Bundesinstitut für Risikobewertung

Report on the 2020 Proficiency Test of the German Reference Laboratory for Mycotoxins and Plant Toxins

Determination of pyrrolizidine and tropane alkaloids in herbal tea and solutions

Anja These
Emanuele Pydde

2020

Impressum

BfR Wissenschaft

BfR-Autoren:
Anja These, Emanuele Pydde

Report on the 2020 Proficiency Test of the German Reference Laboratory for Mycotoxins and Plant Toxins
Determination of pyrrolizidine and tropane alkaloids in herbal tea and solutions

Herausgeber:
Bundesinstitut für Risikobewertung
Pressestelle
Max-Dohrn-Straße 8–10
10589 Berlin

V.i.S.d.P: Suzan Fiack

Berlin 2020 (BfR-Wissenschaft 03/2020)
54 Seiten, 49 Abbildungen, 21 Tabellen

Druck: Inhalt und buchbinderische Verarbeitung
BfR-Hausdruckerei Marienfelde

ISBN 978-3-948484-13-2
ISSN 1614-3841 (Online)

DOI: 10.17590/20200716-112459

Download als kostenfreies PDF unter www.bfr.bund.de

Table of Contents

1 Summary

Pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) are toxins found in a wide variety of plant species. While PAs are mainly produced by flowering plants of Asteraceae, Boraginaceae and Fabaceae, TAs are biosynthesized by species belonging to Solanaceae family like *Datura*, *Atropa* and *Hyoscyamus* spp. Those plants can be co-harvested as botanical impurities during (herbal) tea production and their occurrence in a variety of tea and herbal blends has been reported, stressing the need to control the quality of these products in the EU market. The European Commission and the Member States are currently discussing the introduction of maximum levels for the presence of PAs and TAs in certain foods, such as tea and herbal tea products.

The German National Reference Laboratory (NRL) for Mycotoxins and Plant Toxins organized a proficiency test (PT) for the determination of pyrrolizidine and tropane alkaloids in herbal tea as well as standard solutions. The PT covered all analytes foreseen to be regulated and was designed to provide insight into the measurement capabilities of laboratories at concentrations close to the recommended limit of quantification (LOQ) that is 5 µg/kg for TAs and 10 µg/kg for individual PAs. Some PAs selected for monitoring the maximum levels occur naturally as isomers. Because of co-elution of isomers they are proposed to be analyzed as a sum. Therefore, this PT was designed to assess how the relative reproducibility standard deviation (RSD_R) is influenced if those isomers are analyzed as sum (group).

Two multianalyte standard solutions and two spiked and partially naturally contaminated herbal teas (chamomile and melissa) were provided to the participants. The concentrations of analytes varied from 0.6 to 77 ng/mL in solutions and ranged from 7 to 113 µg/kg in herbal teas.

Twenty-five laboratories reported results within the given time line, with 21 participants from Germany.

For none of the test materials a certified content was available. The robust mean values calculated from the laboratory results were used as assigned values for all materials and a target standard deviation of 25 % was set. The performance of the laboratories was assessed using z-scores. On average, 91 % of the z-scores for TAs and 94 % for individual PAs fell in the acceptable range ($|z| \leq 2$). The success rate of laboratories varied from 57 to 100 % for TAs and from 65 to 100 % for PAs, across the distributed matrices and concentration levels. The RSD_R of the reported results for TAs and PAs were in good agreement with the target standard deviation (25 %) for all materials. The results of this PT indicate that participating laboratories can determine PAs and TAs reliably in herbal tea at levels relevant to the proposed regulatory limits. The analysis of the PA isomers as a sum does not have a significant influence on the RSD_R, indicating that chromatographic separation is not necessary to comply with regulatory requirements. Most PA analytes were spiked into tea samples but some were already present as natural contaminants. Although teas samples were ground to 500 µm and thoroughly shaken, samples contaminated with PA containing plants showed a higher RSD_R because of the still existing inhomogeneous distribution of these naturally occurring contaminants.

2 Introduction

Plant toxins have been recognized as one of the most widespread and potent groups of toxicants. Tropane alkaloids (TAs) occur mainly in *Datura*, *Atropa* and *Hyoscyamus* sp., belonging to the Solanaceae family, besides a variety of other families such as Erythroxylaceae, Brassicaceae, Rhizophoraceae, Proteaceae, Euphorbiaceae, Convolvulaceae and Cruciferae [1]. The ability to form pyrrolizidine alkaloids (PAs) is found in representatives of at least 13 plant families, including in particular representatives of the families Asteraceae, Boraginaceae or Fabaceae. Several hundred of individual structures are known and PAs have been detected in more than 350 plant species so far [2].

In recent years, it has been shown that PAs and TAs in particular occur in food of plant origin in quantities that are relevant to food safety [1, 3]. They can enter the food chain via several routes. In most cases PAs or TAs containing wild herbs and weeds contaminate plant foods during harvest. The European Commission and the Member States are currently discussing the introduction of maximum levels for the presence of PAs and TAs in certain foods, as for instance tea and herbal tea products [4]. The maximum levels for TAs refer to the sum of atropine and scopolamine. Since a large number of individual PA analytes are known, the maximum level must refer to a clearly defined spectrum of individual analytes. The analytes summarized in Table 1 represent the current consensus on the methodological scope. Some of these analytes occur naturally as isomers. By means of LC MS/MS these isomers can hardly be distinguished, since they have similar chromatographic retention times (co-elution) and form the same precursor and product ions in the mass spectrometer, which are often similar in intensity distribution. With the methods currently used, most isomers are difficult to distinguish and unambiguous statements about the exact isomer pattern in a sample are only possible with a high analytical effort. Therefore, foreseen regulation proposes to analyze isomers as a group rather than individually [4]. The PA content is to be determined as the lower bound content. This means that an individual analyte whose content is below the limit of quantification (LOQ), is included in the sum calculation with the numerical value of "zero". Hereby a LOQ of at least 10 µg/kg for individual PAs and 5 µg/kg for individual TAs needs to be achieved in herbal tea [4].

3 Scope and Study design

The German NRL for Mycotoxins and Plant Toxins organized the proficiency test on the determination of PAs and TAs in herbal tea and standard solutions. The samples were sent together with the documents on 14.01.2020 and the deadline for results submission was 28.02.2020. Twenty-seven laboratories from three countries registered for the PT and twenty-five reported within the announced deadline (Table 2). The majority of the laboratories were official laboratories of the Federal states of Germany, as well as contract laboratories involved in food control. The target concentration was set at the LOQ required for monitoring of maximum levels (5 µg/kg respectively 10 µg/kg) and spiking levels covered the range from 0.6 to 77 ng/mL in solutions respectively and varied from 7 to 113 µg/kg in herbal teas. Some PAs selected for monitoring the maximum levels occur in nature as isomers, which should be analyzed as a sum (for grouped isomers please refer to Table 1). Since isomers differ in stereochemistry, they also differ in MS response [5]. This difference will affect RSD_R when individual isomers are quantified indirectly via a representative, depending on the selected isomer and its stereochemistry. Therefore, the participants were asked to report isomer amounts for certain prescribed MRM transitions.

The PT was conducted to assess the proficiency of the laboratories and the fitness for purpose of the methods in use with special focus on:

- required LOQ of 10 µg/kg per individual PA or 5 µg/kg per individual TA
- influence of the analysis of natural isomers as a sum/group on the relative standard deviation (RSD_R)
- reproducibility standard deviation - required $RSD_R \leq 25$ %
- influence of inhomogeneity of naturally contaminated samples on RSD_R

Table 1: Analyte selection currently proposed by the EU commission for monitoring the discussed maximum levels for the occurrence of PA in food as the sum of the individual levels [4]

PA or PA group [abbreviation]	ester form	necine base	natural isomers
Echimidine group [Em_G]	open chained diester	retronecine	Echimidine [Em], Heliosupine [Hs]
Echimidine-*N*-oxide group [EmN_G]	open chained diester	retronecine	Echimidine-*N*-oxide [EmN], Heliosupine-*N*-oxide [HsN]
Europine [Eu]	monoester	heliotridine	
Europine-*N*-oxide [EuN]	monoester	heliotridine	
Heliotrine [Ht]	monoester	heliotridine	
Heliotrine-*N*-oxide [HtN]	monoester	heliotridine	
Intermedine group [Im_G]	monoester	retronecine	Intermedine [Im] Lycopsamine [Ly] Indicine [Id] Echinatine [En] Rinderine [Rn]
Intermedine-*N*-oxide group [ImN_G]	monoester	retronecine	Intermedine-*N*-oxide [ImN] Lycopsamine-*N*-oxide [LyN] Indicine-*N*-oxide [IdN] Echinatine-*N*-oxide [EnN] Rinderine-*N*-oxide [RnN]
Lasiocarpine [Lc]	open chained diester	heliotridine	
Lasiocarpine-*N*-oxide [LcN]	open chained diester	heliotridine	
Retrorsine group [Re_G]	cyclic diester	retronecine	Retrorsine [Re] Usaramine [Us]
Retrorsine-*N*-oxide group [ReN_G]	cycli cdiester	retronecine	Retrorsine-*N*-oxide [ReN] Usaramine-*N*-oxide (UsN]
Senecionine group [Sc_G]	cyclic diester	retronecine	Senecionine [Sc] Senecivernine [Sv] Integerrimine [Ig]
Senecionine-*N*-oxide group [ScN_G]	cyclic diester	retronecine	Senecionine-*N*-oxide [ScN] Senecivernine-*N*-oxide [SvN] Integerrimine-*N*-oxide [IgN]
Seneciphylline group [Sp_G]	cyclic diester	retronecine	Seneciphylline [Sp] Spartioidine [St]
Seneciphylline-*N*-oxide group [SpN_G]	cyclic diester	retronecine	Seneciphylline-*N*-oxide [SpN] Spartioidine-*N*-oxide [StN]
Senkirkine [Sk]	cyclic diester	otonecine	

The laboratories that participated in this exercise, alphabetically listed in Table 2 below, are also sincerely acknowledged.

Table 2 Participating laboratories

AGES - Austrian Agency for Health and Food Safety; Austria
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit; Germany
Federal Institute for Risk Assessment - NRL Mycotoxins and Plant Toxins; Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe; Germany
Chemisches und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper; Germany
Coop Zentrallabor; Switzerland
Eurofins Dr. Specht International GmbH; Germany
Eurofins WEJ Contaminants GmbH; Germany
GBA Gesellschaft für Bioanalytik mbH; Germany
Institut Kirchhoff Berlin GmbH; Germany
Kantonales Labor Zürich; Switzerland
Kantonales Laboratorium Basel; Switzerland
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Rostock; Germany
Landesbetrieb Hessisches Landeslabor (LHL); Germany
Landeslabor Berlin-Brandenburg; Germany
Landesuntersuchungsamt Rheinland-Pfalz - Institut für Lebensmittelchemie; Germany
Landesuntersuchungsanstalt Chemnitz, FG 5.5, Labor 2.19/2.29; Germany
LAV LSA (Landesamt für Verbraucherschutz Sachsen-Anhalt) Halle/Saale; Germany
Lehrstuhl für Lebensmittelsicherheit, LMU München; Germany
LEON Institute of Applied Analytics and Research GmbH; Germany
Max Rubner-Institut- Institut für Sicherheit und Qualität bei Obst und Gemüse; Germany
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Fachbereich 41 (Standort Braunschweig) ; Germany
PhytoLab GmbH & Co. KG; Germany
QSI – Quality Services International GmbH; Germany
SGS Germany GmbH; Germany

4 Test Material

The participating laboratories received spiked samples of chamomile tea and melissa tea of approx. 10 g each. In addition, two standard solutions (LSG1, LSG2) of 1.5 mL with a mixture of the PAs and TAs in different concentrations were sent.

4.1 Preparation

Herbal tea samples were prepared and spiked according to a protocol applied for the PT for “The determination of tropane alkaloids in herbal tea and herbal infusion” [6]. Commercially available chamomile and melissa teas were contaminated in the laboratory with a mixture of individual PAs as well as atropine and scopolamine (Table 3).

Multi-analyte mixtures with target concentrations for each individual substance and each tea were prepared. For this purpose defined volumes of stock solution of each substance were pipetted into an Erlenmeyer flask and made up to 5 ml with methanol. Tert-butylmethylether (tBME) was used for sample contamination. Volumes of tBME required for complete wetting of 500 g of both teas were individually tested and finally 1740 ml for chamomile tea and 1095 ml for melissa tea were mixed with 5 ml of the prepared multi-analyte mixtures. Ten tea samples of 50 g each and 1/10 of tBME multi-analyte solution were effectively mixed by manual stirring in the fume cupboard and spread in a flat aluminium tray as shown Figure 1. After the solvent had evaporated, all ten subsamples were combined to a total amount of 500 g of contaminated tea and mixed for about 12 hours in a Rhoenrad-mixer to obtain homogeneous test material. Table 3 shows the qualitative spiking profile within the samples.

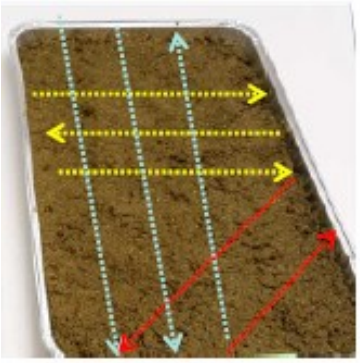

Figure 1: scheme for preparing contaminated herbal tea samples

Table 3: spiking scheme for PT samples. (Table 1 presents naturally occurring isomers per analyte group)

Analyte / analytegroup	LSG1	LSG2	melissa tea	chamomile tea
Atropine [At]	+	+	+	+
Scopolamine [Sco]	+	+	+	+
Echimidine-group	Em+Hs	Em	Em	Em+Hs
Echimidine- *N*-oxide group	EmN+HsN	EmN	EmN	EmN+HsN
Europine	+	+	+	+
Europine-*N*-oxide	+	+	+	+
Heliotrine	+	+	+	+
Heliotrine-*N*-oxide	+	+	+	+
Intermedine-group	Im+Ly+Rn	En+Id+Im	En+Id+Im	Im+Ly+Rn
Intermedine- *N*-oxide group	ImN+LyN+RnN	EnN+IdN+ImN	EnN+IdN+ImN	ImN+LyN+RnN
Lasiocarpine	+	+	+	+
Lasiocarpine-*N*-Oxide	+	+	+	+
Retrorsine-group	Re	Re	Re	Re
Retrorsine- *N*-oxide group	ReN	ReN	ReN	ReN
Senecionine group	Sc+Ig	Sc+Sv	Sc+Sv	Sc+Ig
Senecionine- *N*-oxide group	ScN+IgN	ScN+SvN	ScN+SvN	ScN+IgN
Seneciphylline group	Sp	Sp	Sp	Sp
Seneciphylline- *N*-oxide group	SpN	SpN	SpN	SpN
Senkirkine	+	+	+	+
concentration range	5-77 ng/mL	0.6-22 ng/mL	12-113 µg/kg	7-52 µg/kg

(+) means spiked

Aliquots of 10 gram per sample were packed in 50 ml plastic tubes, closed with screw caps and additionally sealed with parafilm. The storage up to dispatch took place at room temperature. Both standard solutions (5 % methanol) were prepared accordingly and filled into 1.5 ml glass vials. The solutions were stored in the refrigerator at 5 °C until dispatch. The laboratories were asked to store the samples in a similar way until analysis. All samples were sent cooled in polystyrene boxes.

