value
U_{ref} – expanded uncertainty of assigned value
s_{rob} – robust standard deviation
σ_p – target standard deviation

Table 6-3: Results submitted by participants for total PA determination
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

	Sample						
Lab. Code	**STD**, µg/kg	**SNH**, µg/kg	z(SNH)	**SAH**, µg/kg	z(SAH)	**NCH**, µg/kg	**CPM**, µg/kg
101	1457.00	70	-0.8	88	0.2	123	1624
102	987.37	70.7	-0.8	78.54	-0.4	123.22	n/a
103	850.54	64.64	-1.1	63.16	-1.2	95.9	2511.81
104	907.90	98.4	0.7	95.3	0.5	154.4	3924.6
105	44.00	95	0.5	88	0.2	165	188
106a	945.00	103	1.0	104	1.0	96	1802
106b	938.00	131	2.4	107	1.2	10100	19200
107	1635.64	267.043	**9.7**	206.419	**6.5**	196.62	4081.88
108	1131.72	73.3	-0.6	70	-0.8	107.5	3051
109	880.00	66.8	-1.0	65.8	-1.0	118.7	4107
111	974.10	86	0.0	54.7	-1.6	161.7	10468.4
112	389.89	56.89	-1.5	46.74	-2.1	103.15	2909.48
113	1447.99	108.51	1.2	95.39	0.6	2146.03	9920.48
114	1214.39	85.31	0.0	86.54	0.1	128.33	1723.02
116	1113.08	94.49	0.5	103.7	1.0	156.41	1635.17
117	27.57	127.63	2.3	178.15	**5.0**	138.57	2028.59
118a	1310.81	96.16	0.6	102.86	0.9	149.89	2012.16
118b	1154.58	67.15	-1.0	69.31	-0.9	167.86	9869.53
119	384.80	156.1	**3.8**	146.1	**3.3**	166.2	1718.1
120	996.00	63	-1.2	74.5	-0.6	97.7	5586
121	1177.44	64.1	-1.1	63.2	-1.2	105	n/a
122	832.20	63.1	-1.2	58.6	-1.4	104.7	615.1
123	2180.00	120	1.9	113	1.5	2137	10858
124	1101.95	94.85	0.5	94.26	0.5	150.58	2085.94
125	2032.26	60.6	-1.3	52.9	-1.7	91.8	2434.5
126	14052.47	819.21	**39.2**	758.15	**35.9**	1340.19	40985.02
127	901.82	64.29	-1.1	56.84	-1.5	82.25	4370.47
128	1058.01	62.32	-1.2	65.29	-1.1	107.79	n/a

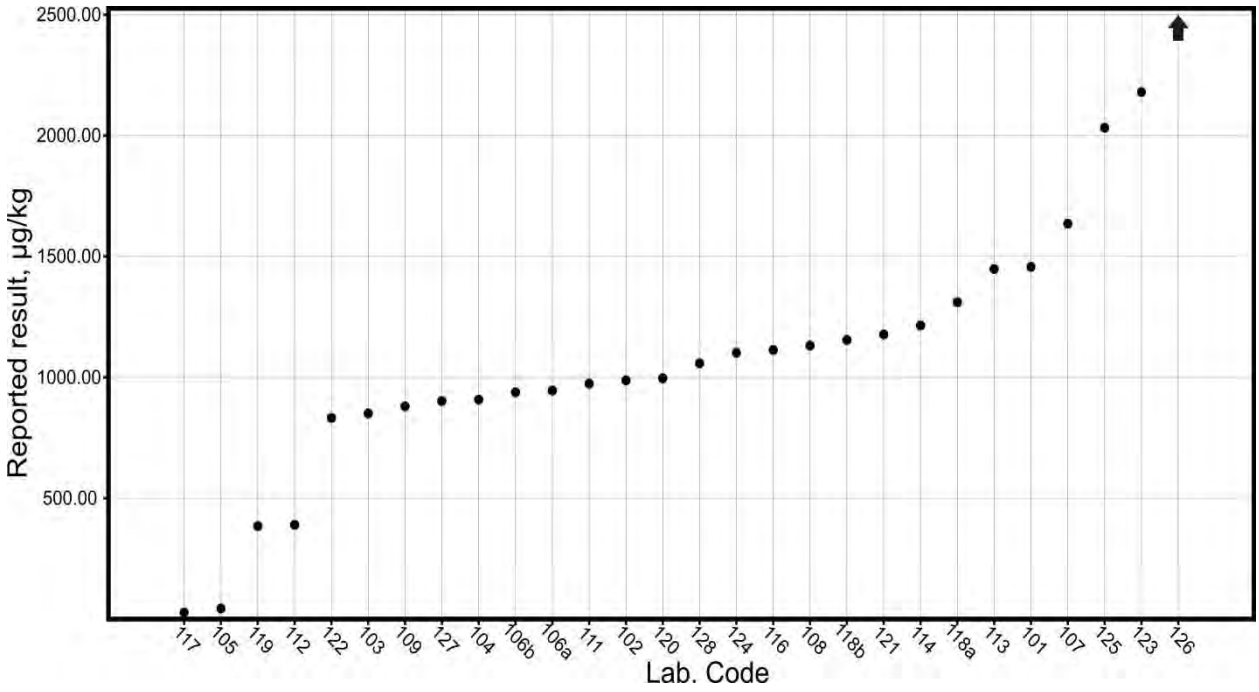

Figure 3: Results for total PA in sample **STD**.

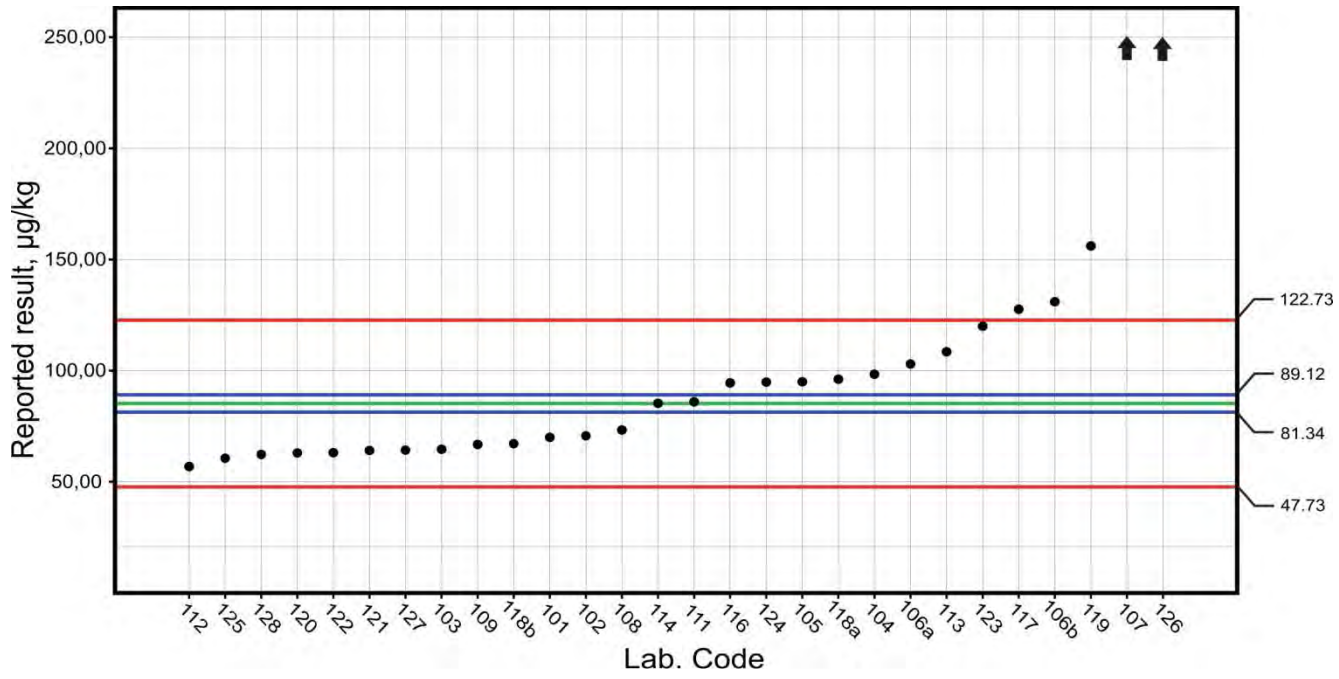

Figure 4: Results for total PA in sample **SNH**. Green line – assigned value, blue lines – uncertainty range of assigned value, red lines result acceptance range for z-score criteria

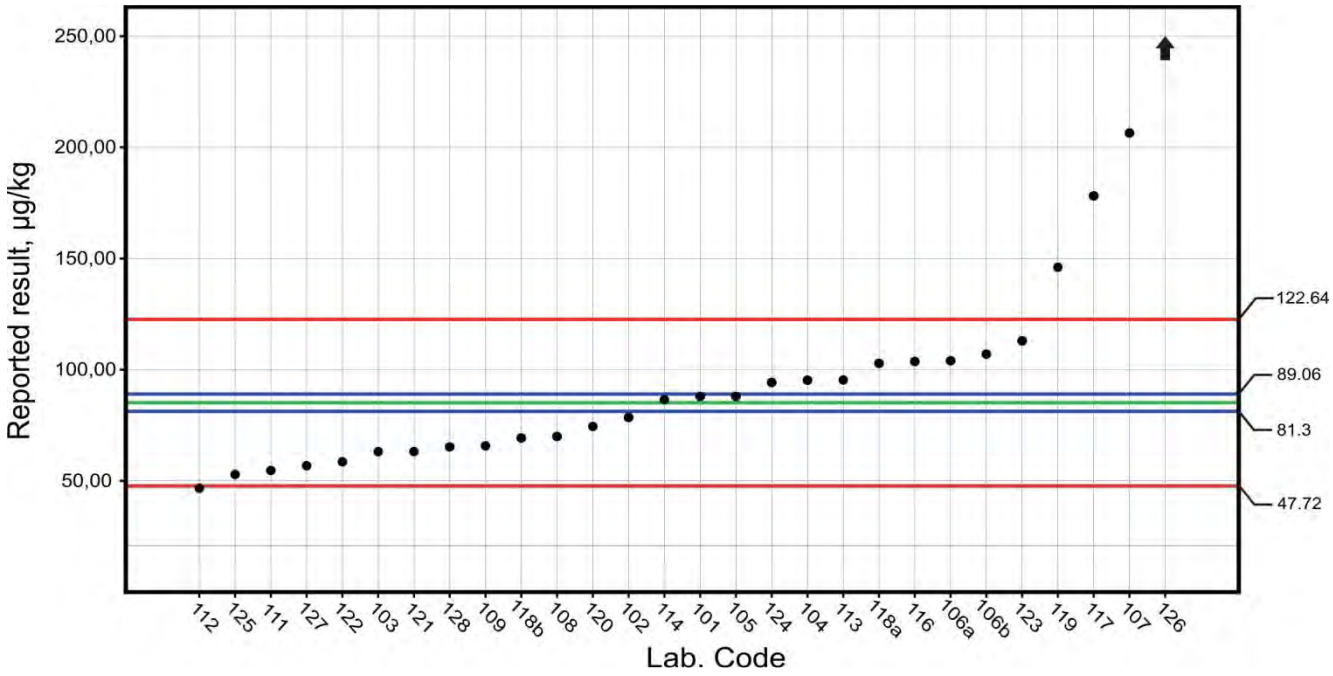

Figure 5: Results for total PA in sample **SAH**. Green line – assigned value, blue lines – uncertainty range of assigned value, red lines - result acceptance range for z-score criteria.

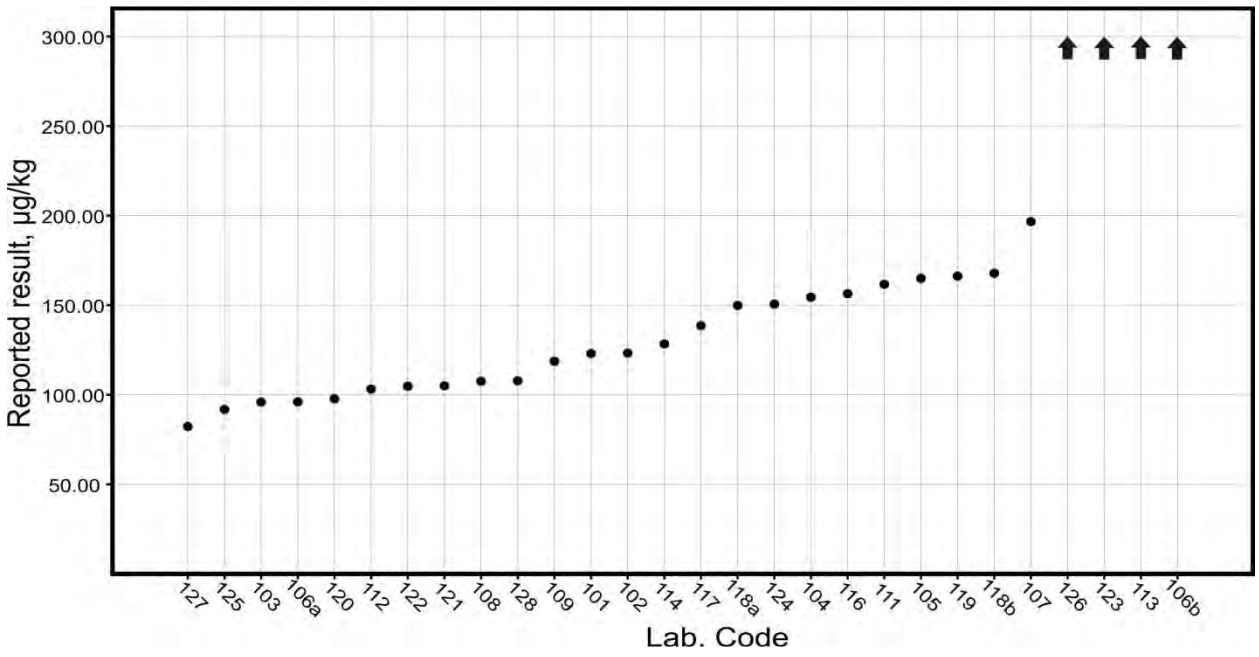

Figure 6: Distribution of results of total estimation of PA's submitted by participants for sample **NCH**.

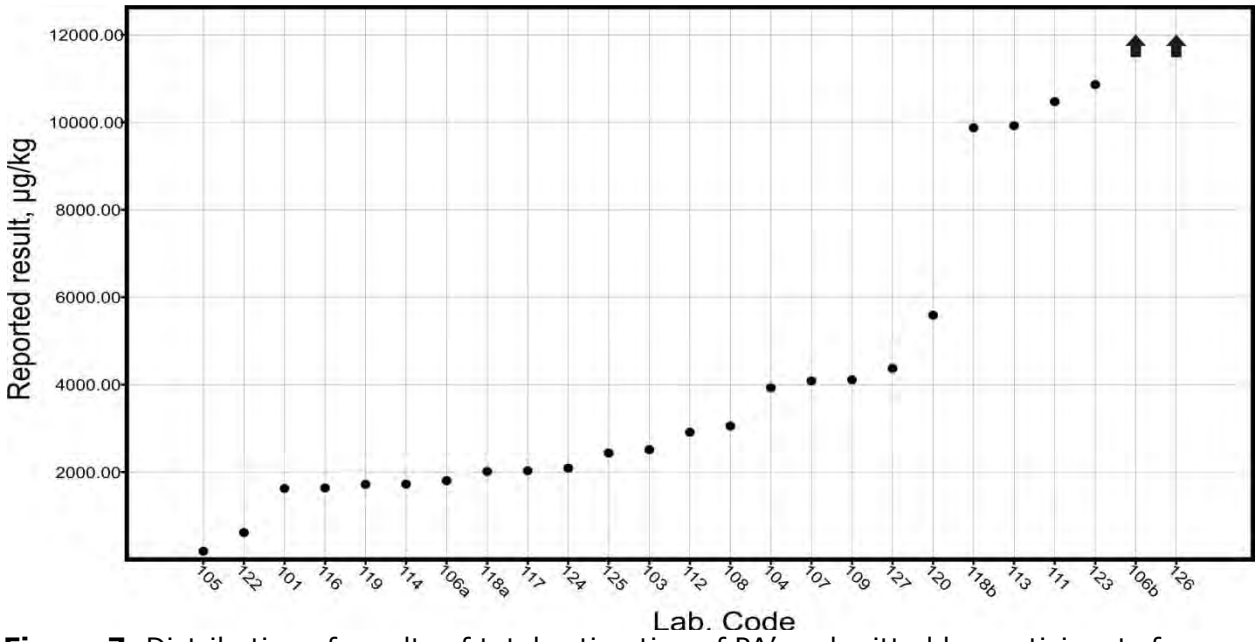

Figure 7: Distribution of results of total estimation of PA's submitted by participants for sample **CPM**. Laboratories 102, 121, 128 did not analyse hay.

Laboratories 106b, 113, 118b, and 123 have been excluded from the following evaluations till chapter 6.3.2, because it concerns only laboratories analysing individual PA's.

Table 6-4: Summary statistics for retrorsine: **CPM**(NO) indicates retrorsine-N-oxide content in the hay sample. **CPM**(total) - total content of retrorsine and retrorsine-N-oxide in the hay sample, expressed as retrorsine equivalent.

Sample		**STD**	**SNH**	**SAH**	**NCH**	**CPM**	**CPM**(NO)	**CPM**(total)
No. Results		22	22	22	22	18	10	18
Min	µg/kg	3.95	12.30	9.16	25.20	23.00	159.64	23.00
Max	µg/kg	3626.00	220.00	189.50	465.90	756.13	354.10	1032.29
x_m	µg/kg	254.2	21.6	21.5	44.4	125.6	304.9	303.7
x_r	µg/kg	249.2	21.3	21.2	44.5	155.0	265.5	309.5
X_{ref}	µg/kg	326.18	20.86	20.84	n/a	n/a	n/a	n/a
U_{ref}	µg/kg	11.50	0.73	0.73	n/a	n/a	n/a	n/a
s_{rob}	µg/kg	69.8	6.2	6.8	13.2	109.8	92.5	161.4
σ_p	µg/kg	61.77	4.59	4.58	n/a	n/a	n/a	n/a
NR /z/>2		n/a	2	3	n/a	n/a	n/a	n/a

x_m – median of results
x_r – robust mean of results
X_{ref} – assigned (nominal) value
U_{ref} – expanded uncertainty of assigned value
s_{rob} – robust standard deviation
σ_p – target standard deviation

Table 6-5: Results submitted by participants for retrorsine:
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

	Sample								
Lab. Code	**STD**, µg/kg	**SNH**, µg/kg	z(SNH)	**SAH**, µg/kg	z(SAH)	**NCH**, µg/kg	**CPM**, µg/kg	**CPM**(NO), µg/kg	**CPM**(Total), µg/kg
101	313.00	15	-1.3	19	-0.4	45	250	n/a	250
102	263.57	20.16	-0.2	22.61	0.4	45.36	n/a	n/a	n/a
103	186.56	14.09	-1.5	12.93	-1.7	25.68	63.86	159.64	216.54
104	239.40	25.7	1.1	23.6	0.6	51.9	59.2	354.1	397.88
105	20.00	30	1.99	25	0.9	65	23	<LOD	23
106a	245.00	26	1.1	26	1.1	44	316	n/a	316
107	446.75	140.67	**26.1**	57.37	**8.0**	84.08	110.8	349.2	444.79
108	266.73	19.1	-0.4	18.4	-0.5	32.8	133	168	293.68
109	229.60	16.2	-1.0	16.2	-1.0	35.8	125.5	321	432.52
111	229.60	20.0	-0.2	12.4	-1.8	57.0	121.0	324.3	431.18
112	205.53	14.63	-1.4	9.16	-2.5	30.68	125.73	196.87	314.03
114	315.48	23.57	0.6	21.53	0.2	42.02	160.91	n/a	160.91
116	263.33	23.48	0.6	26.01	1.1	48.39	203.77	n/a	203.77
117	3.95	23.04	0.5	28.81	1.7	42.26	557.46	n/a	557.46
118a	289.60	24.76	0.9	27.98	1.6	44.36	<LOD	n/a	<LOD
119	76.60	23.4	0.6	21.4	0.1	55.8	48.8	169.8	211.21
120	226.00	12.3	-1.9	13.4	-1.6	25.2	99.9	323.6	409.41
121	321.35	18.8	-0.4	18.6	-0.5	37.4	n/a	n/a	n/a
122	195.10	15.7	-1.1	17.2	-0.8	31.3	67.7	n/a	67.7
124	278.25	25.4	1.0	24.48	0.8	47.6	251.08	n/a	251.08
125	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
126	3626.00	220	**43.4**	189.5	**36.8**	465.9	<LOD	<LOD	<LOD
127	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	756.13	288.73	1032.29
128	270.00	17.53	-0.7	17.61	-0.7	44.5	n/a	n/a	n/a

The results are written as reported by the laboratories. SUM* - means, that laboratory uses the method involving conversion of retrorsine-N-oxide to retrorsine prior to detection

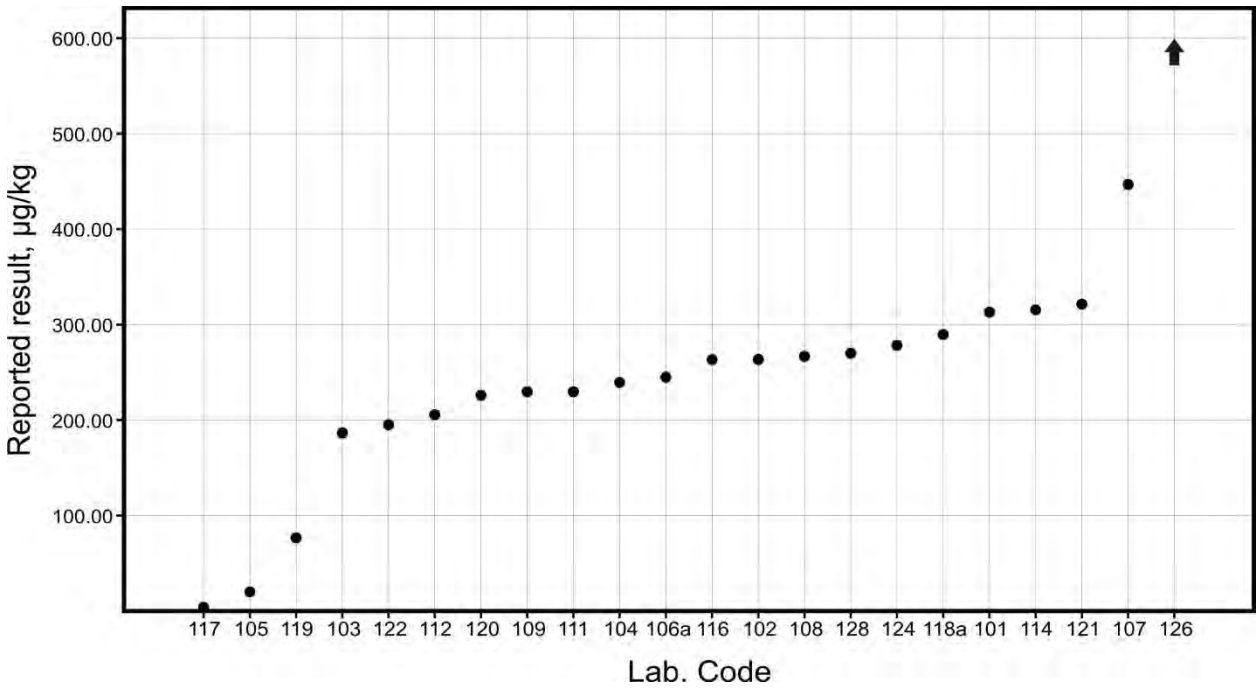

Figure 8: Retrorsine results for sample **STD**. Laboratory 125 did not analyse, laboratory 127 did not detect retrorsine.

