German Federal Institute for Risk Assessment

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Report on the 2024 Proficiency Test of the German Reference Laboratory for Mycotoxins and Plant Toxins

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Determination of pyrrolizidine alkaloids in honey – Report on the 2024 Proficiency Test of the German Reference Laboratory for Mycotoxins and Plant Toxins

The NRL for Mycotoxins and Plant Toxins organised a proficiency test for the determination of pyrrolizidines in honey in which 26 laboratories participated. Three honey samples and one standard solution were analysed and the tested concentration ranged from 0.8 to 84.3 µg/kg. The reproducibility standard deviation varied from 12 to 42 % for PAs and from 44 to 77 % for the PA-*N*-oxides. On average, 88 % of the z-scores fell within the acceptable range ($|z| \leq 2$).

All proficiency test materials were prepared by spiking with either the free bases of the PAs or their *N*-oxides or a combination of both. The materials were stored for twelve or six weeks before dispatch. This approach allowed the stability of individual analytes in honey to be assessed. The data show that the PA-*N*-oxides were rapidly degraded and reduced to a certain percentage to the respective free tertiary base. The data also show that the free tertiary bases in honey are also degraded, albeit more slowly than the *N*-oxides.

The pilot project to investigate the stability of PAs during storage beyond the shelf life guaranteed by the supplier gave first results. After two years of storage a high stability of analytes was demonstrated. Results obtained for standard solution samples were evaluated according to the standard supplier and the respective lot number. Laboratories can use this data to assess the quality of their own calibration standards and also for metrological traceability of their calibration standards in the context of accreditation.

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1 Introduction

Since 1 July 2022, maximum levels for pyrrolizidine alkaloids in certain foods such as dried herbs and (herbal) teas have been in force under Commission Regulation (EU) 2023/915 [1]. The maximum levels refer to the sum of 35 pyrrolizidine alkaloids, whereby isomers can be determined as a sum or as a group [2]. The complete analytical scope for monitoring maximum levels is shown in Table 1.

Honey is often discussed as a contributing factor to consumer exposure to PAs. Compared to plant-based foods, the PA content in honey is relatively low. One possible explanation could be that the *N*-oxide forms, which make up the largest proportion in the PA plant, are degraded or are not stable in honey [3]. The aim of this PT was therefore to verify the performance of existing methods and laboratories with regard to the determination of PAs in honey and to provide additional data on the stability of PAs and their *N*-oxides in honey.

In addition, a pilot project was started in 2022 to investigate the stability of PAs during storage beyond the shelf life guaranteed by the supplier [4]. These standards stored at the NRL-DE were re-shipped and analysed in this PT.

Table 1: Analytical scope for monitoring the PA maximum levels in food. Natural occurring isomers can be summarized as group. The maximum level refers to the lower bound sum of the given PA and/or PA groups [1].

PA or PA group [abbreviation]	**Natural isomers**
Echimidine group [Em-G]	Echimidine [Em], Heliosupine [Hs]
Echimidine-*N*-oxide group [EmN-G]	Echimidine-*N*-oxide [EmN], Heliosupine-*N*-oxide [HsN]
Europine [Eu]	
Europine-*N*-oxide [EuN]	
Heliotrine [He]	
Heliotrine-*N*-oxide [HeN]	
Intermedine group [Im-G]	Intermedine [Im], Lycopsamine [Ly], Indicine [Id], Echinatine [En], Rinderine [Rn]
Intermedine-*N*-oxide group [ImN-G]	Intermedine-*N*-oxide [ImN], Lycopsamine-*N*-oxide [LyN], Indicine-*N*-oxide [IdN], Echinatine-*N*-oxide [EnN], Rinderine-*N*-oxide [RnN]
Lasiocarpine [Lc]	
Lasiocarpine-*N*-oxide [LcN]	
Retrorsine group [Re-G]	Retrorsine [Re], Usaramine [Us]
Retrorsine-*N*-oxide group [ReN-G]	Retrorsine-*N*-oxide [ReN], Usaramine-*N*-oxide (UsN]
Senecionine group [Sc-G]	Senecionine [Sc], Senecivernine [Sv], Integerrimine [Ig]
Senecionine-*N*-oxide group [ScN-G]	Senecionine-*N*-oxide [ScN], Senecivernine-*N*-oxide [SvN], Integerrimine-*N*-oxide [IgN]
Seneciphylline group [Sp-G]	Seneciphylline [Sp], Spartioidine [St]
Seneciphylline-*N*-oxide group [SpN-G]	Seneciphylline-*N*-oxide [SpN], Spartioidine-*N*-oxide [StN]
Senkirkine [Sk]	

2 Scope and study design

2.1 Participating laboratories

A total of 26 laboratories took part in the proficiency test and submitted results. The laboratories are active in food control, either as official laboratory of the federal states or as contract laboratories or were laboratories of the EURL/NRL network (Table 2).

Table 2: Participating laboratories (in alphabetical order)

Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Erlangen, Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL) – AöR, Muenster, Germany
Environmental and other food contamination and Natural & Plant toxins Laboratory – State General Laboratory, Nicosia, Cyprus
Eurofins Dr. Specht International GmbH, Hamburg, Germany
Eurofins WEJ Contaminants, Hamburg, Germany
Group for Contaminant and Special Analysis, Austrian Agency for Health and Food Safety, Vienna, Austria
ILVO-T&V, Melle, Belgium
Institut für Hygiene und Umwelt, Hamburg, Germany
Institut Kirchhoff Berlin GmbH, Berlin, Germany
Institute of Food Safety, Animal Health and Environment BIOR, Riga, Latvia
Intertek Food Services GmbH, Bremen, Germany
IZSLER Chemical Department of Bologna – Italy (National Reference Laboratory for plant toxins in food), Bologna, Italy
Kantonales Laboratorium, Bern, Switzerland
Labor Friedle GmbH, Tegernheim, Germany
Laboratoire Nationale de Santé (LNS) of Luxembourg, Dudelange, Luxembourg
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Rostock, Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt, Fachbereich Lebensmittelsicherheit, Halle (Saale), Germany
Landesbetrieb Hessisches Landeslabor (LHL), Kassel, Germany
Landesuntersuchungsamt Rheinland-Pfalz, Trier, Germany
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Chemnitz, Germany
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Braunschweig, Germany
Norwegian Institute of Bioeconomy Research (NIBIO), Aas, Norway
NRL DE, Berlin, Germany
SGS Germany GmbH, Hamburg,
University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Ljubljana, Slovenia
Wageningen Food Safety Research (EURL), Wageningen, The Netherlands

2.2 Test materials and time frame

2.2.1 Material

Three honey samples and one standard solution were prepared for the proficiency test.

Sample 1 (Preparation on 09/08/2023):

1.0 kg of a freshly harvested PA-free polyfloral honey was heated to 30°C and a mixture of PA standards was added while stirring. Homogenisation was completed after 2 hours and sample quantities of about 40 g were filled into falcon tubes and stored at room temperature until dispatch. Sample 1 was spiked with the free tertiary bases of the pyrrolizidine alkaloids only. The spike profile and concentrations are shown in Table 3. The homogeneity was tested and confirmed on 10/08/2023.

Sample 2 (Preparation on 15/08/2023):

1.0 kg of a freshly harvested PA-free polyfloral honey was heated to 30°C and a mixture of PANO standards was added while stirring. Homogenisation was completed after 2 hours and sample quantities of about 35 g were filled into falcon tubes and stored at room temperature until dispatch. Sample 2 was spiked with the *N*-oxide forms of the pyrrolizidine alkaloids only. The spike profile and concentration are given in Table 3. The homogeneity was tested and confirmed on 16/08/2023.

Sample 3 (Preparation on 17/10/2023):

1.5 kg of blossom honey purchased from the retail market containing trace amounts of a natural contamination with echimidine and intermedine was heated to 30°C and a mixture of PA and PANOs was added while stirring. Homogenisation was completed after 2 hours and sample quantities of about 50 g were filled into falcon tubes and stored at room temperature until dispatch. Sample 3 was spiked with the free tertiary base as well as the *N*-oxide forms of the pyrrolizidine. The spike concentrations are given in Table 3. The homogeneity was tested and confirmed on 23/10/2023.

Table 3: Spiking profile of the PT materials. For the isomer groups, only one isomer per group was spiked. For each group, the name-giving isomer was spiked.

PA group	Honey 1 [µg/kg]	Honey 2 [µg/kg]	Honey 3 [µg/kg]	Standard [ng/mL]
Echimidine-group	3.7	/	*	10
Echimidine-*N*-oxide group	/	800	/	16
Europine	6.7	/	120	24
Europine-*N*-oxide	/	100	37	40
Heliotrine	1.1	/	7.2	32
Heliotrine-*N*-oxide	/	725	35	16
Intermedine-group	17	/	*	40
Intermedine-*N*-oxide group	/	480	/	10
Lasiocarpine	17	/	1.5	24
Lasiocarpine-*N*-oxide	/	390	41	16
Retrorsine-group	17	/	13	32
Retrorsine-*N*-oxide group	/	580	17	20
Senecionine group	14	/	65	40
Senecionine-*N*-oxide group	/	170	14	16
Seneciphylline group	6.4	/	37	32
Seneciphylline-*N*-oxide group	/	400	14	20
Senkirkine	0.9	0.5	1.7	10
Atropine	/	/	/	22
Scopolamine	/	/	/	38

* natural contamination

2.2.2 Homogeneity and stability

The homogeneity of samples was determined according to Annex B, Point B. 1 in ISO 13528:2022 [5]. For this purpose, 10 units per test sample were randomly selected and analysed in duplicates under repeatability conditions. The homogeneity of the samples was confirmed at the NRL on the basis of a sample weight of 2 g.

The analyte distribution (c) was considered as sufficiently homogeneous if at least the extended condition for homogeneity according to point B.2.3 in ISO 13528 is fulfilled.

$$s_s = \sqrt{\max(0, \quad s\frac{2}{x} - \frac{{s_w}^2}{2}} \qquad \text{Equation 1}$$

$$s_w = \sqrt{\sum_{t=1}^{10} w_t^2 / 2} \qquad \text{Equation 2}$$

$$c = F1 \times (0{,}3\sigma_{PT})^2 + F2 \times {s_w}^2) \qquad \text{Equation 3}$$

where:

s_s:	between-samples standard deviation
σ_{PT}:	target standard deviation (here: 25 %)
s_w:	within-sample standard deviation (here: duplicate analysis)
w_t:	between-sample ranges
F1 und F2:	from Table B.1 in Annex B, see ISO 13528:2022

The stability of the analytes during the PT period was checked by analysing the three test materials before shipment and after the deadline for submission of the results. Comparable results were obtained between the analyses. In addition, the participants were asked about the date of sample analysis. No correlation was found between the date of analysis and the PT results.

2.2.3 Shipment and instructions

Samples were sent with the documents on 04/12/2023, and the deadline for submitting the results was 12/02/2024. An Excel reporting sheet was provided.

3 Statistical evaluation

3.1 Blunder removal and treatment of data below the reported Limit of Quantification

A visual pre-evaluation of the data was carried out to identify systematically deviating laboratories (obvious blunders) [5]. A systematic deviation of a laboratory is present if a consistent trend can be determined for the majority of the analytes in the test materials (Appendix A). Laboratories 11 and 25 showed systematic deviations for the majority of analyte-matrix-combinations, and laboratory 25 also deviated for the majority of analytes in the standard solution. The data from laboratories 11 and 25 were not used for the statistical evaluation to determine the assigned value and precision data of the honey test materials. In addition, the data from laboratory 25 were excluded for the statistical evaluation of the standard solution.

After calculating the assigned values for the respective analyte-matrix-combinations, z scores were determined for all participating laboratories.

Some laboratories submitted data below their reported limits of quantification. These data were not included in the calculation of the assigned value, but z scores were calculated and reported to the laboratories.

3.2 Assigned value and reproducibility standard deviation

The statistical evaluation was carried out in accordance with the "Statistical methods for use in proficiency testing by interlaboratory comparison" published in ISO 13528:2022 [5]. Further specific procedures were adopted from the background document "Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed" [6].

The calculation of the assigned value (consensus value) was based on the results of participants and was determined by using robust statistics (algorithm A, Huber estimator in Annex C.3 [5]). This algorithm yields robust estimates of the mean (assigned value, av) and reproducibility standard deviation (s_R) of the submitted data in which deviating laboratory results are given less weight. A further advantage of robust statistics is that data do not have to be normally distributed in contrast to conventional outlier elimination methods [6]. To determine the assigned value, the number of results received for an analyte per PT material must be at least seven [6].

Depending on the number of participating laboratories or the number of received results and the dispersion between the laboratory results, the **assigned value** is subject to **uncertainty**. A high uncertainty of the assigned value will lead to a high uncertainty of the calculated participants z-scores. If the uncertainty of the consensus value and thus the uncertainty of the z-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. To avoid drawing inappropriate conclusions about the performance of the participating laboratories from the calculated z-scores, the uncertainty of the consensus value is taken into account [6], see section 3.3.

3.2.1 Uncertainty of the assigned value

The uncertainty of the assigned value was calculated from the estimation of the standard deviation of the assigned value and the number of values used for the calculation of the assigned value:

$$u_{av} = 1.25 \times \frac{s_R}{\sqrt{n}} \qquad \text{Equation 4}$$

where:

u_{av}: uncertainty of the assigned value

n: number of values used to calculate the assigned value

s_R: robust reproducibility standard deviation of the laboratory results

Note: According to ISO 13528 Chapter 7.7.7 the factor 1.25 is based on the standard deviation of the median, or the efficiency of the median as an estimate of the mean, in a large set of results drawn from a normal distribution.

In accordance with ISO/IEC 13528 Chapter 9.2.1, the uncertainty of the assigned value (u_{av}) may be considered to be negligible if:

$$u_{av} \leq 0.3 \times \sigma_{PT} \qquad (\sigma_{PT} = 0.25 \text{ x assigned value})$$

$$u_{av} \leq 0.3 \times 0.25 \times \text{assigned value}$$

3.3 Performance characteristics of the laboratories with regard to the accuracy

3.3.1 Calculation of z-scores and z'-scores

One of the basic objectives of proficiency testing is to evaluate the performance of laboratories and the methods they use, also known as fit for purpose testing. For this evaluation, the difference between the measured value of laboratory and the assigned value is considered and calculated as z-score. Commission Regulation (EU) 2023/2783 [2] specifies a target standard deviation of 25 %, so a deviation of 25 % from the assigned value corresponds to a z-score of 1.

$$z = \frac{(x - \text{assigned value})}{\sigma_{PT}} \qquad \text{Equation 5}$$

As described in section 3.2, the uncertainty of the assigned value can be high in some cases and must be taken into account in the evaluation of the laboratories. If the uncertainty of the assigned value is not negligible, z'-scores have to be calculated instead of z-scores [6].

$$z'\text{-score} = \frac{x - \text{assigned value}}{\sqrt{\sigma_{PT}^2 + u^2}} \qquad \text{Equation 6}$$

where:

z'-score:	z-score taking into account the uncertainty of the assigned value
x:	the result of the laboratory
σ_{PT}:	standard deviation for proficiency assessment (here 25 %)
u:	uncertainty of the assigned value.

As can be seen from Equation 6, taking into account the uncertainty of the assigned value means that even laboratory results deviating more than 25 % from the assigned value still lead to z'-scores of 1.

The z-score/z'-score is interpreted as follows [5]:

$\|z\| \leq 2$	result is considered to be acceptable
$2 < \|z\| < 3$	result is considered to be questionable (or warning signal)
$\|z\| \geq 3$	result is considered to be unacceptable (or action signal)

3.3.2 Calculation of the laboratory bias and precision

Participation in proficiency tests is a good opportunity to estimate the laboratory/method bias (also called systematic error). The assigned value derived for an analyte in a test material is a sufficiently good estimate of the true value. Therefore, the laboratory's deviation from the assigned value – calculated as a z-score – can be used in the same way as the laboratory's deviation when analysing a certified reference material.

According to ISO 11352 Chapter 8.3.3, a laboratory should have analysed at least six samples in one or two proficiency test rounds to estimate the bias with confidence. For many laboratories/methods, this approach would have the effect that bias estimation would only be possible after long periods of time. This way of estimating the bias is correct if the bias is an intrinsic factor, e.g. an analyte loss during sample processing. However, it would be wrong if the bias is not an intrinsic factor, a laboratory recognises an error based on the deviation in the proficiency test and corrects it immediately. This is the case, for example, if the concentration of the standard solution is incorrect.

Since up to 17 analytes per PT sample are tested for pyrrolizidine alkaloids (Table 1), the confidence in the bias estimate appears to be sufficiently high even with fewer than the recommended six samples. This approach is already used in the field of pesticides for bias estimation from proficiency testing data, see SANTE/11312/2021, Annex C, Approach 2 [7].

The bias can be calculated in different ways:

(1) Calculating the Root Mean Squares of the sum of the squared bias divided by the number of PT results [7].

This approach has the disadvantage that information about the bias is lost, e.g. whether the laboratory underestimated or overestimated the assigned value (recovery below or above 100 %). In other words, this procedure calculates a precision parameter rather than a bias.

(2) A direct method for assessing a laboratory's bias is to use the deviation of an analyte-matrix-combination from the assigned values (true value), similar to analysing a reference material. The mean deviation of all tested analyte-matrix-combinations reflects the mean bias.

The disadvantage of this approach is that inaccurate methods that both underestimate and overestimate the assigned value (recovery below or above 100 %) can lead to a bias of zero. Therefore, this type of bias estimation must be supported by an additional precision estimation that enables the laboratory to distinguish between bias and (im)precision.

The mean percent bias is calculated as follows:

$$\mathrm{bias_{mean}}\,(\%) = \frac{\sum_1^{\mathrm{n}} \left(\frac{\mathrm{x_m} - \mathrm{av_m}}{\mathrm{av_m} \times 100} \right)}{\mathrm{n}} \qquad \text{Equation 7}$$

where:

x_m: the result of the laboratory for analyte m

av_m: the assigned value of analyte m

n: the number of analytes

The mean z-score of all analyte-matrix-combinations tested can be calculated as follows (mean deviation of a laboratory from the assigned value for all analyte-matrix-combination tested):

$$\mathrm{z-score_{mean}} = \frac{\sum_1^{\mathrm{n}} \mathrm{z-scores}}{\mathrm{n}} \qquad \text{Equation 8}$$

where:

n: number of z-scores (evaluated analyte-matrix-combinations)

z-score: for calculation refer to Equation 5.

For each laboratory, the dispersion of the individual analyte-matrix-bias results around the mean bias (mean deviation from the assigned values) can be used as a measure of precision, as it is an indicator of how the laboratory reproduces its measurements. The standard deviation of the individual analyte-matrix-bias results was calculated according to the following equation and reported as the precision of the laboratory:

$$\mathrm{z-score_{precision}} = \sqrt{\frac{1}{\mathrm{n}\ -\ 1}\sum_{\mathrm{m}}^{\mathrm{n}}(\mathrm{z-score_{m}}\ -\ \mathrm{z-score_{mean}})^2} \qquad \text{Equation 9}$$

where:

$z\text{-score}_m$: z-score of the laboratory for analyte m

$z\text{-score}_{mean}$: mean z-score of the laboratory for all analytes. For calculation refer to Equation 8.

Remark:

Calculating the bias as the mean percentage bias is equivalent to calculating the mean of the z-scores multiplied by 25. The same applies to the standard deviation.

3.3.3 Calculation of the laboratory absolute mean z-score

According to Commission Regulation (EU) 2023/915 [1], a laboratory may report an expanded standard measurement uncertainty of 50 %, provided that the laboratory meets the specified intra-laboratory precision requirements and successfully participates in proficiency testing programmes. For this purpose, an average absolute z-score $|z| \leq 2$ must be achieved, which proves that the required precision under reproducibility conditions (RSD_R) is fulfilled.

$$|\mathrm{z-score_{mean}}| = \frac{\sum_{1}^{\mathrm{n}}|\mathrm{z-scores}|}{\mathrm{n}} \qquad \text{Equation 10}$$

3.4 Dealing with false positives, '<LOQ', and false negatives

The results submitted by the laboratories were evaluated for false positive and false negative results. The assessment of whether a false positive or false negative result is present was carried out on the basis of the criteria set out in the background document of the EURL-MP [6].

False positives (FP): A false positive is a quantitative result reported by the participant while the toxin is not detected in the PT material by the majority of the other participants. In those cases, no assigned value can be calculated (n<7). To evaluate the laboratory result, a factor was calculated that indicates by how much the laboratory result exceeds the robust LOQ of all participating laboratories. The higher this value is, the more likely it is to be a false positive measurement.