4.2 Homogeneity and stability

The homogeneity of samples was determined according to ISO 13528 [7]. For this purpose, 10 units per tea sample each were randomly selected and examined in duplicate analyses under repeatability conditions via the BfR method (https://www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-pa-in-plant-material.pdf). As herbal tea presents a solid, coarse-grained material, the analyte was considered as sufficiently homogeneous if at least the extended condition for homogeneity according to ISO 13528 point B.2.3 is fulfilled:

$$s_s = \sqrt{c} \qquad \text{(Equation 1)}$$

$$c = F_1 * (0.3 * \sigma_{PT})^2 + F_2 * s_w^2 \qquad \text{(Equation 2)}$$

s_s: Standard deviation between samples
σ_{PT}: Target standard deviation
s_w: Standard deviation within the sample (duplicate analysis)
F_1 und F_2: from standard statistical tables see ISO 13528

All analytes in melissa tea were found to be sufficiently homogenous according to the criteria of DIN 13528 (B.2.3). Chamomile tea was naturally contaminated with the *N*-oxides (as group) of intermedine (ImN) retrorsine (ReN) and senecionine (ScN). Despite comminution to 500 µm these analytes were not found to be sufficiently homogenous and consequently laboratories were not evaluated for these analyte-matrix-combinations. Sufficient homogeneity was confirmed for all other analytes in the sample. Results of homogeneity testing are summarized in Table 11 and Table12 in the appendix. The stability was not tested, as previous ring trials did not provide any indication of instability.

5 Statistical evaluation

Twenty-five laboratories submitted the analytical results on time (Table 14 to Table 17)). Laboratory L-021 only determined TAs, while Laboratories L-025, L-026 and L-029 analyzed only for PAs.

5.1 Procedure for statistical evaluation

Mandel's statistics was used to check whether the results of the laboratories differed significantly from those of the other laboratories. If there were significant deviations, these laboratories were excluded before the statistical calculation.

The statistical evaluation of the PT was carried out with robust evaluation methods according to ISO 13528 [7]. A robust evaluation can also be applied in cases of not normally distributed measurement results. Results with right-skewed distributions and a break point of 30 to 50 % can also be evaluated. The reproducibility standard deviation was determined according to the Q method and the robust mean value by ”Hampel-Schätzer” using the ProLabPlus software (version 2019.1.23.0). When calculating the robust mean value, the estimation procedure according to Hampel et al. [8] does not take into account laboratory results that deviate from the mean value by more than 4.5 times of the reproducibility standard deviation. The standard error (u_x) of the robust mean value according to Hampel et al. [8] was calculated using Equation 3.

$$u_x = \sqrt{\left(\frac{s_R^2 - s_r^2}{p} + \frac{s_r^2}{p+n}\right)} = \sqrt{\left(\frac{s_R^2}{p}\right)} \qquad \text{(Equation 3)}$$

s_R: reproducibility standard deviation according to Q method
s_r: repeatability standard deviation according to Q method (here: 0)
p: Number of laboratory mean values
n: average number of laboratory measurements per analyte-sample combination (here: 1)

For none of the test materials a certified content was available. Therefore, assigned values per analyte-matrix-combinations were calculated from as robust mean values based on laboratory results. This procedure was also applied for assigned values of the standard solution, since a) in some cases non certified standards were used and b) random errors during preparation may cause deviations. The target standard deviation (σ_{PT}) of 25 % was applied for each analyte-matrix-combination in accordance to the proposed amendments of the European regulation laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. For some analyte-matrix-combinations an increased uncertainty of the calculated robust mean was found (quotient of the standard error of the mean and the target standard deviation was greater than 0.3), an expanded target deviation σ'_{PT} was applied, which is calculated according to Equation (4) [7].

$$\sigma'_{PT} = \sqrt{\sigma_{PT}^2 + u_x^2} \qquad \text{if } u_x/\sigma_{PT} > 0.3 \qquad \text{(Equation 4)}$$

σ'_{PT}: expanded target deviation [µg/kg]or [ng/mL]
σ_{PT}: target standard deviation [here 25% [µg/kg] or [ng/mL]
u_x: standard error of robust mean [µg/kg] or [ng/mL]

Due to the insufficient homogeneity of *N*-oxide groups of intermedine, retrorsine and senecionine in chamomile tea, the respective analyte-matrix-combinations were not included in the evaluation of the laboratories. These statistical parameters are only given to estimate the influence of inhomogeneity on RSD_R in naturally contaminated samples.
The Horwitz ratio [9] is used to evaluate the comparative laboratory test calculated according to equation 5.

$$\mathrm{HorRat} = s_R / \sigma_{PT} \qquad \text{(Equation 5)}$$

HorRat: Horwitz ratio
s_R: *reproducibility standard deviation [µg/kg or ng/mL]*
σ_{PT}: *target standard deviation [µg/kg or ng/mL]*

The assessment of laboratory performance is based on the z-score [10] according to Equation (6)

$$z = \frac{(x_i - x_{PT})}{\sigma_{PT}} \qquad \text{(Equation 6)}$$

x_i: *measurement result reported by the participant [µg/kg or ng/mL]*
x_{PT}: *assigned value [µg/kg or ng/mL]*
σ_{PT}: *target standard deviation [µg/kg or ng/mL]*

For some analyte-matrix-combinations with an increased uncertainty of the calculated robust mean ($0.3 < u_x/\sigma_{PT}$) a z'-score according to ISO 13528 was determined using equation (7).

(Equation 7)

$$z' = \frac{(x_i - x_{PT})}{\sigma'_{PT}}$$

x_i: *measurement result reported by the participant [µg/kg or ng/mL]*
x_{PT}: *assigned value [µg/kg or ng/mL]*
σ'_{PT} : *expanded target deviation [µg/kg or ng/mL]*

The upper and lower tolerance limits (TL) for the evaluation of the laboratories were calculated according to equation 8 and for analyte-matrix- combinations with expanded uncertainty of the calculated robust mean value according to equation 9.

$\mathrm{TL} = x_{PT} \pm (2*\sigma_{PT})$ *[µg/kg or ng/mL] or* (Equation 8)
$\mathrm{TL'} = x_{PT} \pm (2*\sigma'_{PT})$ *[µg/kg or ng/mL]* (Equation 9)

The z-score or z´-score (in cases $u_x/\sigma_{PT} > 0.3$) compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, σ_{PT}/σ'_{PT}. The z-score/z´-score is interpreted as follows:

$|z| \leq 2$ 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)

5.2 Results of the graphic compliance test according to Mandel

Mandel's h-statistics was used to evaluate deviations of an individual laboratory mean values in comparison to the mean values of the other laboratories. If the h-values of a laboratory for one analyte in several samples or for several analytes in one sample are above the corresponding critical h-values of respective analyte-matrix-combinations, it can be concluded that there are systematic deviations of the mean values of this laboratory in the sample or for this particular analyte in all samples. The graphical reports of Mandel's h-statistics can be found in Figure 9 to Figure 12 in the appendix. Here the critical values for the significance level of 5 % are shown as a yellow line and for the significance level of 1 % as a red line. Statistically deviating values of the laboratories are accordingly marked as yellow bars (significance level 5 %) or red bars (significance level 1 %). No laboratory showed significant deviations in more than 75 % of the sample-analyte-combinations or 75 % of the analytes in a sample.

6 Proficiency test results

The statistical evaluation of the results was performed as described in Section 5.1. The evaluated values of the laboratories are given per sample in Table 4 to Table 7, Table 13 and Figure 2 to Figure 5.

With a few exceptions, the laboratories achieved the required LOQ of 10 µg/kg for PA in herbal tea. Acceptable z-scores within the concentration range at the required LOQ demonstrated reliable measurement capabilities of participating laboratories. Only one laboratory reported a LOQ of 20 µg/kg for two PAs in herbal tea, while two laboratories reported a LOQ of 5 µg/kg for retrosine-*N*-oxide, but failed to quantify the respective content in melissa tea.

z-score of all particpants

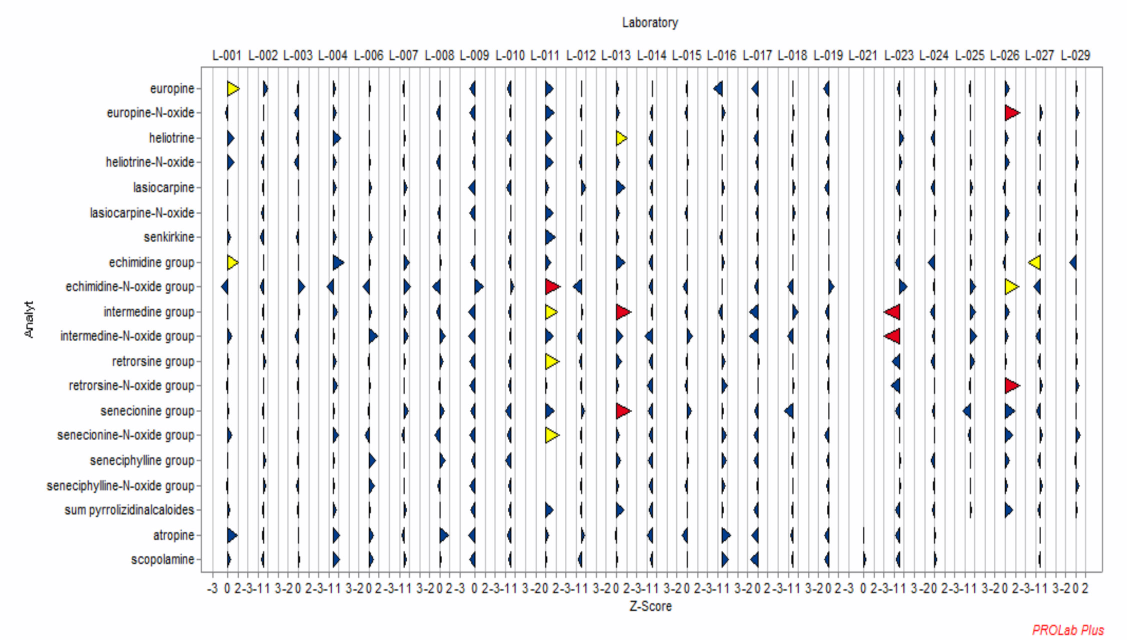

Figure 2: z-score results for standard mix 1 (blue triangle: z-score ≤ 2. yellow triangle: 2 < z-score < 3. red triangle z-score ≥ 3)

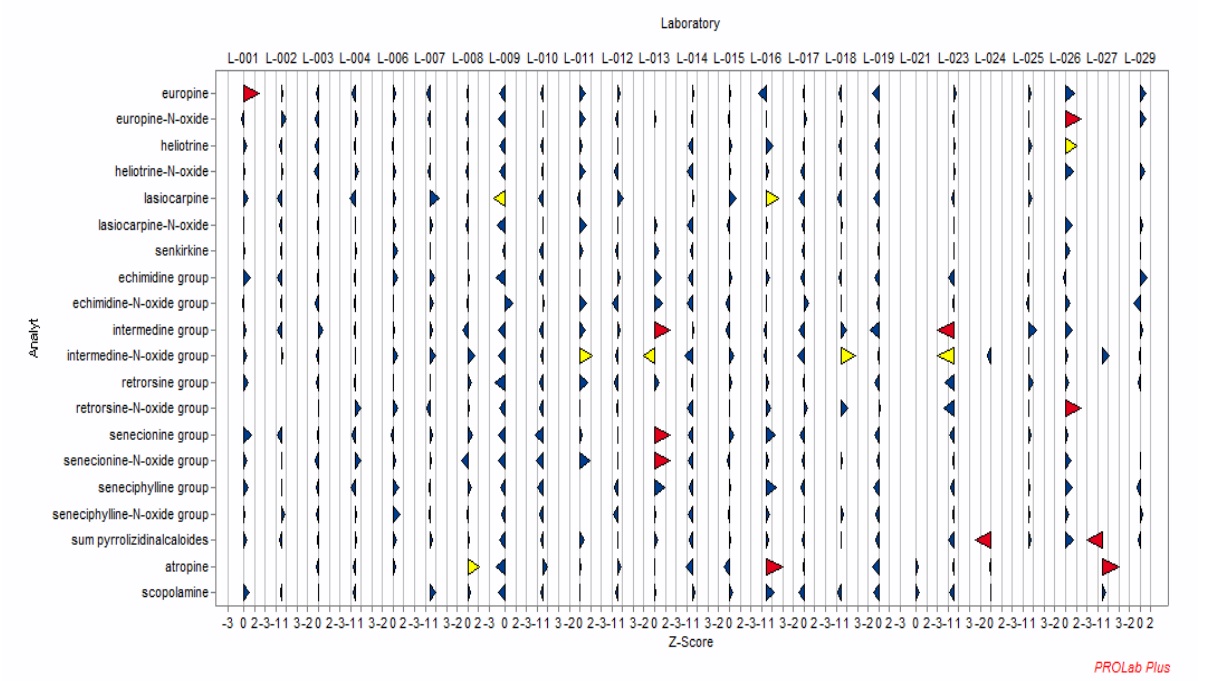

Figure 3: z-score results for standard mix 2 (blue triangle: z-score ≤ 2. yellow triangle: 2 < z-score < 3. red triangle z-score ≥ 3)