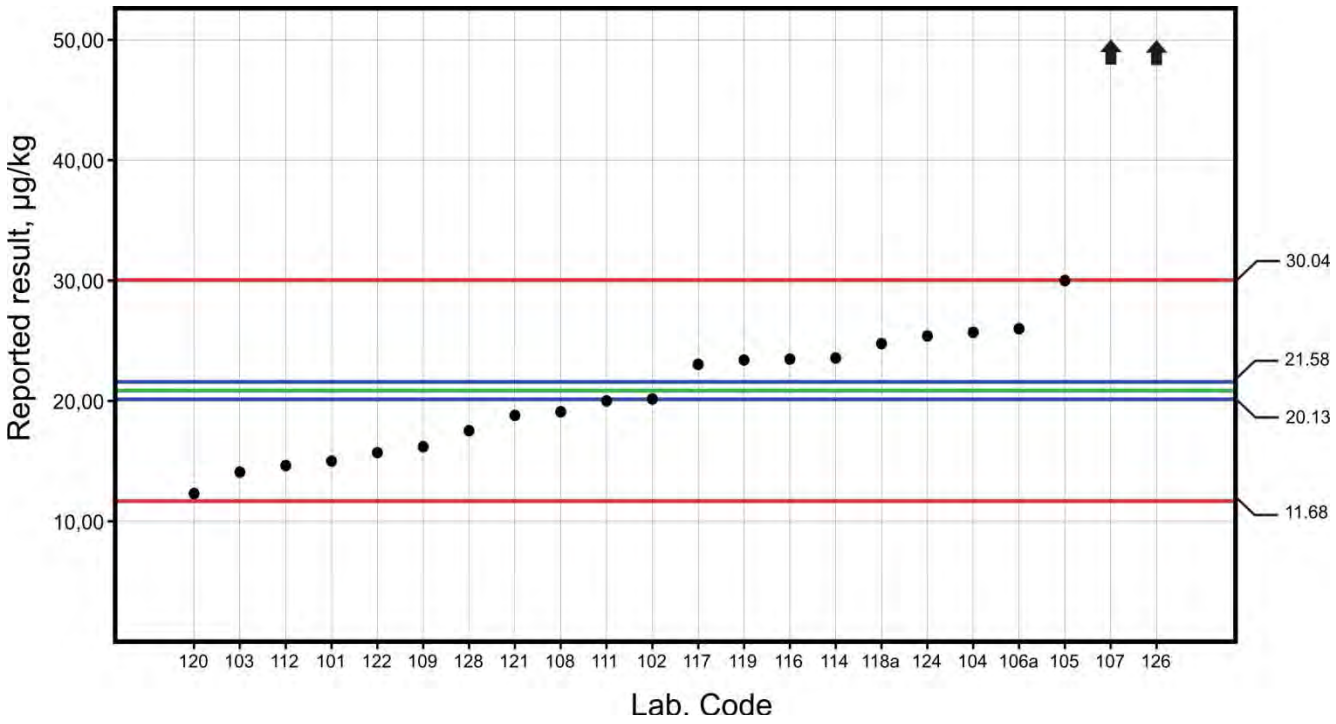

Figure 9: Retrorsine results for sample **SNH**. Laboratory 125 did not analyse, laboratory 127 did not detect retrorsine. Green line is assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria

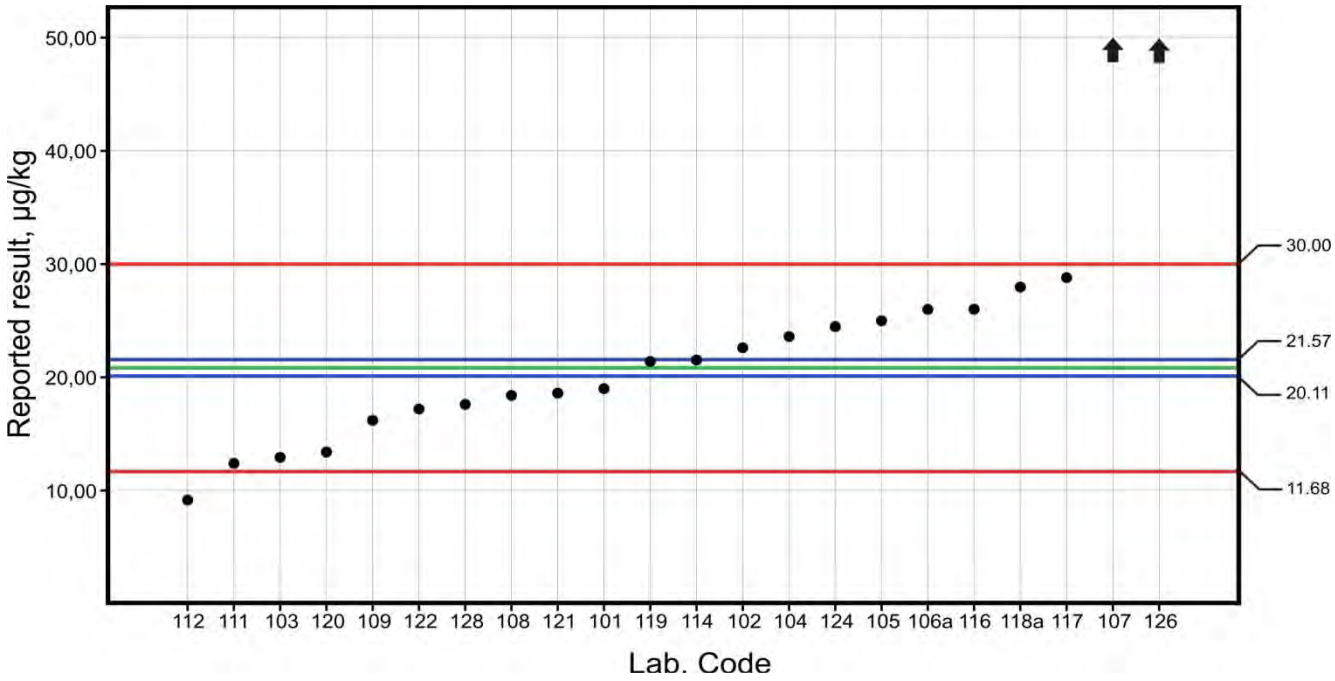

Figure 10: Retrorsine results for sample **SAH**. Laboratory 125 did not analyse, laboratory 127 did not detect retrorsine. Green line is assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria

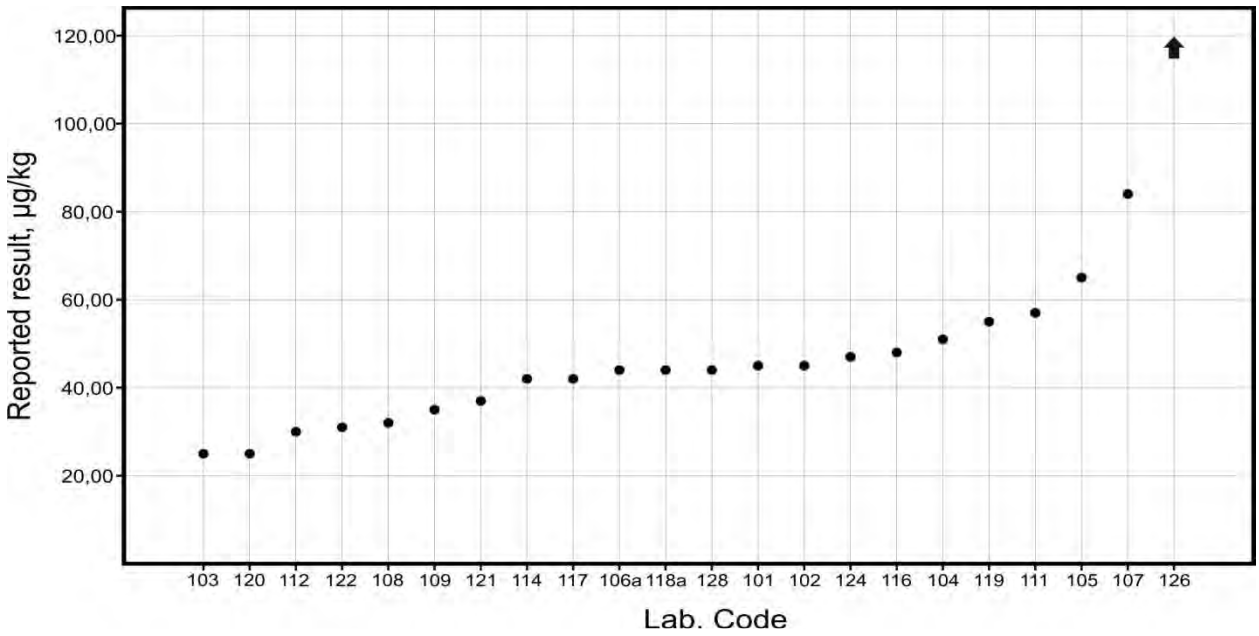

Figure 11: Retrorsine results for sample **NCH**. Laboratory 125 did not report and laboratory 127 did not detect retrorsine.

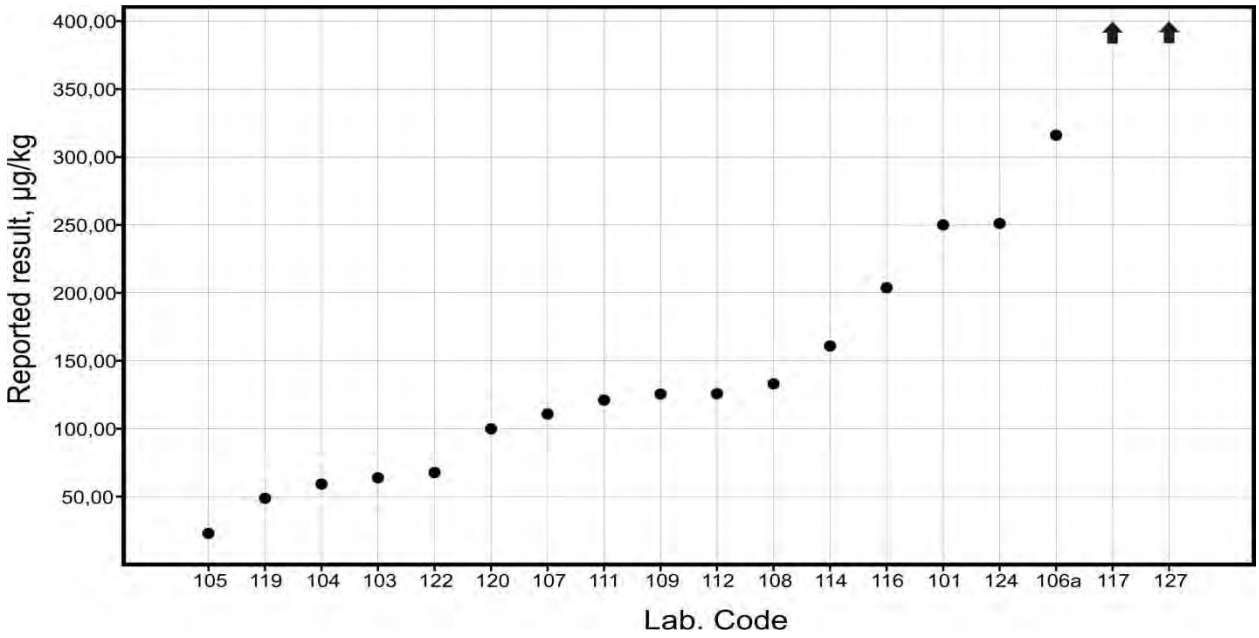

Figure 12: Retrorsine results for sample **CPM**. Laboratories 102, 121, 125, 128 did not report and laboratories 118a, 126 did not detect retrorsine. Due to the methodology used by laboratory 106a, their result is presented as total retrorsine.

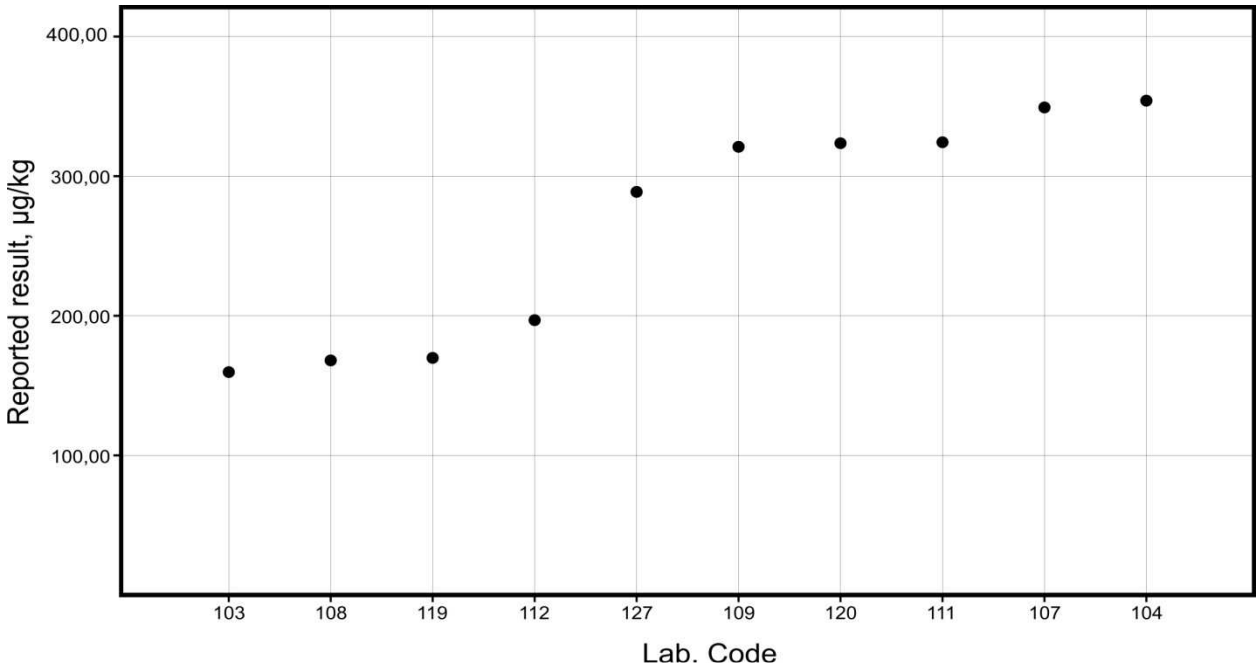

Figure 13: Retrorsine-N-oxide results for sample **CPM**. Only those laboratories are indicated which reported numeric figures.

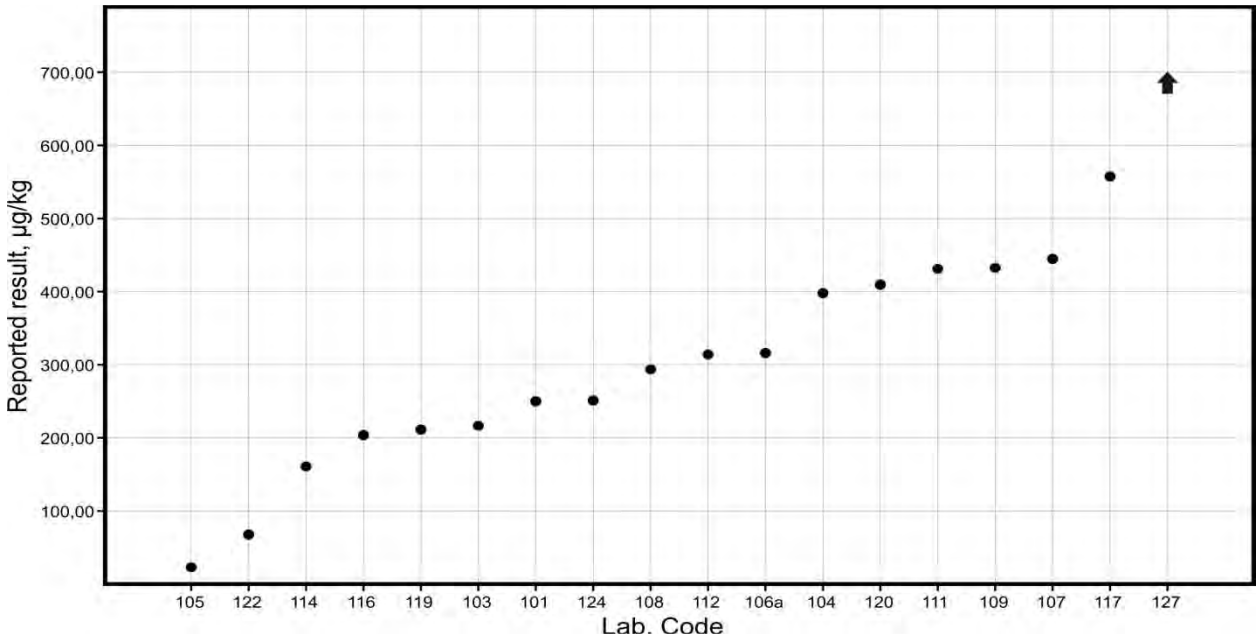

Figure 14: Total Retrorsine results for sample **CPM**. Laboratories 102, 121, 125, 128 did not analyse retrorsine in the sample.

Table 6-6: Summary statistics for seneciphylline. CPM(NO) indicates seneciphylline-N-oxide content in the hay sample. CPT(total) - total content of seneciphyline and seneciphylline-N-oxide, expressed as seneciphylline equivalent

Sample		**STD**	**SNH**	**SAH**	**NCH**	**CPM**	**CPM**(NO)	**CPM**(total)
No. Results		24	24	24	24	21	14	21
Min	µg/kg	13.45	25.97	24.22	20.00	80.00	300.13	80.00
Max	µg/kg	7438.10	452.30	429.10	440.50	5096.80	8588.00	13291.40
x_m	µg/kg	492.3	41.8	39.8	33.9	484.5	798.5	932.3
x_r	µg/kg	488.3	41.4	40.1	35.0	467.6	747.8	986.3
X_{ref}	µg/kg	647.62	40.68	40.65	n/a	n/a	n/a	n/a
U_{ref}	µg/kg	7.55	0.47	0.47	n/a	n/a	n/a	n/a
s_{rob}	µg/kg	221.6	12.3	13.0	11.3	157.8	404.1	538.4
σ_p	µg/kg	110.62	8.95	8.94	n/a	n/a	n/a	n/a
NR z>2		n/a	4	4	n/a	n/a	n/a	n/a

x_m – median of results
x_r – robust mean of results
X_{ref} – assigned (nominal) value
U_{ref} – expanded uncertainty of assigned value
s_{rob} – robust standard deviation
σ_p – target standard deviation

Table 6-7: Results submitted by participants for seneciphylline
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

	Sample								
Lab. Code	**STD**, µg/kg	**SNH**, µg/kg	z(SNH)	**SAH**, µg/kg	z(SAH)	**NCH**, µg/kg	**CPM**, µg/kg	**CPM**(NO), µg/kg	**CPM**(total), µg/kg
101	737.00	40	-0.1	46	0.6	32	483	n/a	483.00
102	422.90	29.95	-1.2	33.92	-0.8	29.58	n/a	n/a	n/a
103	418.84	32.25	-0.9	32.7	-0.9	27.02	318.92	505.58	801.35
104	372.50	39.6	-0.1	39.9	-0.1	32.6	327	718.6	1012.69
105	15.00	45	0.5	40	-0.1	38	80	<LOD	80.00
106a	456.00	47	0.7	47	0.7	20	745	SUM*	745.00
107	875.00	89.39	**5.4**	108.37	**7.6**	55.03	378	1069.2	1398.24
108	609.68	43.3	0.3	39.7	-0.1	39	334	410	725.22
109	391.90	29	-1.3	30.3	-1.2	28.2	484.5	824.5	1271.24
111	497.60	44.0	0.4	26.0	-1.6	38.6	531.0	1214.4	1689.79
112	97.52	25.97	-1.6	24.22	-1.8	24.46	487.21	504.86	968.95
114	522.51	36.97	-0.4	39.26	-0.2	35.09	572.58	n/a	572.58
116	510.83	41.89	0.1	45.95	0.6	36.03	473.32	n/a	473.32
117	13.45	68.4	**3.1**	70.97	**3.4**	45.3	557.46	1175.47	1679.10
118a	679.54	45.95	0.6	47.15	0.7	42.1	534.59	305	825.62
119	192.30	100.3	**6.7**	90.6	**5.6**	54.4	336.8	789.3	1089.95
120	487.00	33.3	-0.8	39.6	-0.1	27.4	495.8	1123	1567.37
121	458.53	27.7	-1.5	27.2	-1.5	24.2	n/a	n/a	n/a
122	383.10	27.5	-1.5	24.8	-1.8	24.9	162	n/a	162.00
124	536.25	45.32	0.5	45.68	0.6	37.48	609.71	n/a	609.71
125	1790.93	49.97	1.0	24.52	-1.8	66.68	645.95	300.13	932.34
126	7438.10	452.3	**46.0**	429.1	**43.4**	440.5	5096.8	8588	13291.51
127	596.98	41.74	0.1	40.57	0.0	31.6	379.13	807.73	1149.87
128	516.35	28.75	-1.3	30.07	-1.2	20.63	n/a	n/a	n/a

The results are written as reported by the laboratories. SUM* - means, that laboratory uses the method involving conversion of seneciphylline-N-oxide to seneciphylline prior to detection

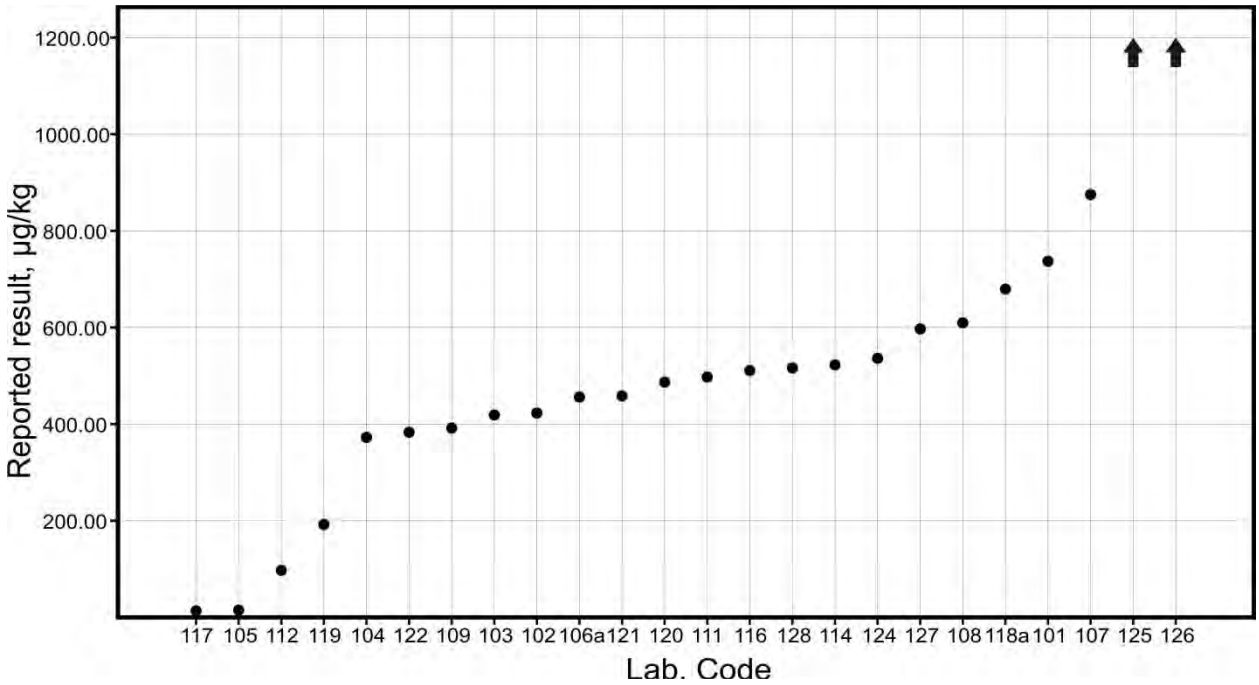

Figure 15: Seneciphylline results for sample **STD**.