False negative or results below LOQ: Participants that analyse the PT material for a certain analyte, either report a quantitative result or, when the toxin was not detected or below the LOQ. In this case, 'proxy z-scores' are calculated as a way to assess possible false negatives. Proxy z-scores are calculated using:

$$\text{proxy-z} = \frac{\text{x} - \text{assigned value}}{\sigma_{\text{PT}}}$$

where:

proxy-z: value to classify <LOQ results

x: the LOQ of the laboratory

σ_{PT}: standard deviation for proficiency assessment (here 25 %)

Proxy z-scores are indicated in the PT report as a value between brackets and are for information only. They are not included in the graphical representations of z-scores of the participants. The interpretation is as follows [6]:

Proxy-z ≤ -3: based on the LOQ provided, the laboratory should have been able to detect and quantify the analyte. The result is classified as a false negative (FN). A false negative is interpreted as 'unsatisfactory' performance.

-3 < proxy-z < -2 based on the LOQ provided, it is highly likely that the laboratory should have been able to detect and quantify the analyte. The result is classified as a false negative (FN) and should be interpreted as 'questionable'.

Proxy-z ≥ -2 based on the assigned value and the LOQ provided, the result cannot be classified as false negative.

4 Proficiency test results

The statistical evaluation of the results was performed as described in section 3. Laboratory results and statistical characteristics for each sample are given in Table 4 to Table 10. A graphical overview on relative reproducibility standard deviation depending on the analyte concentration is shown in Figure 1.

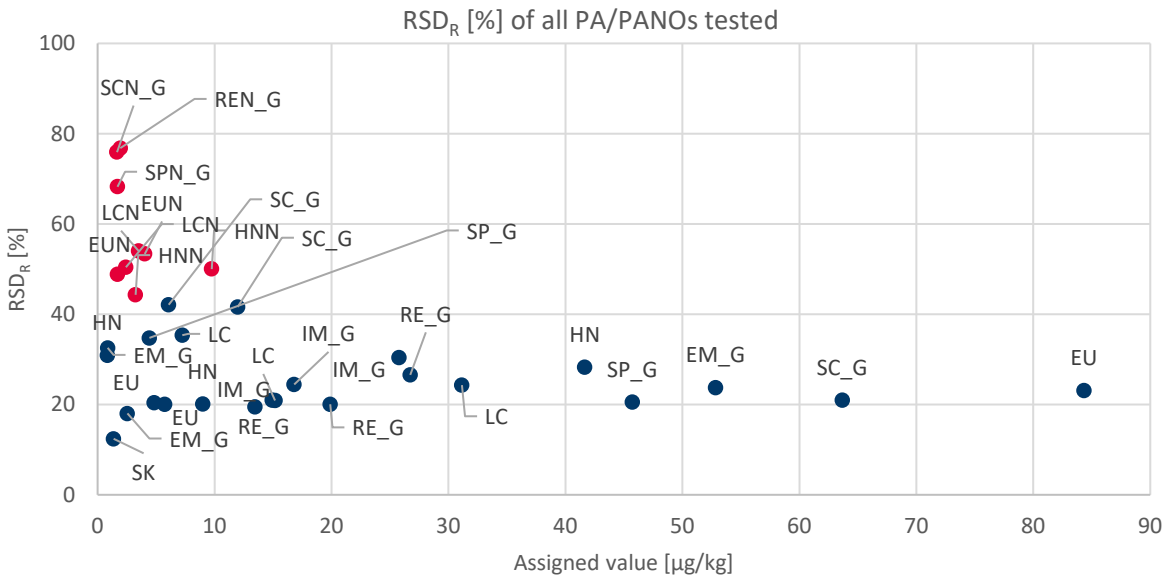


Figure 1: relative reproducibility standard deviation of all analytes as function of their content in the sample. PANOs are shown in red and the free tertiary bases in blue.

Honey sample 1 was spiked with echimidine, europine, heliotrine, intermedine, lasiocarpine, retrorsine, senecionine, seneciphylline and senkirkine in concentrations between 0.9 to 17.4 µg/kg (refer to Table 3). The spiked analytes were also detected in the sample by the participants, but the respective assigned values, i.e. the consensus values of the laboratories for the individual PAs, were approx. 30 % lower, indicating a significant reduction of PAs in the honey after three months (Table 4).

Table 4: Analytical results and statistical characteristics for honey sample 1

PA	Em group	Eu	Hn	Im group	Lc	Re group	Sc group	Sp group	Total PA
Lab	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
01	2.5	5.2		14.8	14.8	9.5	5.2	1.3	53.3
02	3.8	3.8	1.0	13.5	17.6	16.,0	16.0	7.3	79.1
03	2.4	3.6	0.5	10.1	14.9	14.5	12.8	4.45	63.6
04	2.7	6.2	<LOQ	15.2	14.1	15	13.7	3.9	70.8
05	2	4	<LOQ	19	9	8	3	3	48
06	2.24	5.2	<LOQ	13.35	13.9	12.6	7.4	3.5	58.2
07	2.4	5.1	1	16.2	13.9	13.9	11.3	6	69.8
08	<LOQ	5.8	<LOQ	14.4	17.6	13.7	18.7	12.0	82.1
09	2.2	5.3	<LOQ	18.3	17.8	15.2	10.4	4.8	74
10	2.9	4.6	<LOQ	14.3	14.1	12.9	8.1	4.5	61.3
11	1.1	0.8	<LOQ	1.6	3.9	1.5	<LOQ	<LOQ	8.9
12	2.3	2.7	<LOQ	9.9	12.0	9.6	10.7	2.1	49.2
13	<LOQ	7.7	<LOQ	20	22	20	18	5.7	93.4
14	2.8	5.3	<LOQ	17.3	14.6	6.3	3.8	2.5	52.61
15	2.1	4.8	0.6	12.9	11.24	10.9	10.1	3.5	56.7
16	<LOQ		<LOQ	16.3	16	16.8	19.2	<LOQ	68.3
17	4.1	6.4	<LOQ	18.2	14.2	14.7	11.4	4.5	73.5
18	2.2	3.8	<LOQ	15.6	19.5	15	13.8	4.4	74.3
19	2.9	5.3	1.0	16.3	16.8	13.8	14.6	5.5	100.5
20	<LOQ	4.2	<LOQ	5.41	10.4	12.7	42.0	4.6	84.7
21	2.6	4.4	<LOQ	12.0	12.0	13.9	8.8	5.0	62.7
22	1.9	4.5	<LOQ	12.5	16	12.1	9.5	3.1	59.6
23	2.9	5.3	0.8	16.2	20.5	15.8	13.1	5.3	80.6
24	<LOQ	<LOQ	<LOQ	17	15	13	12	<LOQ	57
25	<LOQ	0.8	0.4	0.3	13.2	5.6	9.0	2.96	32.6
26	2.9	4.2	1.0	11.0	17.4	15.2	14.1	5.6	73.0
No. of labs	26	26	26	26	26	26	26	26	26
No. of results used for stat. evaluation	19	22	7	24	24	24	24	22	24
Assigned value	2.6	4.9	0.8	14.9	15.2	13.5	12.0	4.4	67.9
rel. target std.	25	25	25	25	25	25	25	25	25
rel. reprod. std.	18.0	20.4	30.9	21.0	20.9	19.5	41.6	34.8	20.6
|z|≤ 2 [%]	90	88	100	88	96	88	90	91	92

z'-scores were calculated for Hn, Sc group and Sp group due to a non-negligible uncertainty of the assigned value: ($0.3 \times \sigma_{PT} \leq u_{assigned\ value} \leq 0.7 \times \sigma_{PT}$)

For the eight analyte-matrix-combinations tested the RSD_R values ranged from 18 % for echimedine group (only Em was spiked) to 42 % for senecionine group (only Sc was spiked) and was 21 % for the total PA content (Table 4). The success rate of laboratories (|z| score ≤ 2) varied from 0 to 100 % (Figure 1).

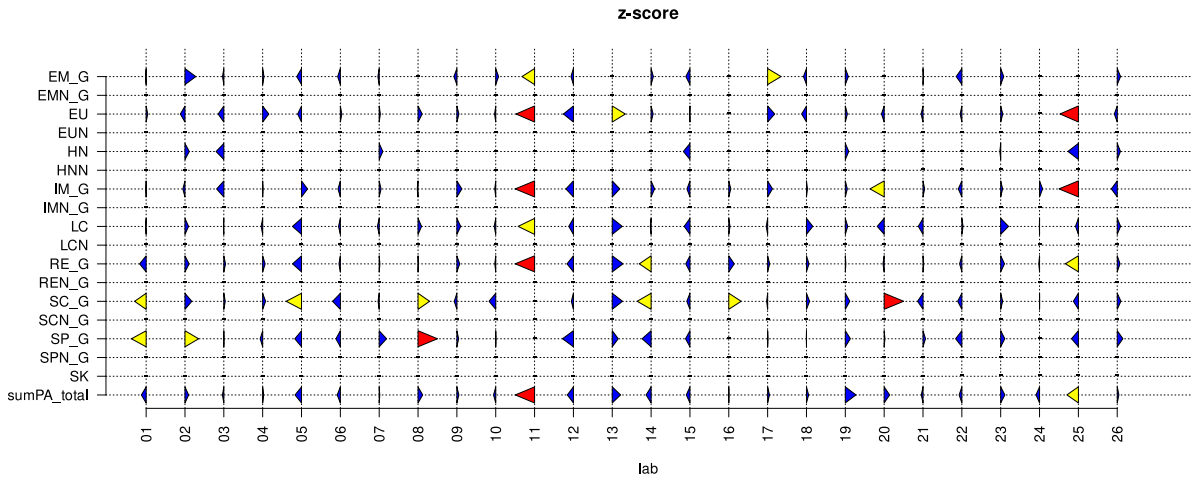


Figure 2: z-score results for honey sample 1, rel. target standard deviation is 25 % and corresponds to |z|-score = 1 (blue triangle: |z|-score ≤ 2, yellow triangle: 2 < |z|-score < |3|, red triangle |z|-score ≥ 3)

Honey sample 2 was spiked with echimidine-*N*-oxide, europine-*N*-oxide, heliotrine-*N*-oxide, intermedine-*N*-oxide, lasiocarpine-*N*-oxide, retrorsine-*N*-oxide, senecionine-*N*-oxide, seneciphylline-*N*-oxide in concentrations between 102 to 799 µg/kg and with senkirkine in a concentration of 0.5 µg/kg (refer to Table 3). Except europine-*N*-oxide, heliotrine-*N*-oxide and lasiocarpine-*N*-oxide that were still detectable in low concentrations all other PANOs were completely degraded after three months. A certain proportion of the spiked PANOs were converted to their corresponding tertiary free base, as they were detected by all participants even though they were not originally added to the sample.

Table 5: Analytical results and statistical characteristics for honey sample 2

PA	Em group	Eu	EuNO	Hn	HnNO	Im group	Lc	LcNO	Re group	Sc group	Sp group	Total PA
Lab	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
01	47.4	6.7	1.7	9.1	5.9	17.2	36.4	2.7	16.2	3.4	4.9	151.6
02	74.2	4.8		49.2	1.5	15.5	39.2	1.0	32.7	8.8	28.0	254.9
03	45.4	4.2	0.6	33.9	4.0	11.1	30.2	1.6	31.8	6.5	17.1	186.9
04	54.7	6.6	1.8	38.2	10.6	16.0	24.5	4.1	28.2	6.6	15.7	207.0
05	41.0	5.0	2.0	27.0	8.0	21.0	19.0	2.0	17.0	1.0		143.0
06	47.4	6.0	<LOQ	45.0	11.3	15.0	31.5	3.3	23.6	4.4	14.0	201.4
07	51.3	5.4	1.9	46.9	14.2	16.8	22.5	4.4	29.0	5.4	21.9	219.7
08	53.0	6.1	<LOQ	51.6	13.4	16.3	30.6		21.7	10.3	36.2	239.2
09	35.7	4.8	1.5	36.0	8.3	15.1	26.4	2.6	23.5	4.3	16.3	174.5
10	62.7	5.5	1.6	38.0	10.1	16.1	30.9	3.1	24.9	3.7	17.1	213.6
11	19.2	1.1	<LOQ	8.4	5.7	2.0	8.1	2.1	3.0	<LOQ	2.4	52.0
12	42.4	2.8	<LOQ	13.3	<LOQ	9.4	22.2	<LOQ	18.5	5.5	8.5	122.6
13	54.0	7.0	<LOQ	47.0	16.0	18.0	34.0	6.9	36.0	7.7	20.0	246.6
14	60.8	6.0	3.3	32.6	8.7	22.8	34.3	4.7	12.4	2.0	8.8	198.0
15	34.9	5.0	0.7	26.8	3.8	12.9	19.4	1.4	19.6	4.9	12.6	142.6
16	85.0	<LOQ	<LOQ	97.1	23.1	25.8	44.3	<LOQ	46.5	10.3	21.2	353.3
17	69.7	6.6	3.6	47.5	13.0	19.5	28.9	6.0	29.8	6.5	18.1	250.2
18	63.5	4.3	1.2	48.2	9.2	17.9	34.3	2.6	31.8	6.5	19.4	238.9
19	54.0	6.2	<LOQ	45.7	24.1	19.5	32.7	7.5	27.5	6.9	18.8	285.4
20	44.9	6.2	<LOQ	41.1	10.8	5.4	26.0	5.5	31.3	19.6	17.2	207.9
21	64.3	7.2	<LOQ	49.2	3.4	22.5	31.3	<LOQ	33.8	5.5	20.6	237.7
22	35.7	5.0	<LOQ	33.9	5.0	12.9	31.7	2.0	24.3	4.6	15.1	170.2
23	61.2	7.6	2.2	54.6	12.1	19.8	45.2	3.6	28.4	6.8	20.5	265.6
24	49.0	5.0	<LOQ	41.0	11.0	18.0	33.0	<LOQ	26.0	5.0	18.0	206.0
25	2.3	0.9	0.2	21.7	4.4	0.3	27.2	2.1	11.1	5.3	12.6	88.4
26	51.6	6.8	1.1	61.5	9.1	15.4	46.9	3.7	32.6	8.7	24.4	264.4
No. of labs	26	26	26	26	26	26	26	26	26	26	26	26
No. of results	24	23	13	24	23	24	24	19	24	24	23	24
Assigned	52.8	5.7	1.7	41.7	9.8	16.8	31.2	3.5	26.7	6.1	17.8	213.9
rel. targ.	25	25	25	25	25	25	25	25	25	25	25	25
rel. reprod.	23.8	20.1	48.8	28.3	50.0	24.4	24.3	54.0	26.6	42.1	28.7	23.9
|z|≤ 2	88	88	71	85	73	85	92	80	85	80	76	88

z'-scores were calculated for EuN, HnN, LcN and Sc group due to a non-negligible uncertainty of the assigned value: ($0.3\ \sigma_{PT} \leq u_{assigned\ value} \leq 0.7\ \sigma_{PT}$)

For the eight analyte-matrix-combinations tested the RSD_R values ranged from 20 % for europine to 54 % for lasiocarpine-*N*-oxide and was 24 % for the total PA content (Table 5). The success rate of laboratories (|z|-score ≤ 2) varied from 20 to 100 % (Figure 3).

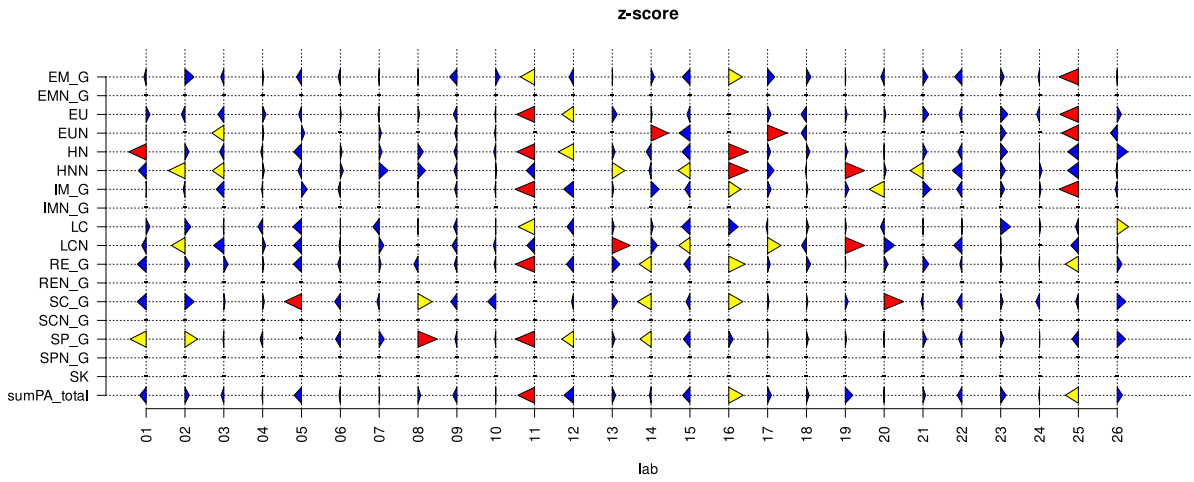


Figure 3: z-score results for honey sample 2, rel. target standard deviation is 25 % and corresponds to |z|-score = 1 (blue triangle: |z|-score ≤ 2, yellow triangle: 2 < |z|-score < |3|, red triangle |z|- score ≥ 3)

Honey sample 3 was spiked with all PA/PANOs at concentrations between 1.5 to 118 µg/kg and with senkirkine at a concentration of 1.7 µg/kg (refer to Table 3), except for the groups Em/EmNO and Im/ImNO, which were present in the sample as natural contaminants. For the spiked *N*-oxides, a reduction of 90 % was determined from the consensus values after six weeks of storage.