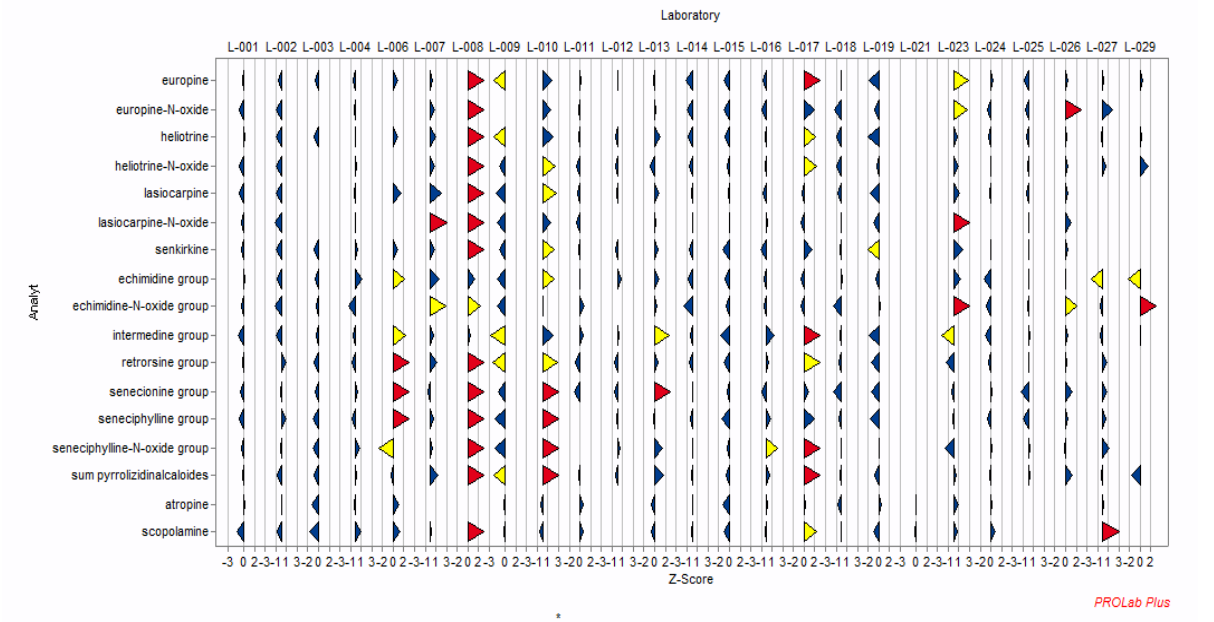

Figure 4: z-score results for chamomile tea (blue triangle: z-score ≤ 2. yellow triangle: 2 < z-score < 3. red triangle z-score ≥ 3)

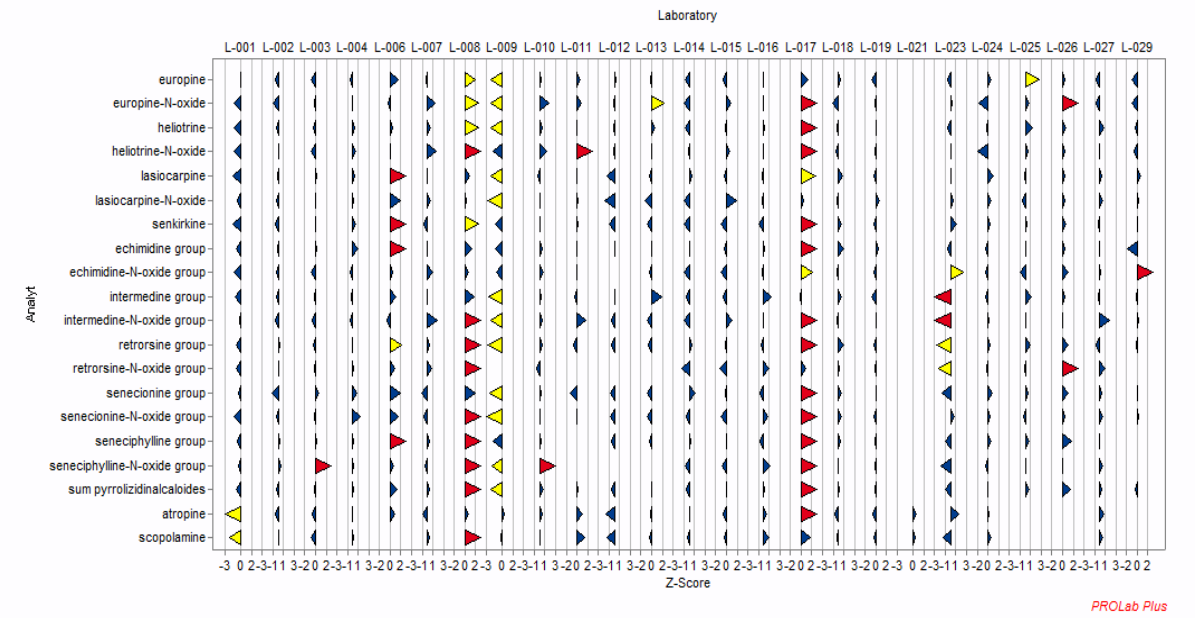

Figure 5: z-score results for melissa tea (blue triangle: z-score ≤ 2. yellow triangle: 2 < z-score < 3. red triangle z-score ≥ 3)

Table 4: Statistical characteristic values for standard solution 1

	Eu	*EuN*	*Ht*	*HtN*	*Lc*	*LcN*	*Sk*	*Em_G*	*EmN_G*	*Im_G*	*ImN_G*	*Re_G*	*ReN_G*	*Sc_G*	*ScN_G*	*Sp_G*	*SpN_G*	*PA_GES*	*At*	*Sco*
Assigned value [µg/kg]	**5.46**	**23.19**	**18.95**	**19.89**	**18.07**	**22.77**	**29.04**	**42.84**	**39.41**	**56.78**	**77.22**	**30.80**	**14.47**	**50.29**	**75.76**	**25.73**	**28.00**	**577.17**	**11.18**	**19.52**
Target-std. dev. [ng/ml]	1.36	5.80	4.74	4.97	4.52	5.69	7.26	10.71	9.85	14.20	19.30	7.70	3.62	12.57	18.94	6.43	7.00	**144.29**	2.79	4.88
Reprod.-std. dev. s_R[ng/ml]	1.05	3.75	2.76	2.51	3.33	2.24	2.82	9.15	13.14	15.34	23.53	5.41	1.88	14.27	15.81	4.26	3.26	**88.27**	2.76	3.46
Rel. target-std. dev. [%]	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	**25.00**	25.00	25.00
Rel. reprod.-std. dev. [%]	**19.29**	**16.17**	**14.54**	**12.62**	**18.42**	**9.83**	**9.72**	**21.37**	**33.35**	**27.01**	**30.48**	**17.57**	**12.96**	**28.38**	**20.87**	**16.54**	**11.64**	**15.29**	**24.64**	**17.73**
Reproducibility Limit, R (2.80 x sR) [ng/ml]	2.95	10.50	7.72	7.03	9.32	6.27	7.90	25.63	36.79	42.95	65.90	15.15	5.25	39.96	44.27	11.92	9.13	**247.14**	7.71	9.69
Rel. Reprod. Limit [%]	54.02	45.28	40.72	35.34	51.58	27.53	27.20	59.84	93.37	75.64	85.34	49.20	36.30	79.45	58.44	46.30	32.59	**42.82**	69.00	49.65
Lower tol. Limit [ng/ml]	2.73	11.60	9.48	9.94	9.03	11.39	14.52	21.42	19.70	28.39	38.61	15.40	7.23	25.14	37.88	12.87	14.00	**288.58**	5.59	9.76
Upper tol. Limit [ng/ml]	8.19	34.78	28.43	29.83	27.10	34.16	43.57	64.25	59.11	85.17	115.83	46.19	21.70	75.43	113.63	38.60	42.01	**865.75**	16.77	29.28
Stand. uncertainty of the assigned value [ng/ml]	0.22	0.77	0.56	0.51	0.68	0.46	0.58	1.87	2.68	3.13	4.80	1.10	0.38	2.91	3.23	0.89	0.68	**18.02**	0.59	0.74
standard error of robust mean /target-std. dev.	0.16	0.13	0.12	0.10	0.15	0.08	0.08	0.17	0.27	0.22	0.25	0.14	0.11	0.23	0.17	0.14	0.10	**0.12**	0.21	0.15
Lower confid limit [ng/ml]	5.02	21.66	17.83	18.86	16.71	21.86	27.89	39.10	34.04	50.52	67.61	28.59	13.70	44.46	69.30	23.96	26.65	**541.13**	10.00	18.05
Upper confid limit [ng/ml]	5.90	24.72	20.08	20.91	19.43	23.69	30.20	46.57	44.77	63.04	86.83	33.01	15.23	56.11	82.21	27.51	29.36	**613.20**	12.35	21.00
No of labs with rep. res.	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	23	23	**24**	22	22
No of labs with quant. res	23	24	24	24	24	24	24	24	24	24	24	24	24	24	24	23	23	**24**	22	22
No of labs with results outside the tol. limits	1	1	1					2	2	3	1	1	1	1	1					
HorRat value	**0.77**	**0.65**	**0.58**	**0.50**	**0.74**	**0.39**	**0.39**	**0.85**	**1.33**	**1.08**	**1.22**	**0.70**	**0.52**	**1.14**	**0.83**	**0.66**	**0.47**	**0.61**	**0.99**	**0.71**

Table 5: Statistical characteristic values for standard solution 2

	Eu	*EuN*	*Ht*	*HtN*	*Lc*	*LcN*	*Sk*	*Em_G*	*EmN_G*	*Im_G*	*ImN_G*	*Re_G*	*ReN_G*	*Sc_G*	*ScN_G*	*Sp_G*	*SpN_G*	*PA_GES*	*At*	*Sco*
Assigned value [ng/ml]	**2.23**	**2.39**	**1.86**	**2.49**	**1.70**	**2.71**	**3.68**	**3.28**	**3.98**	**6.89**	**10.88**	**2.60**	**1.72**	**4.57**	**6.25**	**2.98**	**3.62**	**62.04**	**0.60**	**22.42**
Target-std. dev. [ng/ml]	0.56	0.60	0.47	0.62	0.43	0.68	0.92	0.82	0.99	1.72	2.72	0.65	0.43	1.14	1.56	0.75	0.90	15.51	0.15	5.61
Reprod.-std. dev. s_R[ng/ml]	0.60	0.35	0.35	0.48	0.48	0.36	0.37	0.69	0.71	2.21	3.80	0.52	0.50	1.25	1.41	0.67	0.61	12.77	0.22	5.24
Rel. target-std. dev. [%]	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Rel. reprod.-std. dev. [%]	**27.13**	**14.71**	**18.54**	**19.15**	**27.98**	**13.23**	**10.15**	**21.08**	**17.90**	**32.07**	**34.88**	**19.79**	**29.04**	**27.36**	**22.59**	**22.34**	**16.88**	**20.59**	**36.59**	**23.39**
Reproducibility Limit, R (2.80 x s_R) [ng/ml]	1.69	0.99	0.97	1.34	1.33	1.01	1.05	1.93	1.99	6.18	10.63	1.44	1.40	3.50	3.96	1.87	1.71	35.76	0.61	14.68
*Rel. Reprod. Limit [%]	75.96	41.18	51.90	53.61	78.35	37.05	28.42	59.03	50.13	89.79	97.67	55.42	81.30	76.62	63.24	62.54	47.27	57.64	102.44	65.48
Lower tol. Limit [ng/ml]	1.11	1.20	0.93	1.25	0.85	1.36	1.84	1.64	1.99	3.44	5.44	1.30	0.86	2.29	3.13	1.49	1.81	31.02	0.30	11.21
Upper tol. Limit [ng/ml]	3.34	3.59	2.80	3.74	2.55	4.07	5.52	4.91	5.97	10.33	16.32	3.91	2.58	6.86	9.38	4.47	5.43	93.06	0.89	33.63
Stand. uncertainty of the assigned value [ng/ml]	0.13	0.08	0.08	0.10	0.11	0.08	0.08	0.15	0.15	0.47	0.78	0.12	0.12	0.28	0.30	0.15	0.13	2.61	0.05	1.14
standard error of robust mean/target-std. dev.	0.24	0.13	0.17	0.17	0.26	0.11	0.09	0.18	0.15	0.27	0.28	0.18	0.27	0.24	0.19	0.20	0.15	0.17	0.34	0.20
Lower confid limit [ng/ml]	1.96	2.24	1.71	2.29	1.48	2.56	3.52	2.98	3.67	5.94	9.33	2.37	1.48	4.01	5.65	2.68	3.35	56.83	0.49	20.13
Upper confid limit [ng/ml]	2.49	2.54	2.02	2.70	1.92	2.87	3.84	3.57	4.28	7.83	12.43	2.83	1.95	5.13	6.86	3.28	3.88	67.26	0.70	24.71
No of labs with rep. res.	24	24	24	24	24	24	24	24	24	24	24	23	23	24	24	23	23	24	21	21
No of labs with quant. res	21	22	20	21	19	22	22	22	22	22	24	20	18	20	22	20	21	24	18	21
No of labs with results outside the tol. limits	1	1	1		2					2	4		1	1	1			2	3	
HorRat value	**1.09**	**0.59**	**0.74**	**0.77**	**1.12**	**0.53**	**0.41**	**0.84**	**0.72**	**1.28**	**1.40**	**0.79**	**1.16**	**1.09**	**0.90**	**0.89**	**0.68**	**0.82**	**1.46**	**0.94**

quotient of standard error of robust mean / target-std. dev. > 0.30

Table 6: Statistical characteristic values for chamomile tea

	Eu	*EuN*	*Ht*	*HtN*	*Lc*	*LcN*	*Sk*	*Em_G*	*EmN_G*	*Im_G*	*ImN_G*	*Re_G*	*ReN_G*	*Sc_G*	*ScN_G*	*Sp_G*	*SpN_G*	*PA_GES*	*At*	*Sco*
Assigned value [µg/kg]	**16.78**	**7.02**	**15.04**	**11.18**	**11.60**	**9.52**	**15.18**	**36.16**	**16.29**	**34.02**	**46.91**	**13.76**	**18.87**	**23.71**	**42.84**	**13.88**	**16.32**	**328.15**	**8.67**	**18.90**
Target-std. dev. [µg/kg]	4.19	1.75	3.76	2.80	2.90	2.38	3.80	9.04	4.07	8.51	11.73	3.44	4.72	5.93	10.71	3.47	4.08	82.04	2.17	4.72
Reprod.-std. dev. s_R[µg/kg]	4.94	1.86	4.31	2.90	3.17	2.75	5.12	12.63	5.67	13.80	26.60	4.65	8.12	8.26	11.91	5.15	5.69	92.41	1.31	6.04
Rel. target-std. dev. [%]	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Rel. reprod.-std. dev. [%]	**29.46**	**26.44**	**28.67**	**25.90**	**27.35**	**28.84**	**33.70**	**34.92**	**34.82**	**40.57**	**56.70**	**33.83**	**43.04**	**34.84**	**27.81**	**37.07**	**34.88**	**28.16**	**15.08**	**31.94**
Reproducibility Limit, R (2.80 x sR) [µg/kg]	13.84	5.19	12.08	8.11	8.89	7.69	14.32	35.36	15.88	38.65	74.48	13.03	22.74	23.14	33.35	14.41	15.94	258.74	3.66	16.90
Rel. Reprod. Limit [%]	82.48	74.02	80.28	72.51	76.57	80.76	94.36	97.77	97.50	113.61	158.76	94.73	120.51	97.56	77.86	103.79	97.66	78.85	42.21	89.44
Lower tol. Limit [µg/kg]	8.39	3.51	7.52	5.59	5.80	4.76	7.59	18.08	8.14	17.01	23.46	6.88	9.44	11.86	21.42	6.94	8.16	164.08	4.34	9.45
Upper tol. Limit [µg/kg]	25.16	10.53	22.56	16.78	17.41	14.28	22.77	54.25	24.43	51.03	70.37	20.64	28.31	35.57	64.26	20.82	24.48	492.23	13.01	28.34
Stand. uncertainty of the assigned value [µg/kg]	1.01	0.43	0.88	0.62	0.69	0.65	1.09	2.58	1.21	2.82	5.43	0.97	1.77	1.72	2.54	1.10	1.21	18.86	0.30	1.32
standard error of robust mean /target-std. dev.	0.24	0.24	0.23	0.22	0.24	0.27	0.29	0.29	0.30	0.33	0.46	0.28	0.38	0.29	0.24	0.32	0.30	0.23	0.14	0.28
Lower confid limit [µg/kg]	14.76	6.17	13.28	9.95	10.22	8.23	13.00	31.01	13.87	28.38	36.05	11.82	15.33	20.27	37.76	11.69	13.89	290.43	8.07	16.26
Upper confid limit [µg/kg]	18.79	7.87	16.80	12.42	12.99	10.82	17.36	41.32	18.70	39.66	57.77	15.70	22.42	27.16	47.92	16.08	18.75	365.88	9.27	21.53
No of labs with rep. res.	24	22	24	22	23	22	24	24	23	24	24	24	23	24	23	23	23	24	22	22
No of labs with quant. res	24	19	24	22	21	18	22	24	22	24	24	23	21	23	22	22	22	24	19	21
No of labs with results outside the tol. limits	4	3	3	3	2	3	3	4	5	5	9	5	3	4	4	3	5	4		3
HorRat value	**1.18**	**1.06**	**1.15**	**1.04**	**1.09**	**1.15**	**1.35**	**1.40**	**1.39**	**1.62**	**2.27**	**1.35**	**1.72**	**1.39**	**1.11**	**1.48**	**1.40**	**1.13**	**0.60**	**1.28**