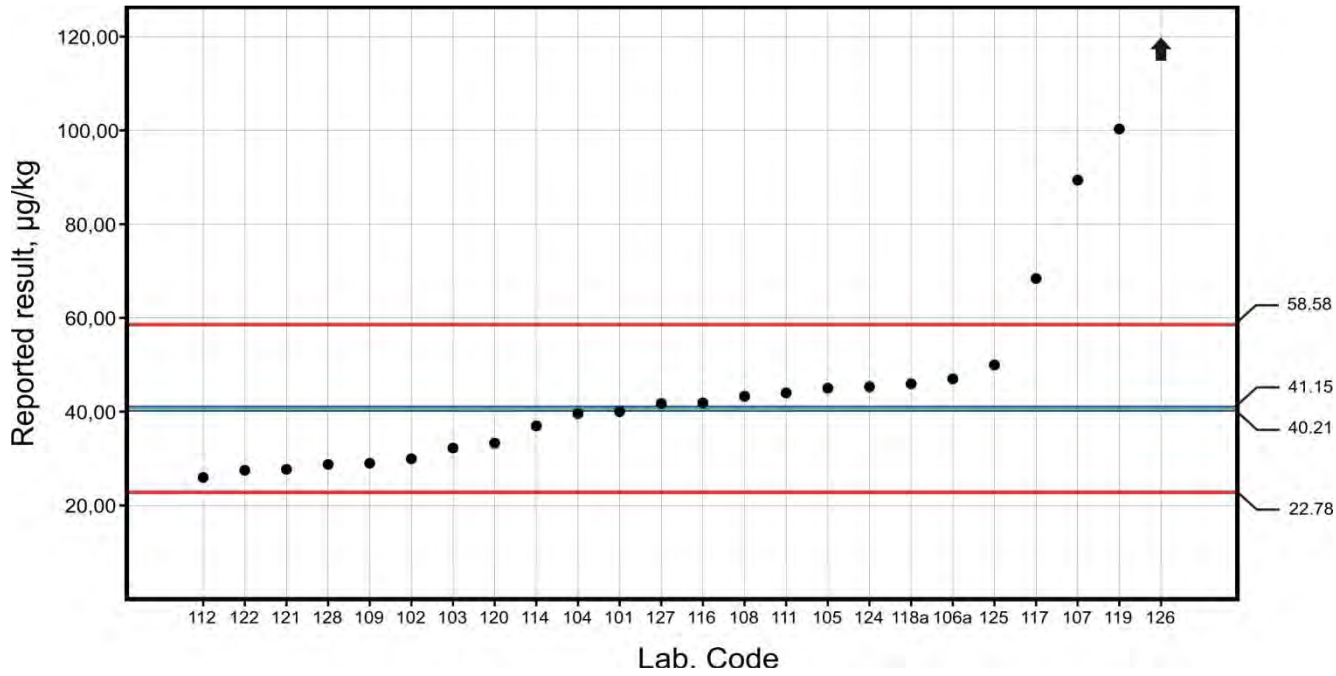

Figure 16: Senecipylline results for sample **SNH**. Green line is assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria

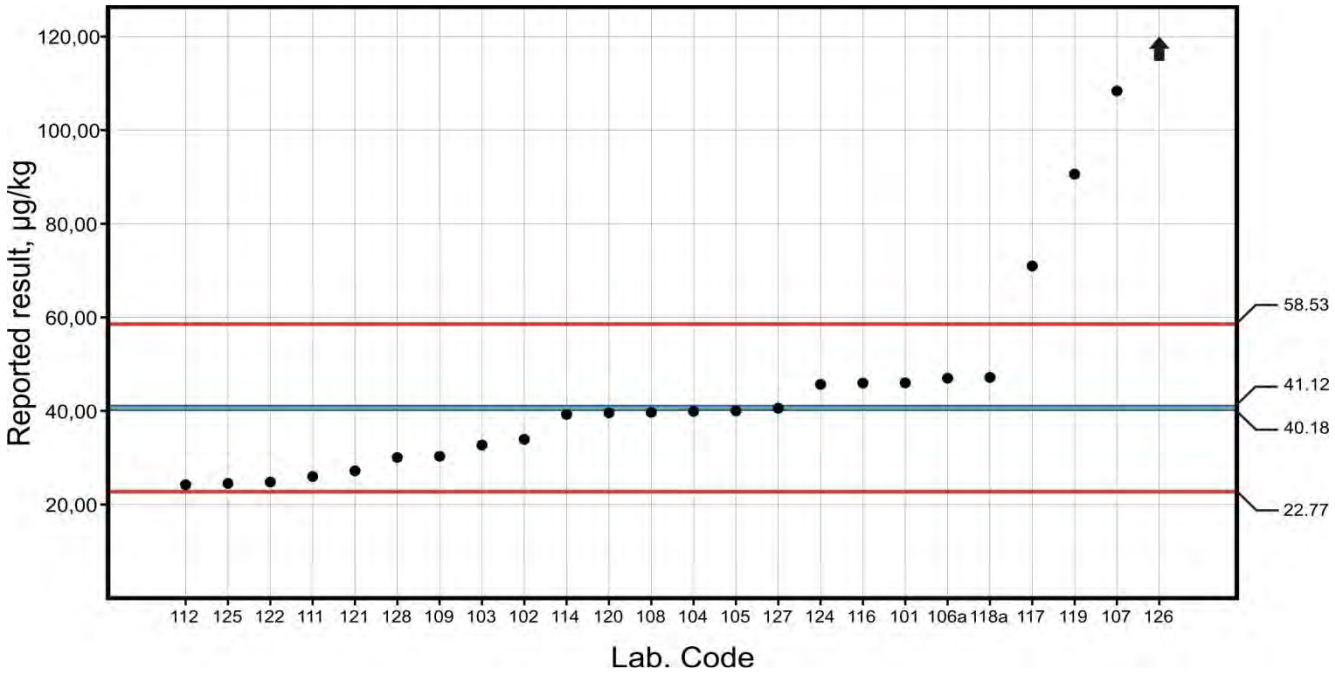

Figure 17: Seneciphylline results for sample **SAH**. Green line is assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria

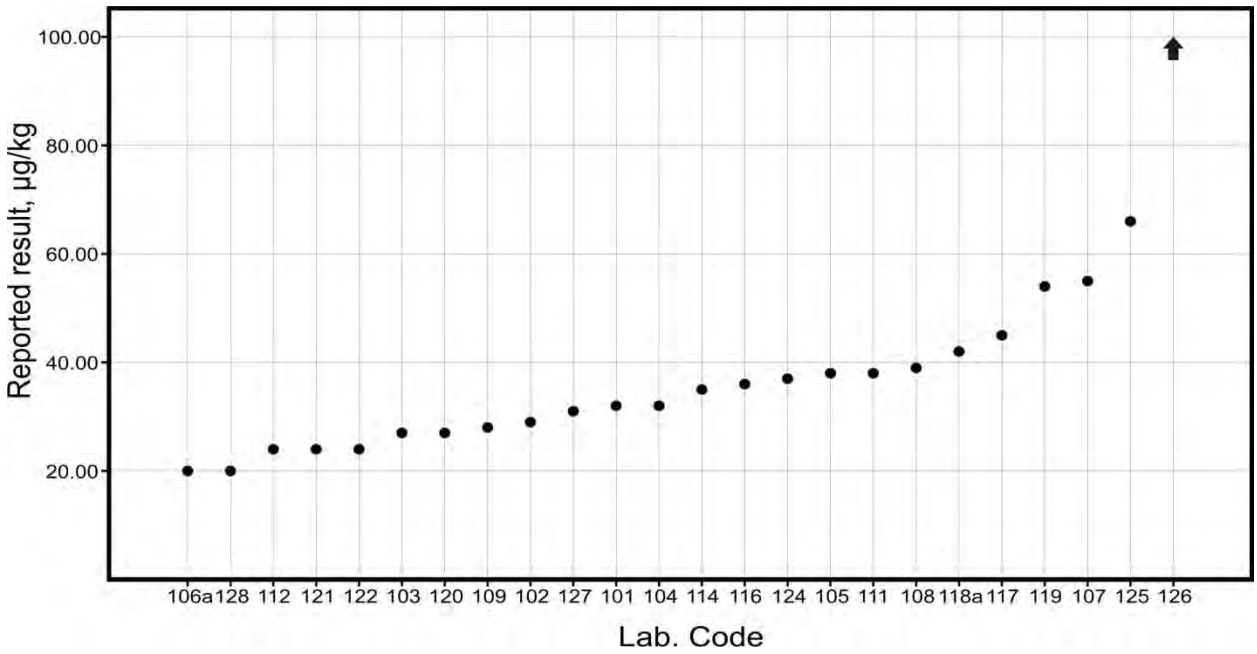

Figure 18: Seneciphylline results for sample **NCH**.

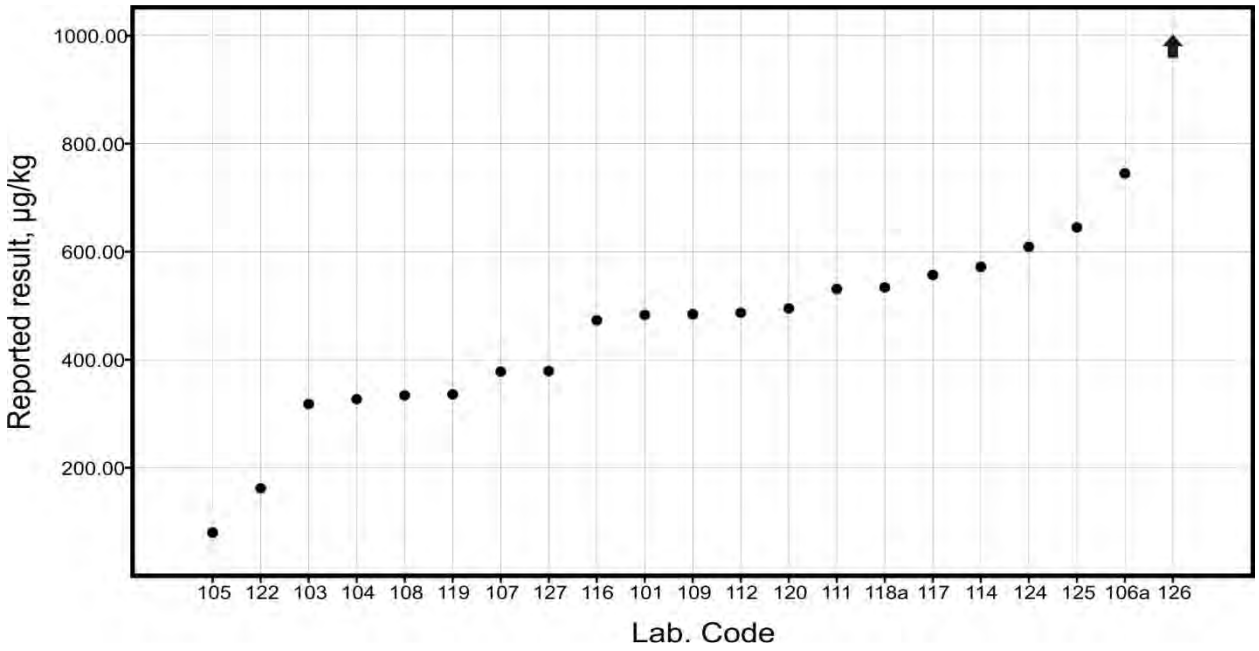

Figure 19: Seneciphylline results for sample **CPM.** Laboratories 102, 121 and 128 did not report. Due to the methodology used by laboratory 106a, their result is presented as total seneciphylline.

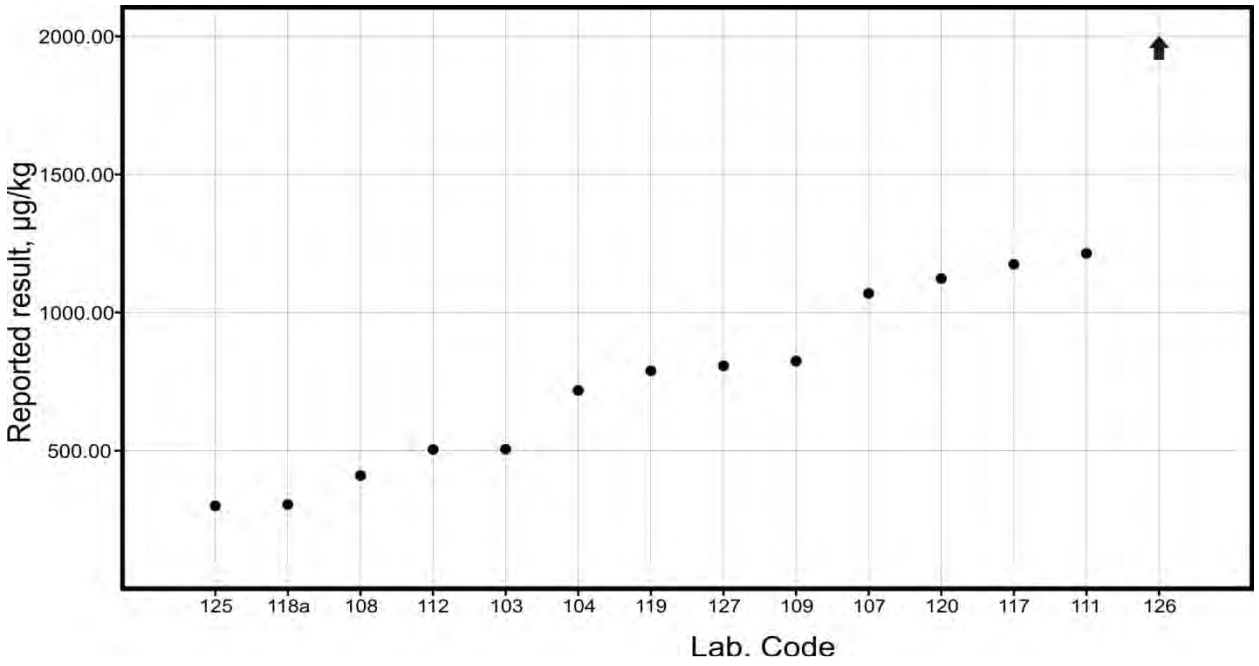

Figure 20: Seneciphylline-N-oxide results for sample **CPM**. Results are only indicated for laboratories which reported numeric figures.

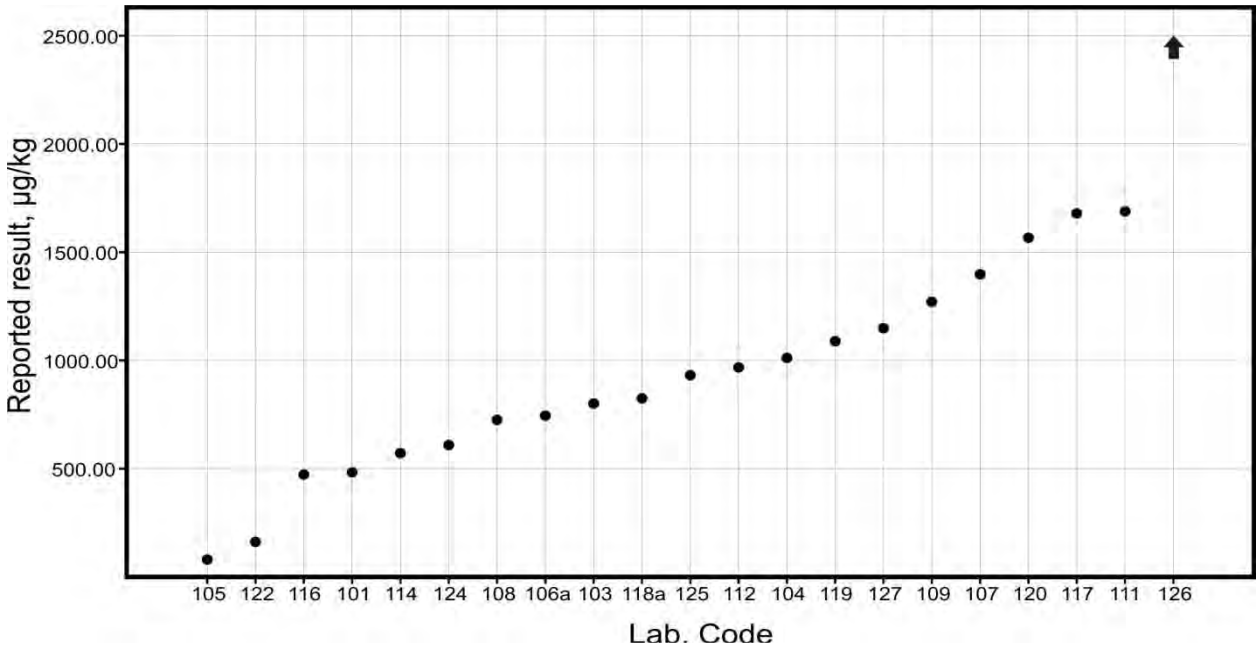

Figure 21: Total seneciphylline results for sample **CPM**. Laboratories 102, 121, 128 did not report results.

Table 6-8: Summary statistics for senecionine. **CPM**(NO) indicates senecionine-N-oxide content in the hay sample. **CPM**(total) - total content of senecionine and senecionine-N-oxide in the hay sample, expressed as senecionine equivalent

Sample		**STD**	**SNH**	**SAH**	**NCH**	**CPM**	**CPM**(NO)	**CPM**(total)
No. Results		24	24	24	23	21	13	21
Min	µg/kg	9.00	1.69	0.38	20.00	80.00	300.13	80.00
Max	µg/kg	2460.00	116.20	110.60	440.50	5096.80	8588.00	13291.40
x_m	µg/kg	238.9	19.3	18.8	33.9	484.5	1496.3	1307.5
x_r	µg/kg	225.5	19.0	19.2	41.1	636.1	1369.5	1387.1
X_{ref}	µg/kg	321.28	20.52	20.51	n/a	n/a	n/a	n/a
U_{ref}	µg/kg	8.55	0.55	0.55	n/a	n/a	n/a	n/a
s_{rob}	µg/kg	86.4	6.4	7.6	12.2	394.0	841.2	951.6
σ_p	µg/kg	60.99	4.51	4.51	n/a	n/a	n/a	n/a
NR /z/>2		n/a	5	5	n/a	n/a	n/a	n/a

x_m – median of results
x_r – robust mean of results
X_{ref} – assigned (nominal) value
U_{ref} – expanded uncertainty of assigned value
s_{rob} – robust standard deviation
σ_p – target standard deviation

Table 6-9: Results submitted by participants for senecionine
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

	Sample								
Lab. Code	**STD**, µg/kg	**SNH**, µg/kg	z(SNH)	**SAH**, µg/kg	z(SAH)	**NCH**, µg/kg	**CPM**, µg/kg	**CPM**(NO), µg/kg	**CPM**(total), µg/kg
101	351.00	12	-1.9	19	-0.3	31	825	n/a	825
102	256.80	18.11	-0.5	19.26	-0.3	44.56	n/a	n/a	n/a
103	211.37	15.94	-1.0	14.59	-1.3	30.94	464.31	970.24	1390.37
104	262.70	27.2	1.5	28.2	1.7	55.5	743.2	1678.2	2344.99
105	9.00	20	-0.1	23	0.6	50	85	<LOD	85
106a	210.00	26	1.2	27	1.4	30	669	SUM*	669
107	254.64	30.72	2.3	33.26	2.8	52.07	369.8	1776	2064.93
108	212.12	8.1	-2.8	9.4	-2.5	23.7	528	1440	1902.43
109	226.40	19.1	-0.3	16.8	-0.8	42	783.4	1514	2228.46
111	210.00	18.8	-0.4	12.1	-1.9	33.9	690.2	1670.1	2284.26
112	58.38	10.64	-2.2	8.91	-2.6	22.7	506.12	839.57	1307.46
114	293.38	21.73	0.3	22.55	0.5	47.09	914.96	n/a	914.96
116	297.17	25.64	1.1	27.93	1.6	55.93	890.09	n/a	890.09
117	9.02	19.93	-0.1	22.89	0.5	35.26	223.94	<LOD	223.94
118a	290.87	21.91	0.3	24.03	0.8	55.27	464.2	655.65	1090.00
119	100.80	19.9	-0.1	17	-0.8	45.1	190.4	158	341.21
120	245.00	14.9	-1.2	18.5	-0.4	41.6	746.9	2742	3364.05
121	247.68	15.4	-1.1	15.2	-1.2	29.6	n/a	n/a	n/a
122	222.60	17.6	-0.6	14.6	-1.3	31.8	359.5	n/a	359.5
124	269.25	22.64	0.5	22.6	0.5	48.08	1189.23	n/a	1189.23
125	101.61	1.69	**-4.2**	0.38	**-4.5**	<LOD	996.37	441.46	1417.73
126	2460.00	116.2	**21.2**	110.6	**20.0**	390.5	6480	20160	25722.06
127	266.73	19.56	-0.2	13.6	-1.5	37.14	610.27	1496.53	2038.66
128	232.70	13.83	-1.5	15.26	-1.2	39.65	n/a	n/a	n/a

The results are written as reported by the laboratories. SUM* - means, that laboratory uses the method involving conversion of senecionine-N-oxide to senecionine prior to quantification.

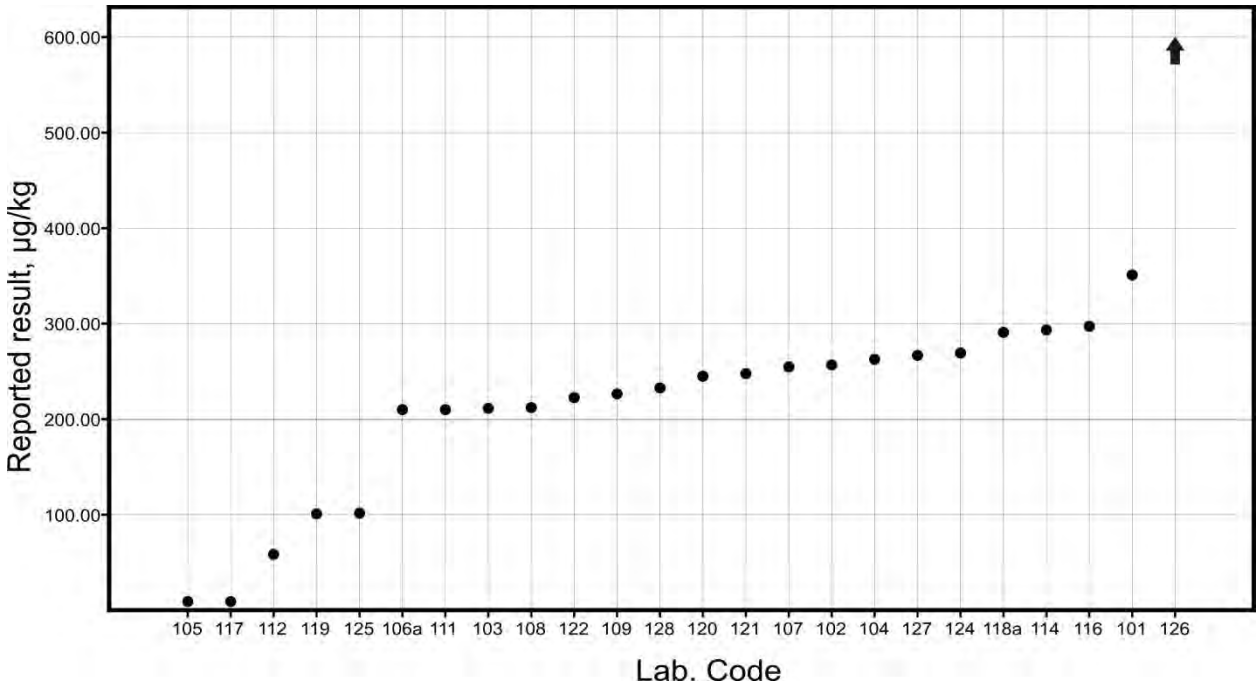

Figure 22: Senecionine results for sample **STD**.

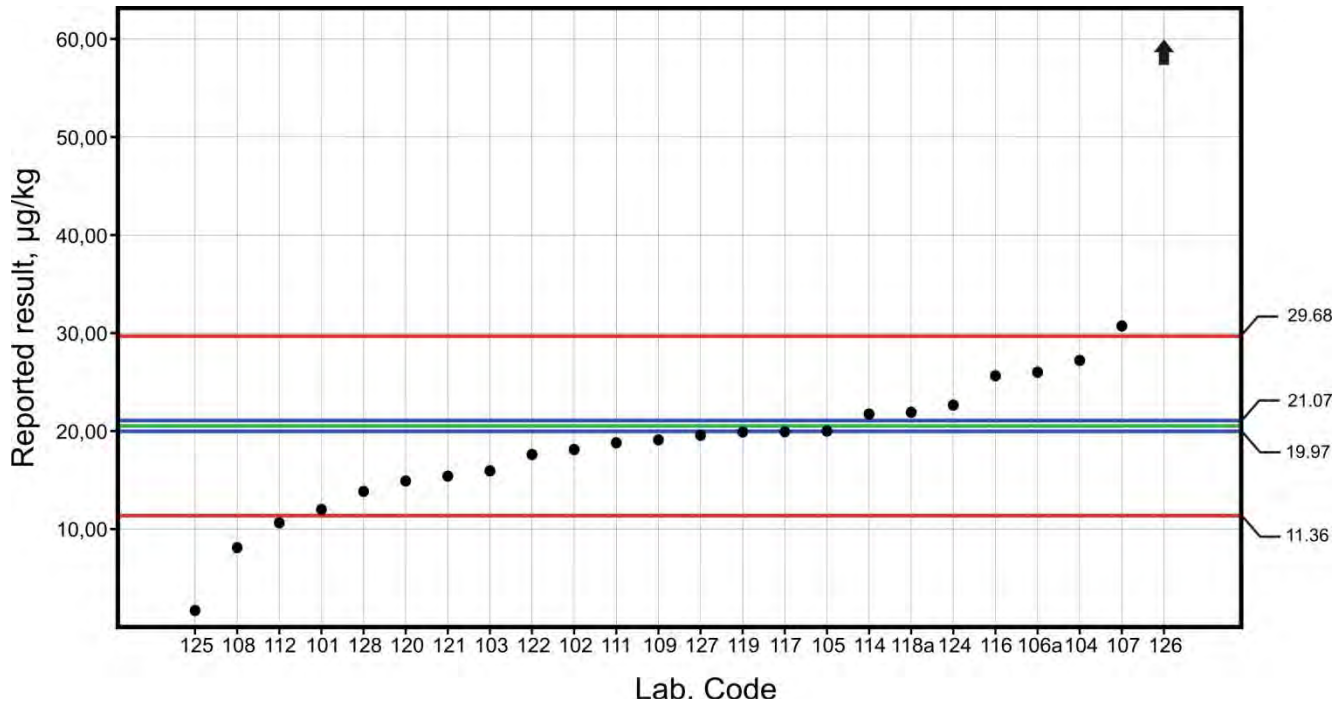

Figure 23: Senecionine results for sample **SNH**. The green line shows the assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria.