Table 6: Analytical results and statistical characteristics for honey sample 3

PA	Em gr	Eu	EuNO	Hn	HnNO	Im gr	Lc	LcNO	Re gr	ReNO gr	Sc gr	ScNO gr	Sp gr	SpNO gr	Sk	Total PA
Lab	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
01	<LOQ	79.2	4.4	8.2	4.0	29.0	9.6	2.1	23.6	2.4	51.4	1.0	44.2	1.3	1.4	261.8
02	1.2	69.4	1.1	8.0	1.7	<LOQ	10.1	1.4	22.0	<LOQ	75.9	<LOQ	63.0	<LOQ	1.4	255.3
03	0.6	70.6	1.3	6.9	1.4	17.6	9.8	1.1	17.7	0.3	69.4	0.2	45.0	0.2	1.3	243.4
04	<LOQ	106.8	3.7	8.2	3.3	28.0	5.0	7.4	19.4	<LOQ	62.8	<LOQ	36.2	<LOQ	1.2	282.0
05	1.0	70.0	4.0	8.0	3.0	18.0	5.0	2.0	17.0	2.0	32.0	<LOQ	40.0	<LOQ	<LOQ	202.0
06	<LOQ	103.2	3.9	8.5	3.9	33.2	5.9	2.3	16.4	1.3	52.3	<LOQ	35.9	<LOQ	<LOQ	266.7
07	<LOQ	72.6	2.0	12.3	3.8	24.4	6.3	2.2	17.7	1.6	57.8	1.9	45.7	2.0	1.2	251.5
08	<LOQ	91.9	<LOQ	10.6	<LOQ	24.7	6.8	<LOQ	15.5	<LOQ	99.3	<LOQ	92.9	<LOQ	<LOQ	341.8
09	<LOQ	86.9	2.6	8.3	2.2	21.5	7.6	1.3	17.0	<LOQ	51.1	<LOQ	41.4	<LOQ	1.3	241.2
10	<LOQ	67.7	6.4	6.6	7.1	2.9	4.8	5.6	15.6	4.6	50.3	2.5	36.7	3.6	1.1	215.5
11	<LOQ	37.3	2.2	5.5	3.6	0.9	2.5	<LOQ	10.1	0.6	20.3	<LOQ	21.1	0.7	<LOQ	104.8
12	<LOQ	56.3	<LOQ	11.0	3.6	24.6	4.2	1.2	20.2	<LOQ	79.6	<LOQ	61.5	1.1	1.5	264.8
13	<LOQ	110.0	5.3	10.0	<LOQ	45.0	5.2	5.1	26.0	<LOQ	83.0	<LOQ	50.0	<LOQ	<LOQ	339.6
14	1.1	90.1	6.3	9.9	5.0	33.7	5.1	3.3	26.0	<LOQ	63.2	1.0	60.2	1.2	1.7	307.7
15	0.6	97.0	1.7	6.4	1.7	18.6	4.5	1.3	16.5	<LOQ	57.2	<LOQ	34.6	<LOQ	1.4	241.4
16	<LOQ	130.2	92.9	14.7	<LOQ	54.5	<LOQ	<LOQ	28.9	<LOQ	72.4	<LOQ	55.3	<LOQ	<LOQ	448.9
17	1.0	86.7	4.1	10.9	2.9	27.5	10.6	1.9	19.3	1.5	61.0	2.1	40.5	1.7	1.4	273.1
18	<LOQ	87.2	2.9	10.3	3.1	27.9	8.6	1.9	25.0	<LOQ	76.7	<LOQ	49.7	<LOQ	1.2	294.5
19	<LOQ	80.3	19.5	8.0	5.1	30.6	5.5	3.4	20.4	5.7	60.9	4.6	38.6	3.9	1.4	295.7
20	<LOQ	123.3	5.5	7.9	<LOQ	21.2	9.7	<LOQ	20.7	<LOQ	246.7	<LOQ	41.9	<LOQ	<LOQ	476.9
21	<LOQ	91.5	2.5	10.0	<LOQ	34.9	7.8	<LOQ	21.4	<LOQ	51.5	2.8	49.6	<LOQ	<LOQ	272.0
22	<LOQ	65.0	<LOQ	7.3	1.1	11.9	6.0	<LOQ	19.0	<LOQ	61.4	<LOQ	42.9	<LOQ	1.4	216.0
23	0.6	87.4	5.2	10.5	3.7	27.2	10.2	2.6	22.8	1.2	64.4	1.4	50.4	1.2	1.5	290.3
24	<LOQ	72.0	<LOQ	8.0	<LOQ	27.0	7.0	<LOQ	18.0	<LOQ	57.0	<LOQ	41.0	<LOQ	<LOQ	230.0
25	<LOQ	65.7	1.1	7.9	1.2	16.8	6.5	0.8	16.9	0.2	55.3	0.2	36.6	0.2	1.2	210.6
26	0.8	49.9	3.2	8.8	3.7	20.4	11.1	2.8	14.5	0.8	57.8	0.2	38.4	1.4	1.6	215.5
No. of labs	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
No. of results used for stat. evaluation	8	24	20	24	18	23	23	18	24	10	24	10	24	10	16	24
Assigned value [µg/kg]	0.9	84.3	4.0	9.0	3.3	25.8	7.2	2.4	19.9	1.9	63.7	1.7	45.7	1.7	1.4	270.3
rel. target std. dev [%]	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
rel. reprod. std. dev. [%]	32.6	23.1	53.3	20.1	44.3	30.4	35.4	50.4	20.1	76.8	21.0	75.9	20.6	68.2	12.4	17.9
$\|z\| \leq 2$	100	92	67	96	75	77	92	75	100	62	88	58	92	62	100	88

z'-scores were calculated for Em, EnN, HnN, Im group, Lc, LcN, ReN group, ScN group, SpN group due to a non-negligible uncertainty of the assigned value: ($0.3 \times \sigma PT \leq u_{assigned\ value} \leq 0.7 \times \sigma_{PT}$)

The data from samples 1 and 2, which were spiked with either free bases (sample 1) or *N*-oxides (sample 2), show that a certain proportion of the *N*-oxides is reduced to tertiary bases, which are then further degraded. Consequently, the PA levels determined by the participants in sample 3 were in comparable ranges to those spiked six weeks ago (sample 1 and 2 were stored for three month).

For the analyte-matrix-combinations tested the RSD_R values ranged from 12 % for senkirkine to 77 % for retrorsine-*N*-oxide and was 18 % for the total PA content (Table 6). The success rate of laboratories (|z| score ≤ 2) varied from 20 to 100 % (Figure 6).

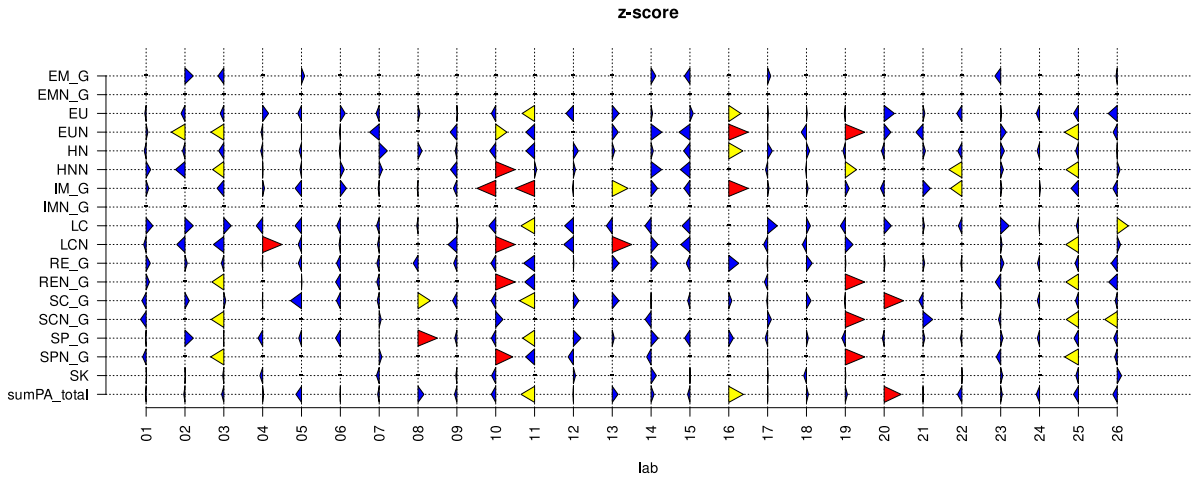


Figure 4: z-score results for honey sample 3, rel. target standard deviation is 25 % and corresponds to |z|-score = 1 (blue triangle: |z|-score ≤ 2, yellow triangle: 2 < |z|-score < |3|, red triangle |z|-score ≥ 3)

4.1 Evaluation of the laboratory performance in terms of bias, precision and absolute mean z-score

One of the main objectives of proficiency testing is to evaluate the performance of laboratories and the methods they use, often referred to as fit for purpose testing. Proficiency testing results provide an estimate of laboratory or method bias (also known as systematic error), as the assigned value for an analyte in a test material serves as a reliable estimate of the true value and is similar to bias estimation using reference materials.

The laboratory's bias was calculated as the mean deviation of all tested analyte-matrix-combinations from the respective assigned values (see section 3.3.2). The mean bias of the laboratories for the honey samples are shown in Figure 5. A laboratory can check the influence of individual PAs on the bias using the figures shown in Appendix B. The figures show the extent to which individual PAs in the samples and in the standard have contributed to underestimates or overestimates. Systematic deviations can be recognised due to equal deviations. The dispersion of the bias results of the individual analyte-matrix-combination around their mean bias is shown in Figure 5 and can be used as a measure of precision. Here again a laboratory can check the influence of individual PAs on the precision using the figures shown in Appendix B.

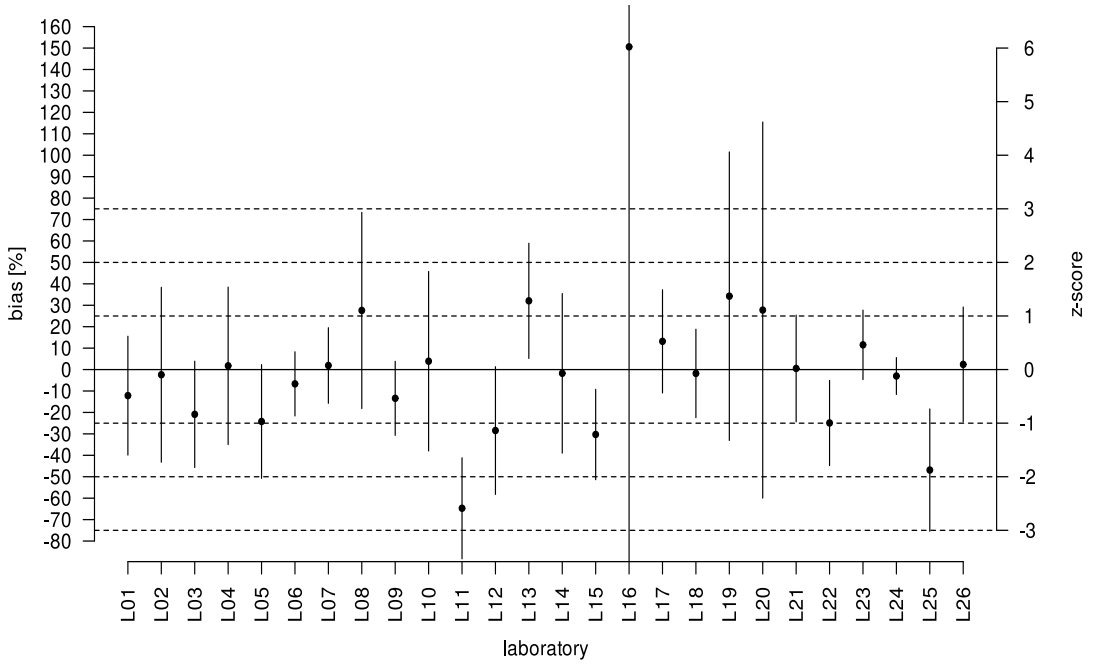


Figure 5: Laboratory performance in terms of bias and precision. The evaluation of the bias is based on the percentage deviation of the laboratory from the assigned value (Equation 7). Shown is the mean deviation of all tested analyte-matrix-combinations of honey samples 1–3 (black circle). The assigned values represent the true value, i.e. the deviation is zero or the baseline at y = 0 corresponds to the assigned value. The distance of a black circle from zero indicates both the magnitude and the direction of the bias (systematic deviation) of a laboratory. The mean bias (left y-axis) corresponds to the mean z-score of the laboratory (right y-axis). For example, since the target standard deviation of 25 % corresponds to a z-score of 1, a mean negative deviation of 12.5 % from the assigned value corresponds to a mean z-score of -0.5. The vertical lines indicate the standard deviation of the individual analyte-matrix-combinations around their mean bias and reflect the precision of a laboratory (see Appendix B). The precision value for L16 is truncated in this figure.

According to Regulation (EU) 2023/915 [1], a laboratory can report a default expanded measurement uncertainty of 50 % if it meets the specific requirements for intra-laboratory precision and successfully participates in proficiency tests. A mean z-score $|z| \leq 2$ has to be achieved, demonstrating that the required precision under reproducibility conditions (RSD_R) is met. Table 7 shows the mean absolute z-scores obtained by the laboratories for honey samples 1–3.

Table 7: Mean absolute z-score of laboratories calculated from honey samples 1–3 according to Equation 10

Laboratory	mean |z-score|
L01	0.86
L02	1.38
L03	1.03
L04	0.76
L05	1.17
L06	0.56
L07	0.54
L08	1.32
L09	0.73
L10	1.09
L11	2.61
L12	1.39
L13	1.37
L14	1.22
L15	1.25
L16	6.02
L17	0.72
L18	0.68
L19	1.52
L20	1.96
L21	0.80
L22	1.02
L23	0.66
L24	0.30
L25	1.88

4.2 Evaluation of methods applied by laboratories

Laboratories were asked to give information on sample preparation and detection with special focus on calibration. All laboratories analysed the samples using liquid chromatography in combination with tandem mass spectrometry.

Seven out of 26 laboratories carried out a direct analysis of the extracts (“dilute and shoot”). Solid phase extraction (SPE) was applied by 16 participants, either using strong cation exchange material or with polymeric sorbents. A few laboratories used dispersive SPE such as QuEChERS.

To obtain indications of whether specific steps in the sample preparation of the methods used might correlate with any bias or precision, the methods (laboratories) were arranged according to their respective performance in this PT. For this purpose, the mean bias (Equation 7) was calculated and ordered from the (absolute) lowest to the highest value

(Table 8). In addition, the precision (Equation 9) was calculated and ordered from the (absolute) lowest to the highest value Table 9).

These results show that satisfactory performance does not depend on a particular procedure and that different procedures lead to satisfactory results. Rather, applying more or less the same procedure can either lead to very low mean z-scores implicating a high precision and accuracy as well to certain degree of questionable results (Table 8 and Table 9).

Table 8: Summary of method information ordered by the mean bias (Equation 7) obtained for the individual PA/PANOs in the three samples (a low bias indicates a satisfactory performance)

Mean bias [%]	Sample weight [g]	Extracting agent	Further clean up	Clean up	Injection volume	Cal. Type	Number of cal. Level	Force through origin	Weighed calibr.
	Sample preparation				LC-MS/MS detection				
0.5	2	0.2% HCOOH	yes	SPE (Strata X)	5	STA	5	no	no
-1.8	5	0.05M H_2SO_4	yes	SPE (HRX)	5	MMS	7	no	1/x
1.8	2	H_2O:MeOH:ACN 1:1:1 (v:v:v)	no	NA	20	STA	6	no	1/x
-1.8	2	0.2% HCOOH	no	NA	10	MMS	8	no	1/x
1.9	5	0.05M H_2SO_4	Yes	SPE (SCX)	3	external	10	yes	no
2.4	10	0.05M H_2SO_4	yes	SPE	2	external	9	no	yes
-2.4	1	2 % HCOOH	yes	dilution	10	external	7	no	1/x
-3.0	10	0.1M H_2SO_4	yes	SPE (SCX)	1	external, with ISTD	8	no	1/x
3.9	1	H_2O/MeOH (9/1 v/v) + 0.07 % HCOOH	no	NA	3	external	8	yes	yes
-6.7	2	0.05M H_2SO_4	no	NA	2	STA	1	yes	no
11.5	2	0.2% HCOOH	yes	SPE (Strata X)	2.5	STA	2	yes	no
-12.2	5	citrated buffer solution	yes	SPE	NA	NA	NA	NA	NA
13.2	4	H2O/ACN	yes	QuEChERS: PSA	2	external	8	yes	1/x
-13.4	2.5	0.05M H_2SO_4	yes	SPE (SCX)	10	external	10	yes	no
-20.9	10	0.05M H_2SO_4	yes	SPE (SCX)	5	MMS	6	no	1/x
-24.2	2	ACN	no	NA	25	STA	1	no	no
-24.9	2.5	0.2% HCOOH	yes	QuEChERS: EN	NA	MMS	NA	NA	NA
27.6	2	2 % HCOOH	no	dilution	NA	NA	NA	NA	NA
27.8	2	0.2% HCOOH	yes	SPE	7.5	MMS	7	no	No
-28.5	5	0.05M H_2SO_4	yes	SPE (HRX)	5	MMS	7	no	1/x
-30.3	10	0.05M H_2SO_4	yes	SPE (SCX)	10	external	6	no	1/x
32.1	2	water with HCOOH	no	-	20	external	5	yes	1/x
34.2	2	0.05M H_2SO_4	yes	SPE (OASIS MCX)	5	STA	2	no	1/X
-46.9	10	2% HCOOH	yes	SPE (SCX)	2	MMS	7	no	Yes
-64.7	5	0.05M H_2SO_4	yes	SPE	2	MMS	5	no	1/x
150.6	2	0.2% HCOOH	yes	SPE	5	STA	1	no	No

Table 9: Summary of method information ordered by the precision (Equation 9) obtained for the individual PA/PANOs in the three samples

Precision [%]	Sample weight [g]	Extracting agent	Further clean up	Clean up	Injection volume	Cal. Type	Number of cal. Level	Force through origin	Weighed calibr.
	Sample preparation				LC-MS/MS detection				
8.6	10	0.1M H_2SO_4	yes	SPE (SCX)	1	external, with ISTD	8	no	1/x
15.0	2	0.05M H_2SO_4	no	NA	2	STA	1	yes	no
16.2	2	0.2% HCOOH	yes	SPE (Strata X)	2.5	STA	2	yes	no
17.3	2.5	0.05M H_2SO_4	yes	SPE (SCX)	10	external	10	yes	no
17.6	5	0.05M H_2SO_4	Yes	SPE (SCX)	3	external	10	yes	no
19.9	2.5	0.2% HCOOH	yes	QuEChERS: EN	NA	MMS	NA	NA	NA
20.6	2	0.2% HCOOH	no	NA	10	MMS	8	no	1/x
21.1	10	0.05M H_2SO_4	yes	SPE (SCX)	10	external	6	no	1/x
23.6	5	0.05M H_2SO_4	yes	SPE	2	MMS	5	no	1/x
24.1	4	H_2O/ACN	yes	QuEChERS: PSA	2	external	8	yes	1/x
24.8	10	0.05M H_2SO_4	yes	SPE (SCX)	5	MMS	6	no	1/x
24.9	2	0.2% HCOOH	yes	SPE (Strata X)	5	STA	5	no	no
26.5	2	ACN	no	NA	25	STA	1	no	no
26.8	10	0.05M H_2SO_4	yes	SPE	2	external	9	no	yes
26.9	2	water with HCOOH	no	-	20	external	5	yes	1/x
27.7	5	citrated buffer solution	yes	SPE	NA	NA	NA	NA	NA
28.5	10	2% HCOOH	yes	SPE (SCX)	2	MMS	7	no	Yes
29.8	5	0.05M H_2SO_4	yes	SPE (HRX)	5	MMS	7	no	1/x
36.7	2	H2O:MeOH:ACN 1:1:1 (v:v:v)	no	NA	20	STA	6	no	1/x
37.2	5	0.05M H_2SO_4	yes	SPE (HRX)	5	MMS	7	no	1/x
40.8	1	2 % HCOOH	yes	dilution	10	external	7	no	1/x
41.9	1	H_2O/MeOH (9/1 v/v) + 0.07 % HCOOH	no	NA	3	external	8	yes	yes
45.8	2	2 % HCOOH	no	dilution	NA	NA	NA	NA	NA
67.3	2	0.05M H_2SO_4	yes	SPE (OASIS MCX)	5	STA	2	no	1/X
87.7	2	0.2% HCOOH	yes	SPE	7.5	MMS	7	no	No
423.7	2	0.2% HCOOH	yes	SPE	5	STA	1	no	No

Table 10: Analytical results and statistical characteristics for the standard solution