quotient of standard error of robust mean / target-std. dev. > 0.30

insufficient homogeneity of the material

Table 7: Statistical characteristic values for melissa tea

	Eu	*EuN*	*Ht*	*HtN*	*Lc*	*LcN*	*Sk*	*Em_G*	*EmN_G*	*Im_G*	*ImN_G*	*Re_G*	*ReN_G*	*Sc_G*	*ScN_G*	*Sp_G*	*SpN_G*	*PA_GES*	*At*	*Sco*
Assigned value [µg/kg]	**99.74**	**64.34**	**56.17**	**45.00**	**25.75**	**21.19**	**45.08**	**30.49**	**14.99**	**92.88**	**107.33**	**45.35**	**12.36**	**42.91**	**46.51**	**15.39**	**11.40**	**765.22**	**60.67**	**77.21**
Target-std. dev. [µg/kg]	24.94	16.09	14.04	11.25	6.44	5.30	11.27	7.62	3.75	23.22	26.83	11.34	3.09	10.73	11.63	3.85	2.85	191.31	15.17	19.30
Reprod.-std. dev. s_R[µg/kg]	23.23	30.00	11.72	11.96	5.42	4.40	12.68	5.98	3.97	20.47	30.11	12.43	4.38	13.78	13.52	3.63	3.77	152.02	15.57	22.01
Rel. target-std. dev. [%]	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Rel. reprod.-std. dev. [%]	**23.29**	**46.63**	**20.86**	**26.58**	**21.03**	**20.74**	**28.13**	**19.60**	**26.46**	**22.04**	**28.06**	**27.41**	**35.39**	**32.11**	**29.06**	**23.61**	**33.09**	**19.87**	**25.67**	**28.51**
Reproducibility Limit, R (2.80 x sR) [µg/kg]	65.03	84.01	32.81	33.50	15.16	12.31	35.51	16.73	11.11	57.31	84.32	34.80	12.25	38.58	37.85	10.17	10.56	425.65	43.60	61.64
Rel. Reprod. Limit [%]	65.20	130.57	58.41	74.44	58.87	58.06	78.77	54.88	74.08	61.70	78.56	76.74	99.10	89.91	81.38	66.11	92.64	55.62	71.87	79.84
Lower tol. Limit [µg/kg]	49.87	32.17	28.09	22.50	12.88	10.60	22.54	15.24	7.50	46.44	53.66	22.68	6.18	21.45	23.26	7.69	5.70	382.61	30.34	38.60
Upper tol. Limit [µg/kg]	149.61	96.51	84.26	67.51	38.63	31.79	67.61	45.73	22.49	139.32	160.99	68.03	18.55	64.36	69.77	23.08	17.10	1147.83	91.00	115.81
Stand. uncertainty of the assigned value [µg/kg]	4.74	6.12	2.39	2.44	1.11	0.90	2.59	1.22	0.83	4.18	6.15	2.54	1.00	2.81	2.76	0.77	0.84	31.03	3.32	4.80
standard error of robust mean/target-std. dev.	0.19	0.38	0.17	0.22	0.17	0.17	0.23	0.16	0.22	0.18	0.23	0.22	0.32	0.26	0.24	0.20	0.30	0.16	0.22	0.25
Lower confid limit [µg/kg]	90.26	52.09	51.39	40.12	23.54	19.40	39.90	28.05	13.34	84.52	95.03	40.28	10.36	37.28	41.00	13.84	9.71	703.16	54.03	67.60
Upper confid limit [µg/kg]	109.22	76.59	60.96	49.89	27.96	22.99	50.25	32.93	16.65	101.24	119.62	50.43	14.37	48.53	52.03	16.94	13.09	827.28	67.31	86.81
No of labs with rep. res.	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	23	22	24	22	21
No of labs with quant. res	24	24	24	24	24	24	24	24	23	24	24	24	19	24	24	22	20	24	22	21
No of labs with results outside the tol. limits	3	5	3	3	3	1	3	2	3	2	4	5	3	2	3	3	5	3	2	2
HorRat value	**0.93**	**1.87**	**0.83**	**1.06**	**0.84**	**0.83**	**1.13**	**0.78**	**1.06**	**0.88**	**1.12**	**1.10**	**1.42**	**1.28**	**1.16**	**0.94**	**1.32**	**0.79**	**1.03**	**1.14**

quotient of standard error of robust mean / target-std. dev. > 0.30

Table 8: Achieved z-scores or z'-scores (in cases of ux/σPT > 0.3) for individual PA and TA analytes

individual PA analytes (without PA_GES)						individual TA analytes					
Lab-code	**no of z-scores**	**|z| ≥ 3**	**2 < |z| < 3**	**|z| ≤ 2**	**percentage of z-scores ≤ 2**	**Lab-code**	**no of z-scores**	**|z| ≥ 3**	**2 < |z| < 3**	**|z| ≤ 2**	**percentage of z-scores ≤ 2**
L-001	64	1	2	61	95.3	L-001	7	0	2	5	71.4
L-002	63	0	0	63	100.0	L-002	7	0	0	7	100.0
L-003	61	1	0	60	98.4	L-003	8	0	0	8	100.0
L-004	65	0	0	65	100.0	L-004	8	0	0	8	100.0
L-006	61	7	4	50	82.0	L-006	6	0	0	6	100.0
L-007	65	1	1	63	96.9	L-007	6	0	0	6	100.0
L-008	65	18	5	42	64.6	L-008	7	2	1	4	57.1
L-009	63	0	16	47	74.6	L-009	8	0	0	8	100.0
L-010	65	4	5	56	86.2	L-010	8	0	0	8	100.0
L-011	56	2	4	50	89.3	L-011	8	0	0	8	100.0
L-012	59	0	0	59	100.0	L-012	6	0	0	6	100.0
L-013	58	6	4	48	82.8	L-013	8	0	0	8	100.0
L-014	65	0	0	65	100.0	L-014	8	0	0	8	100.0
L-015	65	0	0	65	100.0	L-015	8	0	0	8	100.0
L-016	65	0	2	63	96.9	L-016	8	1	0	7	87.5
L-017	65	14	5	46	70.8	L-017	8	1	1	6	75.0
L-018	62	0	1	61	98.4	L-018	7	0	0	7	100.0
L-019	65	0	1	64	98.5	L-019	8	0	0	8	100.0
L-021	0	0	0	0		L-021	8	0	0	8	100.0
L-023	65	5	9	51	78.5	L-023	8	0	0	8	100.0
L-024	48	0	0	48	100.0	L-024	8	0	0	8	100.0
L-025	65	0	1	64	98.5	L-025	0	0	0	0	
L-026	64	7	3	54	84.4	L-026	0	0	0	0	
L-027	45	0	2	43	95.6	L-027	8	2	0	6	75.0
L-029	50	2	1	47	94.0	L-029	0	0	0	0	

6.1 Results on LOQ requirements

The reliability of results within the range of the required LOQ were assessed based on RSD_R for the 38 analyte matrix combinations obtained in chamomile and melissa tea in relation to the analyte amounts Figure 6-A-left). To increase the statistical significance of the limited number of data we included the results for 484 PA-TA-analyte/matrix combinations obtained in formerly conducted ring trials (Figure 6-B-right). Ring trial data for PAs and TAs did not indicate a concentration dependency of RSD_R. Therefore, results obtained so far for plant toxins are comparable to mycotoxins. For these analytes RSD_R is more or less independent of concentration, toxin and matrix as long as suitable validated methods are used by experienced labs and identical (homogenous) material was investigated [11].

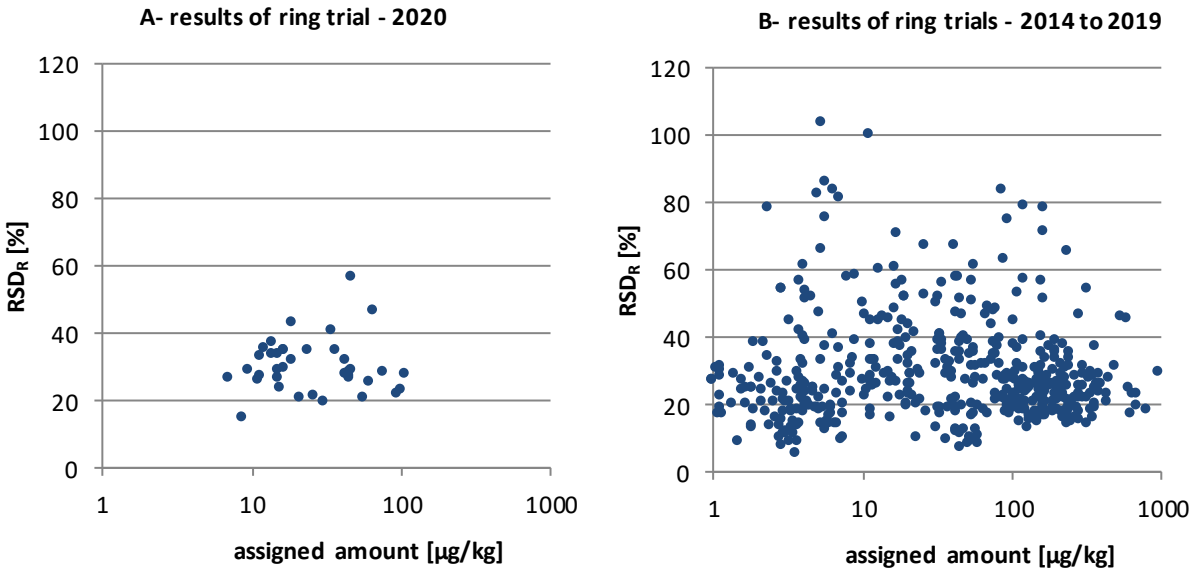

Figure 6: relative reproducibility standard deviation (RSD_R) in relation to the assigned analyte amounts obtained with the PT (left) in comparison to former ring trials (right)

6.2 Results on analysis of isomer groups

The analytical scope selected for monitoring maximum levels of PAs includes several analytes that naturally occur as isomers (Table 1). By using LC-MS/MS these isomers can hardly be distinguished as they are prone to co-elution and form the same precursor and product ion in comparable intensity ratios. Consequently, unambiguous statements on isomeric profiles are only possible with advanced analytical effort and the EU commission suggested that those isomers can be analyzed as groups. PT materials were spiked with combinations of several isomers per group (Table 3) and laboratories were requested to report the sum of concentrations per isomer group. The laboratories were asked to perform measurements for the analyte groups Em_G, EmN_G, Im_G, ImN_G, Sc_G and ScN_G with specified MRM transitions in addition to their in-house MRMs. For these recommended MRM transitions, comparable intensity ratios between the isomers, independent of their stereochemistry, were observed. The majority of laboratories did already use these recommended MRM transitions and therefore only a limited number of 7 to 12 laboratories (depending on the group of isomers to be tested) submitted data with different MRM transitions (Table 9). Therefore, a comparison of the results was only possible for Im_G, Sc_G and ScN. A detailed compilation of applied MRMs can be found in the Appendix (Table 19).

The influence of sum analysis of isomers on interlaboratory variability was assessed for six isomer groups that were present in the samples with a variable combination of isomers (Ta-

ble 3). All other analytes represent a single target analysis. To avoid the inhomogeneity af-fecting the RSD_R only data for standard solutions (Std.1 and 2) are shown but herbal teas spiked with comparable isomer combinations revealed similar results (Table 6 and Table 7). In Figure 7 results are shown for all analytes and for Im_G, Sc_G and ScN results obtained for in-house and recommended MRM transitions are reported. In this PT those PAs that were analyzed as sums of several isomers exhibited a slightly increased RSD_R compared to single target analysis (Figure 7). The RSD_R for isomer sum analyses was about 5-7 % higher (using in-house MRMs) but was still considered acceptable. The decrease of RSD_R using pre-scribed MRMs could improve interlaboratory variability for isomer analysis but as only a lim-ited number of results was evaluable in this PT no reliable conclusions can be drawn.

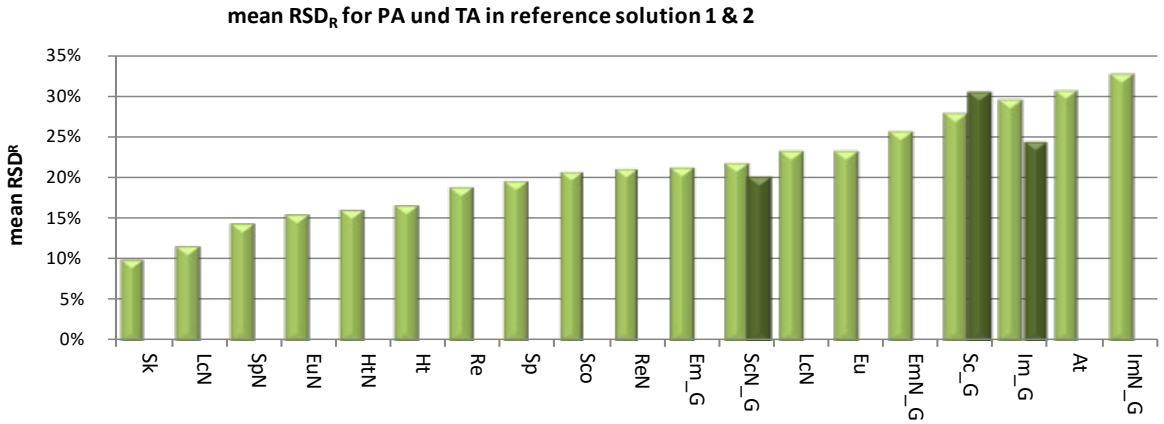

Figure 7: Mean RSD_R [%] per analyte in standard solutions arranged from smallest to highest. Analyte groups present as a mixture of isomers and reported as sum are marked as xx_G while all other analytes represent a single target analysis. The RSD_R in the case of applying recommended MRMs is shown for ScN_G, Sc_G and Im_G as dark green bars.