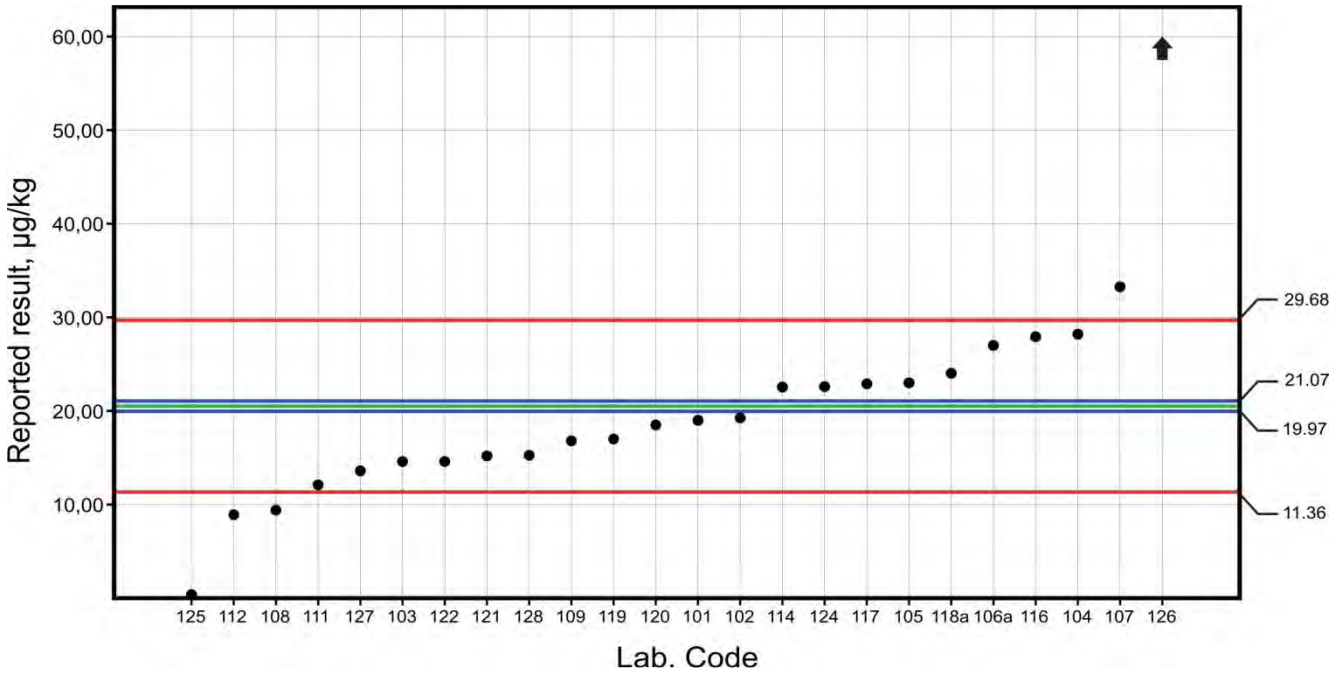

Figure 24: Senecionine results for sample **SAH**. The green line shows the assigned value, blue lines - expanded uncertainty of assigned value, red lines - result acceptance range for z-score criteria.

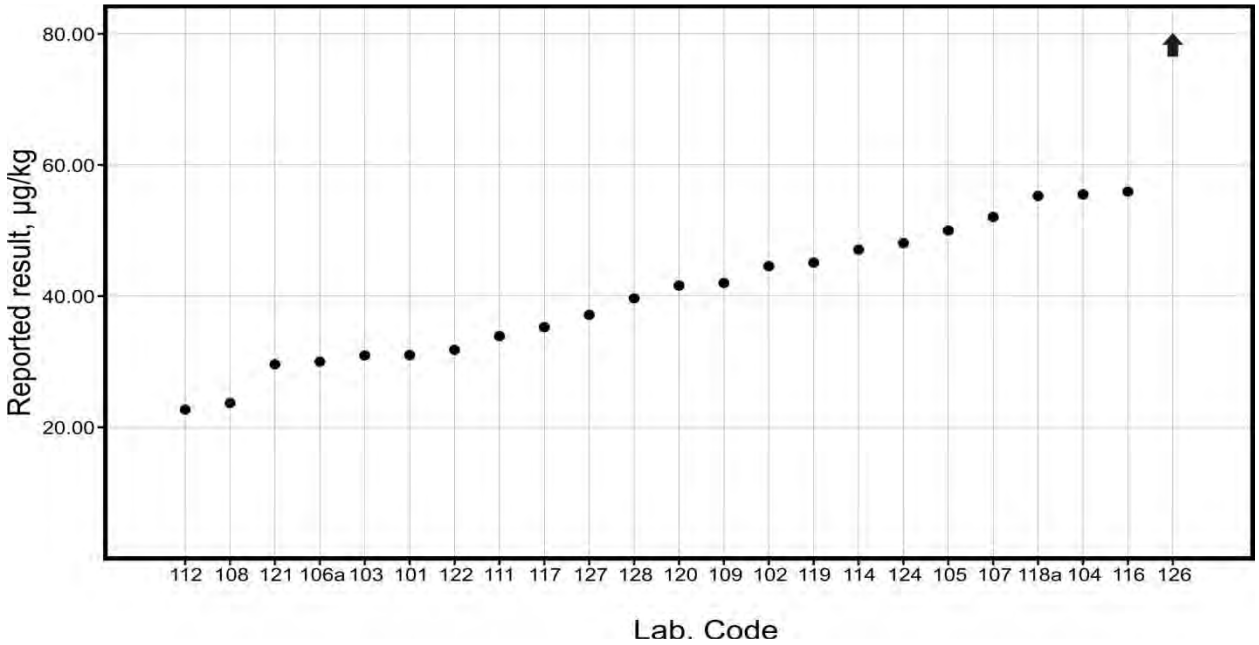

Figure 25: Senecionine results for sample **NCH**. Laboratory 125 did not detect senecionine in the samples.

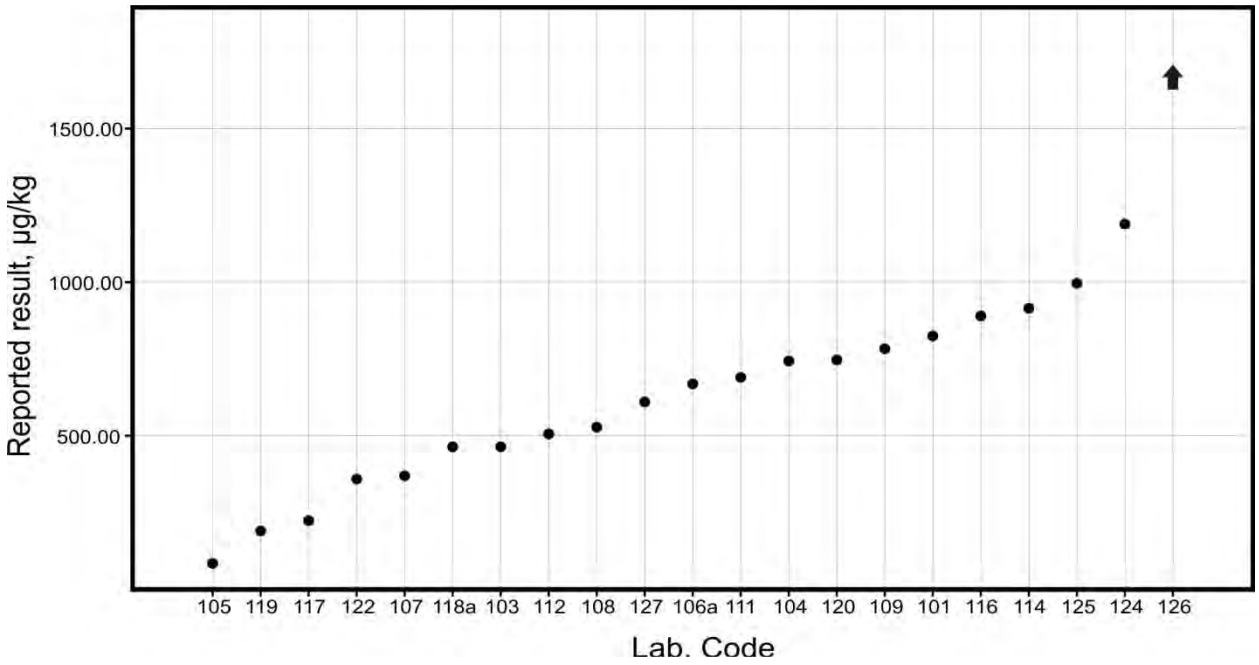

Figure 26: Senecionine results for sample **CPM** Laboratories 102, 121 and 128 did not report due to their methodology. The result of laboratory 106a is presented as total senecionine.

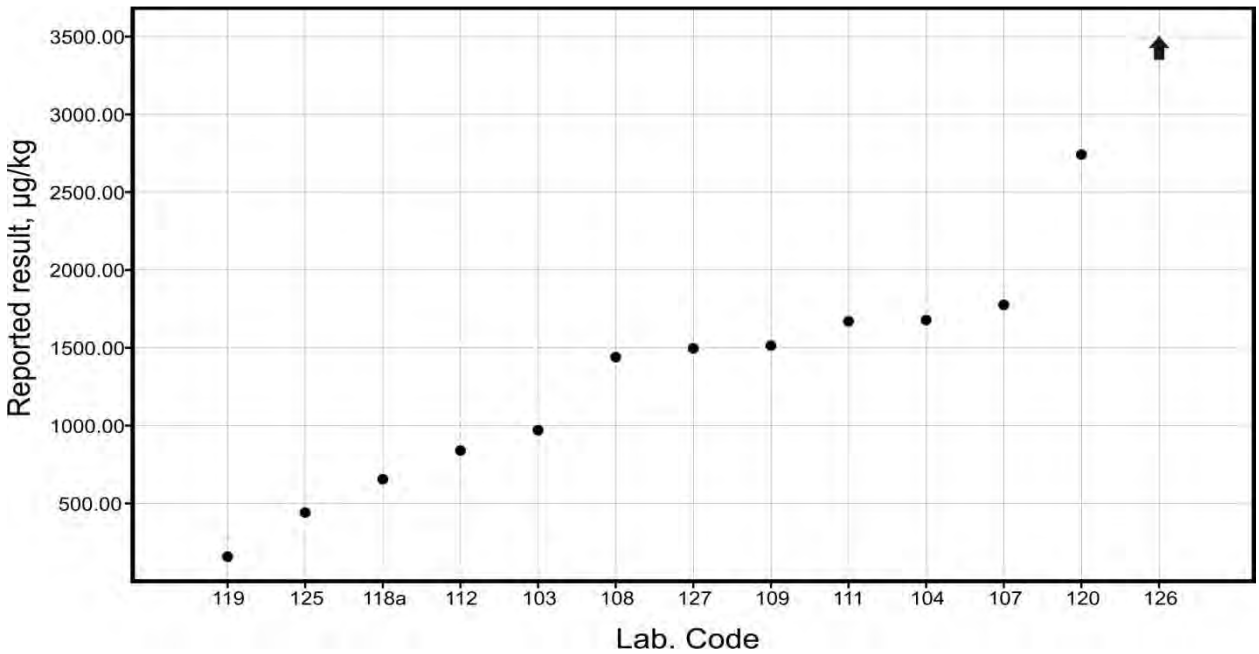

Figure 27: Senecionine-N-oxide results for sample **CPM**. Only laboratories which reported numeric figures are shown.

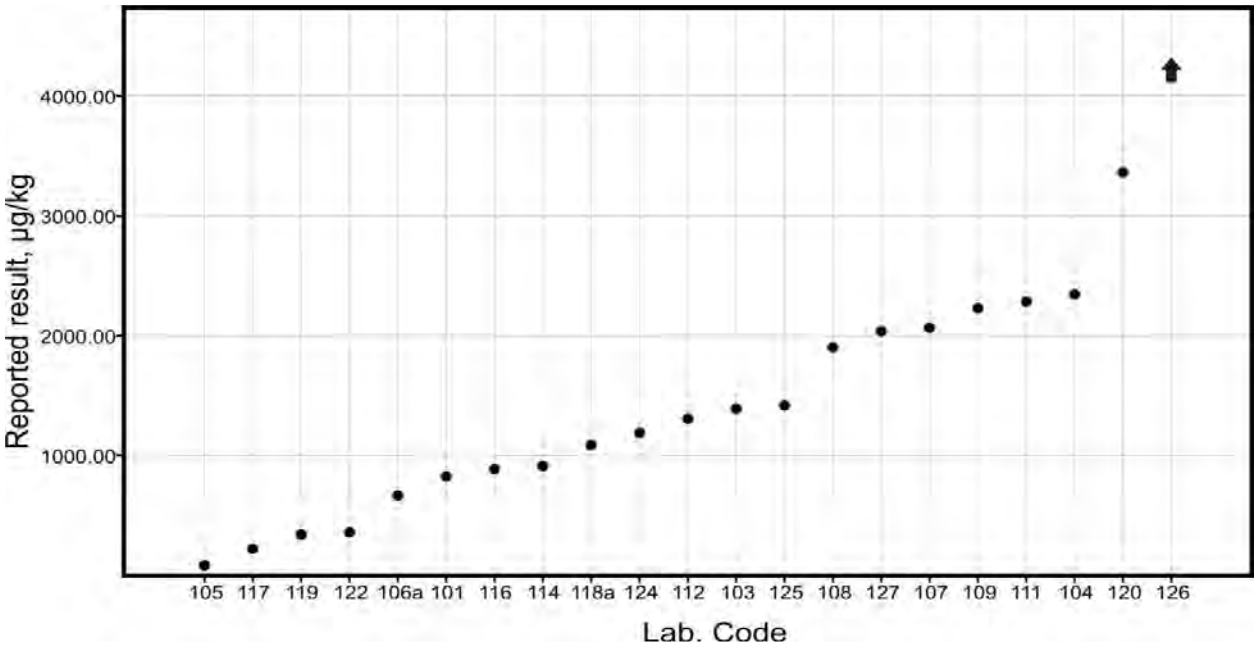

Figure 28: Total senecionine results for sample **CPM.** Laboratories 102, 121, and 128 did not report results.

Laboratory 125 used mainly self-isolated PAs for calibration. The relatively high number of unsatisfactory results for individual PAs reported by this laboratory suggests that the quality of their standard materials might be a cause for this observation. Laboratory 125 got however satisfactory z-scores for evaluation of total PAs in both samples. This might be explained by the fact of incorrect identification of PAs (**Tables 6-12, 6-13**).

Laboratory 126 submitted results several fold higher than the assigned values for samples. Aliquotation or calculation errors might be the cause for this, as these sources were identified as leading factors for such observations in other PTs.

The content of N-oxides in honey materials was reported to range from 'non detectable' to 50% of the base compound [12]. In this study N-oxides were however a minor contributor to the total PA content in honey. N-oxides are however an important contributor to the total PA content in the hay sample. This is illustrated in **Figure 29.**

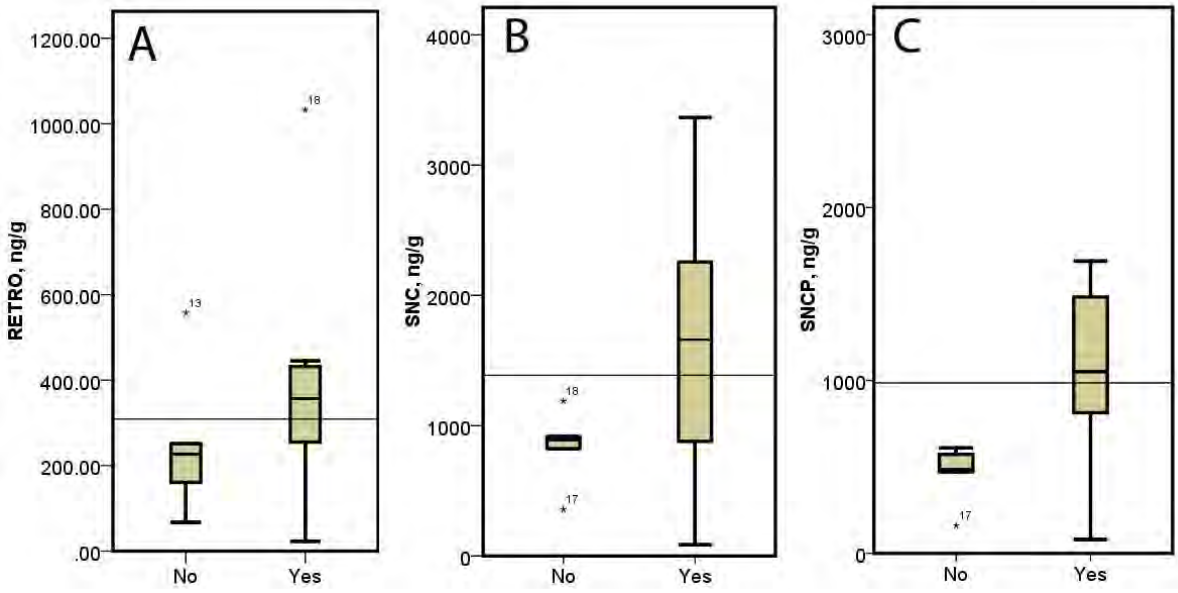

Figure 29: Results for analysis for total retrorsine (A), senecionine (B) and seneciphylline (C) in plant material for which N-oxides have been considered (Yes) or not considered (No). Horizontal lines indicate robust means.

6.3.2 Incorrect identification of PAs

Table 6-10: Results of participants identified as false positive

Lab. Code	Sample	Analyte	Result, µg/kg
112	**STD**	Retrorsine N-oxide	6.3
112	**STD**	Ridelliine	6.68
117	**SAH**	Lycopsamine	11.54
117	**SNH**	Senecionine-N-oxide	5.54
117	**SNH**	Seneciphilline-N-oxide	11.55
117	**SAH**	Seneciphilline-N-oxide	19.02
117	**CPM**	Senkirkine	12.11
119	**SNH**	Seneciphilline-N-oxide	6.88
121	**STD**	Senecionine-N-oxide	20.7
121	**STD**	Seneciphilline-N-oxide	38
125	**NCH**	Seneciphilline-N-oxide	13.82
125	**SAH**	Senkirkine	22.3
125	**CPM**	Lycopsamine	196.6

Table 6-11: Results of participants identified as false negative

Labcode	Sample	Analyte	Result, µg/kg
105	**CPM**	Seneciphilline-N-oxide	<LOD
105	**CPM**	Senecionine-N-oxide	<LOD
117	**CPM**	Senecionine-N-oxide	<LOD
125	**NCH**	Senecionine	<LOD
125	**NCH**	Lycopsamine	<LOD
127	**STD**	Retrorsine	<LOD
127	**SNH**	Retrorsine	<LOD
127	**SAH**	Retrorsine	<LOD
127	**NCH**	Retrorsine	<LOD

A number of laboratories had difficulties identifying compounds correctly in the samples. Due to the low probability of oxidation of PA bases to N-oxides it has been decided to put a threshhold of 5 µg/kg to all N-oxides detected in the standard solution and the spiked samples (**STD**, **SNH** and **SAH**). Other compounds detected by participants but being not spiked in to the samples were treated in the same way. It was more challenging to point out false negative results. For judging the correctness of identification the LOD/LOQ of the laboratory in question was considered. Thus the result of laboratory 118a for retrorsine in the sample **CPM** (< LOD) has not been judged false negative, because the laboratory declared a LOQ of 351 µg/kg for this substance, which was, however, significantly higher than median of results provided by participants (125.2 µg/kg).

The correct identification of the isomer pair intermedine/lycopsamine present in sample **NCH** was also difficult to assess. Laboratories using an alkaline mobile phase for HPLC separation were not able to separate these isomers, and therefore reported both (in e.g. 103, 111) as single result. Laboratory 122 did not use an alkaline mobile phase, but did not include lycopsamine into the scope of the method. This participant identified intermedine in the sample. Since one of the isomers has been identified it cannot be considered as false identification.

The summary of false identification is reported in the **Tables 6-12, 6-13**.

6.4 Evaluation of the questionnaire

6.4.1 Scope of the method

Thirty two different compounds were detect in the test material; on average 10 analytes were reported per laboratory. Some laboratories (111, 118a) indicated just the most relevant analytes, due to lack of sufficient reporting fields in the questionnaire. Four laboratories reported only the sum of PAs.

For analysing the relationship between the number of analytes in the scope of the method and the total PA content reported, participants have been clustered into 4 groups according to the number of PAs included in the method: 1-9, 10-15 and 16-20 as well as a group of laboratories who used a method that converts all retronecine/heliotridine-based PA esters as well as their corresponding N-oxides into the core structures, i.e., retronecine and/or heliotridine, resulting in a sum parameter for all PAs present.

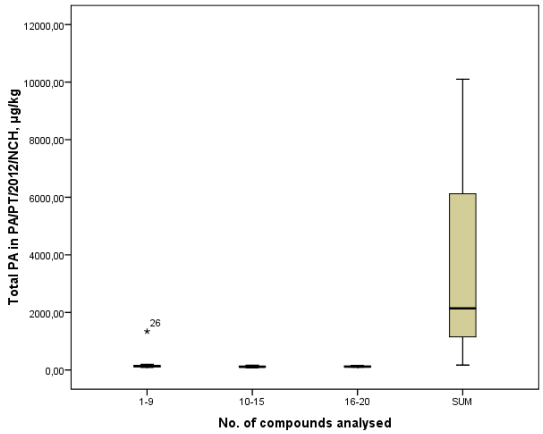

Figure 30: Effect on the amount of analytes in the scope of the method for naturally contaminated honey.

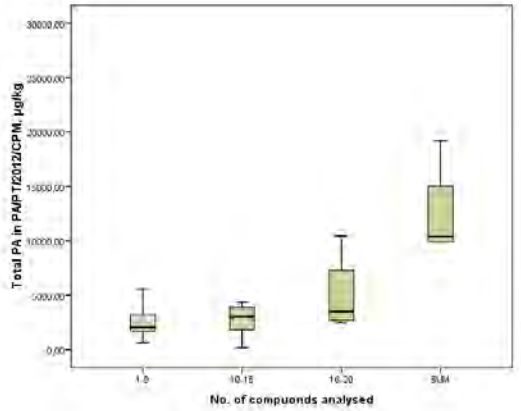

Figure 31: Effect on the amount of analytes in the scope of the method for contaminated hey.

Concerning spiked materials no significant difference was observed with respect to the number of analytes in the scope. This is due to the fact that a limited number of PAs was added, which were in the scope for the majority of laboratories. For naturally contaminated honey the relationship between the number of analytes in the method scope and the total content of PA's reported was not observed (**Figure 30**),

However for naturally contaminated hey a weak relationship exists between the total amount of PAs reported (**Figure 31)** and the number of PAs in the scope of the method used for determination.. Only *Senecio* spp. has been blend in to the hay and the dominant PAs such as retrorsine, senecionine, seneciphyline were quantified by most laboratories. More than half of the participants included the corresponding N-oxides. Other important PAs produced by S*enecio* spp. are erucifoline and the corresponding N-oxide [2] which have been detected by laboratory 111. Additionally laboratory 106a, identified acetylerucifoline, but due to a lack of standard materials it was not quantified.

As a matter of fact, lycopsamine and very small amounts of echimidine detected by a few laboratories suggest that the honey was collected from various PA producing plant species. Furthermore, it is remarkable that the sum parameter methods gave much higher results than the summing up of individually determines PAs, which indicates that not all PAs present have been quantified by the latter methods.

Another possibility is that the honey contained other substances which were also converted into retronecine, which is measured with the "sum parameter" methods. To come to a final conclusion, there is clear need for more standard substances to provide better coverage of relevant PAs.

6.4.2 Sample preparation

Extraction by simple agitation (shaker) has been found a sufficient technique for the extraction of analytes from hay (17 laboratories) and honey (22 laboratories). As an extraction solvent most laboratories used aqueous acid solutions (formic acid, sulphuric acid and hydrochloric acid). Solid phase extraction implementing SCX or mixed mode cation exchange sorbents were utilised by the majority of participants (17 for honey matrix, 15 for plant matrices) for clean-up. Double or more clean-up steps have been applied in several cases (lab 112, 118b). Participants, who used mass spectrometry compatible extraction solvents were able to do direct analysis without any further clean-up (103, 114, 120, 127 in case of honey). There is a clear relation between the amount of sample taken for analysis and the capacity of clean-up columns used; most participants using less than 5 g sample intake went for small (60mg/3ml) columns (107, 111, 112, 119, 122, 126, 128 in case of honey), and the ones using more than 5 g used larger columns. This tendency is even more pronounced in case of the hay sample; because of the higher concentrations present in the samples, most laboratories used a lower sample intake.