PA	Em	EmNO	Eu	EuNO	Hn	HnNO	Im	ImNO	Lc	LcNO	Re	ReNO	Sc	ScNO	Sp	SpNOr	Sk	Total-PA	Atr	Sco
Lab	ng/mL																			
01	3.1	6.0	9.1	14.9	11.3	6.7	13.9	4.7	8.3	5.2	13.1	7.5	14.3	5.8	10.8	7.8	3.6	146.3	6.2	12.8
02	4.0	5.2	8.0	14.5	13.4	6.3	13.7	3.2	9.0	5.1	16.0	6.9	15.4	5.7	15.6	7.1	3.8	152.8	n.r.	n.r.
03	2.8	5.7	7.5	13.6	11.3	6.4	13.4	3.9	8.5	5.2	11.9	6.4	14.7	5.8	10.9	6.9	3.5	138.3	n.r.	n.r.
04	3.0	5.2	7.0	13.3	10.9	5.4	12.1	3.8	7.6	5.4	10.6	6.4	12.4	5.7	10.6	6.5	3.3	129.1	5.9	10.7
05	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
06	2.4	6.8	9.2	14.6	12.0	5.9	11.5	3.7	6.9	4.8	11.4	8.2	10.2	6.2	9.5	10.0	3.7	137.0	7.7	11.8
07	3.2	5.8	7.2	13.0	13.8	6.4	12.9	3.6	9.8	5.4	12.9	6.7	13.8	5.8	11.6	7.6	3.5	143.0	n.r.	n.r.
08	2.9	5.8	7.8	14.9	12.4	6.0	14.0	3.8	8.4	5.5	12.9	5.9	24.6	12.0	23.2	6.8	3.7	170.4	n.r.	n.r.
09	2.2	5.7	7.6	15.5	11.9	6.6	14.2	2.1	8.5	5.2	12.2	8.8	11.4	5.6	10.5	7.1	3.5	138.6	5.3	11.2
10	3.7	5.4	7.8	14.7	12.7	6.9	15.0	4.2	9.1	5.9	10.0	7.3	11.9	4.7	11.7	6.8	4.1	141.8	n.r.	n.r.
11	3.7	6.7	8.6	14.0	13.1	7.0	15.8	3.4	10.7	6.5	14.9	6.5	14.6	5.7	11.9	7.7	4.5	155.3	6.5	12.9
12	4.1	6.1	7.5	16.0	14.9	5.8	16.3	4.6	9.6	5.3	14.3	7.8	20.0	6.0	12.4	7.7	4.1	162.5	8.3	14.3
13	3.6	8.7	12.5	21.8	15.7	8.2	17.0	4.1	7.4	7.7	18.0	10.7	19.6	8.6	14.5	9.9	4.8	192.7	n.r.	n.r.
14	2.1	8.6	8.7	15.3	16.4	7.1	15.4	2.6	8.8	5.1	14.1	7.5	13.5	5.6	12.2	7.2	3.5	153.7	n.r.	n.r.
15	2.9	5.2	8.3	13.0	9.2	5.9	12.7	3.7	7.8	4.6	11.2	5.9	13.2	4.9	10.2	6.2	3.3	128.1	n.r.	n.r.
16	<LOQ	6.6	7.1	14.1	10.2	7.6	14.4	<LOQ	7.9	5.2	15.0	6.0	9.8	5.5	8.9	6.0	<LOQ	124.3	7.9	14.1
17	4.8	5.8	10.1	18.0	14.0	7.5	18.4	5.5	11.8	6.5	13.4	8.0	29.5	14.1	12.1	8.3	4.5	192.3	n.r.	n.r.
18	2.6	5.3	6.6	12.8	11.1	6.3	12.2	3.8	8.5	4.6	11.3	6.2	12.7	5.2	3.3	9.5	6.3	128.2	n.r.	n.r.
19	2.4	5.4	7.0	12.4	11.0	5.7	13.6	3.6	7.7	4.9	11.2	5.6	14.9	5.0	10.5	5.7	3.6	130.2	n.r.	n.r.
20	5.5	4.4	5.5	10.0	10.7	4.9	5.5	5.0	5.3	5.2	10.1	4.8	31.2	5.6	5.1	10.9	5.2	134.8	n.r.	n.r.
21	3.6	7.5	9.2	18.7	12.6	5.4	17.0	4.8	7.9	3.4	13.7	8.8	12.5	7.4	13.6	7.8	3.4	156.9	7.3	15.9
22	3.6	5.5	7.6	15.3	11.5	7.1	13.4	4.9	11.3	6.3	14.4	8.2	15.2	7.1	12.2	7.7	3.8	155.1	4.1	10.0
23	2.8	5.5	9.3	14.5	13.2	5.5	13.7	3.4	8.5	5.9	11.5	6.2	13.5	6.5	10.2	7.1	3.3	140.6	5.6	14.2
24	2.5	4.9	6.6	11.2	9.7	5.7	12.2	3.2	7.5	4.5	10.2	5.5	11.6	5.1	9.4	6.0	3.2	119.0	n.r.	n.r.
25	<LOQ	<LOQ	4.0	6.4	5.2	2.9	3.4	2.1	2.8	2.4	5.4	3.5	6.4	3.2	4.3	3.3	1.6	56.9	n.r.	n.r.
26	3.5	5.5	7.4	13.9	11.9	6.0	12.4	3.6	8.8	4.8	12.3	6.5	13.8	5.7	12.4	8.0	3.5	140.1	7.8	15.2
No. of labs	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
No. used for st. ev.	23	24	25	25	25	25	25	24	25	25	25	25	25	25	25	25	24	25	11	11
Ass.value [ng/mL]	3.2	5.8	8.0	14.4	12.2	6.3	13.9	3.9	8.5	5.3	12.7	6.9	14.5	5.9	11.3	7.5	3.8	144.3	6.6	13.0
target-RSD_R. [%]	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
RSD_R. [%]	24.1	13.6	14.6	11.7	14.7	13.1	13.9	19.8	13.1	12.8	15.9	18.3	24.2	15.2	18.0	16.2	13.7	11.6	21.4	16.6
$\|z\| \leq 2$ [%]	91	96	96	92	96	96	92	100	96	96	96	96	84	92	84	96	92	96	100	100

n.r.: not reported

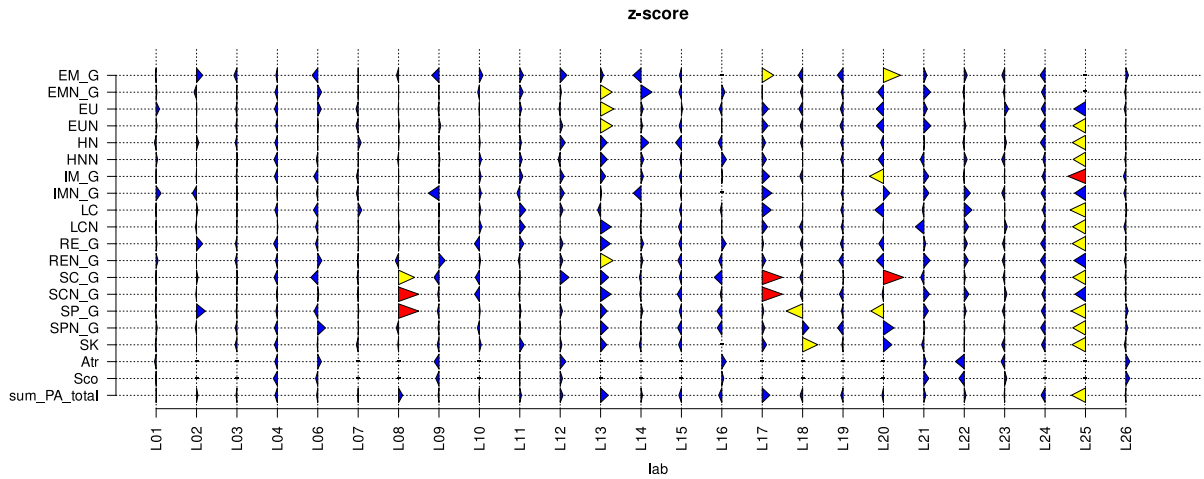


Figure 6: z-score results the standard solution, rel. target standard deviation is 25 % and corresponds to |z| score = 1 (blue triangle: |z| score ≤ 2, yellow triangle: 2 < |z| score < |3|, red triangle |z| score ≥ 3)

5 Pilot project on standard stability of PA/PANO and TA

5.1 Introduction

As part of the 2022 PT on “Determination of pyrrolizidine alkaloids and tropane alkaloids in herbs and spices”, a pilot study was started to test the storage stability of PA and TA standard solutions beyond the expiry date specified by the manufacturer [4]. A multi-toxin solution was prepared at the NRL, tested by the participants in 2022 and stored in a freezer since then. This solution is to be sent out regularly in various dilution levels as part of the NRL’s PT programme. The participants then analyse the unknown dilutions with their “non-expired” calibration standards. Based on the assigned values of the PT conducted in 2022, all results from subsequent PTs can serve as a trend for storage stability.

5.2 Results and conclusion for storage stability

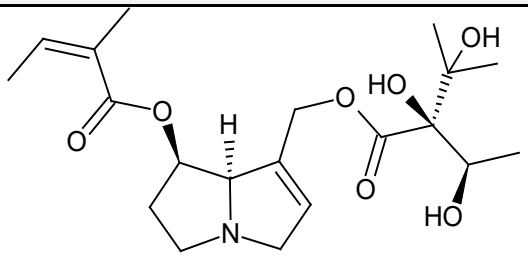
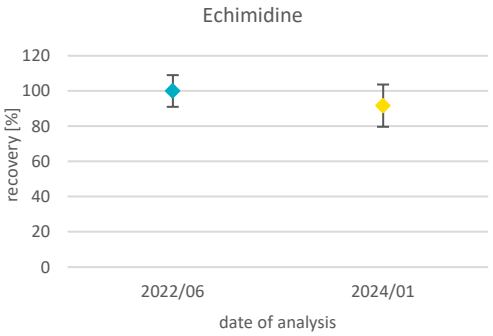
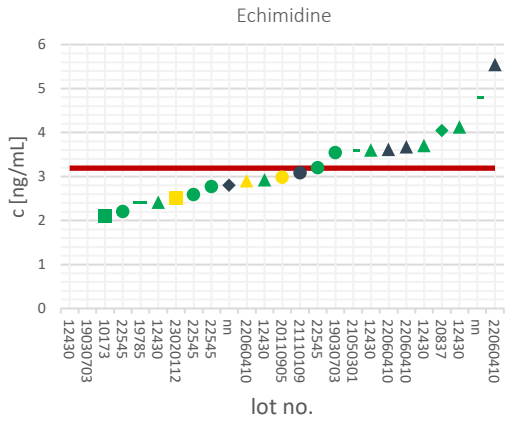
For almost all analytes, the concentrations determined in relation to the expected value (assigned value of the PT performed in 2022) were 95 % and above. Exceptions were retrorsine-*N*-oxide, europine and echimidine with recoveries of 91, 94 and 92 %. Since all assigned values are subject to uncertainty, future results will have to show whether these results represent a trend towards degradation of the analytes or whether it is just a temporary increased spread between the laboratories.

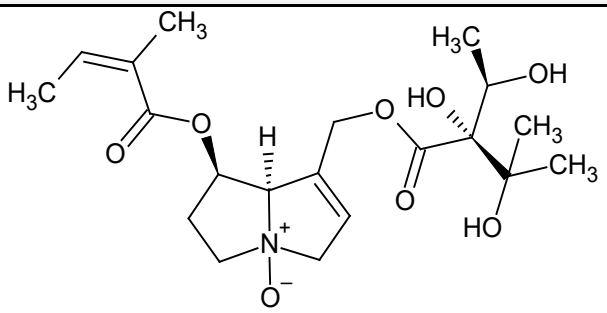
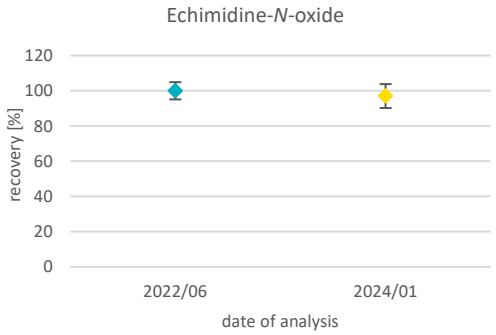
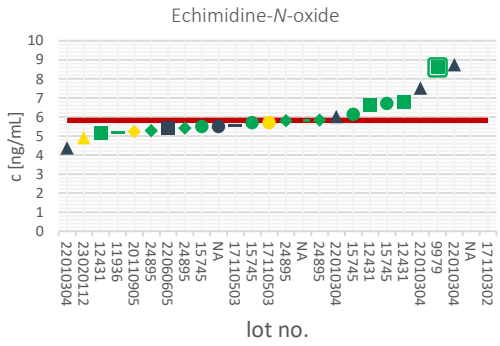
However, the first results clearly show that PAs and TAs have a relatively high storage stability. Degradation rates of more than 10 % are not to be expected when stored in the freezer for two years.

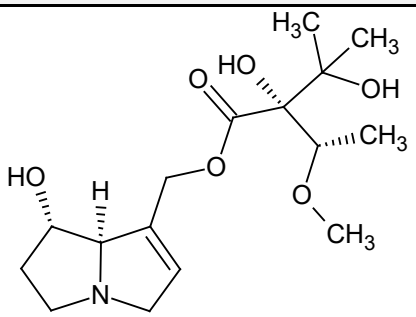
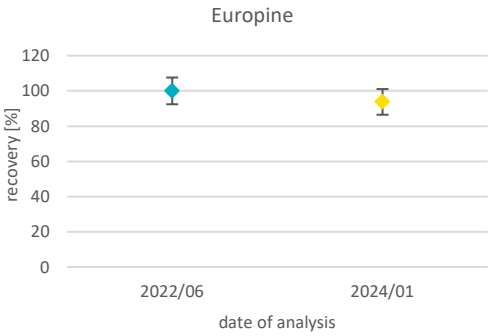
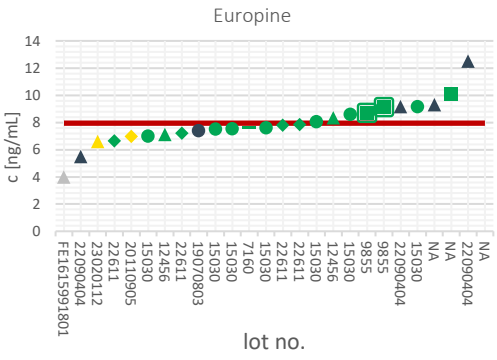
5.3 Results and certificates for individual PA/PANOs and TAs

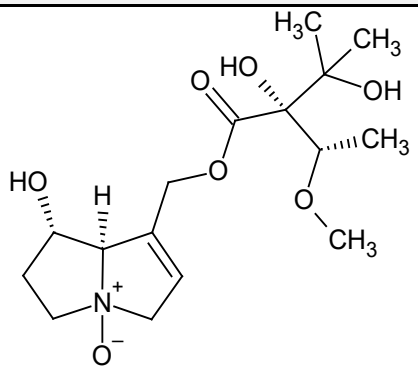
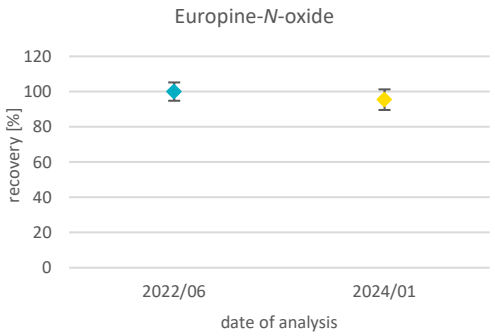
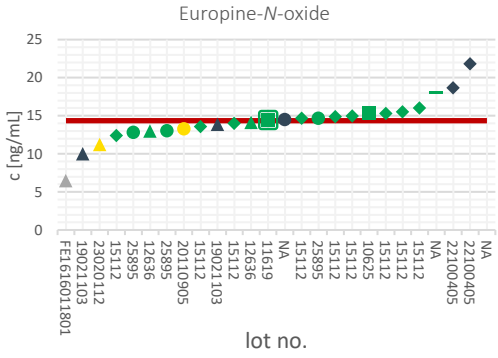
The graphs below show the individual results obtained by the laboratories in the standard solution, whereby the reference standard used (supplier and lot) is shown for each value. This makes it possible to estimate the influence of the calibration solution on the results.

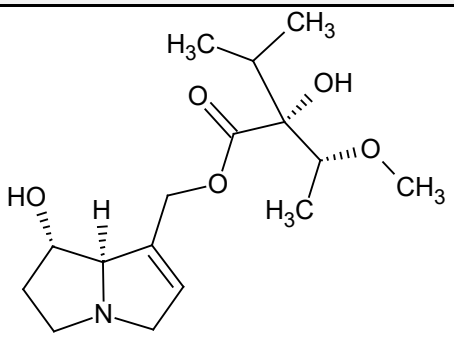
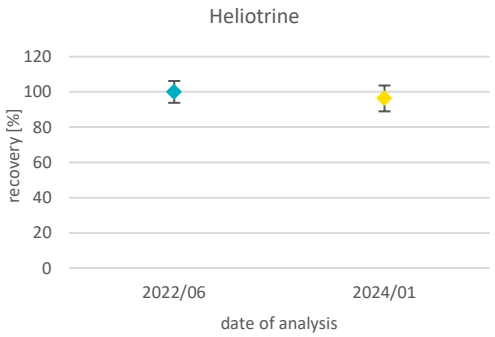
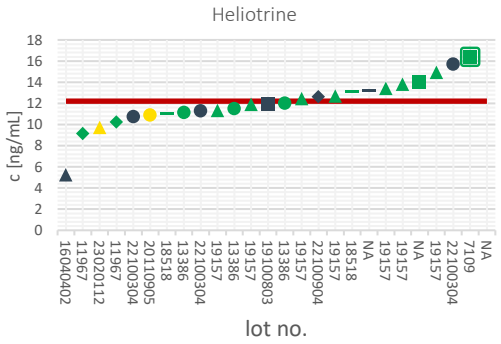
The data show that different lots/batches from one supplier lead to comparable results in most cases. Furthermore, in most cases, deviating results cannot be explained by the quality of the standard, as this supplier or this lot/batch was often also used by other laboratories and these did not achieve deviating results. In some exceptions, laboratories with deviating results have used reference standards that were not used by any other laboratory. In these cases, the influence of the standard is less clear and should be checked (e.g. europine, lasiocarpine and their *N*-oxides).

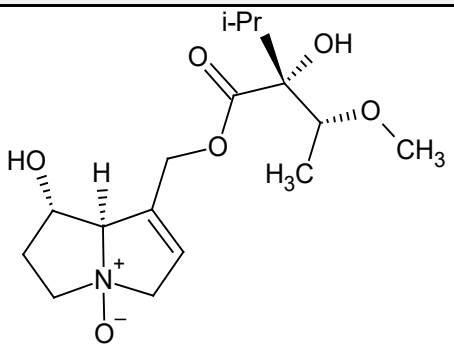
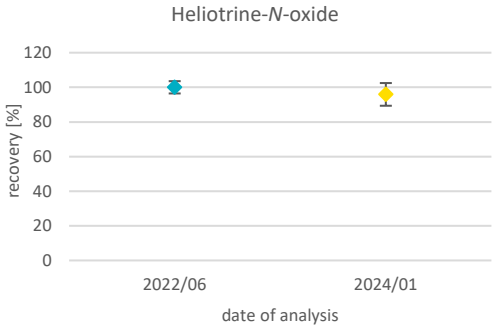
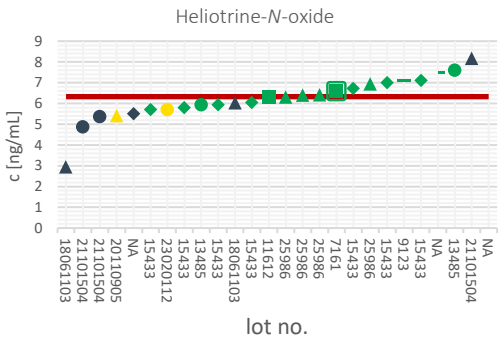
Certificate Of Proficiency Test Analysis		
compound	echimidine $C_{20}H_{31}NO_7$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 1:** Results of the Em concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	3.2	
number of individual laboratory results	23	
number of unique lots	6	
relative standard deviation between laboratory results [%]	24.1	**Info 2:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here: lots 22060410 and 19030703).

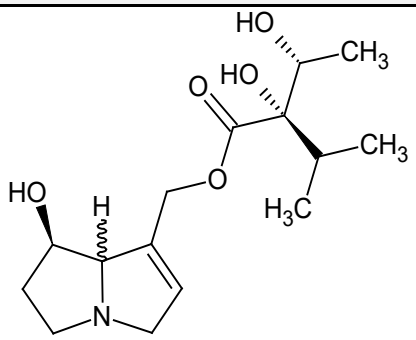
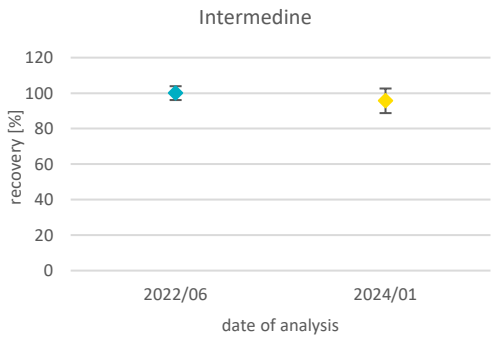
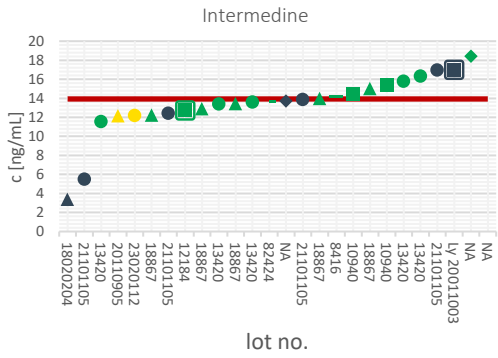
Certificate Of Proficiency Test Analysis		
compound	echimidine-*N*-oxide $C_{20}H_{31}NO_8$	
Start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 3:** Results of the EmNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	5.8	
number of individual laboratory results	24	
number of unique lots	11	
relative standard deviation between laboratory results [%]	13.6	**Info 4:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

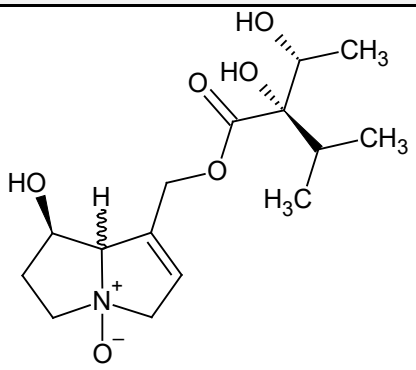
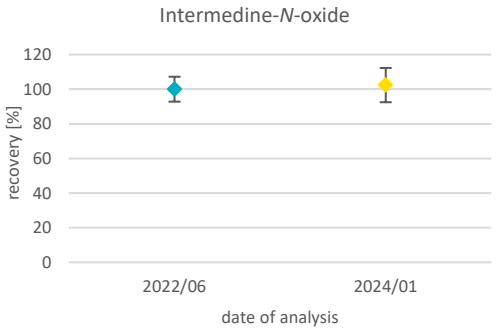
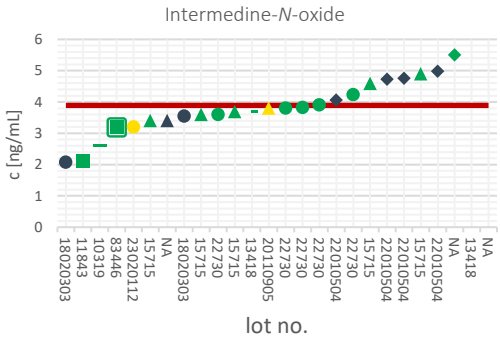
Certificate Of Proficiency Test Analysis		
compound	europine $C_{16}H_{27}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 5:** Results of the Eu concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	8.0	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	14.6	**Info 6:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, CarboSynth: grey. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