Table 9: Number of laboratories whose in-house methods diverged from the recommended target MRM

	Em_G					
chamomile	1	0	12	1	8	7
melissa	1	0	12	1	9	7
Ref. solution 1 (Std.1)	1	0	12	1	8	7
Ref. solution 2 (Std.2)	1	0	12	1	8	7

6.3 Results on reproducibility standard deviation

Bases on PT results the main factors influencing the interlaboratory variability of PA and TA analyses may be summarized as follows:

- For PAs (and not for TAs) the reference standards have to be isolated from plant material which often hampers the quality of reference solutions. Almost all laboratories obtained satisfactory results for all PAs in both standard solutions. Thus, an improved quality of the currently available reference solutions can be concluded (Figure 2 and Figure 3).
- Three strategies were used by participants to compensate matrix effects, which influence quantification by LC-MS/MS, like matrix-matched calibration, matrix dilution or standard

addition. In this PT methods using standard addition or matrix-dilution exhibited lower RSD_R than methods with matrix-matched calibration (Table 10).

- The quality of calibration can be checked by back-calculation of calibration level using the calibration curve. The deviation from the true concentration should not be more than ± 20 % (accuracy) [12]. Results of this PT indicate that calibration curves forced through origin and/or applying a weighed calibration showed lower RSD_R values. These types of calibration primarily improve the accuracy of low calibration level (Table 10).
- RSD_R of plant toxins analysis is influenced by analytical factors summarized as measurement uncertainty and in addition by the inhomogeneity of sample material. The inhomogeneity can be explained as only a comparatively small number of PA or TA plant particles with very high toxin content contaminates a sample and those particles have to be evenly distributed in a large number of toxin free sample particles. The difficulty is increased by the fact that PA and TA plants contain different toxin levels within their respective plant parts. This means that even the particles contaminating the sample differ in their content, depending on whether flower, leaf or stem particles are present in the sample. A satisfactory accuracy for naturally contaminated samples can therefore only be achieved if, after homogenization, the same number and type of PA plant particles are present in each subsample. This is hardly possible in practice, so that the inevitably heterogeneous distribution of parts of PA-containing plants in contaminated samples influences the comparability of the analytical results of subsamples. The influence of inhomogeneity on RSD_R can be clearly seen in the results obtained in this PT as well as in earlier conducted ring trials (Figure 8). While RSD_Rs that are solely influenced by measurement uncertainty like in spiked material, are around the required 25 % the RSD_R is higher for naturally contaminated samples.

Table 10: Performance of methods in relation to type of quantification and calibration. Methods applied by participants were divided into two groups depending on performance (in some laboratories various types of quantification or calibrations are used in parallel)

	methods with 95 % of z-scores < 2 n=13	other n=12
Type of quantification		
standard addition	6 of 13	3 of 12
matrix-dilution	6 of 13	0 of 12
matrix-matched calibration	3 of 13	11 of 12
Type of calibration		
Requirements for calibration level accuracy	9 of 13	6 of 12
forcing through origin	4 of 13	1 of 12
weighed calibration	9 of 13	2 of 12

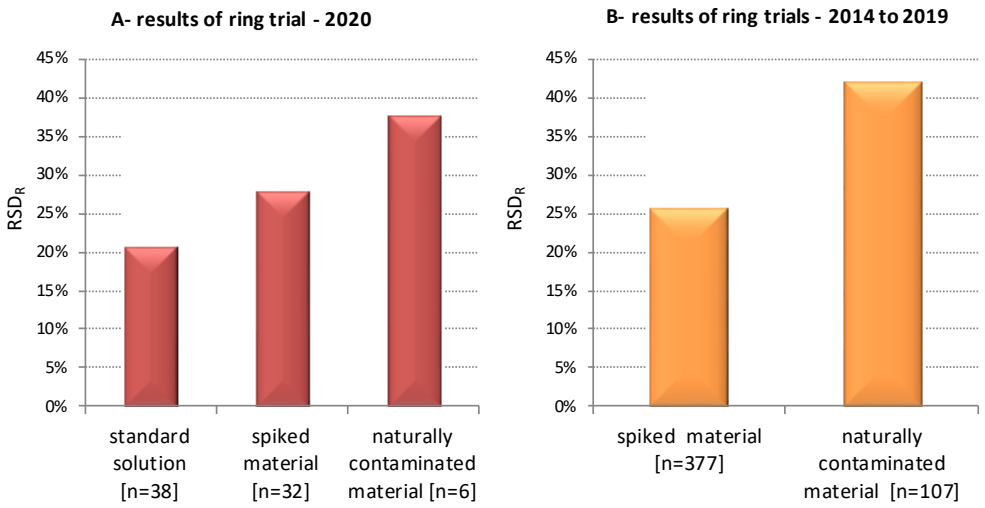

Figure 8: Relative reproducibility standard deviation (RSD_R) in relation to the matrix and homogeneity of sample material obtained by this PT (A-left) in comparison to former ring trails (B-right)

7 Conclusions

The NRL for Mycotoxins and Plant Toxins organized a PT aiming at assessing the measurement capability of laboratories regarding the determination of pyrrolizidine (PA-21 including related isomers) and tropane alkaloids (atropine and scopolamine) in herbal tea and standard solutions. Twenty-seven laboratories registered for this PT and twenty-five delivered results within the requested time.

Two herbal teas (chamomile and melissa) and two standard solutions were prepared in the concentration range from 0.6 to 77 ng/mL and 7 to 113 µg/kg, respectively, which further covered the entire analytical scope proposed to control maximum levels.

As no certified content was available, the robust mean values calculated from the laboratory results were used as assigned values for all materials. The performance of the laboratories was assessed using z-scores and a target standard deviation of 25 % was set.

On average, 91 % of the z-scores for TAs and 94 % for individual PAs fell in the acceptable range ($|z| \leq 2$). The success rate of laboratories varied from 57 to 100 % for TAs and from 65 to 100 % for PAs, across the distributed matrices and concentration levels. The RSD_R of the reported results for TAs and PAs were in good agreement with the target standard deviation (25 %) for all materials. RSD_R for the total PA amount ranged between 15 to 21 % in standard solutions and 20 to 28 % in herbal tea samples. The results of the PT support the conclusion that PAs and TAs can be reliably determined in herbal tea at the LOQs foreseen to be set up in EU Regulation. The satisfactory results for the standard solutions indicate an improved quality of the standard reference solutions currently commercially available. Mean RSD_R per analyte in both standard solutions (Std.1 and 2) ranged from 10 % for senkirkine to 33 % for intermedine N-oxide group. For the analysis of isomeric groups as a sum compared to the measurement of single target analytes higher RSD_R of around 5-7 % were obtained, indicating that variability between laboratories for isomer analysis is still satisfactory. A positive effect on RSD_R from the recommendation of MRM transitions can be assumed, but the number of results is too limited to allow a statistically sound conclusion.

A variety of analytical protocols used by laboratories has shown to be adequate for the determination of PA and TA in tea. Most laboratories analyzed PAs and TAs together. This procedure is highly recommended due to the same pathways of contamination and therefore the same matrices affected. Highly satisfactory performance was achieved, when: (a) matrix effects were compensated by matrix dilution or standard addition, (b) the accuracy of calibration curves is controlled by back-calculation of target concentration (primarily for lower concentrated level) as well as using weighted calibration function or forcing through the origin. The outcome of this PT supports results of earlier PA and TA ring trials in tea, which show that RSD_R is influenced by the inhomogeneity of the sample material. Despite much effort in sample preparation like grinding and shaking the RSD_R in naturally contaminated samples is exceeding the proposed limit of 25 %.

8 References

1. EFSA, Tropane alkaloids in food and feed. EFSA Journal, 2013.
2. Wiedenfeld, H., et al., Pyrrolizidine Alkaloids - Structure and Toxicity. 2008: V&R unipress, Bonn University Press, Goettingen, Germany.
3. EFSA, Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. The EFSA Journal, 2017. 15(7):4908: p. 34.
4. EC, European Commission Working Group of Agricultural Contaminants. Draft commission regulation amending regulation (EC) 1881/2006 as regards maximum levels of pyrrolizidine alkaloids in certain foods - from protocol. 2020.
5. Duebecke, A., Luellmann, C., Beckh, G., Pyrrolizidinalkaloid-Isomere - Eine Herausforderung für die Analytik. DLR, 2017. Sonderthema: Kontaminanten in Lebensmitteln.
6. EURL for mycotoxins-JRC, Report on the 2016 Proficiency Test of the European Union Reference Laboratory for Mycotoxins for the Network of National Reference Laboratories - Determination of tropane alkaloids in tea and herbal infusions. 2016.
7. ISO, DIN ISO 13528 - Statistical methods for use in proficiency testing by intelaboratory comparison. 2016.
8. Hampel, F., Ronchetti, E., Rousseeuw, P., Stahel, W., Robust Statistics. The Approach Based on Influence Functions. 1986: Wiley and Sons,New York.
9. Horwitz, W., Albert, R., The Horwitz Ratio (HorRat): A useful index of method performance with respect to precision. J.of AOAC International, 2006. 89(4): p. 1095-1109.
10. Thompson, M. and R. Wood: The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories (Technical Report). Pure Appl.Chem., 1993. 65(9): p. 2123-2144.
11. EURL for mycotoxins & plant toxins-WUR, Presentation: AQC session: method performance criteria mycotoxin and plant toxins in food & feed. 2019.
12. SANTE/11813/2017, Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.

9 List of figures

10 List of tables

11 Annex

11.1 Results of homogeneity testing

Table 11: Results of homogeneity testing for chamomile tea

	Em_G	EmN_G	Eu	EuN	Ht	HtN	Im_G	ImN_G	Lc	LcN	Re	ReN	Sc_G	ScN_G	Sk	Sp	SpN	At	So
mean [µg/kg]	39.2	12.3	12.4	6.5	12.3	10.1	35.4	40.3	10.5	9.9	11.1	13.7	16.6	24.0	8,4	10,1	10,3	6,34	11,7
Std. dev. [µg/kg] s_S	4.0	1.3	0.6	0.2	1.3	0.5	1.5	11	0.6	0.2	1.2	4.8	1.6	3.0	0.2	0.6	1.1	0.3	0.7
test value F	47.2	14.1	6.9	5.7	29.1	5.3	7.4	306	10.0	1.3	9.1	137.	23.6	20.9	2.1	12.3	11.5	7.1	4.5
F_{krit} 95 %	3.0	3.0	3.5	3.5	3.2	3.0	3.5	3.0	3.0	3.	3.0	3.0	3.0	3.0	3.0	3.0	3.5	3.0	3.5
homogenous, if $F < F_{krit}$	no	no	no	no	no	no	no	no	no	yes	no	no	no	no	yes	no	no	no	no
(DIN 13582 B2.2) $0.3*s_{Soll}$	2.9	0.9	0.9	0.5	0.9	0.7	2.7	3.0	0.8	7.4	0.8	1.0	1.2	1.8	0.6	0.8	0.8	0.4	0.8
homogenous, if $s_s \leq 0,3*s_{Soll}$	no	no	yes	yes	no	yes	yes	no	yes	yes	no	no	no	no	yes	yes	no	yes	yes
(DIN 13582 B2.3) $\sqrt{c}$	4.1	1.4	1.4	0.7	1.4	1.1	3.9	4.2	1.1	1.1	1.3	1.5	1.8	2.7	0.9	1.1	1.2	0.7	1.4
homogenous, if $s_s < \sqrt{c}$	yes	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	no	yes	no	yes	yes	yes	yes	yes

Insufficient homogeneity for N-oxide groups of intermedine, retrorsine and senecionine

Table 12: Results of homogeneity testing for melissa tea

	Em_G	EmN_G	Eu	EuN	Ht	HtN	Im_G	ImN_G	Lc	LcN	Re	ReN	Sc_G	ScN_G	Sk	Sp	SpN	At	So
mean [µg/kg]	37.0	15.8	46.2	44.3	52.5	25.6	87.6	89.6	25.5	14.0	52.0	14.6	46.7	39	32.0	20.0	13.6	35.9	74.6
Std. dev. [µg/kg] s_S	0.6	0.4	4.9	2.9	1.3	2.9	0.0	4.583	0.7	0.5	5.3	0.4	2.0	1.7	0.0	1.3	1.0	2.1	2.5
Prüfgröße F	3.2	3.0	17.3	21.9	1.8	7.6	0.7	16.9	3.7	5.5	41.1	2.7	4.9	11.2	1.0	6.0	17.2	3.2	3.6
F_{krit} 95 %	3.0	3.0	3.2	3.2	3.0	3.0	3.0	3.0	3.0	3.2	3.2	3.0	3.2	3.0	3.0	3.0	3.0	3.0	3.0
homogenous, if $F < F_{krit}$	no	yes	no	no	yes	no	yes	no	no	no	no	yes	no	no	yes	no	no	no	no
(DIN 13582 B2.2) $0.3*s_{Soll}$	2.8	1.2	3.5	3.3	3.9	1.9	6.6	6.7	1.9	1.0	3.9	1.1	3.5	2.9	2.4	1.5	1.0	2.7	5.6
homogenous, if $s_s \leq 0,3*s_{Soll}$	yes	yes	no	yes	yes	no	yes	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes
(DIN 13582 B2.3) $\sqrt{c}$	3.8	1.7	5.1	4.7	5.8	3.1	9.4	9.3	2.7	1.5	5.6	1.6	5.1	4.1	3.6	2.2	1.4	4.2	8.0
homogenous, if $s_s < \sqrt{c}$	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

11.2 Results of statistical evaluation

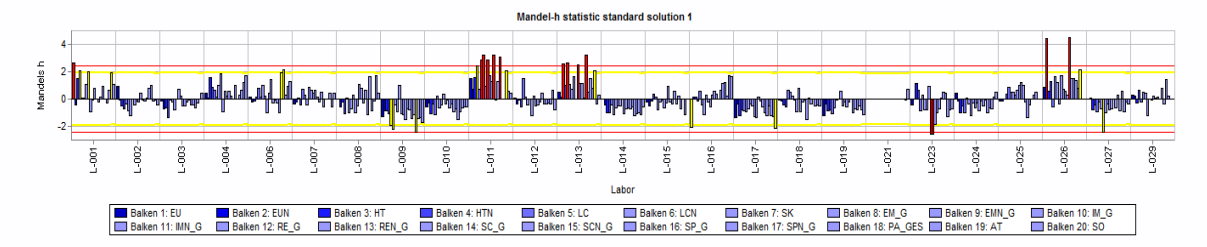

Figure 9: Mandel's-h statistics for standard solution 1 (critical values for the 5 % and 1 % significance level are shown as yellow and red lines respectively. Laboratories that deviate statistically from these values are marked with the same color)