6.4.3 Detection and separation

Most laboratories that used HPLC for separation employed triple quadrupol mass spectrometers for detection/quantification, but there were some (117, 118a, 120) using high resolution instruments. At least two transitions per analyte were monitored; however, labs 117 and 120 used only one ion trace on high resolution instruments. This may result in insufficient identification, because of lack of structural data. Laboratory 118a however did additional fragmentation and measurement of the fragment ions to confirm the presence of analytes. For retronecinic and heliotridinic alkaloids the most common fragments were 94, 120 and 138, which are common for all of the PAs having a fused ring substructure. On the other hand laboratories 128, 105, 125, 108, 126, 122, 109, 101, 102, 104 made use of CO loss transitions, such as 336>308 (senecionine/integerrimine) for identification.

The highest diversity in methods was found for the determination of the sum parameter. Laboratories had very different approaches for the derivatisation of necinic bases before detection. 113 and 123 used classical silylation, 106b used heptrafluorobutyrilimidazole to produce bis-heptafluorbutyric esters of necinic bases. Lab 118b used pyrrolytical methylation of analytes with tetramethylammonium hydroxide, which took place in the GC injector, producing methyl ethers of necinic bases.

Reversed phase separation has been utilised by all laboratories using LC, most of them have used C18 narrow bore columns (≤2.1 mm i.d.). Gradient elution has been utilised by all participants. The majority of laboratories used an acidic mobile phase (17 participants). Alkaline mobile phase should give significantly more retention of analytes on the columns, however some of diastereomeric analytes (lycopsamine/intermedine) cannot be separated, and identified individually as reported by 111, 103.

DB-5 type GC columns can be used to detect silylated or heptafluorobutyrated necinic bases, however a WAX type column must be used to detect methyl ethers, as it was done by laboratory 118b.

6.4.4 Quantification

Participants have been asked to provide relevant information regarding quantification. The calibration range reported by participants varied from one order of magnitude, up to three orders of magnitude. Some participants indicated different calibration ranges for honey and hay. This could be linked to different levels of PAs occurring in the materials. The most common calibration range reported spanned two orders of magnitude. A majority of participants used matrix matched calibration (14 laboratories). External standard calibration was used by 8 participants, while the remaining ones made use of standard addition. A comparison of results for all of these laboratories is presented in figure 32. There is no clear difference between the calibration approaches and results obtained by laboratories in terms of accuracy, but standard addition produced the least spread of results.

Internal standards were utilised by 12 participants. The majority used heliotrine, stating that it was necessary to screen samples for presence of it in advance. Other compounds used as internal standards were D6-isoproturon, D1-retrorsine-bis-butyrate and D2-retrorsine-bis-butyrate. Effects of use of internal standards on the results is given in figure 33.

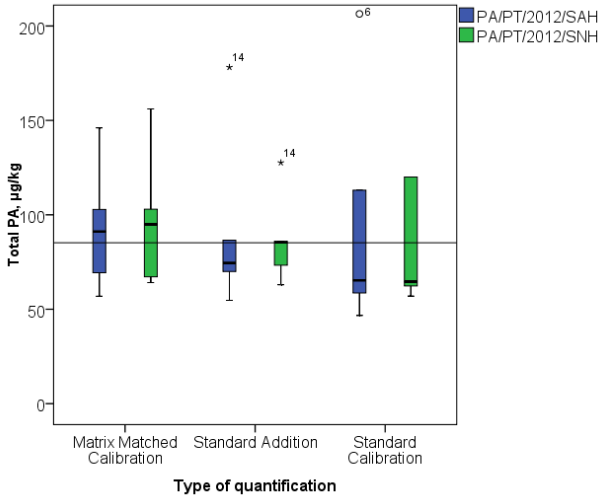

Figure 32: Effect of quantification type used on results for spiked honey samples.

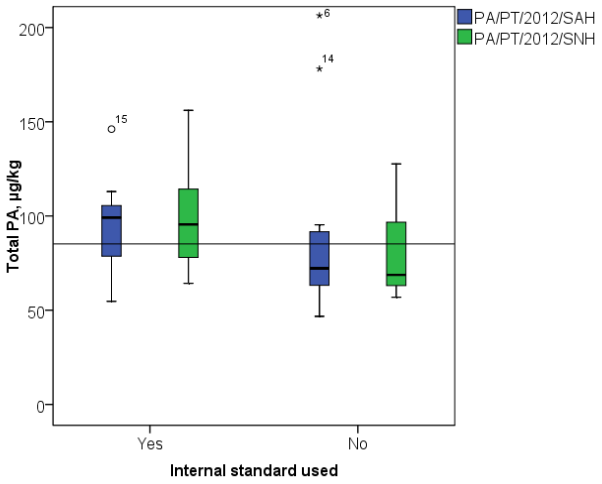

Figure 33: Effect of use of internal standard on results for spiked honey samples.

7 Conclusions

- Twenty-six out of 28 registered participants submitted results for this exercise. Two laboratories quantified PAs individually as well as used a technique that produced a sum parameter for all PAs.
- All laboratories used mass spectrometric detection.
- No significant differences were found between any of the quantification approach used: standard calibration, matrix matched calibration or standard addition. Results obtained using an internal standard (heliotrine in most cases) were not statistically different form the ones obtained without internal standard. Most of the participants using heliotrine noted that additional screening for it must be carried out in the sample before use.
- The results obtained by laboratories using a "sum parameter" approach and laboratories analysing PAs individually did not differ statistically for spiked samples. Both approaches were equally effective when a limited number of known PAs were present in the sample.
- The results for naturally contaminated samples obtained by the two approaches did not agree. Apparently, there is a lack of standard substances on the market to analyse for the majority of PAs produced by different plant species.

8 Acknowledgements

JRC-IRMM would like to acknowledge the support for carrying out this proficiency test:

Gerard van Bruchem, RIKILT – Institute of Food Safety (The Netherlands), for perfoming qNMR measurements on PAs.

Arne Dübecke, Quality Services International GmbH (Germany) - for allocation of honey test material.

Breitsamer und Ulrich GmbH & Co KG (Germany) – for allocation of blank honey test material.

Franz Ulberth and Beatriz de la Calle, JRC-IRMM for their support.

Håkan Emteborg JRC-IRMM for ampouling of the test samples.

www.ak-kreuzkraut.de - for kind permission to use the picture located on the title page.

All the participants for cooperation:

Table 8-1 : Participating laboratories

Company	Country
ICT Prague	Czech Republic
Central Institute for Supervising and Testing in Agriculture (Feed)	Czech Republic
Laboratoire SCL de Strasbourg	France
Quality Services International GmbH	Germany
LAVES Lebensmittelinstitut Braunschweig	Germany
Federal Institute for Risk Assessment	Germany
Technische Universität Braunschweig, Inst. f. Pharm. Biol.,	Germany
CVUA Freiburg	Germany
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL)	Germany
CVUA Stuttgart	Germany
Intertek Food Services GmbH	Germany
Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL)	Germany
Gehrlicher Pharmazeutische Extrakte GmbH	Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	Germany
LUA Sachsen Standort Chemnitz	Germany
LUFA Speyer	Germany
Landesuntersuchungsamt Rheinland-Pfalz, ILC Trier	Germany
Breitsamer und Ulrich GmbH & Co.KG	Germany
PhytoLab GmbH & Co KG	Germany
Technische Universität Dresden	Germany
Public Analyst's Laboratory	Ireland
Cork Institute of Technology	Ireland
The State Laboratory	Ireland
Health Science Authority	Singapore
Federal Office of Public Health, Food Safety Division	Switzerland
RIKILT - Institute of Food Safety	The Nederlands
NVWA	The Nederlands
Food and Environment Research Agency	United Kingdom

9 References

[1] M. Boppré, "The Ecological Context of Pyrrolizidine Alkaliods in Food, Feed and Forage: an owerview," *Food Additives and Contaminants,* vol. 28, no. 3, pp. 260-281, 2011.

[2] EFSA Panel on Contaminants in Food Chain, "Opinion of the scientific panel on contaminants in the Food Chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed," *The EFSA Journal,* vol. 447, pp. 1-51, 2007.

[3] EFSA Panel on Contaminants in Food Chain, "Scientific opinion on pyrrolizidine alkaloids in food and feed," *The EFSA Journal,* vol. 2406, no. 9(11), pp. 1-134, 2011.

[4] M. Kempf, M. Wittig, A. Reinhard, K. von der Ohe, T. Blacqiuere, K. P. Raezke, R. Michel, P. Schreier and T. Beuerle, "Pyrrolizidine Alkaloids in Honey: Comparicon of Analytical Methods," *Food Additives And Contaminants,* vol. 28, no. 3, pp. 325-347, 2011.

[5] International Organization of Legal Metrology, *Weights of Classes E1, E2, F1, F2, M1, M1-2, M2, M2-3, and M3 Part 1: Metrological and Technical Requirements,* 2004.

[6] "Directive 2002/32/EC of the European Parliament and of the Coucil of 7 May 2002 on Undesirable Substances in Animal Feed," *Official Journal of European Communities,* vol. L 140, pp. 10-21, 2002.

[7] ISO 13528:2005, " Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons".

[8] M. Thompson, "Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing," *Analyst,* vol. 125, pp. 385-386, 2000.

[9] I. J. Elbers, P. P. Mulder and M. de Nijs, "Interlaboratory Research Study for Pyrrolizidine Alkaloids in Animal Feed," *RIKILT Report 2012.507,* 2012.

[10] Analytical Methods Committee, *Robust statistics: a method of coping with outliers,* 2001.

[11] M. Thompson, S. L. Ellison and R. Wood, "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories," *Pure Appl. Chem.,* vol. 78, no. 1, pp. 145-196, 2006.

[12] A. Dübecke, G. Beckh and C. Lüllmann, "Pyrrolizidine Alkaloids in Honey and Pollen," *Food Additives and Contaminants,* vol. 28, no. 3, pp. 348-358, 2011.

[13] ISO 17043:2010, "Conformity assessment -- General requirements for proficiency testing".

[14] EU-RL. for. Pesticides, "General Protocol for EU Profitiency Tests for Pesticides Residues in Food and Feed," 2010.

10 Annexes

10.1 Particle size analysis for plant material

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Institute for Reference Materials and Measurements
Reference Materials Unit
Retieseweg 111, 2440 Geel, Belgium

Accredited according to ISO 17 025 (BELAC 268-TEST)

RM Unit Report of Analysis #2386

Particle Size Analysis by
LASER LIGHT DIFFRACTION
According to RM WI/0042

Requested by:	**J.Stroka**
Applicant sample identification:	TM-111109 TM-120320A TM-120320B (see attached request in annex 2)
RM Unit sample(s) ID:	**17043 to 17046**
Sample reception date (dd/mm/yyyy):	21/03/2012
Condition of the samples:	ok (powder)
Due date (dd/mm/yyyy):	30/03/2012

The particle size distribution is given in volume fraction of equivalent sphere diameter in µm. The uncertainties associated with the measurements can be found in Table 1 of page 2.

Results are valid only if **15% <Optical Concentration (C_{opt}) < 25%**

See attached graphs in the annex 1 (6 pages).

Number of pages: 9 (this page included) in 2 annexes

Date and signature
Responsible Analyst

22/03/2012

Date and signature
Laboratory responsible

22/03-12

Data files are stored in the following folders:

PSA computer (Registration number 01RG 2004 00772 04)
D:\PSA\request 2386

J: drive (for archiving)
J:\PSA\request 2386

Note: No feedback within 4 weeks is seen as acceptance of the report. Potential rests of samples will be destroyed after that period.

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Institute for Reference Materials and Measurements
Reference Materials Unit, Processing Lab
Retieseweg 111, 2440 Geel, Belgium

Institute for Reference Materials and Measurements

Annex to RM Unit Report of Analysis #2386 *Laser Light Diffraction*

(Annex 1)

Measurement date and time : 2012-03-22, 10:07:49

sediment, Sample ID: 17043, Replicate: a
Operator: MF. Tumba

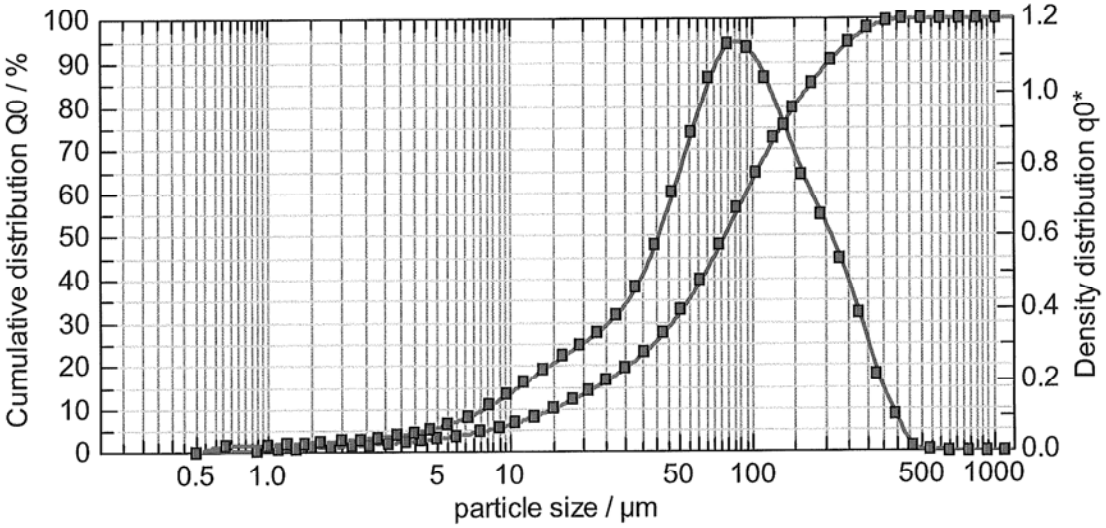
DEVICE: HELOS (H3335) & CUVETTE, R3+R5+R7 Dispersant: 2-Propanol
Dispersing method: 1200 Trigger condition: ref meas
Stirrer rate: 1200.00 Sonication duration: 0.00 s
Comment:

EVALUATION: *WINDOX 5.7.1.0, FREE* Optical concentration: **23.65 %**

RESULTS

x_{10} = 15.31 µm x_{16} = 24.44 µm x_{50} = 76.72 µm x_{84} = 168.71 µm x_{90} = 206.64 µm

The relative expanded uncertainties are given on page 2 in the main part of the Laser Diffraction report



Cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	0.41	3.70	2.23	15.00	9.79	61.00	39.62
1.10	0.57	4.30	2.57	18.00	11.88	73.00	47.72
1.30	0.73	5.00	2.97	21.00	13.85	87.00	56.31
1.50	0.87	6.00	3.55	25.00	16.35	103.00	64.51
1.80	1.09	7.50	4.49	30.00	19.35	123.00	72.49
2.20	1.35	9.00	5.49	36.00	22.96	147.00	79.49
2.60	1.60	10.50	6.55	43.00	27.39	175.00	85.31
3.10	1.89	12.50	7.99	51.00	32.74		

IMPORTANT: Results valid only if **15% < Optical Concentration < 25%**

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Retieseweg 111, 2440 Geel, Belgium

Institute for Reference Materials and Measurements

Annex to RM Unit Report of Analysis #2386 *Laser Light Diffraction*

(Annex 1)

Measurement date and time : 2012-03-22, 10:15:27

ediment, Sample ID: 17043, Replicate: b
Operator: MF. Tumba

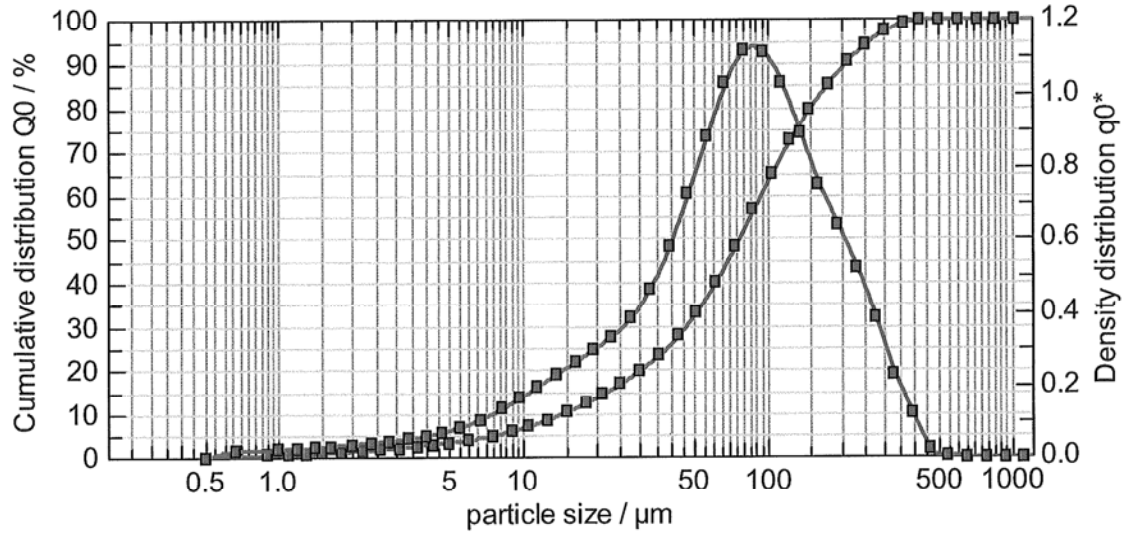
DEVICE: HELOS (H3335) & CUVETTE, R3+R5+R7 Dispersant: 2-Propanol
Dispersing method: 1200 Trigger condition: 10s
Stirrer rate: 1200.00 Sonication duration: 0.00 s
Comment:

EVALUATION: *WINDOX 5.7.1.0, FREE* Optical concentration: **24.05 %**

RESULTS

x_{10} = 14.69 µm x_{16} = 23.74 µm x_{50} = 76.06 µm x_{84} = 168.82 µm x_{90} = 207.99 µm

The relative expanded uncertainties are given on page 2 in the main part of the Laser Diffraction report



Cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	0.41	3.70	2.39	15.00	10.23	61.00	40.15
1.10	0.58	4.30	2.76	18.00	12.31	73.00	48.14
1.30	0.75	5.00	3.19	21.00	14.28	87.00	56.62
1.50	0.91	6.00	3.82	25.00	16.79	103.00	64.75
1.80	1.13	7.50	4.82	30.00	19.83	123.00	72.68
2.20	1.42	9.00	5.86	36.00	23.50	147.00	79.58
2.60	1.69	10.50	6.95	43.00	27.96	175.00	85.25
3.10	2.02	12.50	8.42	51.00	33.31		

IMPORTANT: Results valid only if **15% < Optical Concentration < 25%**

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Annex to RM Unit Report of Analysis #2386 *Laser Light Diffraction*

(Annex 1)

Measurement date and time : 2012-03-22, 10:35:12

sediment, Sample ID: 17044, Replicate: b
Operator: MF. Tumba

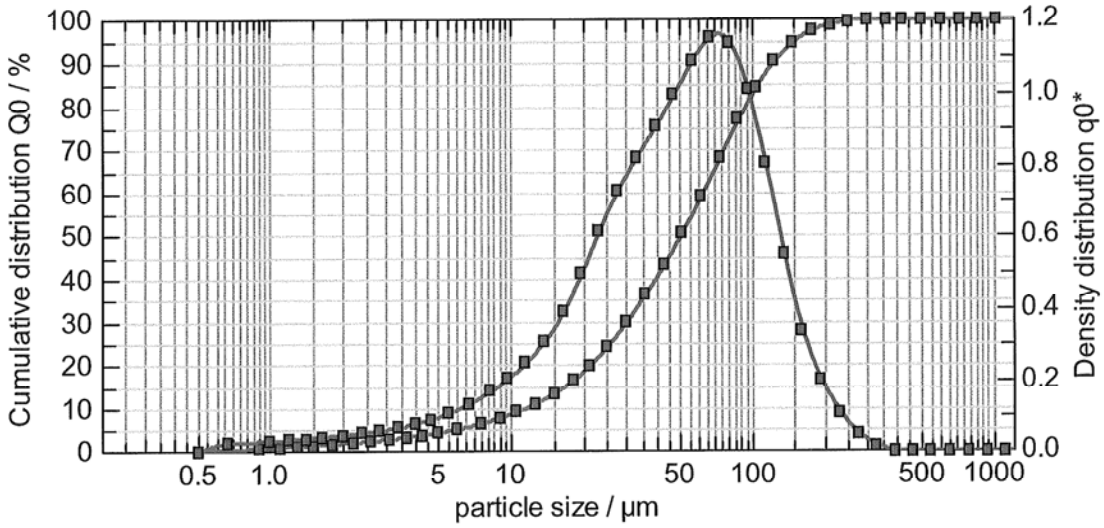
DEVICE: HELOS (H3335) & CUVETTE, R3+R5+R7 Dispersant: 2-Propanol
Dispersing method: 1200 Trigger condition: 10s
Stirrer rate: 1200.00 Sonication duration: 0.00 s
Comment:

EVALUATION: *WINDOX 5.7.1.0, FREE* Optical concentration: **20.86 %**

RESULTS

x_{10} = 11.59 µm x_{16} = 17.66 µm x_{50} = 50.12 µm x_{84} = 102.51 µm x_{90} = 1[illegible]72 µm

The relative expanded uncertainties are given on page 2 in the main part of the Laser Diffraction report



Cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	0.55	3.70	3.16	15.00	13.26	61.00	59.23
1.10	0.78	4.30	3.65	18.00	16.35	73.00	68.18
1.30	1.00	5.00	4.22	21.00	19.66	87.00	76.83
1.50	1.21	6.00	5.04	25.00	24.29	103.00	84.23
1.80	1.51	7.50	6.31	30.00	30.02	123.00	90.39
2.20	1.88	9.00	7.63	36.00	36.50	147.00	94.64
2.60	2.24	10.50	8.98	43.00	43.46	175.00	97.17
3.10	2.67	12.50	10.84	51.00	50.81		

IMPORTANT: Results valid only if **15% < Optical Concentration < 25%**

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Annex to RM Unit Report of Analysis #2386 *Laser Light Diffraction*

(Annex 1)

Measurement date and time : 2012-03-22, 10:45:41

sediment, Sample ID: 17046, Replicate: a
Operator: MF. Tumba

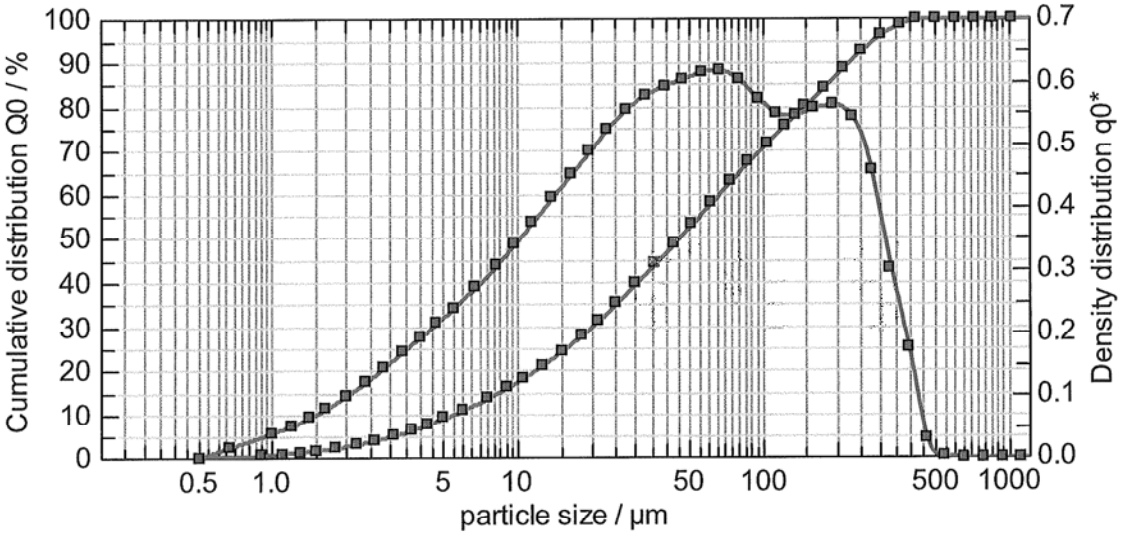
DEVICE: HELOS (H3335) & CUVETTE, R3+R5+R7 Dispersant: 2-Propanol
Dispersing method: 1200 Trigger condition: ref meas
Stirrer rate: 1200.00 Sonication duration: 0.00 s
Comment:

EVALUATION: *WINDOX 5.7.1.0, FREE* Optical concentration: **22.41 %**

RESULTS

x_{10} = 5.56 µm x_{16} = 9.07 µm x_{50} = 45.55 µm x_{84} = 174.72 µm x_{90} = 224.70 µm

The relative expanded uncertainties are given on page 2 in the main part of the Laser Diffraction report



Cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	0.43	3.70	6.29	15.00	24.27	61.00	57.82
1.10	0.77	4.30	7.55	18.00	27.84	73.00	62.61
1.30	1.14	5.00	8.94	21.00	31.10	87.00	67.19
1.50	1.54	6.00	10.83	25.00	35.05	103.00	71.38
1.80	2.16	7.50	13.47	30.00	39.44	123.00	75.61
2.20	3.02	9.00	15.89	36.00	44.01	147.00	79.83
2.60	3.89	10.50	18.17	43.00	48.57	175.00	84.04
3.10	4.99	12.50	21.00	51.00	53.04		

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Annex to RM Unit Report of Analysis #2386 *Laser Light Diffraction*

(Annex 1)

Measurement date and time : 2012-03-22, 10:51:20

sediment, Sample ID: 17046, Replicate: b
Operator: MF. Tumba

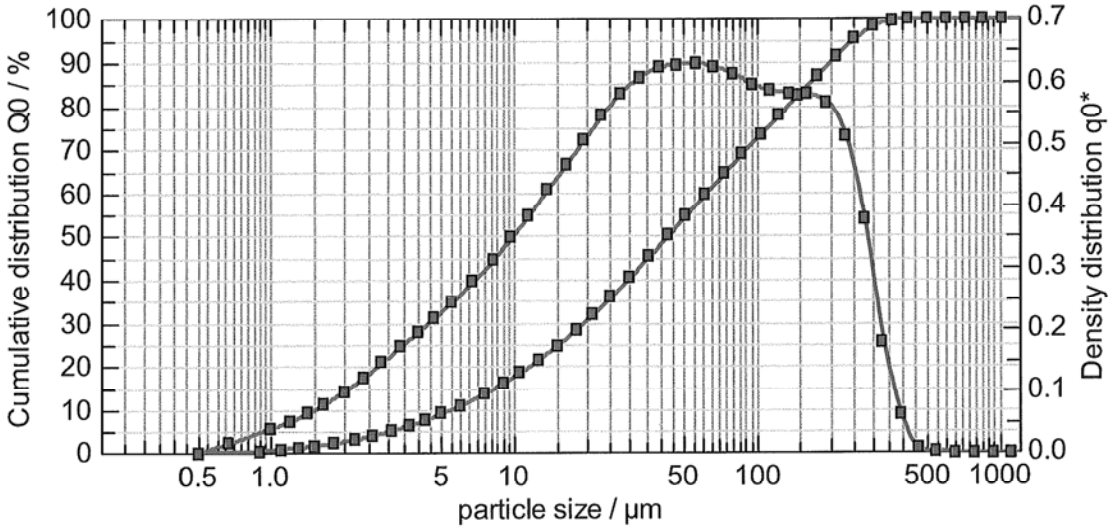
DEVICE: HELOS (H3335) & CUVETTE, R3+R5+R7 Dispersant: 2-Propanol
Dispersing method: 1200 Trigger condition: 10s
Stirrer rate: 1200.00 Sonication duration: 0.00 s
Comment:

EVALUATION: *WINDOX 5.7.1.0, FREE* Optical concentration: **21.99 %**

RESULTS

x_{10} = 5.52 µm | x_{16} = 8.95 µm | x_{50} = 42.91 µm | x_{84} = 157.10 µm | x_{90} = 199.99 µm

The relative expanded uncertainties are given on page 2 in the main part of the Laser Diffraction report



Cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
0.90	0.41	3.70	6.31	15.00	24.68	61.00	59.58
1.10	0.75	4.30	7.59	18.00	28.37	73.00	64.42
1.30	1.12	5.00	9.01	21.00	31.76	87.00	69.07
1.50	1.51	6.00	10.93	25.00	35.88	103.00	73.43
1.80	2.13	7.50	13.60	30.00	40.47	123.00	77.93
2.20	3.00	9.00	16.08	36.00	45.27	147.00	82.42
2.60	3.89	10.50	18.41	43.00	50.06	175.00	86.80
3.10	5.00	12.50	21.32	51.00	54.70		

IMPORTANT: Results valid only if **15% < Optical Concentration < 25%**

Annex 2

RM-Unit Request for Analysis # 2386

From: J. Stroka Unit FSQ Action: 0

Results due: 30/03/2012 and to be sent to J. Stroka

Sample ID	Sample description	Amount
17043	TM-111109	~ 10 g

Analyses	replicates	Comment
Particle size analysis	2	

Sample ID	Sample description	Amount
17044	TM-120320A	~ 10 g

Analyses	replicates	Comment
Particle size analysis	2	

Sample ID	Sample description	Amount
17046	TM-120320B	~ 10 g

Analyses	replicates	Comment
Particle size analysis	2	

21/03/2012 **Signature (applicant):** Vytautas Tamošiūnas

forward to: if mainly biotech: Philippe Corbisier; if mainly inorganic analysis:James Snell; if mainly PSA/Sieve/water analysis: Håkan Emteborg; if mainly organic analysis: Marta Dabrio; if engineering materials: Gert Roebben;if protein characterisation: I. Zegers if only isotopics: S. Richter; if nuclear chemistry: R. Jakopič; if mainly target preparation: G. Sibbens

Laboratory: Request received on 21/03-2012 **accepted by (signature)** .....

10.2 Homogeneity tests

Table 10-1

PA/PT/2012/NCH	Lycopsamine		Retrorsine		Senecionine		Seneciphylline	
Sample	Result a	Result b	Result a	Result b	Result a	Result b	Result a	Result b
TM110901_2	8.347	8.754	47.350	45.850	41.497	41.529	45.589	43.673
TM110901_23	8.310	8.674	46.750	45.750	40.010	42.760	45.677	44.715
TM110901_24	8.815	8.370	52.200	50.150	43.398	41.048	50.510	47.269
TM110901_25	8.327	8.246	50.500	49.200	41.913	42.267	47.036	47.370
TM110901_35	8.936	8.917	50.750	50.350	43.239	44.660	51.330	50.932
TM110901_37	8.598	8.825	50.100	44.350	42.319	42.253	48.077	43.178
TM110901_44	8.131	8.890	44.600	49.400	39.310	43.888	42.043	48.448
TM110901_5	8.627	8.704	48.500	43.300	44.003	42.254	46.801	42.157
TM110901_52	8.690	8.505	49.600	50.500	42.253	43.078	49.487	48.613
TM110901_54	8.630	8.515	51.050	48.700	42.736	41.702	49.867	48.528
mean =	8.591		48.448		42.306		47.065	
σ_t(%)=	0.220		0.220		0.220		0.220	
σ_t=	1.890		10.658		9.307		10.354	
s_x =	0.165		2.021		0.785		2.353	
s_w =	0.243		2.222		1.432		2.286	
s_s =	**0.000**		**1.271**		0.000		1.710	
0,3*σ_t=	**0.567**		**3.198**		2.792		3.106	
Result =	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**

Table 10-2

PA/PT/2012/CPM	Retrorsine		Senecionine		Seneciphylline	
Sample	Result a	Result b	Result a	Result b	Result a	Result b
TM-120404_38	414.585	411.000	1988.012	1920.000	1308.691	1270.000
TM-120404_3	415.170	400.995	2035.928	1900.498	1307.385	1144.279
TM-120404_40	422.886	408.367	1940.299	1922.311	1263.682	1304.781
TM-120404_42	425.150	389.055	1986.028	1840.796	1307.385	1263.682
TM-120404_56	398.414	391.566	1942.517	1877.510	1278.494	1275.100
TM-120404_57	415.187	396.432	1893.491	1952.428	1242.604	1298.315
TM-120404_58	411.298	413.174	1932.607	1966.068	1328.048	1277.445
TM-120404_73	395.792	391.391	1953.908	1881.882	1252.505	1211.211
TM-120404_7	396.825	412.115	1924.603	1976.167	1230.159	1340.616
TM-120404_85	389.610	393.393	1828.172	1921.922	1238.761	1241.241
mean =	404.620		1929.257		1269.219	
σ_t(%)=	18%		14%		15%	
σ_t=	74.183		279.607		196.260	
s_x =	8.510		27.310		26.820	
s_w =	10.940		59.090		50.630	
s_s =	**3.530**		**0.000**		**0.000**	
0,3*σ_t=	**22.255**		**83.882**		**58.878**	
Result =	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**

Table 10-3

PA/PT/2012/SNH	Retrorsine		Senecionine		Seneciphylline	
Sample	Result a	Result b	Result a	Result b	Result a	Result b
TM120514_2	18.135	19.361	18.231	18.181	37.614	37.837
TM120514_9	19.421	20.071	18.829	18.334	38.546	39.466
TM120514_13	20.646	19.666	18.833	19.182	39.480	38.557
TM120514_22	19.120	20.934	18.824	19.956	39.126	40.694
TM120514_28	19.492	19.012	19.590	17.829	40.062	38.713
TM120514_37	18.863	18.903	17.901	17.336	38.303	36.239
TM120514_40	19.650	18.188	19.063	17.317	40.472	36.763
TM120514_42	19.639	18.572	18.955	18.278	37.617	38.128
TM120514_52	19.262	17.813	18.871	16.748	38.916	34.947
TM120514_56	19.120	19.390	19.414	17.815	38.141	36.517
mean =	19.262		18.474		38.307	
σ_t(%)=	22%		22%		22%	
σ_t=	4.238		4.064		8.428	
s_x =	0.547		0.530		1.015	
s_w =	0.770		0.884		1.461	
s_s =	**0.053**		**0.000**		**0.000**	
0,3*σ_t=	**1.271**		**1.219**		**2.528**	
Result =	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**	**Pass**	**s_s < 0,3 σ_t**

Table 10-4

PA/PT/2012/SAH	Retrorsine		Senecionine		Seneciphylline	
Sample	Result a	Result b	Result a	Result b	Result a	Result b
TM120608_14	18.586	18.155	18.586	16.907	38.934	37.464
TM120608_18	18.395	19.136	17.505	20.393	38.075	41.462
TM120608_22	20.179	17.277	18.992	17.468	40.062	36.082
TM120608_36	18.471	17.734	17.317	18.033	36.558	35.967
TM120608_3	18.576	18.361	17.892	17.681	38.033	37.694
TM120608_54	17.373	17.604	17.765	18.588	36.217	38.061
TM120608_60	18.038	19.037	19.202	18.553	38.113	38.556
TM120608_69	18.345	17.829	18.924	18.507	39.006	36.142
TM120608_52	19.168	18.498	19.856	20.081	41.482	44.019
TM120608_65	19.370	19.384	19.468	19.584	42.692	43.464
mean =	18.476		18.565		38.904	
σ_t(%)=	22%		22%		22%	
σ_t=	4.065		4.084		8.559	
s_x =	0.513		0.781		2.295	
s_w =	0.759		0.876		1.560	
s_s =	**0.000**		**0.475**		**2.012**	
0,3*σ_t=	**1.219**		**1.225**		**2.568**	
Result =	**Pass**	**s_s < 0,3*s**	**Pass**	**s_s < 0,3*s**	**Pass**	**s_s < 0,3*s**

s_s – between-sample variance
s_x – standard deviation from averages
s_w – analytical or within-sample variance
σ_{tl} – allowable between-sample variance

10.3 Stability test

Table 10-5

PA/PT/2012/**STD**	Retrorsine			Senecionine			Seneciphylline		
	Storage			Storage			Storage		
Time, weeks	RT	+4	-20	RT	+4	-20	RT	+4	-20
0	11.5	11.5	11.5	12.1	12.1	12.1	24.7	24.7	24.7
0	11.8	11.8	11.8	12.8	12.8	12.8	24.6	24.6	24.6
0	12.9	12.9	12.9	13.6	13.6	13.6	24.8	24.8	24.8
4	12.1	12	12.8	12.5	11.8	12.3	25	25	23.5
4	11.9	12.4	13	11.4	12.3	13.4	24.1	24.4	26.3
8	13.0	12.8	11.9	13.3	12.5	12	24	24.6	23.7
8	11.5	11.6	12.5	12	12.1	13	23.4	23.8	25.2
12	12.3	12.4	13.1	12.1	12.6	12.8	23.7	24	24.7
12	12.1	11.4	11.9	12	11.5	12.3	24.3	22.8	25.6
12	11.0	12.1	11.9	11.3	11.8	12.4	21.9	23.1	25.4
Slope	-0.016	-0.008	0.006	-0.068	-0.063	-0.032	-0.12	-0.117	0.034
Intercept	12.108	12.137	12.294	12.719	12.688	12.86	24.79	24.88	24.688
CI(slope) P=0.95	0.099	0.085	0.094	0.107	0.082	0.083	0.105	0.072	0.132
Conclusion	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Instability**	**Instability**	**Stable**
$\Delta_{(0\text{-}12)}$, %	n/a	n/a	n/a	n/a	n/a	n/a	5.668	5.66802	n/a

Table 10-6

PA/PT/2012/**SNH**	Retrorsine			Senecionine			Seneciphylline		
	Storage			Storage			Storage		
t, weeks	RT	+4	-20	RT	+4	-20	RT	+4	-20
0	21.2	21.2	21.2	21.0	21.0	21.0	38.3	38.3	38.3
0	21.6	21.6	21.6	21.5	21.5	21.5	40.4	40.4	40.4
0	20.6	20.6	20.6	21.0	21.0	21.0	39.3	39.3	39.3
4	22.9	21.0	21.3	23.2	21.8	21.4	40.7	39.8	38.4
4	21.6	20.8	20.8	23.5	22.2	21.1	39.8	41.1	37.6
8	23.8	21.1	22.2	23.9	20.9	24.2	42.5	39.5	41.6
8	20.8	21.7	21.4	20.7	23.4	21.9	38.5	39.0	39.7
12	23.0	20.8	22.0	24.0	20.7	23.6	44.4	39.1	40.6
12	21.6	22.2	21.1	22.3	22.8	21.6	40.7	38.7	40.1
12	21.5	20.1	21.8	22.8	22.9	22.9	38.0	40.3	42.3
Slope	0.072	0.002	0.052	0.126	0.0795	0.149	0.134	0.02	0.171
Intercept	21.420	21.106	21.086	21.627	21.35	21.11	39.47	39.68	38.8
CI(slope) P=0.95	0.156	0.085	0.071	0.177	0.142	0.139	0.301	0.135	0.19
Conclusion	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
$\Delta_{(0\text{-}12)}$, %	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 10-7

PA/PT/2012/**SAH**	Retrorsine			Senecionine			Seneciphylline		
	Storage			Storage			Storage		
t, weeks	RT	+4	-20	RT	+4	-20	RT	+4	-20
0	19.8	19.8	19.8	20.9	20.9	20.9	39.5	39.5	39.5
0	19.9	19.9	19.9	19.9	19.9	19.9	38.2	38.2	38.2
0	18.2	18.2	18.2	17.3	17.3	17.3	35.8	35.8	35.8
4	18.7	18.8	18.1	18.4	18.0	17.2	35.1	36.3	33.4
4	17.7	17.4	18.3	17.4	17.1	17.6	34.1	34.2	34.5
8	18.6	18.1	20.7	18.1	18.2	21.6	35.7	34.4	39.8
8	19.5	18.3	21.6	18.6	17.9	20.6	38.8	34.7	41.0
12	20.2	19.1	18.8	20.4	19.5	18.0	39.4	37.0	36.2
12	20.0	16.8	18.4	19.8	16.8	18.1	37.6	32.3	36.9
12	18.9	18.1	19.0	19.5	17.6	19.8	37.3	35.1	39.1
Slope	0.047	-0.1	0.008	0.049	-0.099	0.009	0.066	-0.247	0.077
Intercept	18.870	19.037	19.224	18.73	18.911	19.04	36.75	37.23	36.982
CI(slope) P=0.95	0.133	0.137	0.19	0.196	0.199	0.261	0.301	0.268	0.39
Conclusion	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
$\Delta_{(0\text{-}12)}$, %	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 10-8

PA/PT/2012/**NCH**	Retrorsine			Senecionine			Seneciphylline			Lycopsamine		
	Storage			Storage			Storage			Storage		
t, weeks	RT	+4	-20	RT	+4	-20	RT	+4	-20	RT	+4	-20
0	44.8	44.8	44.8	35.9	35.9	35.9	35.2	35.2	35.2	11.0	11.0	11.0
0	44.8	44.8	44.8	39.3	39.3	39.3	35.5	35.5	35.5	11.4	11.4	11.4
0	44.5	44.5	44.5	40.2	40.2	40.2	35.4	35.4	35.4	10.9	10.9	10.9
4	46.0	47.0	45.3	40.5	38.4	40.6	36.1	37.1	36.5	11.3	11.1	10.7
4	43.7	44.8	43.8	35.6	38.3	37.5	35.4	36.1	35.6	10.4	10.8	11.1
8	48.4	44.6	46.1	41.3	38.8	38.8	37.3	37.2	34.3	12.3	11.2	11.2
8	44.3	45.1	45.3	36.3	37.8	37.5	30.7	36.1	35.1	11.2	10.9	11.7
12	47.4	48.2	49.2	39.0	40.3	40.6	36.1	36.9	37.5	11.4	11.7	12.4
12	47.2	43.4	46.6	38.1	37.7	36.9	33.9	35.8	35.0	11.0	10.8	10.7
12	43.7	43.9	44.3	34.7	37.7	35.9	31.7	35.8	35.0	10.3	10.7	10.5
Slope	0.137	0.021	0.177	-0.08	0.007	-0.068	-0.141	0.065	0.012	-0.032	-0.004	0.017
Intercept	44.645	44.97	44.403	38.57	38.39	38.72	35.574	35.71	35.436	11.145	11.078	11.064
CI(slope) P=0.95	0.245	0.231	0.205	0.367	0.212	0.289	0.31	0.103	0.143	0.092	0.048	0.088
Conclusion	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
$\Delta_{(0\text{-}12)}$, %	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 10-9

PA/PT/2012/**CPM**	Retrorsine			Senecionine			Seneciphylline		
	Storage			Storage			Storage		
t, weeks	RT	+4	-20	RT	+4	-20	RT	+4	-20
0	394.1	394.1	394.1	1960.4	1960.4	1960.4	1316.8	1316.8	1316.8
0	402.6	402.6	402.6	1978.1	1978.1	1978.1	1272.4	1272.4	1272.4
0	407.3	407.3	407.3	2051.5	2051.5	2051.5	1318.1	1318.1	1318.1
4	379.0	393.4	388.9	2000.0	2061.8	2033.7	1310.0	1374.5	1349.2
4	370.4	395.0	354.5	1837.1	2110.0	1787.5	1350.5	1260.0	1191.7
8	387.7	389.9	392.3	1978.1	2003.9	2055.3	1292.2	1293.2	1393.3
8	392.8	393.2	392.4	1994.0	2035.9	1972.1	1316.1	1337.3	1324.7
12	410.0	377.9	391.3	2079.6	1784.6	1907.6	1482.6	1216.4	1278.3
12	365.8	384.3	380.8	1888.7	2015.9	2027.7	1212.7	1330.7	1276.0
12	406.6	385.8	392.4	2028.0	1939.0	1931.9	1338.7	1289.4	1271.3
Slope	-0.291	-1.493	-0.663	1.323	-7.617	-1.4	2.824	-1.869	-0.589
Intercept	393.371	401.314	393.64	1971.6	2039.82	1979	1304.07	1312.09	1302.71
CI(slope) P=0.95	2.514	0.648	2.23	11.56	12.943	13.077	10.776	7.02	8.735
Conclusion	**Stable**	**Instability**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
$\Delta_{(0\text{-}12)}$, %	n/a	4.64916	n/a	n/a	n/a	n/a	n/a	n/a	n/a

10.4 Opening of registration

EUROPA > European Commission > JRC > Search IRMM Internet
IRMM > EU Reference Laboratories > EURL mycotoxins > Interlaboratory comparisons
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Proficiency Test on

Determination of Pyrrolizidine Alkaloids in Honey and Materials of Plant Origin

Institute for Reference Materials and Measurements organises a proficiency test on determination of pyrrolizidine alkaloids in honey and materials of plant origin.

The objective of this study is to evaluate laboratories' capability to determine the analytes. The study also aims at identifying methodological and/or analyte specific trends in the analysis.

Test materials

One standard solution (of unknown content of PAs. The identity of the PAs will be indicated)

Two honey samples (of unknown PA content)

One plant material (of unknown PA content)

General outline

Participation is free of charge. Participants commit themselves to submit results within the deadlines.

Please note that for evaluation of methodological and/or analyte specific trends, it is crucial to have detailed information on the methodology used.

Registration

To register please follow the link (PA_PT_Registration_distributed.pdf [498Kb]), and send the completed PDF form to vytautas.tamosiunas@ec.europa.eu.

Registration deadline	Sample dispatch	Reporting of results	Report
28 February 2012	End of March 2012	6 weeks from sample dispatch	

News archive
- Environmental analysis
- Nuclear research
- Reference materials and measurements
- Food, biotechnology and health

IRMM catalogue
TrainMiC
ERM
EURL
EUFRAT

10.5 Accompanying letter

Please report all requested results and answer the questionnaire provided with this letter. This will help us to gain as much as possible methodological information that can be evaluated in the report.

Print out the final pdf and return the signed and stamped report sheet NOT later than **10th August 2012** to:

Vytautas Tamosiunas
JRC-IRMM-FSQ
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 852
FAX: +32-14-571 783
E-mail: vytautas.tamosiunas@ec.europa.eu

In case of questions please do not hesitate to contact me.

Vytautas Tamosiunas

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Joerg Stroka

10.6 . Acknowledgement of receipt form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Food Safety and Quality Unit

Geel, 26th June 2012

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:

Institute:

Address:

Laboratory code:

NOTE: store all materials in a refrigerator at +4 °C!

Please ensure that the items listed below have been received undamaged, and then check the relevant statement:

Parcel reception date:

Matrix	Sample code	OK	Damaged
Ampoule with standard solution	PA/PT/2012/STD	☐	☐
Natural honey	PA/PT/2012/NCH	☐	☐
Natural honey	PA/PT/2012/SNH	☐	☐
Artificial honey	PA/PT/2012/SAH	☐	☐
Plant material	PA/PT/2012/CPM	☐	☐

Signature/Stamp: _____

Please fax and e-mail the completed form to:

Vytautas Tamosiunas
EC-JRC-IRMM
Food Safety and Quality Unit
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 852
FAX: +32-14-571 783
E-mail: **vytautas.tamosiunas@ec.europa.eu**

Submit by Email

Print Form

10.7 Questionnaire and instructions

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Food Safety and Quality Unit

Geel, 26th June 2012

Questionnaire for the PT on Pyrrolizidine Alkaloids (PA's) in Honey and Material of Plant Origin

Dear Participant

Please note that we need electronic version of the document. Do not print, scan and then send the form, as it's not possible to extract data from it. Please use "submit by e-mail button, at the end of document. As we need confirmation, please use "Print Form" button, sign, and fax it to us.

Instructions:

In the form You will be asked to calculate total concentration for all PA's quantified and express it as retrorsine equivalent. Please use provided formula:

$$c_{(total\_PA)} = \sum \frac{c_{(PA)i}}{Mr_{(PA)i}} \times 351.39$$

c(total_PA's) - total amount of PA's in the sample expressed as retrorsine equivalent (µg/kg)
c(PA)i - concentration of PA quantified in the sample (µg/kg)
Mr(PA)i - molecular mass of PA quantified in the sample
351,39 - molecular mass of retrorsine

All results must be reported with precision of two decimals.

Results which are below Your LOD, must be reported as: "**<LOD**", the analytes, which are detected, but cannot be quantified, should be reported "**<LOQ**"

For sample preparation techniques as well as detection techniques, please fill only the field which are relevant for Your method. If You do not see proper choice in combo boxes, please do not hesitate to use "other" fields. The information we collect is confidential and is necessary to identify the possible methodological trends.

The **deadline** for analysis and reporting the results is **10 August 2012**

Section 1: General Information

Lab Code

Laboratory Name:

Address:

City: Postal Code: Country: Albania

Measurement responsible:

e-mail: Tel. Nr.:

Date

Signature/ Stamp:

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General method information for honey analysis

Do You detect individual analytes with this method or "Sum Parameter"?

How many Years have You been analyzing honey for PA's?

Is the method applied in routine analysis?

If "yes" how many samples do You analyze per Year?

Is the method accredited according to ISO 17025?