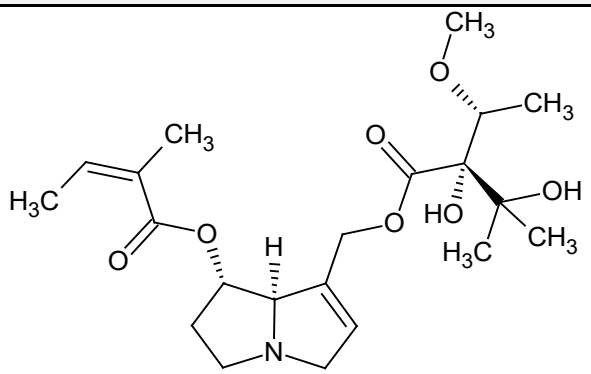
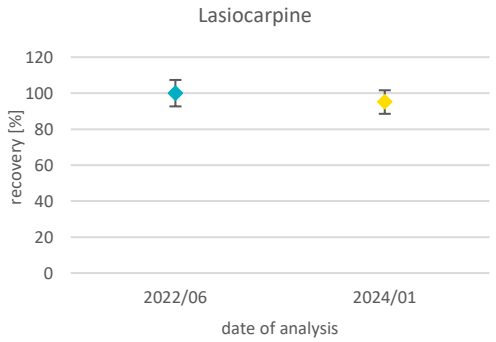
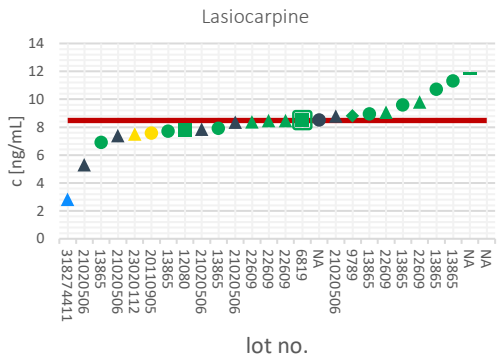
Certificate Of Proficiency Test Analysis		
compound	europine-*N*-oxide $C_{16}H_{27}NO_7$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 7:** Results of the EuNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	14.4	
number of individual laboratory results	25	
number of unique lots	11	
relative standard deviation between laboratory results [%]	11.7	**Info 8:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, CarboSynth: grey. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

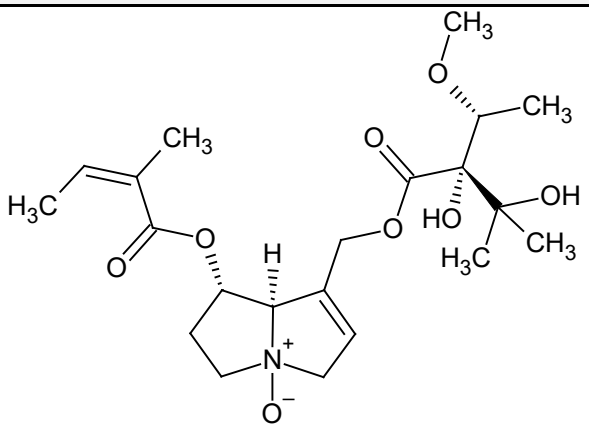
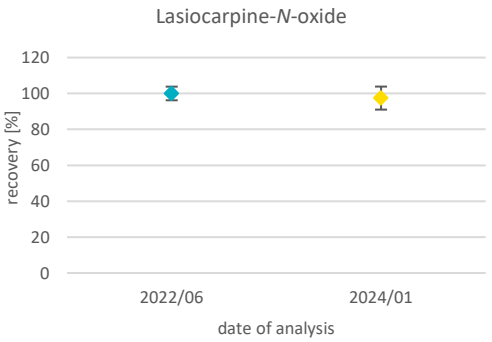
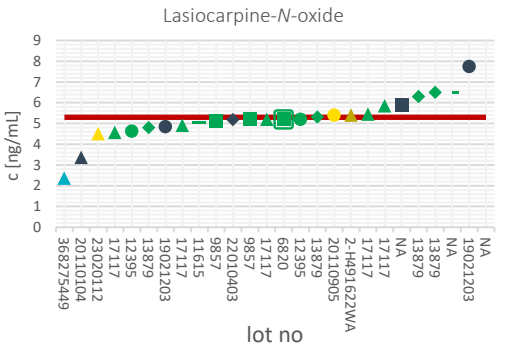
Certificate Of Proficiency Test Analysis		
compound	heliotrine $C_{16}H_{27}NO_5$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 9:** Results of the Hn concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	12.2	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	14.7	**Info 10:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

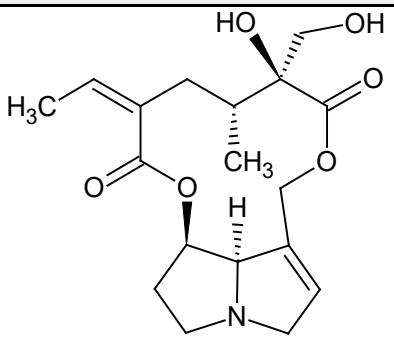
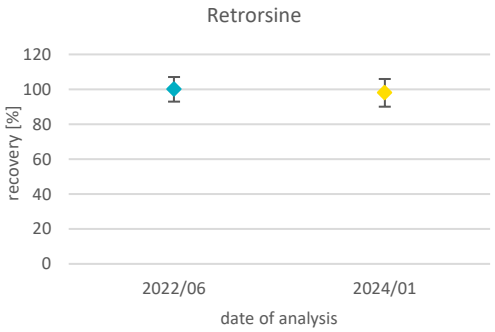
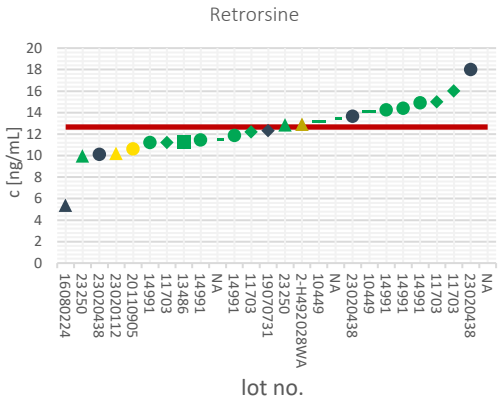
Certificate Of Proficiency Test Analysis		
compound	heliotrine-*N*-oxide $C_{16}H_{27}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	
		Info 11: Results of the HnNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	6.3	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	13.1	**Info 12:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

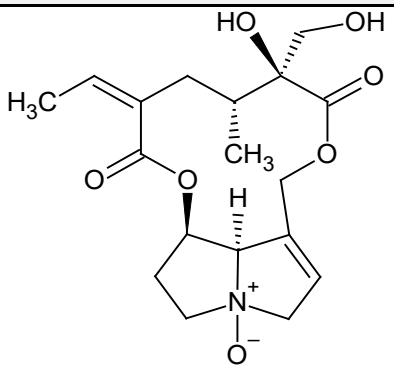
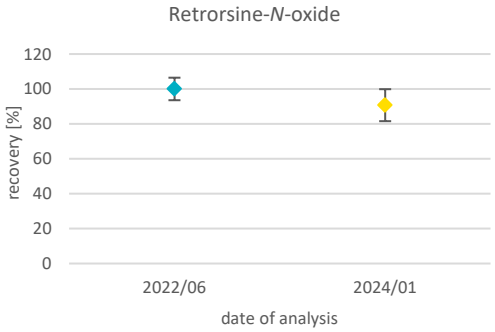
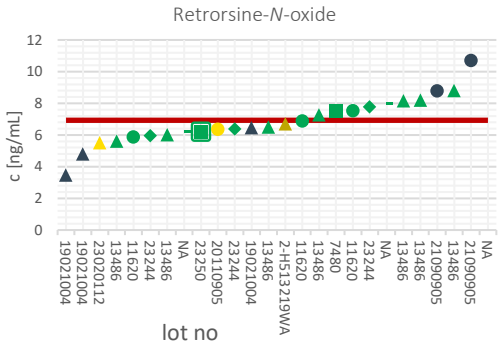
Certificate Of Proficiency Test Analysis		
compound	intermedine $C_{15}H_{25}NO_5$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 13:** Results of the Im concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	13.9	
number of individual laboratory results	25	
number of unique lots	11	
relative standard deviation between laboratory results [%]	13.9	**Info 14:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

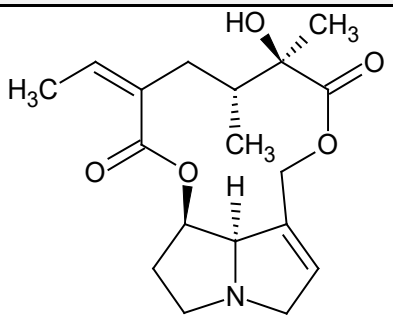
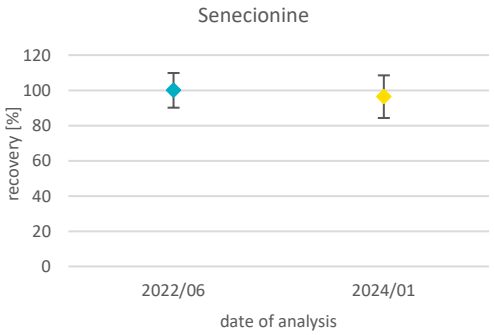
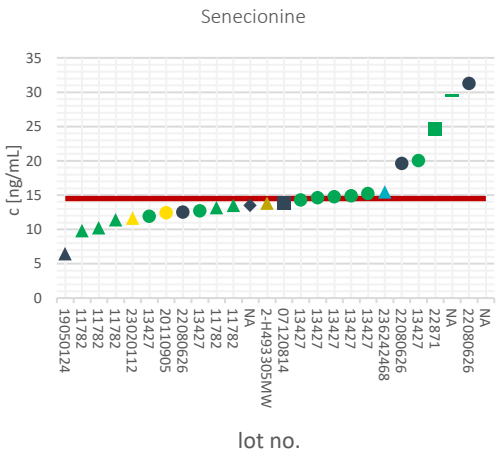
Certificate Of Proficiency Test Analysis		
compound	intermedine-*N*-oxide $C_{15}H_{25}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 15:** Results of the ImNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	3.9	
number of individual laboratory results	24	
number of unique lots	11	
relative standard deviation between laboratory results [%]	19.8	**Info 16:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

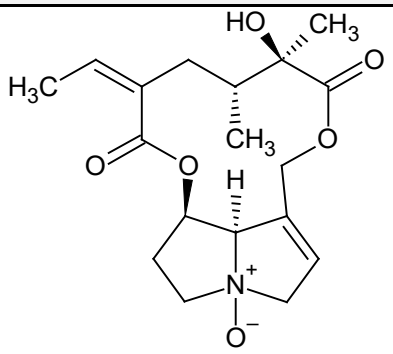
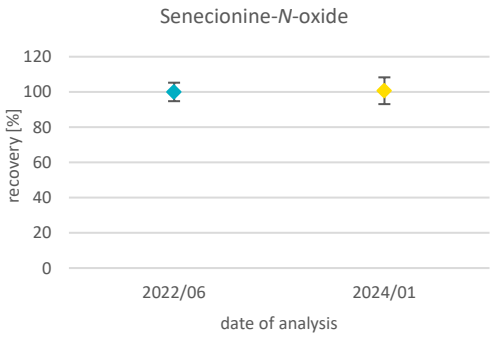
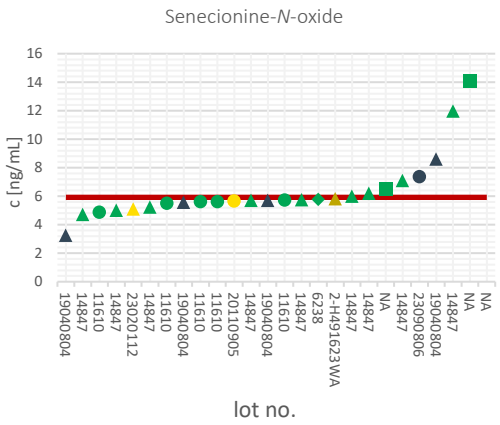
Certificate Of Proficiency Test Analysis		
compound	lasiocarpine $C_{21}H_{33}NO_7$	
start the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 17:** Results of the Lc concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	8.5	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	13.1	**Info 18:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Carl Roth: light blue. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

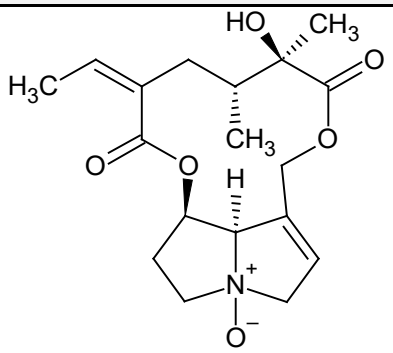
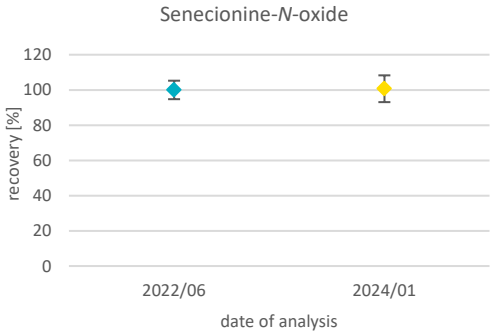
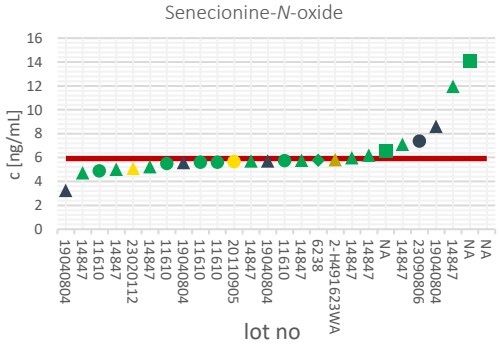
Certificate Of Proficiency Test Analysis		
compound	lasiocarpine-*N*-oxide $C_{21}H_{33}NO_8$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	
		Info 19: Results of the LcNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	5.3	
number of individual laboratory results	25	
number of unique lots	14	
relative standard deviation between laboratory results [%]	12.8	**Info 20:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Carl Roth: light blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

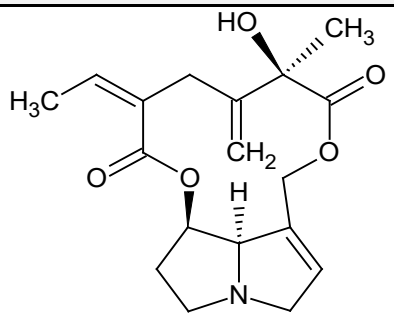
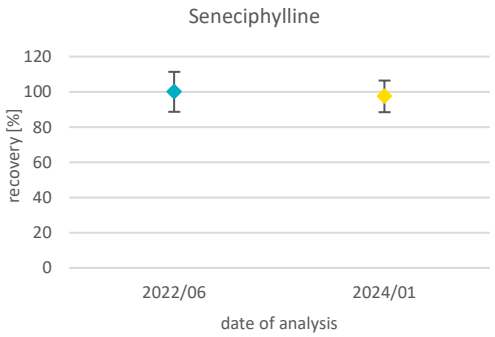
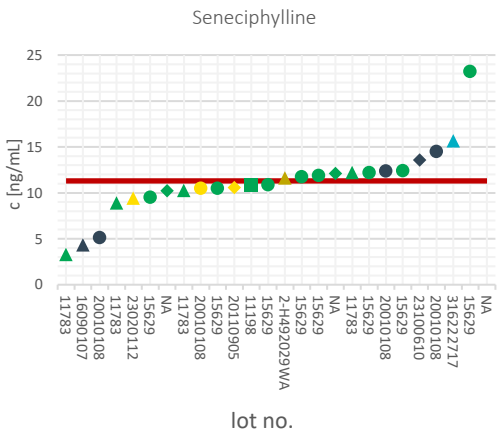
Certificate Of Proficiency Test Analysis		
compound	retrorsine $C_{18}H_{25}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 21:** Results of the Re concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	12.7	
number of individual laboratory results	25	
number of unique lots	11	
relative standard deviation between laboratory results [%]	15.9	**Info 22:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

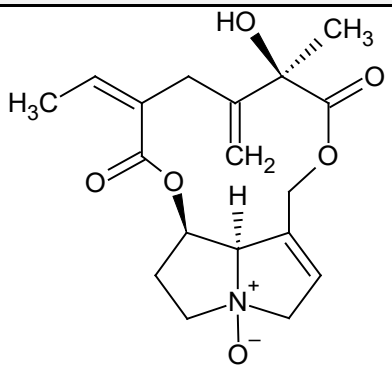
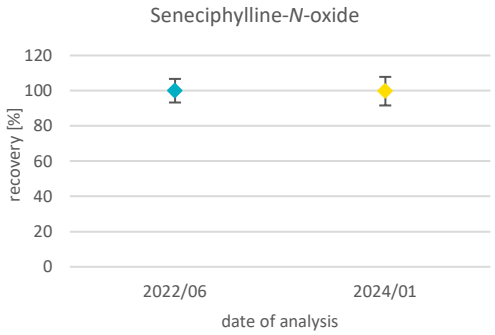
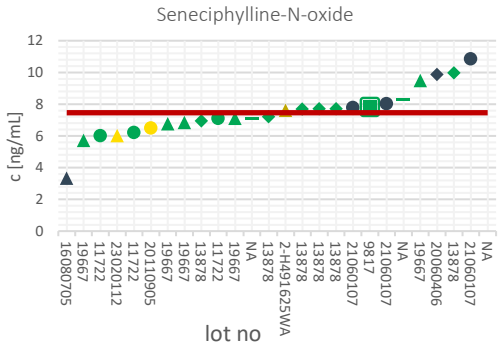
Certificate Of Proficiency Test Analysis		
compound	retrorsine-*N*-oxide $C_{18}H_{25}NO_7$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 23:** Results of the ReNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	6.9	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	18.3	**Info 24:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

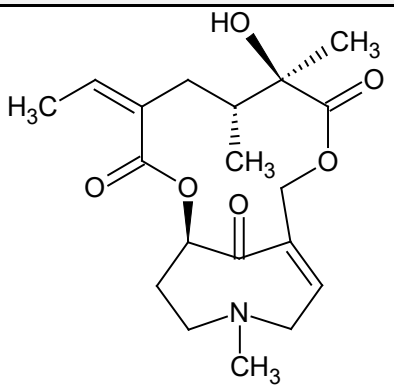
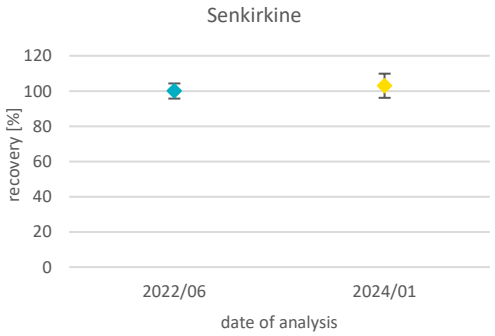
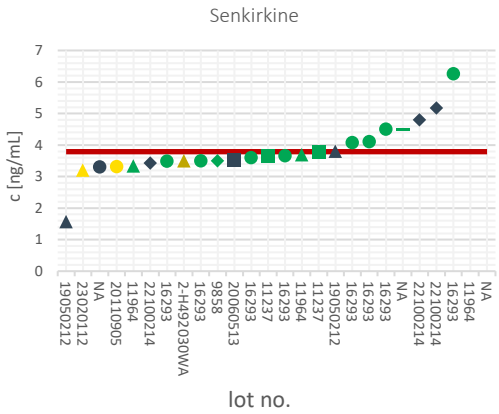
Certificate Of Proficiency Test Analysis		
compound	senecionine $C_{18}H_{25}NO_5$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 25:** Results of the Sc concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	14.5	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	24.2	**Info 26:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Carl Roth: light blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

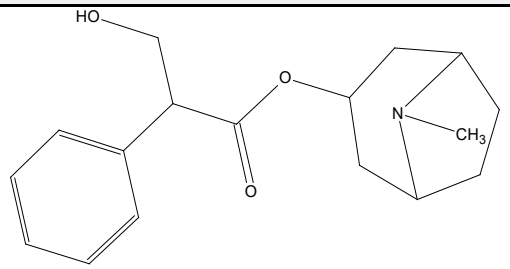
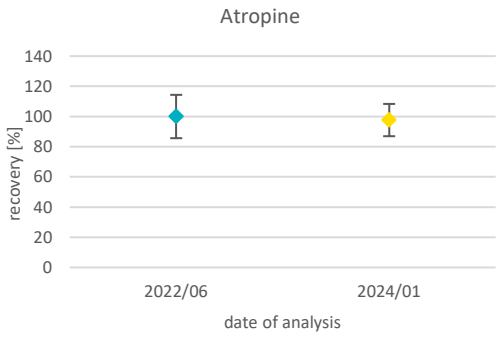
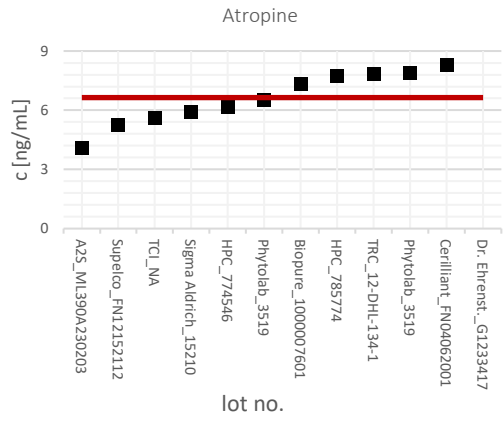
Certificate Of Proficiency Test Analysis		
compound	senecionine-*N*-oxide $C_{18}H_{25}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 27:** Results of the ScNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	5.9	
number of individual laboratory results	25	
number of unique lots	9	
relative standard deviation between laboratory results [%]	15.2	**Info 28:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).