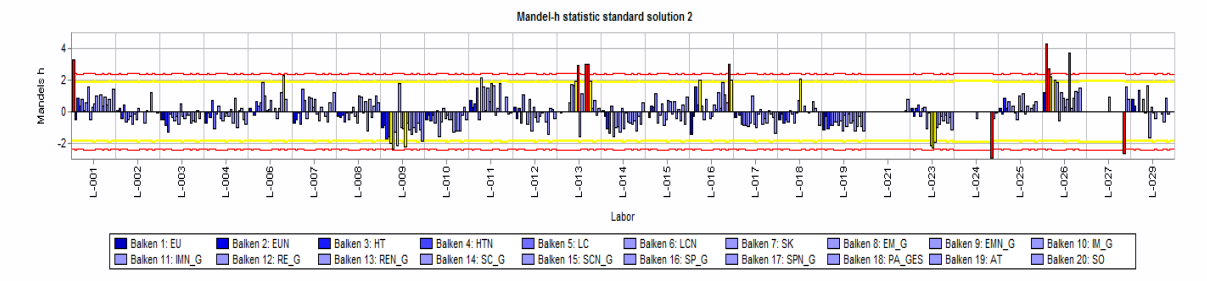

Figure 10: Mandel's-h statistics for standard solution 2 (critical values for the 5 % and 1 % significance level are shown as yellow and red lines respectively. Laboratories that deviate statistically from these values are marked with the same color)

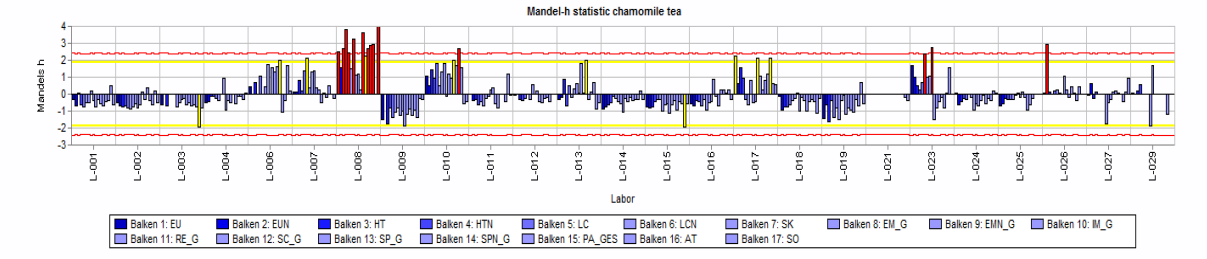

Figure 11: Mandel's-h statistics for chamomile tea (critical values for the 5 % and 1 % significance level are shown as yellow and red lines respectively. Laboratories that deviate statistically from these values are marked with the same color)

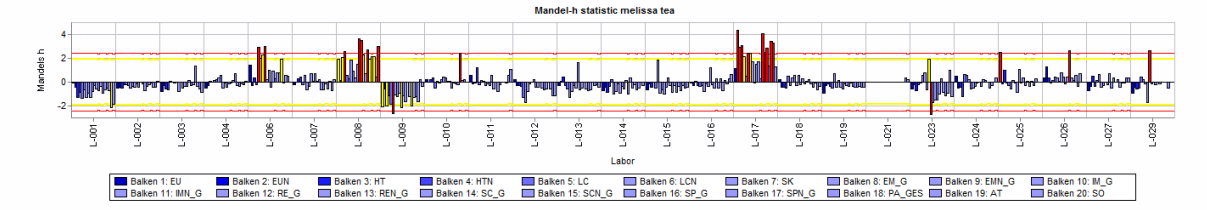

Figure 12: Mandel's-h statistics for melissa tea (critical values for the 5 % and 1 % significance level are shown as yellow and red lines respectively. Laboratories that deviate statistically from these values are marked with the same color)

Table 13: Change of z-score evaluation due to the increased quotient (standard error of robust mean/target-standard. deviation > 0.30)

	chamomile tea		melissa tea		LSG2
	Im_G	**Sp_G**	**EuN**	**ReN_G**	**At**
Rel. reprod.-std. dev. [%]	40.57	37.07	46.63	35.39	36.59
Standard error of robust mean / Standard deviation for PT (u_x/σ_{PT})	0.33	0.32	0.38	0.32	0.34
Expanded Rel. target dev. RSDσ'_{PT} [%]	26.34	26.22	26.75	26.28	26.47
Expanded standard deviation for proficiency assessment σ'_{PT} [µg/kg or ng/mL]	8.96	3.64	17.21	3.25	0.16
Lower limit TL´ [µg/kg or ng/mL]	16.10	6.60	29.92	5.86	0.28
Upper limit TL´ [µg/kg or ng/mL]	51.94	21.16	98.76	18.86	0.91
HorRat	1.54	1.41	1.74	1.35	1.38

Table 14: Result overview for standard solution 1 (*when calculating PA_GES, "< LOQ" is set as 0)

lab code	Eu	EuN	Ht	HtN	Lc	LcN	Sk	Em_G	EmN_G	Im_G	ImN_G	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	PA_GES	AT	SO
	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
L-001	8.69	21.13	24.60	25.90	18.28	23.15	33.66	65.06	27.47	56.20	94.00	31.58	13.93	53.87	93.13	25.84	26.93	**643.42**	16.37	22.91
L-002	6.61	24.20	17.70	18.20	17.20	20.40	25.00	42.90	34.30	55.00	72.20	34.20	15.10	49.60	78.90	29.00	31.50	**572.01**	11.00	18.00
L-003	5.30	18.32	17.23	16.25	17.96	22.49	26.65	43.39	51.13	61.05	65.69	28.32	13.92	50.15	70.37	24.00	25.75	**557.97**	11.34	20.79
L-004	6.03	25.72	24.99	22.61	20.07	23.52	33.32	63.48	26.89	66.65	80.96	34.76	17.17	52.14	93.93	25.92	29.08	**647.24**	14.46	24.91
L-006	5.65	23.20	18.90	20.58	20.42	23.41	33.47	45.41	26.53	64.18	108.07	29.45	15.14	47.96	61.14	33.53	35.48	**612.52**	13.61	23.59
L-007	5.12	23.11	19.82	18.94	20.29	23.93	27.97	52.77	50.28	64.43	90.34	32.10	14.23	59.00	67.34	25.94	27.94	**623.55**	9.75	20.82
L-008	5.54	19.67	18.68	17.08	18.48	20.16	26.94	46.64	25.88	48.78	99.62	36.27	13.85	60.53	58.21	32.47	24.96	**573.74**	15.71	20.89
L-009	3.94	16.69	16.53	17.05	12.39	16.36	28.04	32.92	54.77	38.61	50.24	21.90	10.66	39.81	53.63	21.28	19.67	**454.49**	7.62	14.01
L-010	4.79	22.02	15.66	19.16	14.86	23.60	27.22	38.34	45.42	50.81	74.24	27.55	13.03	38.51	65.45	19.66	24.80	**525.12**	9.81	17.52
L-011	7.32	32.36	24.86	26.89	20.32	31.51	41.80	53.64	81.52	87.62	105.58	51.58	15.40	70.37	128.64			**779.39**	12.78	20.87
L-012	5.62	21.90	19.19	18.49	22.52	23.45	28.06	43.40	23.55	60.89	64.68	28.62	14.16	58.07	71.93	24.45	27.03	**556.01**	12.92	16.88
L-013	6.14	26.76	28.55	22.88	26.02	26.21	32.32	61.06	41.22	101.09	101.47	38.72	15.99	98.25	85.70	31.70	30.67	**774.75**	10.32	20.32
L-014	5.52	20.92	16.02	17.25	16.83	19.70	27.32	37.82	33.43	56.70	52.35	27.03	11.05	41.47	67.40	20.90	24.35	**496.05**	8.26	17.63
L-015	5.25	20.28	19.07	21.05	18.55	20.75	28.80	42.22	31.74	51.74	96.50	30.06	13.22	59.80	71.71	26.69	25.57	**583.00**	8.31	19.36
L-016	3.05	26.42	19.94	19.78	19.93	21.74	25.22	45.95	38.08	46.50	82.20	35.10	18.31	51.16	88.50	30.39	32.11	**604.38**	15.69	24.79
L-017	3.88	21.62	15.30	17.82	15.72	20.32	27.04	38.84	33.68	34.38	45.18	32.28	14.58	43.44	61.00	20.98	26.36	**472.42**	7.92	12.64
L-018	5.40	24.00	18.00	18.50	20.00	24.50	30.50	42.45	27.80	71.00	55.00	30.00	15.00	29.50	78.00	24.50	28.00	**542.15**	10.09	17.90
L-019	4.06	21.97	16.50	18.01	14.90	21.15	29.25	43.20	49.35	47.83	71.68	27.79	14.10	51.65	60.68	22.89	25.16	**540.17**	9.19	15.87
L-021																			11.04	21.65
L-023	5.00	25.00	23.49	22.01	15.69	23.79	26.62	34.20	54.17	12.37	19.30	19.94	8.62	41.58	78.64	27.95	29.41	**467.78**	9.19	17.06
L-024	6.00	23.80	16.00	18.70	15.30	23.30	28.20	29.70	36.90	45.40	69.80	26.10	14.60	44.50	76.00	21.80	25.80	**521.90**	11.70	21.10
L-025	5.38	24.03	19.38	21.15	20.77	24.48	31.64	46.06	50.17	74.06	103.75	37.72	14.39	31.83	69.89	25.72	29.05	**629.47**		
L-026	6.53	70.07	21.43	23.64	16.48	27.82	34.29	39.45	65.78	69.12	88.53	33.03	47.27	73.03	102.18	31.22	30.78	**780.65**		
L-027	< 10.00	25.64	16.81	18.89	15.31	22.45	26.81	16.51	26.86	50.31	58.44	26.92	15.77	42.13	82.26	22.50	29.96	**497.58**	10.67	18.25
L-029	5.88	27.26	18.57	21.96	17.64	24.46	31.31	29.96	40.58	55.78	80.78	31.77	16.66	51.63	90.84	24.27	32.97	**602.30**		

Table 15: Result overview for standard solution 2 (*when calculating PA_GES, "< LOQ" is set as 0)

lab code	Eu	EuN	Ht	HtN	Lc	LcN	Sk	Em_G	EmN_G	Im_G	ImN_G	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	PA_GES*	AT	SO
	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
L-001	4.55	2.08	2.19	2.66	2.07	2.74	3.94	4.31	3.65	7.67	12.57	3.11	< 2.00	6.14	7.07	3.66	3.86	**72.27**	< 2.00	28.15
L-002	2.36	2.90	1.75	2.69	1.41	2.52	3.58	2.75	3.89	5.70	11.50			3.74	6.54	3.02	4.25	**58.60**		22.00
L-003	2.00	2.07	1.59	1.95	1.64	2.58	3.48	3.06	3.45	8.23	9.77	2.34	1.72	4.43	5.34	2.64	3.29	**59.58**	0.53	22.47
L-004	1.85	2.57	1.76	2.82	1.21	2.66	3.85	2.99	3.61	6.32	10.67	2.41	2.10	3.69	7.81	2.31	3.65	**62.28**	0.52	19.18
L-006	2.45	2.61	1.82	2.79	1.86	2.96	4.45	3.92	4.08	7.37	13.26	2.57	2.13	4.16	6.86	3.84	4.79	**71.92**	0.66	
L-007	1.82	2.08	1.86	2.17	2.35	3.01	3.53	3.89	4.63	7.64	13.67	2.51	1.38	5.05	6.45	2.82	3.29	**68.15**	< 1.00	27.33
L-008	2.12	2.22	1.80	2.23	1.67	2.52	3.68	3.43	3.77	5.20	14.31	3.09	1.84	5.55	4.51	3.55	3.46	**64.95**	0.94	24.89
L-009	1.63	1.60	1.31	1.84	0.79	1.74	3.20	1.84	5.31	4.54	7.24	1.37	1.22	3.14	4.13	2.34	2.98	**46.22**	0.32	14.86
L-010	1.98	2.38	1.70	2.42	1.37	2.75	3.07	2.85	4.18	5.93	10.02	2.28	1.45	2.96	4.46	2.20	3.03	**55.03**	0.74	20.41
L-011	2.78	3.00	2.07	3.14	1.48	3.59	4.32	3.32	5.13	8.55	17.13	3.47	1.72	5.04	9.30			**74.03**	0.62	22.00
L-012	2.49	2.14	1.95	2.21	2.19	2.73	3.39	3.77	3.10	7.75	9.71	2.21	1.64	4.64	6.43	2.58	2.91	**61.84**	0.70	20.63
L-013	< 2.50	2.52	< 2.50	< 2.50	< 2.50	2.95	4.39	4.34	5.45	14.28	5.37	3.20	< 2.50	8.77	11.32	4.37	3.75	**70.71**	0.60	23.23
L-014	2.38	2.28	1.53	1.95	1.85	2.07	3.31	2.66	3.04	7.47	7.01	2.52	1.33	3.76	5.21	2.62	3.01	**53.99**	0.42	24.80
L-015	2.31	2.22	2.02	2.62	2.24	2.42	3.61	3.58	3.35	5.86	13.40	2.91	1.76	5.54	5.73	3.11	3.35	**66.03**	0.42	26.21
L-016	1.36	2.32	2.43	2.69	2.62	2.85	3.52	3.79	3.99	6.04	9.76	2.81	2.08	6.38	6.86	4.28	4.15	**67.93**	1.55	30.54
L-017	2.10	2.60	1.82	2.18	1.30	2.38	3.34	2.78	4.76	5.04	7.48	2.48	2.04	3.66	5.76	2.54	3.66	**55.92**	0.56	16.92
L-018	2.00	2.50	1.65	2.30	1.40	2.75	3.65	2.80	3.95	8.75	18.05	< 2.00	2.25	< 5.00	6.40	< 5.00	3.95	**62.40**	< 1.00	18.90
L-019	1.56	2.26	1.52	2.15	1.29	2.46	3.44	2.67	3.56	4.07	10.19	2.08	1.80	3.70	5.55	2.20	3.17	**53.67**	0.39	17.39
L-021																			0.67	25.75
L-023	2.45	2.33	1.96	2.70	1.56	2.81	3.82	2.56	3.94	1.72	2.76	1.50	0.98	3.60	6.01	2.60	3.52	**46.82**	0.56	17.76
L-024	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 10.00	< 5.00	9.32	< 5.00	< 10.00	< 10.00	< 10.00	< 5.00	< 10.00	**9.32**	0.54	22.00
L-025	2.47	2.45	2.18	2.58	1.98	2.72	3.86	3.21	3.63	9.48	11.05	3.19	1.79	5.24	6.46	3.18	3.86	**69.33**		
L-026	3.13	7.73	2.81	3.44	< 2.50	3.55	4.44	2.87	4.89	9.09	11.89	2.98	5.52	4.98	7.90	3.90	4.24	**83.36**		
L-027	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	14.06	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	< 10.00	**14.06**	1.12	25.65
L-029	2.86	3.05	< 2.50	3.08	< 2.50	3.04	3.69	4.37	2.79	7.79	10.51	2.35	< 2.50	< 5.00	6.18	2.56	4.05	**56.31**		