Sample preparation for honey analysis

Amount of sample taken for the analysis in grams:

Extraction technique used: Other:

Extraction solvent: Other:

* - please indicate sorbents, drying agents used in "other" field

** - please indicate concentrations of solutions used in "other" field

Sample cleanup technique: Other:

SPE sorbent type: Other:

SPE column form factor: Other:

SPE column manufacturer: Other:

LLE solvents:

Do You reduce N-Oxides during sample preparation:

Derivatization for PA-NO > PA-base reduction: Other:

Derivatization prior detection: Other:

Additional comments on sample preparation

General method information for plant material

Do You detect individual analytes with this method or "Sum Parameter"?

How many Years have You been analyzing plant materials for PA's?

Is the method applied in routine analysis?

If "yes" how many samples do You analyze per Year?

Is the method accredited according to ISO 17025?

Sample preparation for plant material analysis

Amount of sample taken for the analysis in grams:

Extraction technique used: Other:

Extraction solvent: Other:

* - please indicate concentrations of the solutions used in the "other" field

Sample cleanup technique: Other:

SPE sorbent type: Other:

SPE column form factor: Other:

SPE column manufacturer: Other:

LLE solvents:

Do You reduce N-Oxides during sample preparation:

Derivatization for PA-NO > PA-base reduction: Other:

Derivatization prior detection: Other:

Additional comments on sample preparation

Scope of the method, individual analyte determination approach

Analyte	Plant Material		Honey Material		Source of standards
	LOD, µg/kg	LOQ, µg/kg	LOD, µg/kg	LOQ, µg/kg	

Scope of the method, sum parameter determination approach

Analyte group	Calibration Compound	Plant material		Honey		Source of standard
		LOD, µg/kg	LOQ, µg/kg	LOD, µg/kg	LOQ, µg/kg	
Retronecinic						
Heliotridinic						

Quantification- both approaches

Quantification is done by: Concentration range:

Internal standard used: If "Yes" specify:

Detection techniques:

Detection technique used: Other

LC Detector: Other

GC Detector: Other:

Liquid Chromatography

LC Stationary Phase: Other:

Diameter, mm: Length, mm: Particle size, µm:

Sorbent Manufacturer: Other

LC Elution : Eluent 1: Eluent 2:

Column temperature: Flow rate, ml/min

Please specify wavelengths for optical detection, if used:

Gas Chromatography

GC Stationary Phase: Other:

Diameter, mm: Length, m: Film Thickness, µm:

Column Manufacturer: Other

GC Elution : Carrier Gas: Column flow, ml/min:

Mass Spectrometric detection

Ion traces for Sum Parameter determination approach

Analyte group	Calibration Compound	Ions monitored for "Sum parameter" determination by GC-MS		
		m/z 1	m/z 2	m/z 3
Retronecinic				
Heliotridinic				

Ion traces for individual analyte determination approach

Analyte	Ions (transitions) monitored for LC/GC-MS(MS) experiments		
	Ion (Transition) 1	Ion (Transition) 2	Ion (Transition) 3
Example:	if SIR used, input - 300	if SRM used, input 352 > 132	

Please fax and e-mail the completed form to:

Vytautas Tamosiunas
EC-JRC-IRMM
Food Safety and Quality Unit
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 852
FAX: +32-14-571 783
E-mail: **vytautas.tamosiunas@ec.europa.eu**

Submit by E-mail

Print Form

10.8 Experimental details

10.8.1 List of analytes analysed by laboratories

Table 10-10

Lab. Code	Analytes reported
101	Echimidine, Echimidine-N-Oxide, Heliotrine, Lycopsamine, Monocrotaline, Retrorsine, Senecionine, Seneciphylline, Senkirkine
102	Echimidine, Heliotrine, Intermedine, Lasiocarpine, Lasiocarpine-N-oxide, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
103	Echimidine, Europine, Europine-N-oxide, Heliotrine, Heliotrine-N-oxide, Indicine-N-oxide, Intermedine, Lasiocarpine, Lasiocarpine-N-oxide, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
104	Echimidine, Heliotrine, Intermedine, Lasiocarpine, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine, Trichodesmine
105	Echimidine, Heliotrine, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
106a	Acetyleruciflorine, Echimidine, Echiumine, Heliotrine, Jacobine, Jacozine, Monocrotaline, Retrorsine, Senecionine, Seneciphylline
107	Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
108	Echimidine, Heliotrine, Lasiocarpine, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
109	Echimidine, Echimidine-N-Oxide, Heliotrine, Heliotrine-N-oxide, Lasiocarpine, Lycopsamine, lycopsamine-N-oxide, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
111	Echimidine, Echimidine-N-Oxide, Erucifoline, Erucifoline-N-oxide, Integerrimine, Integerrimine-N-oxide, Jacobine, Jacobine-N-oxide, Lycopsamine/Intermedine, Lycopsamine/Intermedine-N-oxide, Otosenine, Retrorsine, Retrorsine-N-oxide, Riddelliine, Riddelliine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
112	Echimidine, Heliotrine, Heliotrine-N-oxide, Integerrimine, Lasiocarpine, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Riddelliine, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
114	Retrorsine, Senecionine, Seneciphylline
116	Echimidine, Lasiocarpine, Lycopsamine, Monocrotaline, Retrorsine, Senecionine, Seneciphylline, Senkirkine
117	Jacobine, Lycopsamine, Retrorsine, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
118a	Lycopsamine, Retrorsine, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide
119	Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
120	Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide
121	Echimidine, Heliotrine, Intermedine, Lycopsamine, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
122	Intermedine, Retrorsine, Senecionine, Seneciphylline
124	Echimidine, Lycopsamine, Retrorsine, Senecionine, Seneciphylline
125	Echimidine, Lycopsamine, Monocrotaline, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
126	Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide
127	Echimidine, Grayanotoxin III, Heliotrine, Lycopsamine, Monocrotaline, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senkirkine
128	Retrorsine, Senecionine, Seneciphylline, Senkirkine

10.8.2LOD's LOQ's and sources of standards

Entiers given bellow were transferred without modification. Fields where participants did not indicated LOD and or LOQ were left blank.

Table 10-11

Seneciphylline	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
AppliChem				
104	0.3	1	5.7	16.7
Biopure				
114	3	10	3	10
Carl Roth				
101	1	3		
108	0.3	0.5	5	10
121	0.2	1		
106a	0.3	0.5	25	50
Cfm Oskar Tropitzsch				
118a	4	15	113	319
Chiron				
102	5	10		
116	0.2	0.7	0.7	2.5
122	1.2	3.6	12	36
Extrasynthese				
117	5	10	1	5
isolated from plant material				
125	0.13	0.36	20	60
PhytoLab				
103	1	3	3	10
105	1	2		
107	0.28	1.11	0.28	1.11
109	0.5	1	0.5	1
111	0.5		5	
119	0.25	0.5	0.8	1.6
120		5		10
124	0.3	0.15	0.3	0.15
126	6	21		
127	0.35	1.24	0.5	1.85
128	3.77	12.58		
Phytoplan				
112			1	14

Table 10-12

Seneciphylline-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
125	0.14	0.42	50	150
118a	5	20	65	217
Latoxan				
109	0.5	1	0.5	1
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.6	1.7	7.4	21.8
105	10	15		
107	0.27	1.08	0.27	1.08
108	0.3	0.5	5	10
111	0.5		5	
112			5	14.8
119	0.5	1	0.8	1.6
120		5		10
121	0.2	1		
126	6	22		
127	0.83	2.94	0.6	2.2
RIKILT				
117				

Table 10-13

Senecionine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
AppliChem				
104	0.2	0.6	3.9	11.4
Biopure				
114	3	10	3	10
Carl Roth				
108	0.3	0.5	5	10
121	0.2	1		
127	0.11	0.41	1.1	3.9
Chiron				
102	5	10		
116	0.2	0.5	1	5
122	1	3.1	10	31
Extrasynthese				
117	5	10	1	5
isolated from plant material				
125	0.71	0.21	70	210
PhytoLab				
101	1	3		
103	1	3	3	10
105	1	2		
107	0.295	1.18	0.295	1.18
109	0.5	1	0.5	1
111	0.5		5	
119	0.25	0.5	0.8	1.6
120		5		10
124	0.3	0.2	0.3	0.2
126	7	24		
128	2.21	7.35		
118a	3	9	49	164
Phytoplan				
112			1	14
Sigma				
106a	0.3	0.5	50	100

Table 10-14

Senecionine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
In house synthesis				
125	0.81	0.24	65	195
118a	5	20	51	171
Latoxan				
109	0.5	1	0.5	1
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.4	1.1	5.7	16.7
105	10	15		
107	0.27	1.08	0.27	1.08
108	0.3	0.5	5	10
111	0.5		5	
112			2	14.8
119	0.5	1	0.8	1.6
120		5		10
121	0.2	1		
126	2	7		
127	0.36	1.29	0.3	1.15
RIKILT				
117				

Table 10-15

Retrorsine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Biopure				
114	3	10	3	10
Carl Roth				
127	0.09	0.35	0.2	0.8
Cfm Oskar Tropitzsch				
118a	4	14	105	351
Chiron				
102	5	10		
116	0.6	2	1	5
122	0.8	2.4	8	24
PhytoLab				
103	1	3	3	10
107	0.32	1.26	0.32	1.26
109	0.5	1	0.5	1
111	0.5		5	
112			2	14
119	0.25	0.5	0.8	1.6
120		5		10
126	6	19	1	
124	1	0.4		0.4
128	4.03	13.42		
Sigma				
101	1	3		
104	0.4	1.2	5.9	17.2
105	1	2		
108	0.3	0.5	5	10
117				
121	0.2	1		
106a	0.5	1	50	100

Table 10-16

Retrorsine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Latoxan				
109	0.5	1	0.5	1
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.7	2	9.4	27.5
105	10	15		
107	0.26	1.04	0.26	1.04
108	0.3	0.5	5	10
111	0.5		5	
112			10	14.8
119	0.5	1	0.8	1.6
120		5		10
121	0.5	2		
126	7	23		
127	0.47	1.68	0.4	1.5

Table 10-17

Integerrimine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
gift				
111	0.5		5	
RIKILT				
112			1	14

Table 10-18

Integerrimine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
111	0.5		5	

Table 10-19

Monocrotaline	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Carl Roth				
104	0.6	1.8	5	14.7
108	0.3	0.5	5	10
125	0.52	0.15	12	36
Chiron				
102	5	10		
PhytoLab				
103	1	3	3	10
109	20	20	20	20
112			1	14
116	0.6	2	0.3	1
127	0.34	1.25	0.45	1.65
Sigma				
101	1	3		
105	1	2		
121	0.2	1		
106a	1	2	50	100

Table 10-20

Monocrotaline-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Latoxan				
109	20	20	20	20
PhytoLab				
102	10	20		
103	1	3	3	10
104	0.2	0.6	3.7	11
105	10	15		
108	0.3	0.5	5	10
112			5	14.8
121	0.5	2		

Table 10-21

Lycopsamine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
isolated from plant material				
125	0.81	0.24	10	30
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.4	1.2	11.1	32.5
105	1	2		
108	0.3	0.5	5	10
109	0.5	1	0.5	1
112			1	12.5
116	0.2	0.7	0.3	1
121	0.2	1		
124	0.3	0.15	0.3	0.15
127	0.43	1.56	0.1	0.4
118a	1	2	75	251
Planta analytica				
101	1	3		
RIKILT				
117				

Table 10-22

Lycopsamine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Latoxan				
109	0.5	1	0.5	1

Table 10-23

Intermedine/Lycopsamine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
111	0.5		5	

Table 10-24

Intermedine/Lycopsamine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
111	0.5		5	

Table 10-25

Intermedine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.5	1.5	7.5	22.1
121	0.2	1		
122	1.3	3.9	13	39

Table 10-26

Heliotrine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Chiron				
102	5	10		
Latoxan				
101	0.7	2		
104	0.5	1.5	8.4	24.6
108	0.2	0.5	3	6
121	0.2	1		
106a	0.3	0.5	25	50
PhytoLab				
103	1	3	3	10
105	1	2		
109	0.5	1	0.5	1
112	0	0	1	14
127	0.18	0.67	0.1	0.4

Table 10-27

Heliotrine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Latoxan				
109	0.5	1	0.5	1
PhytoLab				
103	1	3	3	10
RIKILT				
112			1	14.8

Table 10-28

Echimidine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
isolated from plant material				
125	0.11	0.34	25	74
no standard available				
106a				
PhytoLab				
102	5	10		
103	1	3	3	10
104	0.2	0.6	8	23.4
105	1	2		
109	0.5	1	0.5	1
111	0.5		5	
112			1	14
116	0.1	0.5	0.6	2
121	0.2	1		
124	0.2	0.1	0.2	0.1
127	0.06	0.24	0.1	0.3
Planta analytica				
101	0.3	1		
108	0.3	0.5	5	10

Table 10-29

Echimidine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
101	0.3	1		
111	0.5		5	
Latoxan				
109	0.5	1	0.5	1

Table 10-30

Senkirkine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Carl Roth				
108	0.3	0.5	5	10
121	0.2	1		
isolated from plant material				
125	0.52	0.15	51	153
PhytoLab				
101	0.3	1		
102	5	10		
103	1	3	3	10
104	0.3	1	7.5	22.1
105	1	2		
107	0.3	1.2	0.3	1.2
109	0.5	1	0.5	1
111	0.5		5	
112			1	14
116	0.3	1	0.5	1.5
119	0.25	0.5	0.8	1.6
127	0.09	0.33	0.15	0.6
128	1.06	3.57		
RIKILT				
117				

Table 10-31

Lasiocarpine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Cfm Oskar Tropitzsch				
104	0.3	0.8	6.5	19
Chiron				
102	5	10		
116	0.1	0.5	0.3	1
PhytoLab				
103	1	3	3	10
108	0.3	0.5	5	10
109	0.5	1	0.5	1
RIKILT				
112			1	14

Table 10-32

Lasiocarpine-N-Oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
102	5	10		
103	1	3	3	10

Table 10-33

Jacobine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
no standard available				
106a				
PRISNA				
111	0.5		5	
RIKILT				
117				

Table 10-34

Jacobine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
111	0.5		5	

Table 10-35

Trichodesmine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Latoxan				
104	0.4	1.2	5	14.5

Table 10-36

Ridelliine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
gift				
111	0.5		5	
RIKILT				
112			2	14

Table 10-37

Ridelliine-N-Oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
gift				
111	0.5		5	

Table 10-38

Erucifoline	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PRISNA				
111	0.5		5	

Table 10-39

Erucifoline-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
in house synthesis				
111	0.5		5	

Table 10-40

Europine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
103	1	3	3	10

Table 10-41

Europine-N-oxide	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
103	1	3	3	10

Table 10-42

Echiumine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
no standard available				
106a				

Table 10-43

Otosenine	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
PhytoLab				
111	0.5		5	

Table 10-44

Grayanotoxin III	Honey		Plant	
Standard source/Lab. Code	LOD	LOQ	LOD	LOQ
Sigma				
127	0.7	2.56		

10.8.3 Detection parameters

Table 10-45 Chromatografic separation

Question	Reply	Lab. Code
Separation technique	Gas Chromatography (GC)	106b, 113, 118b, 123
	Liquid Chromatography (LC)	101, 102, 103, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 117, 118, 119, 120, 121, 122, 124, 125, 126, 127, 128
GC detection	Single quadrupol MS	106b, 113, 118b, 123
LC Detection	Triple quadrupol MS	101, 102, 103, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 119, 121, 122, 124, 125, 126, 127, 128
	High resolution MS	117, 118a, 120
GC stationary phase	DB-5 type	106b, 123
	DB-1 type	113, 123
	WAX	118b
GC column lenght	30 m	106b, 113, 118b, 123,
GC occn diameter	0.25mm	106b, 118b, 123
	0.32mm	113
GC film thickness	0.25µm	106b, 113, 118b
	25µm	123
GC column manufacturer	Agilent	106b, 113, 118b, 123
	Phenomenex	123
GC Elution	Temperature ramp	106b, 113, 118b, 123
GC carrier gas	He	106b, 113, 118b, 123
GC Column flow	1 ml/min	118b, 123
	1.6 ml/min	106b
	1.7 ml/min	113
LC Column phase	C18	101, 103, 104, 105, 106a, 108, 109, 111, 112, 114, 116, 117, 118a, 119, 120, 121, 122, 124, 125, 127, 128
	PFP	102, 107, 126
LC collumn diameter, mm	2	103, 105, 106a, 118a, 124, 128
	2.1	101, 102, 104, 107, 108, 109, 111, 112, 114, 117, 119, 120, 121, 122, 126
	3	116
	4	125, 127
LC column lenght, mm	50	101, 107, 114, 124
	15	121, 122
	100	102, 108, 112, 120, 128
	125	118a
	150	103, 104, 105, 106a, 109, 111, 116, 117, 119, 126, 127
	250	125
LC column particle size, µm	<2	101, 104, 108, 111, 114, 119, 120, 122
	2≤x<3	102, 107, 112, 124, 126
	3≤x≤5	103, 106a, 109, 116, 117, 118a, 121, 125, 127, 128
LC column manufacturer	Phenomenex	102, 103, 105, 106a, 112, 124, 126, 127, 128
	Waters	108, 111, 114, 117, 119
	Thermo Fisher Scientific	101, 104, 109, 120, 122
	Agilent	121
	YMC	116
	ACE	107
	Mashery-Nagel	125
	AkzoNobel	118a
LC separation mode	Gradient elution	101, 102, 103, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 117, 118a, 119, 121, 120, 122, 124, 125, 126, 127, 128
LC mobile phase pH	Acidic	101, 102, 104, 105, 106a, 107, 109, 114, 116, 117, 118a, 119, 122, 124, 127, 126, 128
	Basic	103, 108, 111, 112, 120, 121
	Neutral	125
LC pH modifier	HCOOH	101, 102, 106, 116, 118, 119, 122, 126, 128
	$HCOOH/NH_4COOH$	104, 114, 124, 127
	AcOH	107, 109
	AcOH/NH4OAc	105, 117
	NH_4COOH	112, 120, 121
	NH_4OH	103, 108, 111
	No modifier	125
LC Mobile Phase organic	MeOH	101, 102, 104, 105, 107, 108, 109, 114, 116, 118a, 120

Question	Reply	Lab. Code
modifier	ACN	103, 106a, 111, 112, 117, 119, 121, 122, 125, 126, 127, 128
LC flow rate, ml/min	0.2	102, 107, 109, 112, 118a, 119, 124
	0.3	101, 103, 104, 106a, 108, 120, 121, 126, 128
	0.35	116, 112
	0.4	111, 114, 117
	0.5	125
	0.15	105
LC column temperature	≤30	101, 103, 109, 112, 116, 118a, 121, 122, 124, 125, 128
	30<x≤40	102, 104, 105, 106a, 107, 114, 117, 119, 120, 126, 127
	>40	108, 111

Table 10-46 Ion traces (transitions) used by laboratories using individual analyte approach

Analyte	Transition (Ion) monitored	Lab. Code
Acetyleruciflorine	392 >94	106a
	392 >120	106a
Echimidine	398 > 120	101, 102, 103, 104, 105, 106a, 108, 109, 111, 112, 116, 121, 125, 127,
	398 > 138	101, 106a
	398 > 220	102, 103, 104, 105, 108, 109, 111, 112, 121, 125, 127
	398 > 238	108
	398 > 238	102, 104, 105, 109, 125
	398 > 55	116
	398 > 77	116
	398 > 83	101, 106a
	398	124
	220	124
	120	124
Echimidine-N-oxide	414 > 220	101, 109
	414 > 254	101, 109, 111
	414 > 352	109
	414 > 396	101
Echiumine	382 > 120	106a
	382 > 83	106a
	382 > 138	106a
Erucifoline	350 > 138	111
	350 > 94	111
Erucifoline-N-oxide	366 > 94	111
	366 > 118	111
Europine	330 > 138	103
	330 > 156	103
Europine-N-oxide	346 > 172	103
	346 > 111	103
Heliotrine	314 > 120	101, 102, 104, 105, 109, 112
	314 > 138	101, 102, 103, 104, 105, 106a, 108, 109, 112, 121, 127
	314 > 156	101, 102, 103, 104, 105, 106a, 108, 109, 112, 121, 127
	314 > 94	106a
Heliotrine-N-oxide	330 > 138	109, 112
	330 > 172	103, 109, 112
	330 > 298	109
	330 > 80	103
Indicine-N-oxide	316 > 172	103
	316 > 138	103
Integerimine/Senecionine	336 > 120	101, 102, 103, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 119, 121, 122, 125, 126, 127
	336 > 138	102, 104, 105, 106a, 107, 109, 112, 114, 116, 119, 122, 125, 127, 128,
	336 > 290	128
	336 > 308	101, 102, 104, 105, 108, 109, 122, 125, 126, 128
	336 > 94	101, 103, 106a, 108, 111, 114, 116, 119, 121, 126
	336	124
	120	124
	308	124
	336.1649	117
	336.1802	118a
	336.1805	120
	120.0807	118a
	138.0913	118a

Analyte	Transition (Ion) monitored	Lab. Code
Integerrimine /Senecionine-N-oxide	352 > 118	102, 104, 107, 109, 125, 126
	352 > 120	102, 104, 105, 108, 109, 111, 112, 121, 125, 126, 127
	352 > 136	102, 103, 104, 105, 107, 108, 109, 112
	352 > 138	119, 125
	352 > 94	103, 105, 108, 111, 119, 121, 126, 127
	352.1755	117
	352.1750	118a
	352.1754	120
	136.0757	118a
	120.0807	118a
Intermedine/Lycopsamine	300 > 112	102
	300 > 120	101, 122, 125
	300 > 138	101, 102, 103, 104, 105, 108, 112, 122, 125
	300 > 156	102, 104, 105, 109, 111, 112, 116, 121, 127
	300 > 94	102, 103, 104, 105, 108, 109, 111, 112, 116, 121, 127
	300	124
	156	124
	94	124
	300.1805	117, 118a
	138.0913	118a
	120.0807	118a
Intermedine/Lycopsamine-N-oxide	316 > 111	109
	316 > 138	109, 111
	316 > 172	111
	316 > 94	109
Jacobine	352 > 120	106a, 111
	352 > 138	106a
	352 > 155	111
	352.1577	117
Jacobine-N-oxide	368 > 296	111
	368 > 120	111
Jacozine	350 > 120	106a
	350 > 138	106a
Lasiocarpine	412 > 120	102, 103, 104, 108, 109, 112, 116
	412 > 220	102, 103, 104, 108, 109, 112, 116
	412 > 238	102, 109
	412 > 336	104, 108
	412 > 77	116
Lasiocarpine-N-oxide	428 > 120	103
	428 > 136	102
	428 > 254	102, 103
	428 > 410	102
Monocrotaline	326 > 120	101, 102, 103, 104, 105, 106a, 108, 109, 116, 121, 125, 127
	326 > 121	106a, 109, 112, 121
	326 > 194	101, 102, 105, 108, 112
	326 > 237	102, 104
	326 > 238	125
	326 > 280	104, 125
	326 > 67	116
	326 > 94	101, 103, 105, 106a, 108, 109, 116, 127
Monocrotaline-N-oxide	342 > 118	102, 104, 109
	342 > 119	103
	342 > 120	102, 104, 108, 109, 112
	342 > 136	102
	342 > 137	103, 104, 108, 112, 121
	342 > 236	108
	342 > 94	105, 109, 121
	368 > 136	105
	369 > 120	105
Retrorsine	352 > 120	101, 102, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 119, 121, 122, 126, 127
	325 > 138	101, 102, 103, 104, 105, 106a, 107, 109, 112, 114, 122, 127, 128
	352 > 276	128
	352 > 324	101, 104, 105, 108, 119, 126, 128
	352 > 67	103
	352 > 77	116