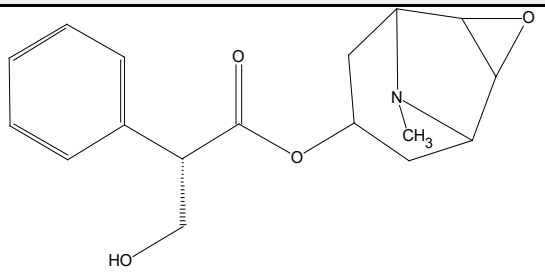
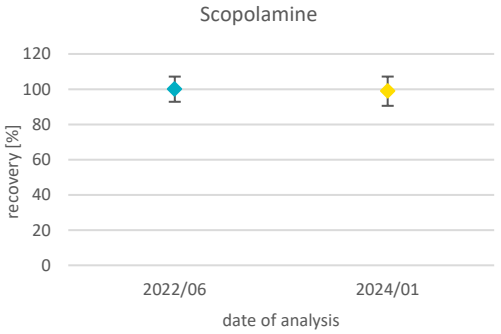
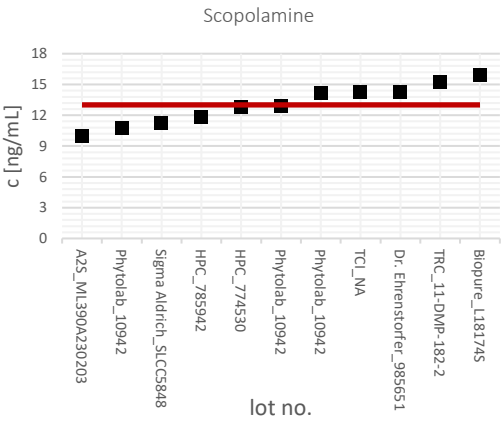
Certificate Of Proficiency Test Analysis		
compound	senecionine-*N*-oxide $C_{18}H_{25}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 29:** Results of the ScNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	5.9	**Info 30:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here /).
number of individual laboratory results	25	
number of unique lots	9	
relative standard deviation between laboratory results [%]	15.2	

Certificate Of Proficiency Test Analysis		
compound	seneciphylline $C_{18}H_{23}NO_5$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 31:** Results of the Sp concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	11.3	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	18.0	**Info 32:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Carl Roth: light blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here: lot 20010108).

Certificate Of Proficiency Test Analysis		
compound	seneciphylline-*N*-oxide $C_{18}H_{23}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 33:** Results of the SpNO concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	7.5	
number of individual laboratory results	25	
number of unique lots	10	
relative standard deviation between laboratory results [%]	16.2	**Info 34:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here: /).

Certificate Of Proficiency Test Analysis		
compound	senkirkine $C_{19}H_{27}NO_6$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 35:** Results of the Sk concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	3.8	
number of individual laboratory results	24	
number of unique lots	10	
relative standard deviation between laboratory results [%]	13.7	**Info 36:** The same supplier is shown in the same colour; PhytoLab: green, Oskar Tropitzsch: orange, Phytoplan: dark blue, Dr. Ehrenstorfer: ochre. Different lots of PAs are shown in different symbols. Note: some lots were distributed by different suppliers (here: /).

Certificate Of Proficiency Test Analysis		
compound	atropine $C_{17}H_{23}NO_3$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 37:** Results of the Atr concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	6.6	
number of individual laboratory results	11	
number of unique lots	10	
relative standard deviation between laboratory results [%]	21.4	

Certificate Of Proficiency Test Analysis		
compound	scopolamine $C_{17}H_{21}NO_4$	
start of the pilot project on standard stability of PA and TA	2022/06	
Number of test rounds	2	**Info 38:** Results of the Sco concentration in a standard solution that has been stored at -24°C since 2022 and analysed in two proficiency test (PT) rounds. The assigned value of the first round represents the expected value. The error bars indicate the RSD_R [%] of the respective PT series.
2024/01		
Assigned value [ng/mL]	13.0	
number of individual laboratory results	11	
number of unique lots	10	
relative standard deviation between laboratory results [%]	16.6	

6 Conclusions

The NRL for Mycotoxins and Plant Toxins organised a proficiency test to evaluate the performance of laboratories regarding the determination of pyrrolizidines in honey. This proficiency test was also used to obtain results on the stability of PA/PANOs in honey. In addition, the pilot programme to assess the storage stability of PA/PANOs and TAs in standard solutions was continued.

A PA/PANO concentration range between 0.8 to 84.3 µg/kg was tested, and the relative standard reproducibility deviation between laboratories ranged between 12 to 77 %, with a significant higher RSD_R for the PA *N*-oxides (Figure 1). A total of 1201 values for analyte-matrix-combinations were evaluated by z-scores, of which 86 % were satisfactory ($|z| \leq 2$) for sample 1, 82 % for sample 2, 84 % for sample 3 and 93 % for the standard solution.

All the honeys analysed were originally PA-free and were spiked with PA/PANO in different ways: Sample 1 was spiked exclusively with PAs, sample 2 exclusively with *N*-oxides and sample 3 contained a mixture of both. While samples 1 and 2 were stored for twelve weeks before shipping, sample 3 was spiked later, so that PA/PANOs only had six weeks to degrade.

Data show that *N*-oxide forms degrade very quickly. For example, sample 2 was spiked with 800 µg/kg of echimidine-*N*-oxide, but no laboratory detected echimidine-*N*-oxide in the sample after 12 weeks of storage. Instead, the corresponding tertiary base was detected with an assigned value of 53 µg/kg, which shows that the PANOs are reduced to a certain percentage to the respective free tertiary base. This observation also applied to all other PA/PANOs. The data also show that the free tertiary bases are also degraded in honey, albeit at a slower rate than the *N*-oxides.

The first results of the pilot project launched in 2022 to assess the storage stability of PAs and TAs are available after almost two years of storage. Although the assigned values are subject to certain uncertainties, these data clearly show that PAs and TAs have a relatively high storage stability. Degradation rates of more than 10 % are not to be expected after two years of storage in the freezer.

7 References

1. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 Official Journal of the European Union, 2023.

2. Commission Regulation (EU) 2023/2783 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of plant toxins in food and repealing Regulation (EU) 2015/705. Official Journal of the European Union, 2023.

3. Gottschalk, C., et al., Uncertainties in the determination of pyrrolizidine alkaloid levels in naturally contaminated honeys and comparison of results obtained by different analytical approaches. Food Additives and Contaminants Part A, 2018. 35(7): p. 1366-1383.

4. These, A., Pydde, E., Weiß, M. Report on the 2022 Proficiency Test of the German Reference Laboratory for Mycotoxins and Plant Toxins: Determination of pyrrolizidine alkaloids and tropane alkaloids in herbs and spices. BfR-Wissenschaft 02/2023.

5. ISO 13528:2022, Statistical methods for use in proficiency testing by interlaboratory comparison. 2022.

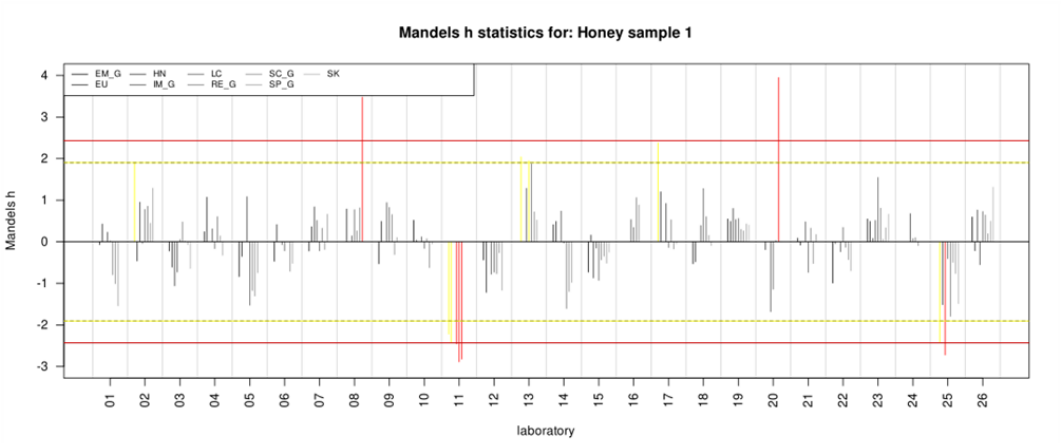
6. Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed v1.1, 2023. EURL mycotoxins & plant toxins, Wageningen Food Safety Research, part of Wageningen University & Research.

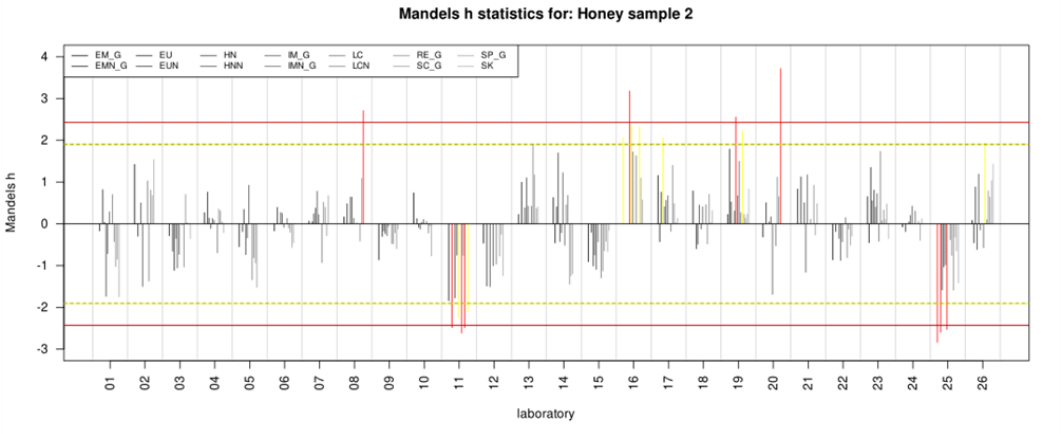
7. SANTE/11312/2021 v2 “Analytical quality control and method validation procedures for pesti-cide residues analysis in food and feed”, implemented 01/01/2024