Table 16: Result overview for chamomile tea (*when calculating PA_GES, "< LOQ" is set as 0)

lab code	Eu	EuN	Ht	HtN	Lc	LcN	Sk	Em_G	EmN_G	Im_G	ImN_G	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	PA_GES*	AT	SO
	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	µg/kg	µg/kg	µg/kg	µg/kg
L-001	15.60	5.53	15.46	8.89	9.01	8.70	13.18	38.37	14.69	24.34	42.43	12.92	14.92	19.60	39.98	10.58	14.37	**308.57**	9.30	13.00
L-002	14.58	5.52	11.76	8.80	8.82	6.86	11.56	29.67	11.61	25.57	25.88	16.79	10.31	22.91	31.37	17.65	15.45	**275.11**	8.90	15.00
L-003	13.82		12.11				11.66	30.33	15.01	31.10	44.19	10.74	12.88	20.29	30.87	10.41	12.13	**255.54**	6.15	11.05
L-004	14.46	6.47	14.82	11.60	11.00	9.39	16.07	46.80	10.61	29.29	39.00	11.43	17.14	26.00	51.59	11.23	18.98	**345.88**	8.21	23.14
L-006	20.54		18.64		16.21		18.48	56.16		55.45	12.69	26.17		45.12		28.82	5.71	**303.99**	10.78	24.69
L-007	18.49	8.28	18.98	13.51	17.28	20.92	18.27	51.37	27.54	39.43	71.67	17.59	23.07	20.62	38.99	15.67	17.95	**439.61**	< 2.000	19.06
L-008	33.30	13.31	27.82	33.18	21.51	18.11	34.69	48.70	26.03	37.88	163.59	44.80	39.49	52.52	117.44	33.65	60.91	**806.93**	< 10.00	78.05
L-009	8.11	< 5.00	7.25	7.97	6.67	6.15	11.34	21.98	10.20	8.80	20.91	5.98	< 5.00	15.80	10.72	6.82	8.21	**156.91**	8.29	17.53
L-010	24.25	9.70	22.13	17.61	19.16	13.34	23.70	56.59	16.74	50.93	78.76	23.12	43.79	49.39	66.24	26.58	58.20	**600.23**	7.94	16.34
L-011	17.80	6.60	13.93	9.05	10.23	7.94	14.49	34.94	19.37	39.17	41.10	10.88	13.01	16.89	45.40			**300.81**	10.13	21.43
L-012	17.41	< 10.00	13.71	10.21	10.94	< 10.00	14.15	42.41	< 10.00	36.61	35.22	12.10	15.23	20.60	42.95	13.50	18.33	**303.37**	< 10.00	< 10.00
L-013	15.65	7.25	19.25	8.79	14.08	10.21	17.83	42.99	18.08	59.11	82.05	16.03	22.64	49.47	48.76	13.05	22.28	**467.52**	7.47	15.44
L-014	12.22	5.43	12.25	9.70	11.52	9.90	13.52	29.82	9.87	30.92	52.53	12.13	12.87	24.02	43.55	12.63	16.90	**319.77**	8.83	17.95
L-015	12.84	5.17	11.64	9.97	10.81	9.49	10.12	29.32	13.35	19.14	53.58	10.31	10.75	21.52	29.45	8.65	14.63	**280.74**	6.15	14.35
L-016	13.93	5.54	13.59	10.11	9.40	9.63	10.56	30.69	14.18	46.67	62.05	14.78	17.13	18.22	54.12	16.98	24.84	**372.42**	8.98	18.00
L-017	31.60	10.20	22.70	17.60	10.70	8.10	20.80	30.40	14.10	63.00	277.30	23.90	20.80	29.20	46.20	20.80	37.50	**684.90**	9.40	30.10
L-018	16.67	4.77	11.67	8.40	9.57	9.20	14.33	37.00	10.20	32.16	27.07	12.33	19.33	14.33	45.33	12.33	16.33	**301.02**	7.16	18.93
L-019	8.68	5.67	7.65	10.37	6.61	7.34	7.46	31.85	17.15	18.39	46.13	9.12	16.94	15.21	33.16	7.73	16.40	**265.86**	9.50	13.90
L-021																			8.42	17.24
L-023	28.18	11.46	17.75	13.87	14.71	22.33	21.67	48.08	37.44	13.96	15.16	9.13	9.89	21.32	65.09	13.75	9.93	**373.72**	10.59	21.97
L-024	18.10	5.79	13.30	11.00	10.80	< 10.00	15.00	25.20	13.20	25.20	41.60	12.30	24.90	25.40	40.60	11.30	17.50	**311.19**	8.86	23.00
L-025	13.23	6.10	14.02	10.75	10.72	9.72	15.42	37.10	16.12	36.02	68.15	14.25	18.05	14.97	47.95	10.77	17.13	**360.47**		
L-026	18.19	18.15	15.96	12.48	12.78	12.11	16.45	36.28	25.09	37.48	73.85	14.28	33.81	31.63	43.27	15.58	15.26	**432.65**		
L-027	17.10	10.13	14.22	12.96	< 10.00	< 10.00	< 10.00	16.41	14.00	30.05	36.93	16.57	23.82	28.35	39.67	15.49	21.24	**296.93**	8.77	35.03
L-029	18.21	< 10.00	16.24	15.36	< 10.00	< 10.00	< 10.00	15.15	29.71	34.67	58.12	< 10.00	< 10.00	< 20.00	< 20.00	< 10.00	< 10.00	**187.45**		

Table 17: Result overview for melissa tea (*when calculating PA_GES, "< LOQ" is set as 0)

lab code	Eu	EuN	Ht	HtN	Lc	LcN	Sk	Em_G	EmN_G	Im_G	ImN_G	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	PA_GES*	AT	SO
	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	µg/kg	µg/kg	µg/kg	µg/kg
L-001	99.87	43.11	38.28	31.53	16.49	17.56	28.44	24.08	9.89	67.50	98.78	36.71	9.29	39.29	32.44	13.12	10.10	**616.48**	16.00	35.40
L-002	87.32	47.80	50.46	46.54	25.08	19.55	39.28	29.09	13.65	85.27	92.04	49.25	12.53	30.86	42.59	16.54	13.15	**701.00**	53.00	81.00
L-003	80.45	59.14	48.99	36.41	27.49	21.15	45.62	32.48	11.97	89.69	88.41	41.70	11.39	49.89	44.35	16.47	22.71	**728.31**	50.50	59.40
L-004	88.22	61.64	59.36	51.71	28.20	22.11	51.91	37.52	13.38	86.95	94.16	45.31	12.97	49.88	61.95	15.73	11.45	**792.45**	58.37	81.30
L-006	137.03	59.09	63.41	46.13	50.23	31.73	79.38	60.24	16.54	117.86	92.61	68.17	14.92	64.36	63.24	31.58	13.41	**1009.93**	74.23	
L-007	95.07	90.02	62.84	62.36	25.25	23.93	37.35	29.18	18.73	91.26	154.32	50.16	14.54	31.69	37.33	16.80	10.06	**850.88**	46.82	83.61
L-008	150.26	110.05	90.61	112.12	31.51	22.43	73.49	41.04	17.18	131.77	318.05	123.50	26.71	63.49	98.85	31.86	28.09	**1471.03**	71.77	150.13
L-009	47.74	28.57	26.66	25.10	12.07	6.88	31.13	21.32	11.26	31.76	51.85	13.82	< 5.00	18.52	12.69	8.79	5.61	**353.77**	67.07	71.08
L-010	106.60	95.59	61.67	60.60	23.16	21.19	45.42	34.43	17.57	98.83	119.50	50.16	10.45	45.61	47.03	16.34	30.08	**884.23**	67.55	78.26
L-011	115.03	78.17	56.79	80.14	25.11	21.74	47.48	29.54	14.83	82.37	149.95	36.81	< 10.00	28.21	45.92			**812.09**	76.31	104.04
L-012	106.07	62.24	53.50	43.34	17.03	11.69	35.30	30.93	< 10.00	96.67	92.35	39.31	< 10.00	34.78	37.03	13.63		**673.87**	37.95	51.49
L-013	93.00	104.13	64.65	44.13	22.48	14.22	35.16	29.79	13.29	135.56	81.94	38.64	< 5.00	33.96	35.77	13.37	< 5.00	**760.09**	57.94	69.18
L-014	81.87	48.85	44.70	42.75	28.60	15.75	35.22	31.00	11.48	80.38	83.65	51.25	7.85	54.93	39.73	16.52	9.30	**683.83**	63.73	72.95
L-015	84.55	77.38	51.97	52.36	22.90	30.95	33.20	26.71	11.20	78.40	134.94	42.09	8.38	40.12	34.04	15.52	8.94	**753.65**	51.40	69.48
L-016	99.68	64.28	58.89	44.62	24.10	19.50	35.59	30.90	13.80	123.75	103.21	38.51	15.28	34.94	54.63	12.86	14.68	**789.22**	68.87	94.12
L-017	131.00	585.00	105.00	124.00	44.00	23.50	82.10	54.80	23.20	88.80	200.00	84.90	15.10	139.00	95.50	38.60	22.70	**1857.20**	135.00	110.00
L-018	107.33	44.33	52.00	40.00	30.67	19.33	52.00	37.67	14.33	106.33	102.33	55.33	12.67	48.34	53.67	17.00	10.97	**804.30**	49.45	71.07
L-019	77.77	61.83	55.73	42.41	22.75	24.87	41.19	33.56	13.50	74.95	109.17	42.07	11.61	44.43	42.34	14.82	10.72	**723.72**	53.17	68.13
L-021																			70.53	83.64
L-023	87,09	69,20	47,07	45,80	26,50	23,95	58,18	26,84	24,20	15,20	20,12	15,78	5,09	24,74	55,00	11,56	6,07	**562,39**	84,41	50,92
L-024	114,00	35,20	51,60	23,20	31,80	24,00	50,00	27,20	13,40	80,80	112,00	49,50	11,40	52,20	51,70	17,90	10,40	**756,30**	63,00	87,30
L-025	164,50	75,97	74,51	49,72	24,83	17,88	49,19	31,64	11,77	119,10	110,41	56,67	11,81	48,53	43,03	18,40	11,55	**919,51**		
L-026	110,89	236,16	64,13	49,60	28,36	23,32	51,95	32,97	19,02	101,21	109,01	58,29	28,67	54,95	51,49	22,20	11,12	**1053,34**		
L-027	82,69	53,00	66,48	43,71	< 10,00	24,41	41,13	30,15	15,62	84,88	155,28	38,01	15,47	46,32	53,63	14,84	13,16	**807,14**	71,06	86,83
L-029	76,61	46,57	49,11	39,31	30,39	21,96	44,81	16,71	27,28	86,48	114,59	44,18	< 10,00	43,91	49,82	< 10,00	< 10,00	**691,71**		

11.3 Plots of individual laboratory results reported for analyte-matrix-combinations in herbal tea

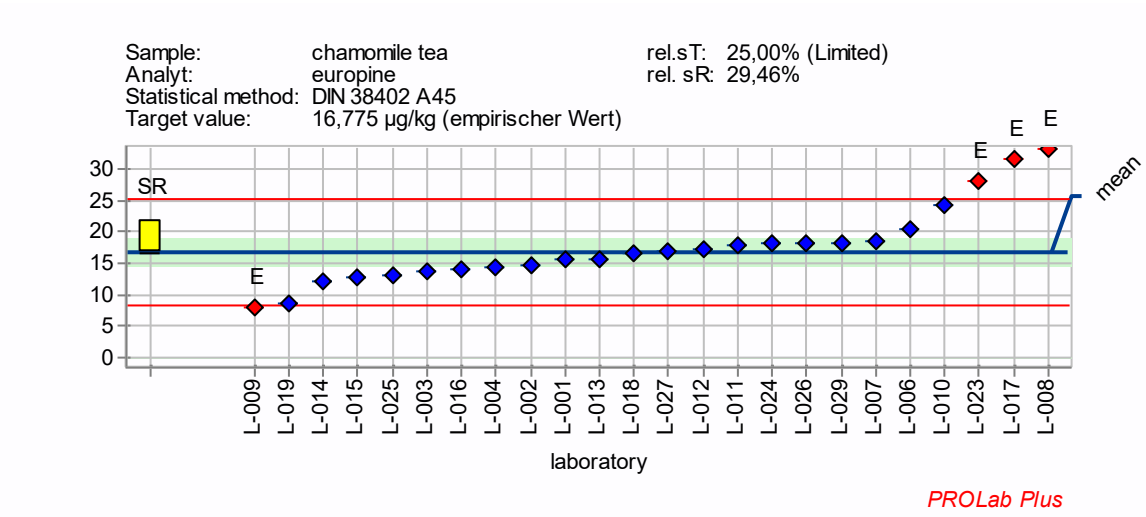

Figure 13: Laboratory results and limits of tolerance for chamomile tea, europine

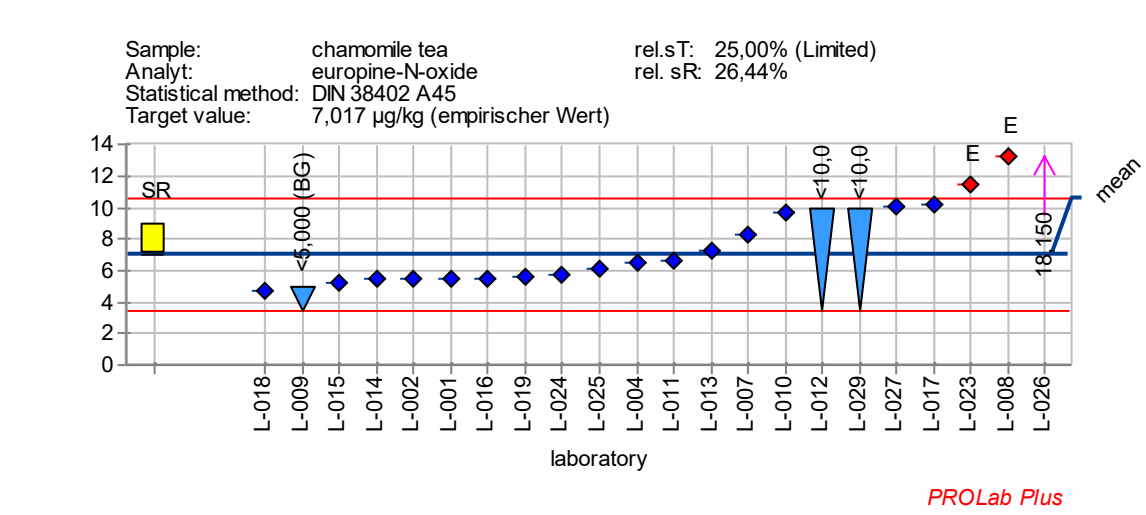

Figure 14: Laboratory results and limits of tolerance for chamomile tea, europine-*N*-oxide