Analyte	Transition (Ion) monitored	Lab. Code
	352 > 94	102, 106a, 108, 109, 111, 114, 116, 119, 121, 122, 126
	352	124
	138	124
	120	124
	352.1751	118a
	352.1755	117
	352.1754	120
	138.0913	118a
	120.0807	118a
Retrorsine-N-oxide	368 > 118	102, 108, 119, 126
	368 > 120	102, 103, 104, 105, 108, 109, 111, 112, 119, 121, 126, 127
	368 > 136	104, 105, 109, 112
	368 > 94	103, 105, 107, 108, 109, 111, 119, 121, 126, 127
	368 > 95	102
	368 > 139	107
	368.1704	120
Seneciphylline	334 > 120	101, 102, 103, 104, 105, 106a, 107, 108, 109, 111, 112, 114, 116, 119, 121, 122, 125, 126, 127
	334 > 138	101, 102, 104, 105, 106a, 107, 109, 111, 112, 114, 116, 119, 122, 125, 127, 128
	334 > 151	109
	334 > 288	128
	334 > 306	101, 102, 104, 105, 108, 125, 126, 128
	334 > 94	103, 106a, 108, 114, 116, 119, 121, 122, 126
	334	124
	306	124
	120	124
	334.1649	117, 120
	334.1647	118a
	138.0913	118a
	120.0807	118a
Seneciphylline-N-oxide	350 > 118	102, 104, 107, 108, 109, 119, 125, 126
	350 > 119	103
	350 > 120	102, 104, 107, 108, 109, 112, 119, 121, 125, 126, 127
	350 > 136	102, 104, 109, 112, 125
	350 > 138	111
	350 > 94	103, 105, 108, 111, 119, 121, 126, 127
	342 > 120	105
	350.1598	117, 120
	350.1596	118a
	136.0757	118a
	120.0807	118a
Senkirkine	366 > 107	101
	366 > 122	101, 102, 103, 104, 105, 108, 109, 111, 119, 125, 127
	366 > 150	102, 104, 105, 107, 108, 109, 112, 119, 121, 125, 128
	366 > 153	128
	366 > 168	101, 102, 103, 104, 105, 107, 108, 109, 111, 112, 116, 119, 125, 127, 128
	366 > 70	116
	366 > 94	116
	366 > 168	121
	366.1911	117
Otosenine	382 > 168	111
	382 > 122	111
Ttrichodesmine	354 > 222	104
	354 > 120	104
	354 > 308	104
Rideliine	350 > 120	111, 112
	350 > 94	111
	150 > 138	112
Rideliine-N-oxide	366 > 94	111
	366 > 118	111
Grayanotoxin III	388 > 317	127
	388 > 299	127

Table 10-47 Ion traces used by laboratories analysing sum parameter

Analyte group	Ion monitored	Lab. Code
Retronecinic PA's	93	106b, 113
	94	118b
	125	118b
	183	118b, 113, 123
	299	113, 123
	106	334
	547	106b
Heliotridinic PA's	93	123
	94	118b
	125	118b
	183	113, 118b, 123
	299	123
	334	106b
	547	106b

Table 10-48 Quantification

Question	Reply	Lab. Code
Calibration type	Matrix Matched calibration	101, 102, 104, 105, 106a, 106b, 109, 113, 118a, 118b, 119, 121, 124, 127
	Standard calibrations	103, 107, 112, 116, 122, 123, 125, 126, 128
	Standard addition	108, 111, 114, 117, 120
Internal standard used	Yes	101, 106a, 106b, 111, 113, 116, 118a, 118b, 119, 123, 124, 127
	No	102, 103, 104, 105, 107, 108, 109, 112, 114, 117, 120, 121, 122, 125, 126, 128
Internal standard	Heliotrine	101, 106a, 106b, 111, 113, 116, 118b, 119, 123
	D1-Retrorsine-bis-butyrate	118a
	D2-Retrorsine-bis-butyrate	118b
	D6-Isoproturon	124, 127
Calibration Range	1-150 µg/kg	101
	1-200	102
	0.01-20 ng/ml	103
	1-50 ng/ml	104
	10-200 µg/kg	105
	0.2-10 µg/ml	106a, 106b
	0-1000	107
	0.5-30 µg/ml	108
	5 µg	109
	0-200 µg/kg for honey, 0-4000 µg/kg for plant material	111
	0.25-4 ng/ml	112
	5-5000	113
	10-1000 ppb	114
	0.2-15ng/ml	116
	10-200 µg/kg	117
	0.05-1 µg/ml	118a
	0.5-10 µg/ml	118b
	1-1000µg/l	120
	1-50	121
	1-50 ppb	122
	0.1-100µg/l	124
	2.5-80 ng/ml 0.1-2µg/ml	125
	0.05-5 µg/ml	126
	0.0005-0.1ng/ml	127
	0-1000 µg/kg	128
	Not Indicated	119, 123

Table 10-49 Sample preparation honey material

Question	Reply	Lab. Code
Detection Approach Used	Individual Analytes	101, 102, 114, 116, 117, 118a, 119, 120, 121, 122, 103, 124, 125 , 126, 127, 128, 104, 105, 107, 108, 109, 111, 112, 120

Question	Reply	Lab. Code
	Sum Parameter	106b, 113, 118b, 123
Experience in the field (Years)	0<y<1	102, 103, 106a, 106b, 107, 114, 116, 118a, 119, 120, 122, 124, 128
	1<y<2	104, 125, 126, 127
	2<y<5	101, 105, 108, 109, 111, 112, 113, 117, 118b, 121, 123
Annual No. of Samples	<100	108, 113, 116, 120, 121, 122, 123, 127
	>500	101, 105, 109
	Not Indicated	102, 103, 104, 106a, 106b, 107, 111, 112, 114, 117, 118a, 118b, 119, 124, 125, 126, 128
Honey Analysed on Routine Basis	No	102, 112, 114, 116, 117, 118a, 118b, 119, 122, 103, 124, 125, 128, 104, 106a, 106b, 107, 111, 120, 126,
	Yes	101, 113, 121, 123, 127, 105, 108, 109
Accreditation	YES	109, 127
	No	102, 103, 104, 105, 106a, 106b, 107, 108, 111, 112, 113, 116, 117, 118a, 118b, 119, 122, 123, 124, 125, 126, 128
	Not Indicated	114, 121
Sample intake per analysis, g	>10	113, 122
	1<x<2	102, 109, 111, 117, 119, 124
	2<x<5	103, 107, 112, 114, 116, 126, 127, 128
	5<x<10	101, 104, 106a, 106b, 108, 118a, 118b, 120, 121, 125
	Not Indicated	105, 123
Extraction technique	Accelerated solvent extraction (with silica)	116
	Shake with solvent	101, 102, 104, 105, 106a, 106b, 107, 108, 109, 111, 112, 113, 114, 117, 118a, 118b, 119, 122, 123, 124, 126, 128
	Ultrasonic assisted solvent extraction	103, 125
	QuEChERs	121, 127
	Modified QuEChERs	120
Extraction Solvent	$HCOOH_{(aq)}$ 2%	102, 103, 113
	$HCOOH_{(aq)}$ 0.1%	111
	$HCl_{(aq)}$ 0.2M	112
	$HCl_{(aq)}$	105
	$H_2SO_{4(aq)}$ 0.05M	101, 106, 107, 118a, 118b, 123, 126, 128
	$H_2SO_{4(aq}$	104, 113, 117
	$H_2SO_{4(aq)}$ /MeOH	125
	MeOH	116
	ACN	108, 121, 127
	ACN/Water	120
	0.1% $HCOOH_{(aq)}$/ACN	114
	Water	109, 122, 124
Clean-Up	No clean-up	103, 114, 120, 127
	Solid Phase Extraction	101, 102, 104, 106, 107, 111, 112, 113, 116, 117, 118a, 118b, 119, 122, 123, 125, 126, 128
	Liquid Liquid Extraction	105, 108, 109, 112, 118b
	Dispersive SPE	121
	QuEChERs	124
Solid Phase Extraction Type	SCX	101, 102, 104, 106a, 106b, 107, 117, 118a, 118b, 123, 125, 126
	Mixed mode cation exchange	112, 113, 122, 126, 128
	Hydrophilic lipophilic bonding	111, 116, 119
Solid Phase extraction column form factor	200mg/6ml	113, 116
	500mg/3ml	101, 117
	500mg/6ml	106a, 106b, 118a, 118b, 123, 125
	60mg/3ml	107, 111, 112, 119, 122, 126, 128
	150mg/6ml	102
	500mg/10ml	104
Solid Phase extraction column manufacturer	Agilent	101, 104, 106, 113, 118, 118, 126
	Phenomenex	107, 111, 116, 117, 119, 123, 126
	Waters	102, 112, 122, 128
	Mashery-Nagel	125
Liquid-liquid extraction solvent	ACN	105
	ACN/Water	109
	ACN/Water+Drying Salts	108

Question	Reply	Lab. Code
	5%NaOH in MeOH/CH_2Cl_2	118b
	0.2M HCl/ CH_2Cl_2	112
N-oxide reduction before detection Applied	No	101, 102, 103, 104, 107, 108, 109, 111, 112, 114, 116, 117, 118a, 119, 120, 121, 122, 124, 125, 126, 127, 128
	Yes	106a, 106b, 113, 118b, 123
	Not Indicated	105
Derivatization PA-NO -> PA	Zn, Acid	106a, 106b, 113, 118b
	Not Indicated	123
Derivatization prior detection	No	101, 102, 103, 107, 109, 111, 114, 116, 118a, 119, 121, 122, 124, 126, 127, 128
	Yes	106b, 113, 118b, 123
	Not Indicated	104, 105, 108, 112, 117, 125
Derivatization Agent prior detection	Tetramethylammonium hydroxide in MeOH	118b
	Heptafluorobutyrylimidazole	106b
	N-methyl-N-(trimethylsilyl)-trifluoracetamide	123
	Not Indicated	113
Additional derivatization	$LiAlH_4$	106b

Table 10-50 Sample preparation plant material

Question	Reply	Lab. Code
Detection Approach Used	Individual Analytes	101, 103, 104, 105, 107, 108, 109, 111, 112, 114, 116, 117, 118a, 119, 122, 124, 125, 126, 127
	Sum Parameter	106b, 113, 118b, 123
Experience in the field (Years)	>5	111, 123
	2<y<5	106a, 106b, 109, 112, 117, 118b, 125, 126
	1<y<2	103, 104, 108, 127
	0<y<1	101, 105, 107, 113, 114, 116, 118a, 119, 122, 124, 128
Annual No. of Samples	<100	104, 106a, 106b, 107, 108, 109, 111, 113, 116, 122, 127
	100<x<200	112
	200<x<500	103
	Not Indicated	101, 105, 114, 117, 118a, 118b, 119, 123, 124, 125, 126, 128
Plant Analysed on Routine Basis	Yes	103, 106a, 106b, 108, 109, 111, 112, 113, 127
	No	101, 104, 105, 107, 114, 116, 117, 118a, 118b, 119, 122, 123, 124, 125, 128
Accreditation	Yes	101, 103, 104, 109, 127
	No	105, 106a, 106b, 107, 108, 111, 112, 113, 116, 117, 118a, 118b, 119, 122, 123, 124, 125, 126, 128
	Not Indicated	114
Sample intake per analysis, g	<1	106a, 106b, 109, 112, 118a, 118b, 124, 125, 127, 128
	1<x<2	101, 107, 108, 111, 117
	2<x<5	103, 113, 114, 116
	5<x<10	122
	Not Indicated	104, 105, 119, 123
Extraction technique	Accelerated solvent extraction	116, 125
	Matrix solid phase dispersion	109
	Shake with solvent	101, 104, 105, 106a, 106b, 107, 108, 111, 112, 113, 114, 117, 119, 122, 123, 124, 126
	Ultrasonic assisted solvent extraction	103, 118a, 118b
	QuEChERs	127
	Method in development	128
Extraction Solvent	$HCOOH_{(aq)}$ 2%	103, 111, 109
	$HCOOH_{(aq)}$	108
	$HCl_{(aq)}$	104, 105
	$HCl_{(aq)}$ 0.2M	112
	$H_2SO_{4(aq)}$ 0.05M	106a, 106b, 107, 118b, 123, 126
	MeOH	116, 118a, 125
	Water	122,
	ACN/Water	124, 109
	$HCOOH_{(aq)}$ 0,1%/ACN	114

Question	Reply	Lab. Code
	ACN	127
Clean-Up	n/a	103, 114, 124, 127
	Solid phase extraction	101, 104, 106a, 106b, 107, 108, 111, 112, 113, 116, 117, 118a, 118b, 119, 122, 123, 125, 126
	Liquid Liquid extraction	105, 109, 112
	QuEChERs	124
Solid phase extraction type	SCX	101, 104, 106a, 106b, 107, 117, 118a, 118b, 123, 126
	Mixed mode cation exchange	112, 113, 116, 122, 126
	Hydrophilic lipophylic bonding	111, 119, 112
	C18	125
Solid Phase extraction column form Factor	200mg/3ml	125
	200mg/6ml	113, 116
	500mg/3ml	101, 117
	500mg/6ml	104, 106a, 106b, 118a, 118b, 123
	60mg/3ml	107, 108, 111, 112, 119, 122
Solid Phase extraction column manufacturer	Agilent	101, 104, 106a, 106b, 113, 118a, 118b, 126
	Phenomenex	107, 108, 111, 116, 117, 119, 123, 126
	Waters	112, 122
	Mashery-Nagel	125
Liquid Liqiud extraction solvents	ACN	105
	ACN/Water	109
	0.2M HCl/0.2M HCl/ CH_2Cl_2 100mM HFBA	112
N-oxide reduction before detection Appllied	No	101, 103, 104, 107, 108, 109, 111, 112, 114, 116, 117, 118a, 119, 122, 124, 125, 126, 127
	Yes	106a, 106b, 113, 118b, 123
	Not Indicated	105
Derivatization PA-NO -> PA	Zn, Acid	106a, 113, 118b
	Not Indicated	123
Derivatization prior detection	No	101, 103, 106a , 107, 109, 111, 114, 116, 118a, 119, 122, 124, 126, 127
	Yes	106b, 113, 118b, 123
	Not Indicated	104, 105, 108, 112, 117, 125, 128
Derivatization Agent prior detection	Tetramethylammonium hydroxide in MeOH	118b
	Heptafluorobutyrylimidazole	106b
	N-methyl-N-(trimethylsilyl)-trifluoracetamide	123
	Not Indicated	113
Additional derivatization	$LiAlH_4$	106b

10.8.4Raw results submitted by participats

Tables presented in this annex are given only for those analytes where at least one participant reported numeric figures or qualitative observation. Other analytes have been omitted. All results are given in µg/kg, unless otherwise stated

Table 10-51

Acetylerucifoline	Result, µg/kg				
Lab.Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
106a	<LOD	<LOD	<LOD	<LOD	positive

Table 10-52

Senkirkine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
117	<LOD	<LOD	<LOD	<LOD	12.11
125	<LOD	1.07	22.3	<LOD	<LOD

Table 10-53

Intermedine/Lycopsamine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	10	<LOD

Table 10-54

Echimidine	Result, µg/kg				
Row Labels	PA/PT/2012/**STD**	PA/PT/2012/**SNH**	PA/PT/2012/**SAH**	PA/PT/2012/**NCH**	PA/PT/2012/**CPM**
101	<LOD	<LOD	<LOD	1.5	<LOD
103	<LOD	<LOD	<LOD	<LOQ	<LOD
104	<LOD	<LOD	<LOD	0.7	<LOD
108	<LOD	<LOD	<LOD	1	<LOD
109	<LOD	<LOD	<LOD	<LOQ	<LOD
112	<LOD	<LOD	<LOD	1.29	<LOD
116	<LOD	<LOD	<LOD	1.38	<LOD
121	<LOD	<LOD	<LOD	1.21	<LOD
124	<LOD	<LOD	<LOD	1.48	<LOD
125	<LOD	<LOD	<LOD	3.21	<LOD
127	<LOD	<LOD	<LOD	1.04	<LOD
106a	<LOD	<LOD	<LOD	positive	<LOD

Table 10-55

Jacobine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	<LOD	25.4
117	<LOD	<LOD	4.07	<LOD	<LOD
106a	<LOD	<LOD	<LOD	<LOD	Positive

Table 10-56

Jacobine-N-oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	<LOD	110.6

Table 10-57

Retrorsine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
101	313	15	19	45	250
102	263.57	20.16	22.61	45.36	n/a
103	186.56	14.09	12.93	25.68	63.68
104	239.4	25.7	23.6	51.9	59.2
105	20	30	25	65	23
107	446.75	140.67	57.37	84.08	110.8
108	210 µg/l	19.1	18.4	32.8	133
109	229.6	16.2	16.2	35.8	125.5
111	229.6	20	12.4	57	121
112	205.53	14.63	9.16	30.68	125.73
114	248.38 µg/l	23.57	21.53	42.02	160.91
116	263.33	23.48	26.01	48.39	203.77
117	3.95	23.04	28.81	42.26	<LOD
119	76.6	23.4	21.4	55.8	48.8
120	226	12.3	13.4	25.2	99.9
121	253 ng/ml	18.8	18.6	37.4	n/a
122	195.1	15.7	17.2	31.3	67.7
124	278.75	25.4	24.48	47.6	251.08
126	3626	220.2	189.5	465.9	<LOD
127	<LOD	<LOD	<LOD	<LOD	756.13
128	270	17.53	17.61	44.5	n/a
106a	245	26	26	44	316
118a	0.228 µg/ml	24.76	27.98	44.36	<LOD

Table 10-58

Lycopsamine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
101	<LOD	<LOD	<LOD	8.9	<LOD
103	<LOD	<LOD	<LOD	8.52	<LOD
104	<LOD	<LOD	<LOD	2.6	<LOD
105	<LOD	<LOD	<LOD	12	<LOD
108	<LOD	<LOD	<LOD	6.8	<LOD
109	<LOD	<LOD	<LOD	7.8	<LOD
112	<LOD	0.39	<LOD	7.17	<LOD
116	<LOD	<LOQ	<LOD	8.71	<LOD
117	<LOD	2.71	11.54	6.93	<LOD
121	<LOD	<LOD	<LOD	8.77	<LOD
124	<LOD	<LOD	<LOD	12.62	<LOD
125	<LOD	<LOD	<LOD	<LOD	196.6
127	<LOD	<LOQ	<LOQ	7.95	<LOD
118a	<LOD	<LOD	<LOD	2.77	<LOD

Table 10-59

Echiumine	Result, µg/kg				
Row Labels	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
106a	<LOD	<LOD	<LOD	Positive	<LOD

Table 10-60

Erucifoline	Result, µg/kg				
Row Labels	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	<LOD	2091.3

Table 10-61

Erucifoline-N-oxide	Result, µg/kg				
Row Labels	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	<LOD	3175.1

Table 10-62

Intermedine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
122	<LOD	<LOD	<LOD	9.33	<LOD

Table 10-63

Integerrimine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	4.8	127.4
112	<LOD	<LOD	<LOD	3.54	166.26

Table 10-64

Integerrimine-N-oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	4.8	348.6

Table 10-65

Retrorsine-N-oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
102	<LOD	<LOD	<LOD	<LOD	n/a
103	<LOD	<LOD	<LOD	<LOD	159.64
104	<LOD	<LOQ	<LOD	<LOQ	354.1
107	<LOD	<LOD	<LOD	<LOD	349.2
108	<LOD	<LOQ	<LOD	<LOD	168
109	<LOD	<LOD	<LOD	<LOD	321
111	<LOD	<LOD	<LOD	<LOD	324.3
112	6.3	0.66	<LOD	<LOD	196.87
119	<LOD	1.7	2.67	2.01	169.8
120	<LOD	<LOD	<LOD	<LOD	323.6
121	32 ng/ml	<LOD	<LOD	<LOD	n/a
127	<LOD	<LOD	<LOD	<LOD	288.73

Table 10-66

Riddelliine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	11.6	30
112	6.68	0.69	0.56	9.58	29.97

Table 10-67

Riddelliine-N-oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
111	<LOD	<LOD	<LOD	11.6	65.4

Table 10-68

Senecionine	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
101	351	12	19	31	825
102	256.8	18.11	19.26	44.56	n/a
103	211.37	15.94	14.59	30.49	464.31
104	262.7	27.7	28.2	55.5	743.2
105	9	20	23	50	85
107	254.65	30.72	33.26	52.07	369.8
108	167 µg/l	8.1	9.4	23.7	528
109	226.4	19.1	16.8	42	783.4
111	210	18.8	12.1	33.9	690.2
112	58.38	10.64	8.91	22.7	506.12
114	231.45 µg/l	21.73	22.55	47.09	914.96
116	297.17	25.64	27.93	55.93	890.09
117	9.02	19.93	22.89	35.26	223.94
119	100.8	19.9	17	45.1	190.4
120	245	14.9	18.5	27.4	495.8
121	195 ng/ml	15.4	15.2	29.6	n/a
122	222.6	17.6	14.6	31.8	359.5
124	269.25	22.64	22.6	48.08	1189.23
125	0.08 µg/ml	1.69	0.38	<LOD	996.37
126	2460	116.2	110.6	390.5	6480
127	210 µg/ml	19.56	13.6	37.14	610.27
128	232.7	13.83	15.26	39.65	n/a
106a	210	26	27	30	669
118a	0.229 µg/ml	21.91	24.03	55.27	464.2

Table 10-69

Senecionine –N-Oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
102	<LOD	<LOD	<LOD	<LOD	n/a
103	<LOD	<LOD	<LOD	<LOD	970.24
104	<LOD	<LOQ	<LOD	1.8	1678.2
105	<LOD	<LOD	<LOD	<LOD	<LOD
107	<LOD	<LOD	<LOD	<LOD	1776
108	<LOD	<LOD	<LOD	<LOD	1440
109	<LOD	<LOD	<LOD	<LOD	1514
111	<LOD	<LOD	<LOD	<LOD	1670.1
112	3.04	0.74	0.59	<LOD	839.57
117	<LOD	<LOD	5.54	<LOD	<LOD
119	<LOD	1.17	1.91	1.24	158.6
120	<LOD	<LOD	<LOD	<LOD	2742
121	20.7	<LOD	<LOD	<LOD	n/a
125	<LOD	0.91	0.66	<LOD	441.46
126	<LOD	<LOD	<LOD	<LOD	20160
127	<LOD	<LOD	<LOD	<LOD	1496.53
118a	<LOD	<LOD	<LOD	<LOD	655.65

Table 10-70

Seneciphylline	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
101	737	40	46	32	483
102	422.9	29.95	33.92	29.58	n/a
103	418.84	32.25	32.7	27.02	318.92
104	372.5	39.3	39.9	32.6	327
105	15	45	40	38	80
107	875	89.39	108.37	55.03	378
108	480 µg/l	43.4	39.7	39	334
109	391.9	29	30.3	28.2	484.5
111	497.6	44	26	38.6	531
112	97.52	25.97	24.22	24.46	487.21
114	3530.97	36.97	39.26	35.09	572.58
116	510.83	41.89	45.95	36.03	473.32
117	13.45	68.4	70.97	45.3	557.46
119	192.3	100.3	90.6	54.4	336.8
120	478	33.3	39.6	27.4	746.9
121	361 ng/ml	27.7	27.2	24.2	n/a
122	383.1	27.5	24.8	24.9	162
124	536.25	45.32	45.68	37.48	609.71
125	1.41 µg/ml	49.97	24.52	66.68	645.95
126	7438.1	452.3	429.1	440.50	5096.8
127	470 µg/ml	41.74	40.57	31.6	379.13
128	516.35	28.75	30.07	20.63	n/a
106a	456	47	47	20	745
118a	0.535 µg/ml	45.95	47.15	42.1	534.59

Table 10-71

Seneciphylline-N-oxide	Result, µg/kg				
Lab. Code	PA/PT/2012/STD	PA/PT/2012/SNH	PA/PT/2012/SAH	PA/PT/2012/NCH	PA/PT/2012/CPM
102	<LOD	<LOD	<LOD	<LOD	n/a
103	<LOD	<LOD	<LOD	<LOD	505.58
104	<LOD	<LOD	<LOD	<LOD	718.6
105	<LOD	<LOD	<LOD	<LOD	<LOD
107	<LOD	<LOD	<LOD	<LOD	1069.2
108	<LOD	<LOD	<LOD	<LOD	410
109	<LOD	<LOD	<LOD	<LOD	824.5
111	<LOD	<LOD	2.3	<LOD	1214.4
112	4.61	1.21	1.55	<LOD	504.86
117	<LOD	11.55	19.02	3.46	1175.47
119	<LOD	3.21	6.88	2.57	789.3
120	<LOD	<LOD	<LOD	<LOD	1123
121	38 ng/ml	<LOD	<LOD	<LOD	n/a
125	<LOD	4.55	4.29	13.82	300.13
126	<LOD	<LOD	<LOD	<LOD	8588
127	<LOD	<LOD	<LOD	<LOD	807.73
118a	<LOD	<LOD	<LOD	<LOD	305.9

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Title: Report on the 2012 Proficiency Test on pyrrolizidine alkaloids in honey and hay

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Abstract

The purpose of this proficiency test was to investigate the current measurement capacities of testing laboratories for pyrroliazidine alkaloids in honey and plant materials. The scheme consisted of two parts: Benchmarking performance of laboratories against known estimates of pyrrolizidine alkaloids in the samples and checking for methodological differences while measuring naturally contaminated materials.

Twenty-eight laboratories expressed their will to participate and analysed multiple analytes in several test samples of honey and plant material.

The analysis of spiked honey showed no statistical differences between determining a common sum parameter for alkaloids and individual determination. A significant difference has been found however for of naturally contaminated materials. Individual alkaloid determination showed significantly lower results, possibly because of the presence of substances contributing to the sum parameter that were not in the scopes of the methods applied as well as lack of standard materials available on the market.

Satisfactory performance for all of analytes has been achieved by more than half of participants analysing for both: sum parameter, and alkaloids analysed individually.

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