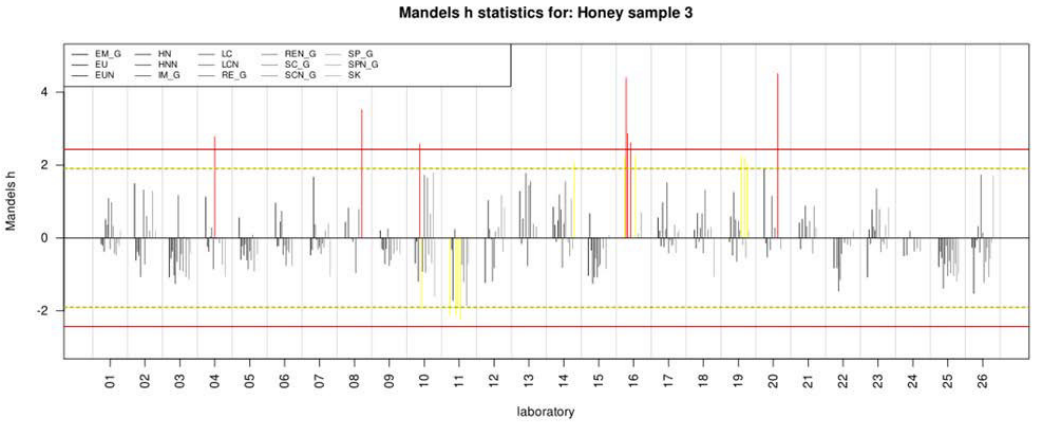
8 List of Tables

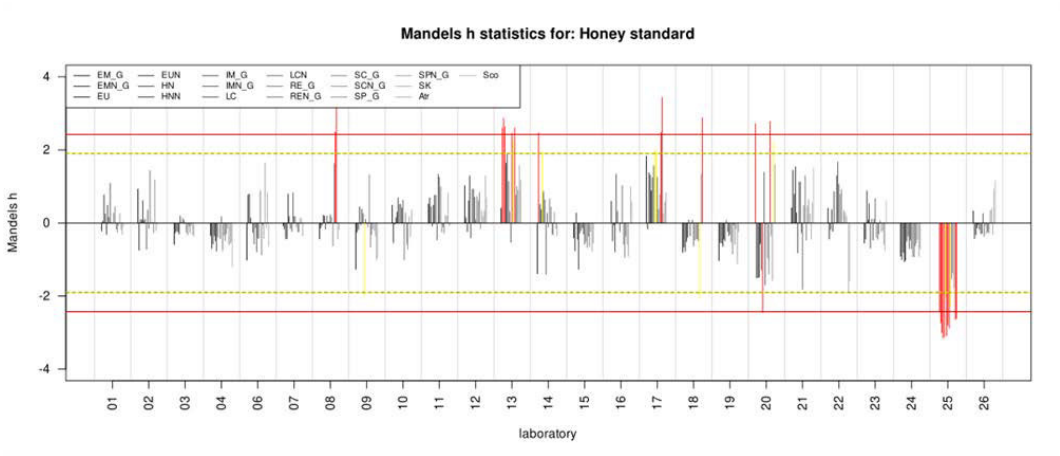
9 List of Figures

10 Appendix A








Mandels h statistics for: Honey standard
EM_G
EMN_G
EU
EUN
HN
HNN
IM_G
IMN_G
LC
LCN
RE_G
REN_G
SC_G
SCN_G
SP_G
SPN_G
SK
Atr
Sco
Mandels h
4
2
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-2
-4
01
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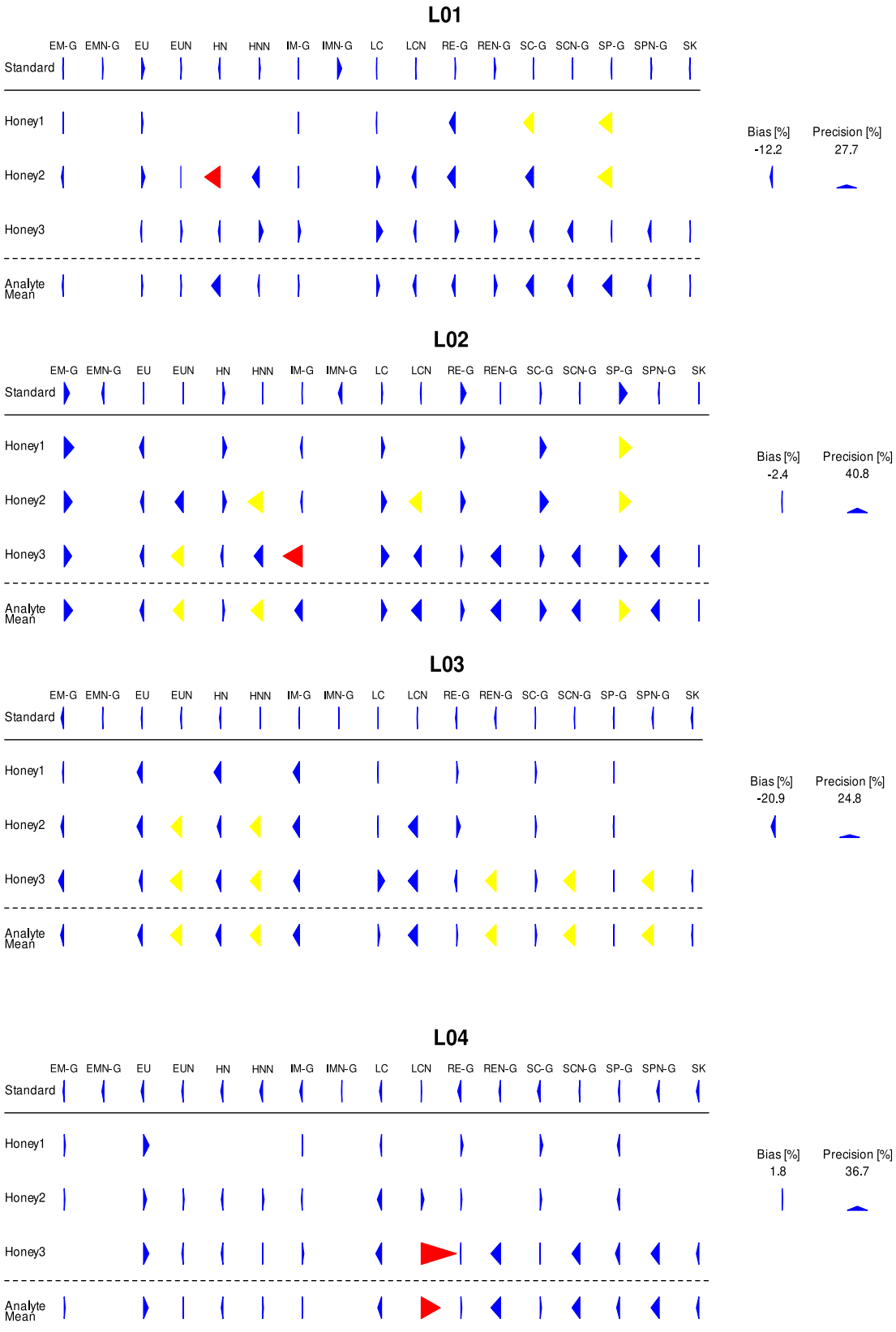
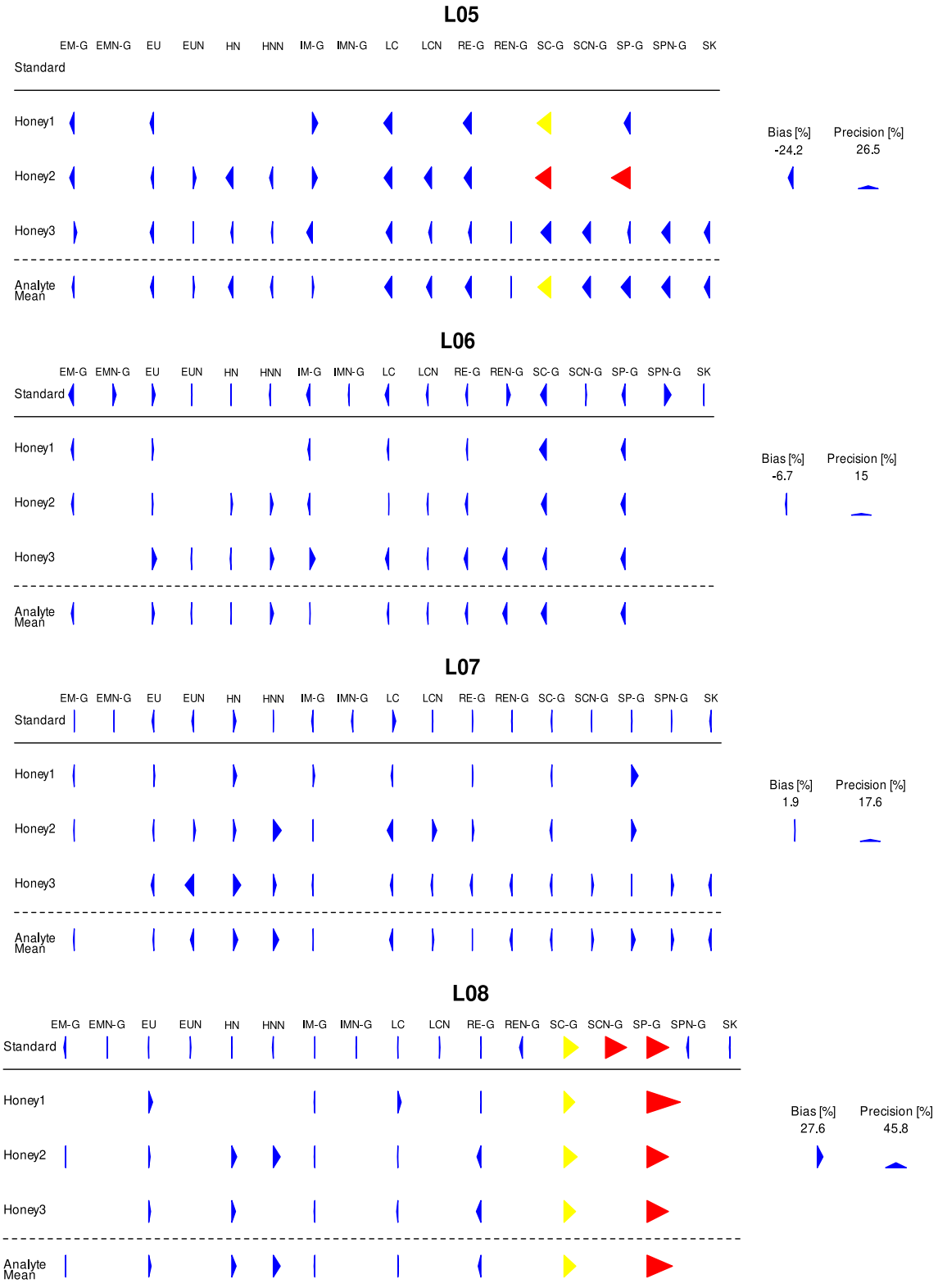
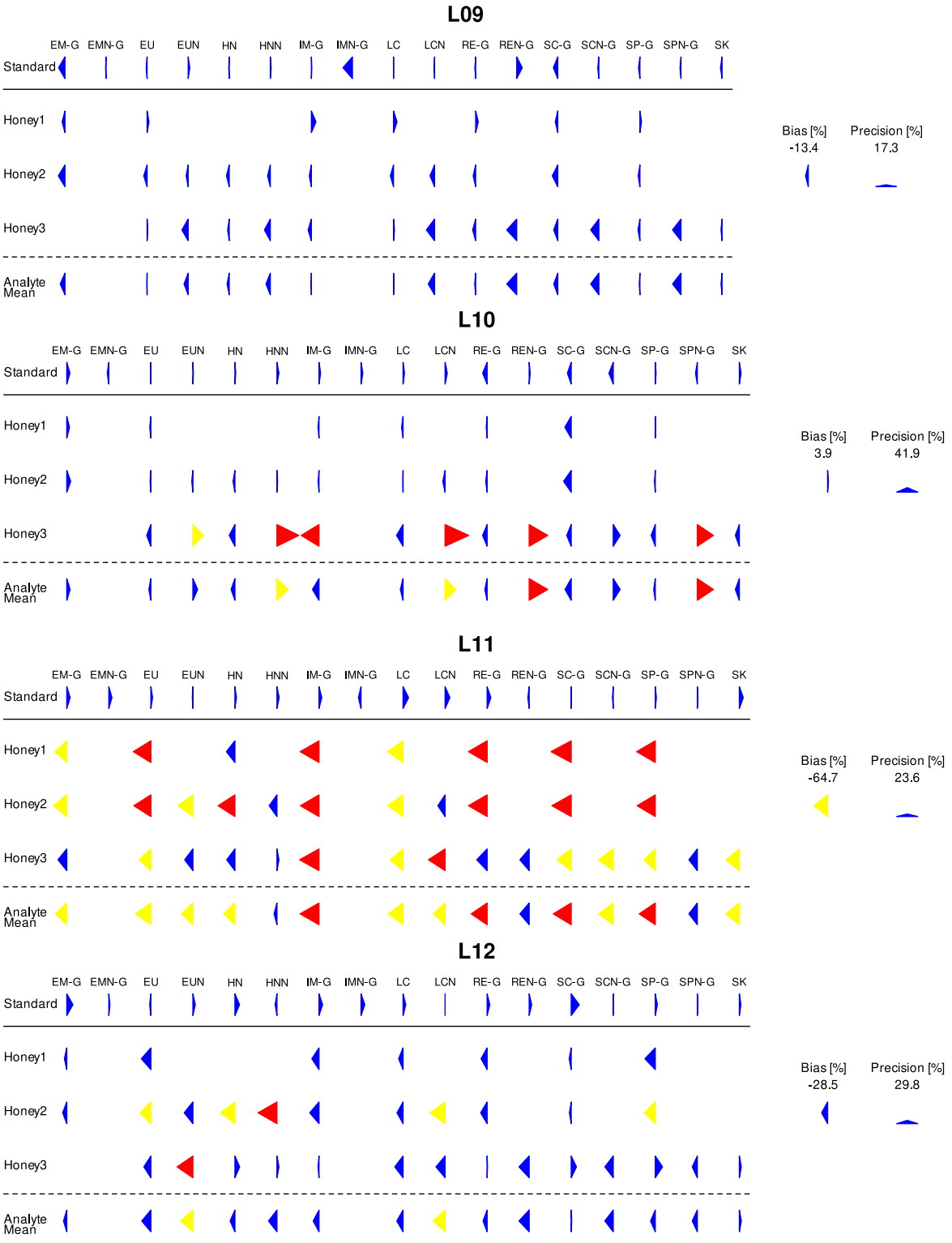
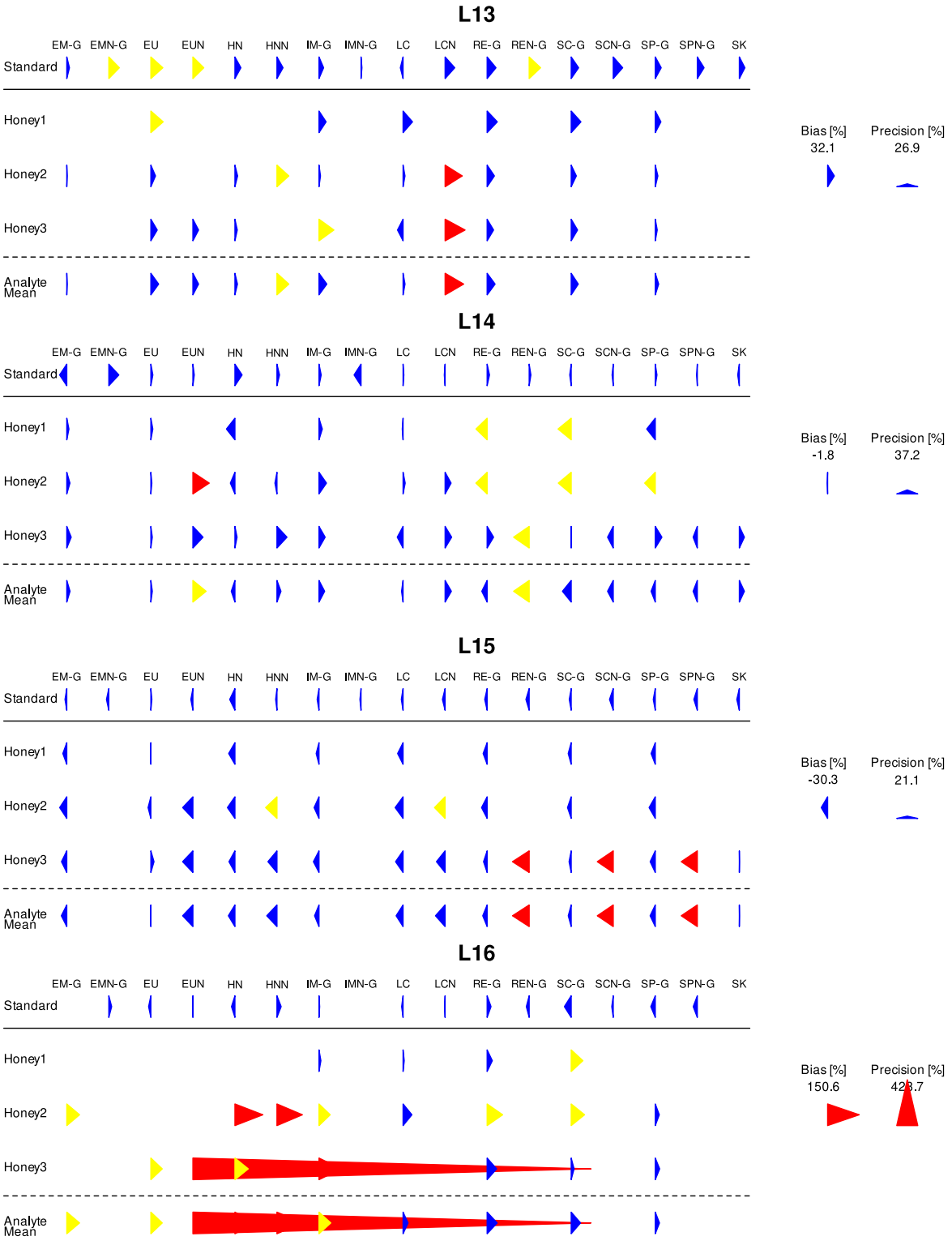
26
laboratory

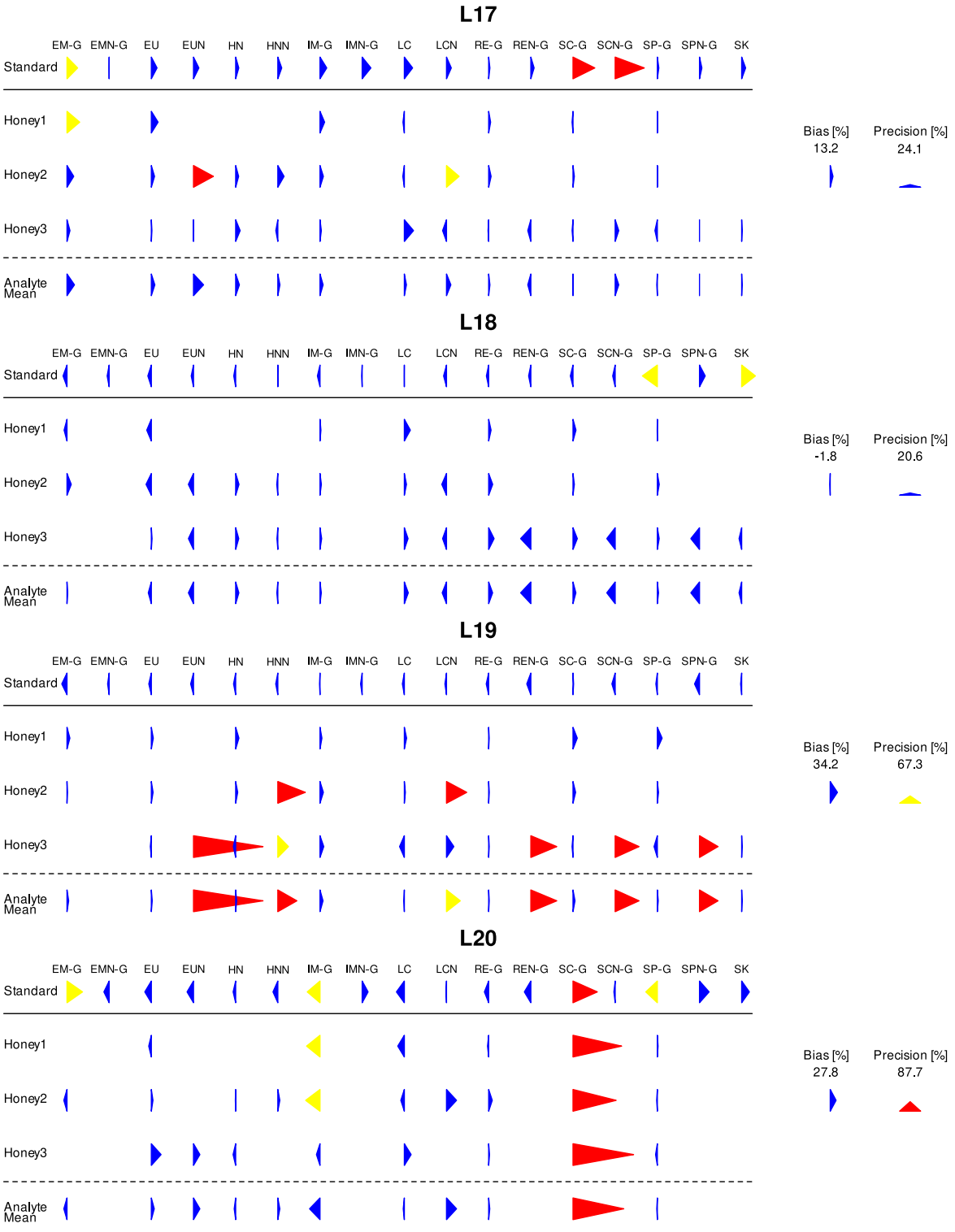
11 Appendix B




L05
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -24.2
Precision [%] 26.5
L06
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -6.7
Precision [%] 15
L07
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 1.9
Precision [%] 17.6
L08
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 27.6
Precision [%] 45.8


L09
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -13.4
Precision [%] 17.3
L10
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 3.9
Precision [%] 41.9
L11
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -64.7
Precision [%] 23.6
L12
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -28.5
Precision [%] 29.8


L13
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 32.1
Precision [%] 26.9
L14
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -1.8
Precision [%] 37.2
L15
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -30.3
Precision [%] 21.1
L16
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 150.6
Precision [%] 428.7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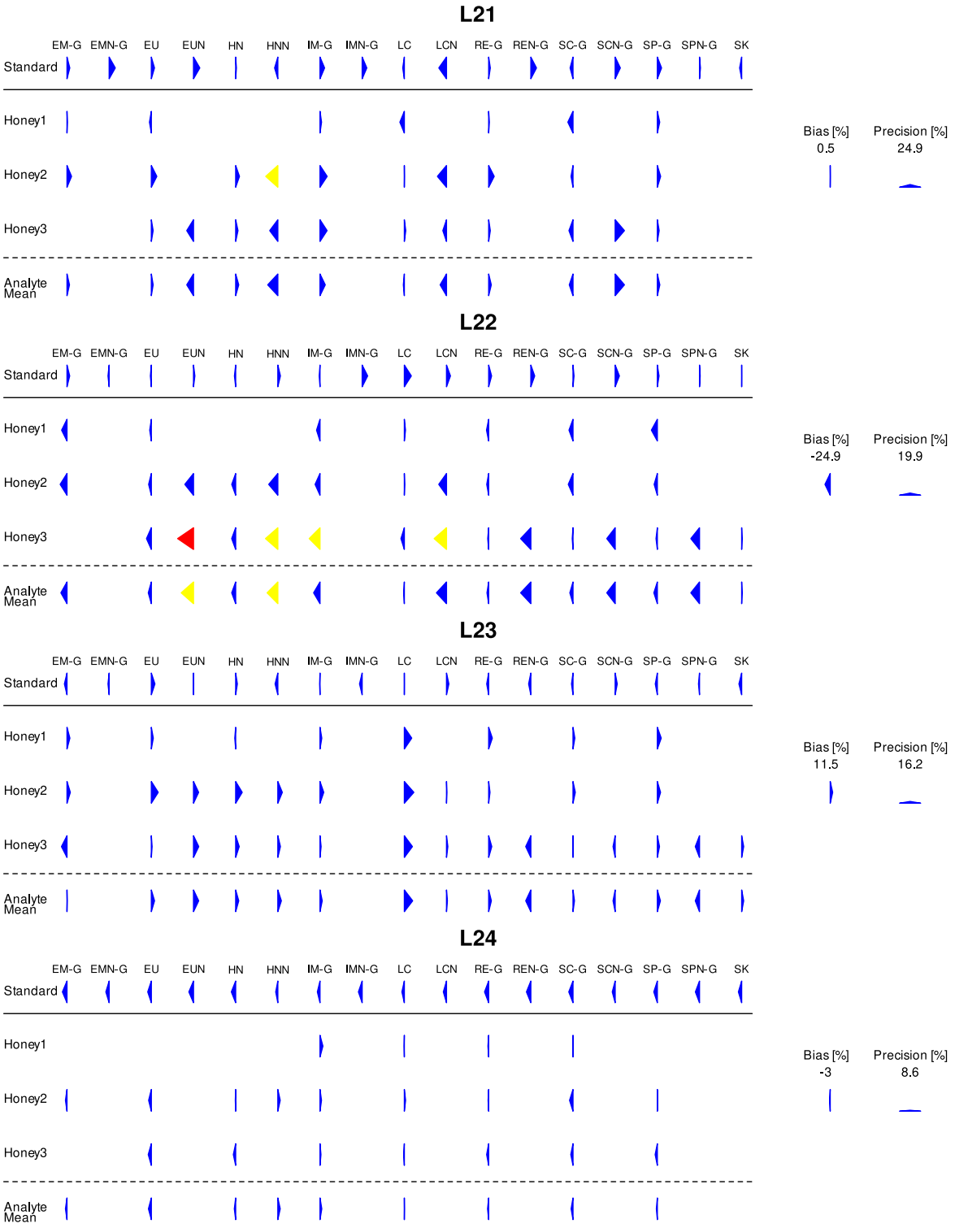
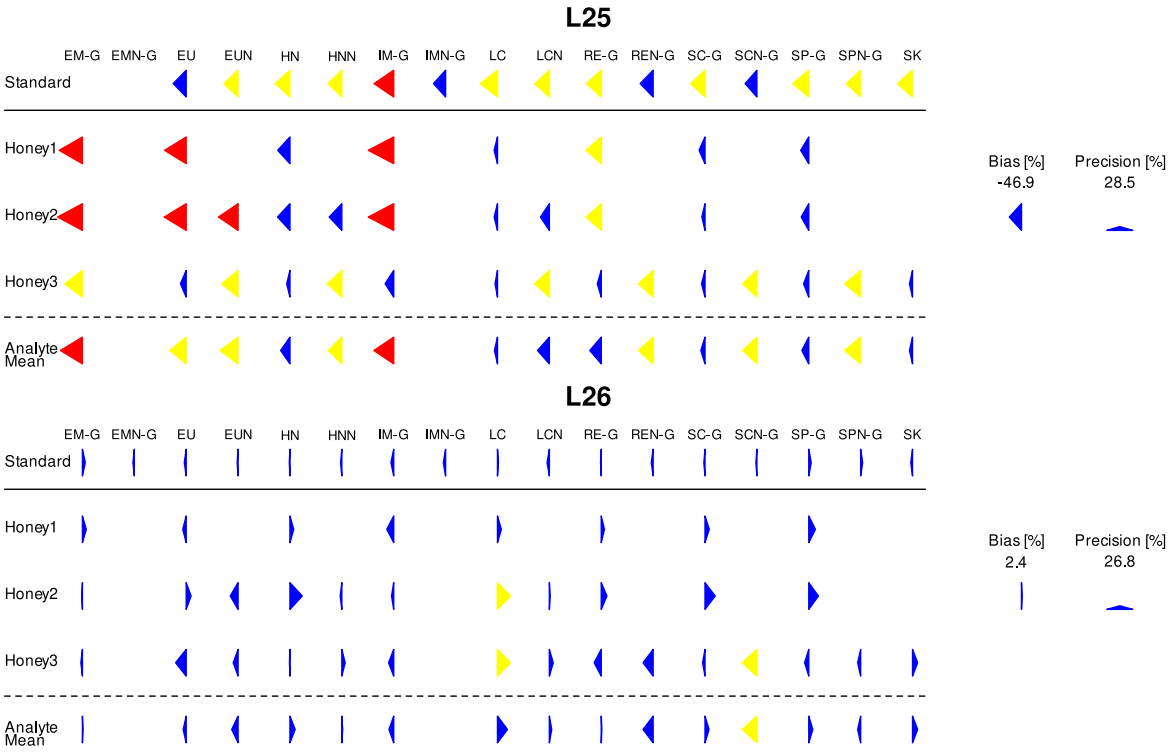
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 13.2
Precision [%] 24.1
L18
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -1.8
Precision [%] 20.6
L19
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 34.2
Precision [%] 67.3
L20
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 27.8
Precision [%] 87.7


L21
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 0.5
Precision [%] 24.9
L22
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -24.9
Precision [%] 19.9
L23
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] 11.5
Precision [%] 16.2
L24
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%] -3
Precision [%] 8.6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EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%]
-46.9
Precision [%]
28.5
L26
EM-G EMN-G EU EUN HN HNN IM-G IMN-G LC LCN RE-G REN-G SC-G SCN-G SP-G SPN-G SK
Standard
Honey1
Honey2
Honey3
Analyte Mean
Bias [%]
2.4
Precision [%]
26.8

12 Appendix C

Table 11: z-scores achieved by the laboratories in sample 1

PA	Em	EmNO	Eu	EuNO	Hn	HnNO	Im	ImNO	Lc	LcNO	Re	ReNO	Sc	ScNO	Sp	SpNO	Sk	Total PA
01	-0.08		0.29		-		-0.04		-0.10		-1.18		-2.08		-2.65			-0.86
02	1.95		-0.84		0.78		-0.39		0.65		0.75		1.23		2.46			0.66
03	-0.24		-1.03		-1.40		-1.30		-0.07		0.31		0.26		0.02		{0.31}	-0.25
04	0.24		1.11		-		0.07		-0.28		0.45		0.53		-0.45			0.17
05	-0.86		-0.70		-		1.09		-1.63		-1.62		-2.76		-1.21			-1.17
06	-0.49		0.27		-		-0.43		-0.33		-0.25		-1.40		-0.80			-0.57
07	-0.24		0.21		0.66		0.34		-0.33		0.13		-0.21		1.33			0.11
08	-		0.75		-		-0.15		0.64		0.05		2.08		6.43			0.84
09	-0.55		0.37		-		0.90		0.69		0.51		-0.48		0.31			0.36
10	0.52		-0.21		-		-0.18		-0.28		-0.17		-1.21		0.04			-0.39
11	-2.27		-3.34		[-1.62]		-3.57		-2.97		-3.55		-		[-3.55]			-3.48
12	-0.45		-1.81		-		-1.36		-0.84		-1.15		-0.38		-1.97			-1.10
13	-		2.35		-		1.35		1.80		1.94		1.85		1.08			1.50
14	0.41		0.37		[-1.62]		0.63		-0.15		-2.13		-2.51		-1.63			-0.90
15	-0.75		-0.05		-1.19		-0.54		-1.04		-0.76		-0.57		-0.80		{0.35}	-0.66
16	-		-		-		0.36		0.22		0.99		2.23		-			0.02
17	2.42		1.28		-		0.87		-0.26		0.37		-0.18		0.06			0.33
18	-0.55		-0.87		-		0.18		1.14		0.45		0.56		-0.03			0.38
19	0.55		0.37		0.62		0.36	{7.85}	0.43	{0.2}	0.10	{2.66}	0.81	{2.26}	0.91	{0.31}	{0.41}	1.92
20	-		-0.49		-	{3.34}	-2.55		-1.25		-0.22		9.23		0.14			0.99
21	0.08		-0.35		-		0.29		-0.85		0.13		-0.98		0.45			-0.31
22	-1.02		-0.31		-		-0.65		0.22		-0.41		-0.76		-1.13			-0.49
23	0.55		0.37		-0.16		0.34		1.41		0.69		0.35		0.74		{0.44}	0.75
24	-		-		-		0.55		-0.04		-0.14		0.01		-			-0.64
25	[-3.58]		-3.32		-1.89		-3.92		-0.52		-2.35		-0.91		-1.24		{0.23}	-2.08
26	0.60	{0.01}	-0.53	{0.02}	0.58	{0.2}	-1.07		0.60	{0.11	0.50	{0.07}	0.66		1.02	{0.04}	{0.5}	0.30

z'-scores were calculated for Hn, Sc group and Sp group due to a non-negligible uncertainty of the assigned value: ($0.3 \times \sigma_{PT} \leq u_{assigned\ value} \leq 0.7 \times \sigma_{PT}$); z-scores in rectangular brackets are proxy z-scores based on the LOQ provided by the laboratory. Values ≤ -3 are classified as a false negative (FN) and should be interpreted as 'unsatisfactory' performance. Values > -3 and < -2 are classified as a false negative (FN) and should be interpreted as 'questionable' and values ≥ -2 are not classified as FN; z-scores in curly brackets were given if no assigned value could be calculated (n<7). They indicate the factor by which the result of the laboratory exceeds the robust LOQ of all participating laboratories. The higher this value is. the more likely it is to be a false positive (FP) measurement.

Table 12: z-scores achieved by the laboratories in sample 2

PA	Em	EmNO	Eu	EuNO	Hn	HnNO	Im	ImNO	Lc	LcNO	Re	ReNO	Sc	ScNO	Sp	SpNO	Sk	Total-PA
01	-0.41		0.68	0	-3.13	-1.40	0.09		0.67	-0.79	-		-1.62		-2.90			-1.16
02	1.62		-0.64	[-1.65]	0.72	-2.99	-0.31		1.03	-2.44	0.89		1.66		2.29			0.77
03	-0.56		-1.07	-2.14	-0.75	-2.09	-1.36		-0.12	-1.86	0.76		0.25		-0.16		{0.31}	-0.50
04	0.14		0.61	0.19	-0.33	0.31	-0.19		-0.85	0.56	0.22		0.31		-0.47			-0.13
05	-0.90		-0.51	0.58	-1.41	-0.64	1.00		-1.56	-1.47	-		-3.07		[-3.78]			-1.33
06	-0.41		0.17	<LOQ	0.32	0.55	-0.44		0.05	-0.23	-		-1.02		-0.86			-0.23
07	-0.12		-0.23	0.39	0.50	1.61	0.00		-1.11	0.85	0.34		-0.41		0.92			0.11
08	0.01		0.28	<LOQ	0.96	1.34	-0.13		-0.08	<LOQ	-		2.54		4.14			0.47
09	-1.30		-0.65	-0.39	-0.54	-0.53	-0.41		-0.61	-0.89	-		-1.08		-0.34			-0.74
10	0.75		-0.15	-0.21	-0.36	0.12	-0.17		-0.03	-0.43	-		-1.44		-0.16			-0.01
11	-2.55		-3.23	[-2.82]	-3.19	-1.48	-3.52		-2.96	-1.37	-		[-		-3.46			-3.03
12	-0.79		-2.05	[-1.65]	-2.73	[-3.59]	-1.75		-1.15	[-2.86]	-		-0.34		-2.10			-1.71
13	0.09		0.89	<LOQ	0.51	2.27	0.28		0.37	3.27	1.39		0.98		0.50			0.61
14	0.60	{0.55}	0.19	3.12	-0.87	-0.39	1.43	{0.38}	0.40	1.14	-		-2.47		-2.02			-0.30
15	-1.36		-0.53	-1.95	-1.43	-2.17	-0.93		-1.51	-2.02	-		-0.70		-1.18		{0.33}	-1.33
16	2.44		<LOQ	<LOQ	5.32	4.85	2.14		1.69	<LOQ	2.96		2.55		0.77			2.61
17	1.28	{0.63}	0.61	3.78	0.56	1.18	0.64		-0.29	2.40	0.46		0.25		0.07			0.68
18	0.81		-1	-0.97	0.63	-0.20	0.26		0.40	-0.89	0.76		0.25		0.36			0.47
19	0.09	{5.96}	0.33	<LOQ	0.39	5.21	0.64	{10.16	0.20	3.85	0.12	{5.23}	0.50	{1.32}	0.23	{1.13}	{0.43}	1.34
20	-0.60		0.31	<LOQ	-0.06	0.38	-2.73		-0.67	1.90	0.68		8.19		-0.13			-0.11
21	0.86		1.04	<LOQ	0.72	-2.30	1.36		0.02	[-1.73]	1.05		-0.37		0.63			0.45
22	-1.30		-0.51	[-1.65]	-0.75	-1.73	-0.93		0.07	-1.47	-		-0.89		-0.60			-0.82
23	0.63	{0.56}	1.31	0.97	1.24	0.85	0.71	{0.85}	1.80	0.08	0.25	{0.35}	0.44		0.61		{0.31}	0.97
24	-0.29		-0.51	<LOQ	-0.06	0.45	0.28		0.24	<LOQ	-		-0.65		0.05			-0.15
25	-3.82		-3.36	-3.02	-1.92	-1.95	-3.93		-0.51	-1.37	-		-0.48		-1.16		{0.21}	-2.35
26	-0.09	{0.56}	0.75	-1.21	1.90	-0.25	-0.33	{0.08}	2.03	0.13	0.87	{0.21}	1.57		1.49	{0.31}	{0.48}	0.94

z'-scores were calculated for EuN, HnN, LcN and Sc group due to a non-negligible uncertainty of the assigned value: ($0.3 \times \sigma_{PT} \leq u_{assigned\ value} \leq 0.7 \times \sigma_{PT}$); z-scores in rectangular brackets are proxy z-scores based on the LOQ provided by the laboratory. Values ≤ -3 are classified as a false negative (FN) and should be interpreted as 'unsatisfactory' performance. Values > -3 and < -2 are classified as a false negative (FN) and should be interpreted as 'questionable' and values ≥ -2 are not classified as FN; z-scores in curly brackets were given if no assigned value could be calculated (n<7). They indicate the factor by which the result of the laboratory exceeds the robust LOQ of all participating laboratories. The higher this value is, the more likely it is to be a false positive (FP) measurement.

Table 13: z-scores achieved by the laboratories in sample 3

PA	Em	EmNO	Eu	EuNO	Hn	HnNO	Im	ImNO	Lc	LcNO	Re	ReNO	Sc	ScNO	Sp	SpNO	Sk	Total PA
01	-		-0.24	0.32	-0.35	0.82	0.48		1.22	-0.45	0.75	0.60	-0.77	-1.02	-0.13	-0.64	0.09	-0.13
02	1.49		-0.71	-2.46	-0.44	-1.71	[-3.84]		1.50	-1.48	0.42	[-1.94]	0.77	[-1.59]	1.51	[-1.65]	0.09	-0.22
03	-1.05		-0.65	-2.33	-0.93	-2.02	-1.21		1.33	-1.88	-0.44	-2.15	0.36	-2.27	-0.06	-2.37	-0.20	-0.40
04	-		1.07	-0.28	-0.35	0.05	0.33		-1.16	7.08	-0.10	[-1.94]	-0.06	[-1.59]	-0.83	[-1.65]	-0.50	0.17
05	0.56		-0.68	-0.03	-0.44	-0.27	-1.15		-1.16	-0.60	-0.58	0.08	-1.99	[-1.59]	-0.50	[-1.65]	[-1.08]	-1.01
06	-		0.89	-0.15	-0.20	0.71	1.09		-0.67	-0.24	-0.70	-0.85	-0.71	-	-0.86	-	-	-0.05
07	-		-0.56	-1.73	1.47	0.60	-0.20		-0.49	-0.31	-0.44	-0.45	-0.37	0.37	0.00	0.48	-0.50	-0.28
08	-		0.36	-	0.72	-	-0.16		-0.24	-	-0.88	-	2.24	-	4.13	-	-	1.06
09	-		0.12	-1.22	-0.31	-1.15	-0.63		0.19	-1.59	-0.58	[-1.94]	-0.79	[-1.59]	-0.38	[-1.65]	-0.20	-0.43
10	-		-0.79	2.02	-1.09	4.16	-3.38		-1.24	4.49	-0.86	3.54	-0.84	1.31	-0.79	3.07	-0.76	-0.81
11	[-1.67]		-2.23	-1.56	-1.55	0.38	-3.68		-2.46	[-3.17]	-1.97	-1.76	-2.72	[-2.8]	-2.15	-1.60	[-2.54]	-2.45
12	-		-1.33	[-3.01]	0.90	0.38	-0.18		-1.58	-1.73	0.07	[-1.94]	1.00	[-1.59]	1.38	-0.96	0.38	-0.08
13	-		1.22	1.08	0.45	-	2.84		-1.06	3.81	1.23	-	1.21	-	0.37	-	-	1.03
14	0.85		0.27	1.94	0.40	1.91	1.17		-1.11	1.25	1.23	[-2.97]	-0.03	-1.08	1.27	-0.80	0.96	0.55
15	-1.01		0.60	-1.96	-1.13	-1.72	-1.07		-1.43	-1.65	-0.68	[-3.18]	-0.41	[-3.04]	-0.97	[-3.06]	0.03	-0.43
16	-		2.18	75.76	2.54	-	4.25		-	-	1.81	-	0.55	-	0.84	-	-	2.64
17	0.56		0.11	0.04	0.85	-0.38	0.25		1.74	-0.74	-0.12	-0.58	-0.17	0.68	-0.46	0	0.09	0.04
18	-		0.14	-0.96	0.58	-0.16	0.31		0.71	-0.74	1.03	[-1.94]	0.82	[-1.59]	0.35	[-1.65]	-0.50	0.36
19	-		-0.19	13.19	-0.44	2.02	0.71	{4.4}	-0.90	1.39	0.10	4.93	-0.17	4.53	-0.62	3.52	0.09	0.38
20	-		1.85	1.21	-0.49	-	-0.67		1.29	-	0.16	-	11.50	-	-0.33	-	-	3.06
21	-		0.34	-1.28	0.44	[-1.54]	1.34		0.27	[-0.69]	0.31	-	-0.77	1.76	0.34	-	-	0.02
22	-		-0.92	[-3.01]	-0.75	-2.35	-2.05		-0.64	[-2.35]	-0.18	[-1.94]	-0.14	[-1.59]	-0.25	[-1.65]	0.09	-0.80
23	-1.05		0.15	1	0.67	0.49	0.21		1.53	0.26	0.59	-0.97	0.05	-0.40	0.41	-0.80	0.38	0.30
24	-		-0.59	-	-0.44	-	0.18		-0.12	-	-0.38	-	-0.42	-	-0.41	-	-	-0.60
25	[-2.74]		-0.88	-2.51	-0.50	-2.23	-1.32		-0.37	-2.27	-0.60	-2.25	-0.53	-2.19	-0.80	-2.48	-0.47	-0.88
26	-0.24		-1.63	-0.75	-0.08	0.51	-0.80		2.01	0.60	-1.09	-1.55	-0.37	-2.25	-0.64	-0.46	0.79	-0.81

z'-scores were calculated for Em, EnN, HnN, Im group, Lc, LcN, ReN group, ScN group, SpN group due to a non-negligible uncertainty of the assigned value: ($0.3 \times \sigma_{PT} \leq u_{assigned\ value} \leq 0.7 \times \sigma_{PT}$); z-scores in rectangular brackets are proxy z-scores based on the LOQ provided by the laboratory. Values ≤ -3 are classified as a false negative (FN) and should be interpreted as 'unsatisfactory' performance. Values > -3 and < -2 are classified as a false negative (FN) and should be interpreted as 'questionable' and values ≥ -2 are not classified as FN; z-scores in curly brackets were given if no assigned value could be calculated (n<7). They indicate the factor by which the result of the laboratory exceeds the robust LOQ of all participating laboratories. The higher this value is the more likely it is to be a false positive (FP) measurement.

Table 14: z-scores achieved by the laboratories in the standard solution

PA	Em	EmNO	Eu	EuNO	Hn	HnNO	Im	ImNO	Lc	LcNO	Re	ReNO	Sc	ScNO	Sp	SpNO	Sk	Total PA	Atr	Sco
Lab											z-score									
01	-0.14	0.14	0.59	0.16	-0.3	0.25	-0.02	0.85	-0.07	-0.08	0.15	0.34	-0.06	-0.11	-0.18	0.19	-0.16	0.05	-0.28	-0.06
02	1.07	-0.44	0.05	0.03	0.39	-0.03	-0.07	-0.72	0.22	-0.18	1.06	-0.03	0.26	-0.13	1.54	-0.2	-0.02	0.24		
03	-0.53	-0.09	-0.21	-0.22	-0.29	0.05	-0.15	0.01	-0.01	-0.08	-0.26	-0.32	0.06	-0.11	-0.15	-0.28	-0.32	-0.17		
04	-0.26	-0.41	-0.50	-0.31	-0.43	-0.58	-0.51	-0.09	-0.43	0.08	-0.65	-0.33	-0.57	-0.18	-0.26	-0.51	-0.51	-0.42	-0.44	-0.70
05																				
06	-0.98	0.67	0.61	0.08	-0.07	-0.25	-0.69	-0.21	-0.74	-0.38	-0.39	0.71	-1.19	0.19	-0.64	1.34	-0.11	-0.2	0.64	-0.37
07	0.01	-0.01	-0.38	-0.38	0.52	0.04	-0.30	-0.30	0.62	0.08	0.08	-0.13	-0.19	-0.08	0.11	0.08	-0.31	-0.04		
08	-0.36	0.01	-0.08	0.14	0.08	-0.18	0.01	-0.07	-0.05	0.11	0.06	-0.57	2.78	4.08	4.21	-0.38	-0.14	0.72		
09	-1.24	-0.08	-0.18	0.32	-0.10	0.17	0.08	-1.84	0.01	-0.08	-0.15	1.08	-0.86	-0.22	-0.28	-0.19	-0.31	-0.16	-0.84	-0.56
10	0.6	-0.29	-0.06	0.09	0.15	0.38	0.31	0.35	0.27	0.42	-0.85	0.2	-0.72	-0.82	0.15	-0.34	0.33	-0.07		
11	0.64	0.60	0.33	-0.10	0.29	0.42	0.54	-0.50	1.05	0.91	0.71	-0.25	0.03	-0.15	0.21	0.13	0.75	0.3	-0.08	-0.03
12	1.17	0.21	-0.22	0.46	0.88	-0.34	0.69	0.72	0.52	0.00	0.51	0.48	1.53	0.04	0.4	0.12	0.30	0.50	1.00	0.4
13	0.51	2.01	2.29	2.08	1.14	1.16	0.88	0.17	-0.52	1.84	1.69	2.18	1.41	1.8	1.13	1.29	1.06	1.34		
14	-1.37	1.91	0.38	0.26	1.37	0.49	0.42	-1.33	0.15	-0.15	0.45	0.33	-0.28	-0.22	0.32	-0.14	-0.31	0.26		
15	-0.34	-0.45	0.19	-0.39	-1.00	-0.25	-0.35	-0.21	-0.33	-0.51	-0.46	-0.62	-0.37	-0.71	-0.38	-0.66	-0.49	-0.45		
16		0.54	-0.43	-0.07	-0.65	0.8	0.13		-0.27	-0.08	0.74	-0.54	-1.3	-0.28	-0.85	-0.78		-0.55	0.76	0.34
17	2.02	-0.01	1.08	1.02	0.59	0.74	1.28	1.66	1.57	0.91	0.23	0.62	4.14	5.53	0.28	0.45	0.75	1.33		
18	-0.75	-0.38	-0.66	-0.43	-0.36	-0.02	-0.49	-0.09	-0.01	-0.55	-0.43	-0.42	-0.50	-0.47	-2.84	1.08	2.61	-0.45		
19	-0.99	-0.29	-0.48	-0.54	-0.4	-0.4	-0.09	-0.3	-0.37	-0.3	-0.46	-0.77	0.11	-0.62	-0.28	-0.94	-0.2	-0.39		
20	2.95	-1	-1.24	-1.22	-0.48	-0.92	-2.42	1.12	-1.5	-0.09	-0.81	-1.23	4.62	-0.24	-2.19	1.82	1.46	-0.27		
21	0.53	1.16	0.6	1.2	0.14	-0.61	0.87	0.88	-0.30	-1.46	0.31	1.07	-0.55	0.98	0.8	0.18	-0.39	0.35	0.41	0.88
22	0.51	-0.22	-0.18	0.26	-0.23	0.49	-0.15	1.04	1.33	0.75	0.55	0.73	0.19	0.8	0.32	0.13	0.01	0.3	-1.53	-0.93
23	-0.49	-0.22	0.68	0.04	0.32	-0.52	-0.07	-0.5	0.01	0.45	-0.37	-0.42	-0.28	0.39	-0.39	-0.19	-0.52	-0.10	-0.63	0.37
24	-0.87	-0.63	-0.68	-0.88	-0.82	-0.40	-0.50	-0.71	-0.46	-0.60	-0.78	-0.83	-0.8	-0.55	-0.67	-0.78	-0.62	-0.70		
25			-2.00	-2.20	-2.28	-2.14	-3.04	-1.87	-2.67	-2.23	-2.30	-2.00	-2.23	-1.81	-2.47	-2.21	-2.34	-2.42		
26	0.44	-0.21	-0.29	-0.14	-0.1	-0.19	-0.43	-0.35	0.15	-0.35	-0.11	-0.28	-0.18	-0.15	0.38	0.31	-0.27	-0.12	0.70	0.67

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