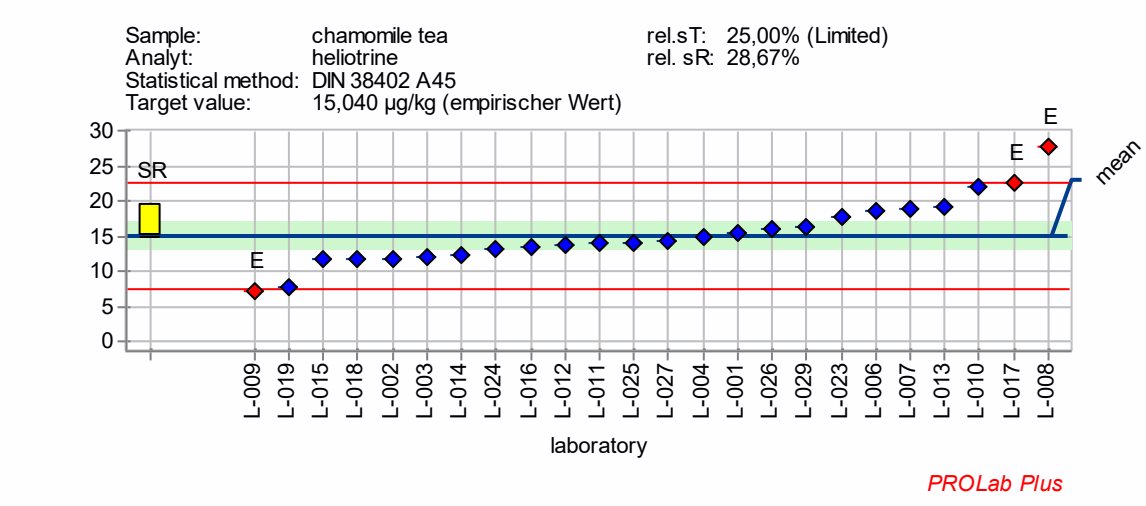

Figure 15: Laboratory results and limits of tolerance for chamomile tea, heliotrine

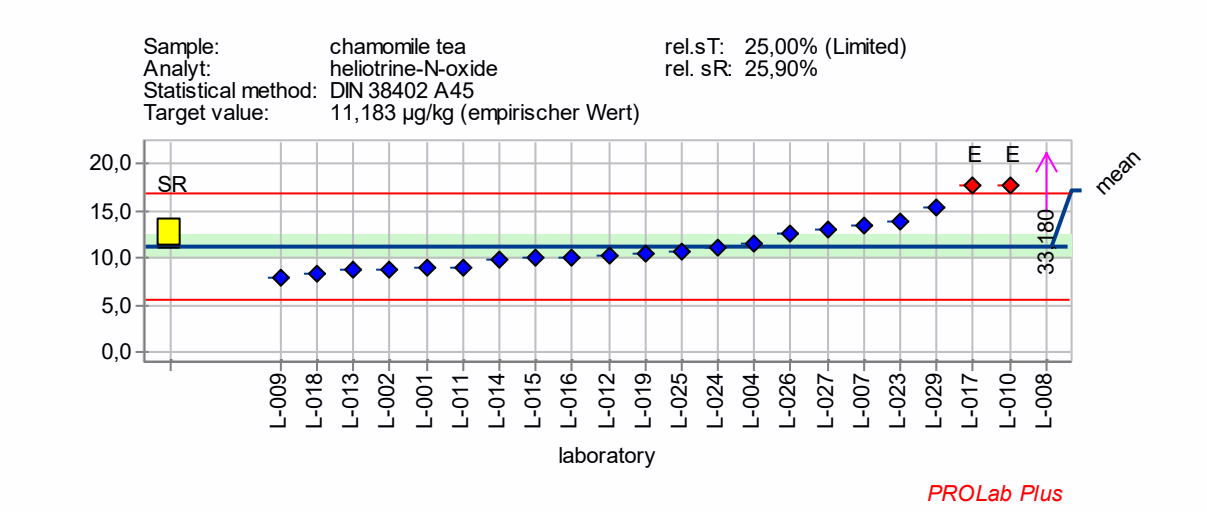

Figure 16: Laboratory results and limits of tolerance for chamomile tea, heliotrine-*N*-oxide

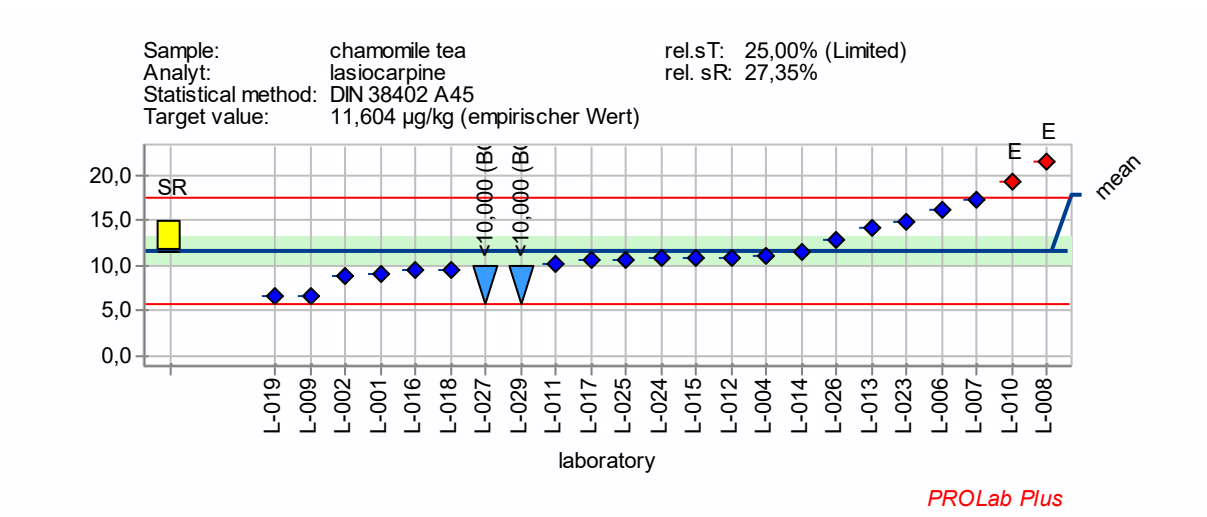

Figure 17: Laboratory results and limits of tolerance for chamomile tea, heliotrine-*N*-oxide

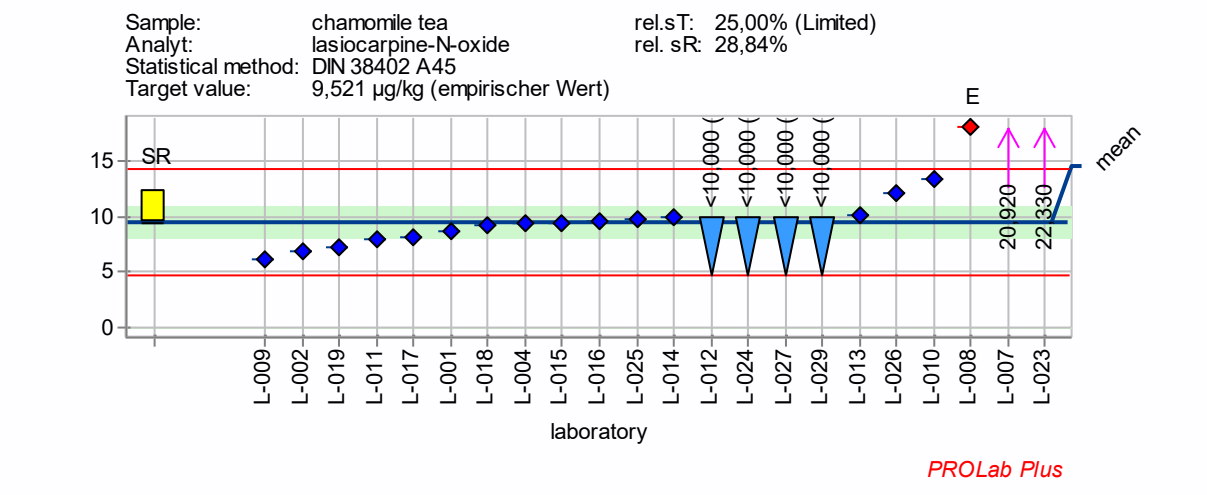

Figure 18: Laboratory results and limits of tolerance for chamomile tea, lasiocarpine-*N*-oxide

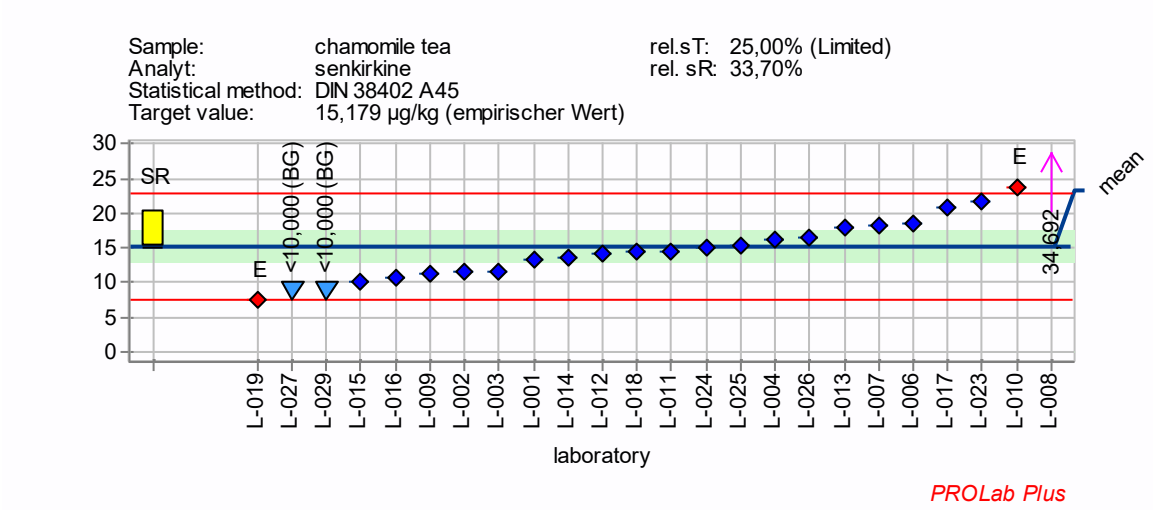

Figure 19: Laboratory results and limits of tolerance for chamomile tea, senkirkine

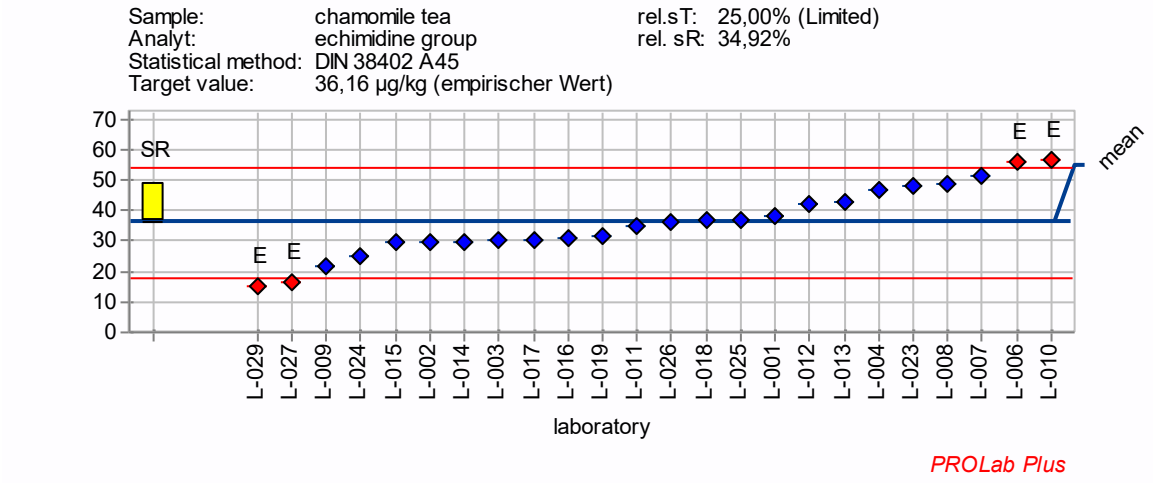

Figure 20: Laboratory results and limits of tolerance for chamomile tea, echimidine group

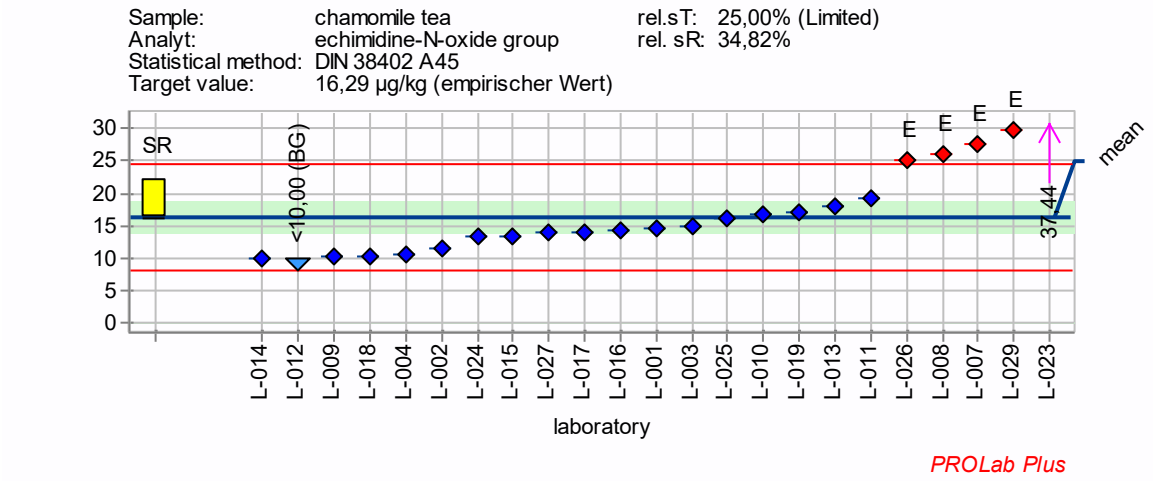

Figure 21: Laboratory results and limits of tolerance for chamomile tea, echimidine-*N*-oxide group

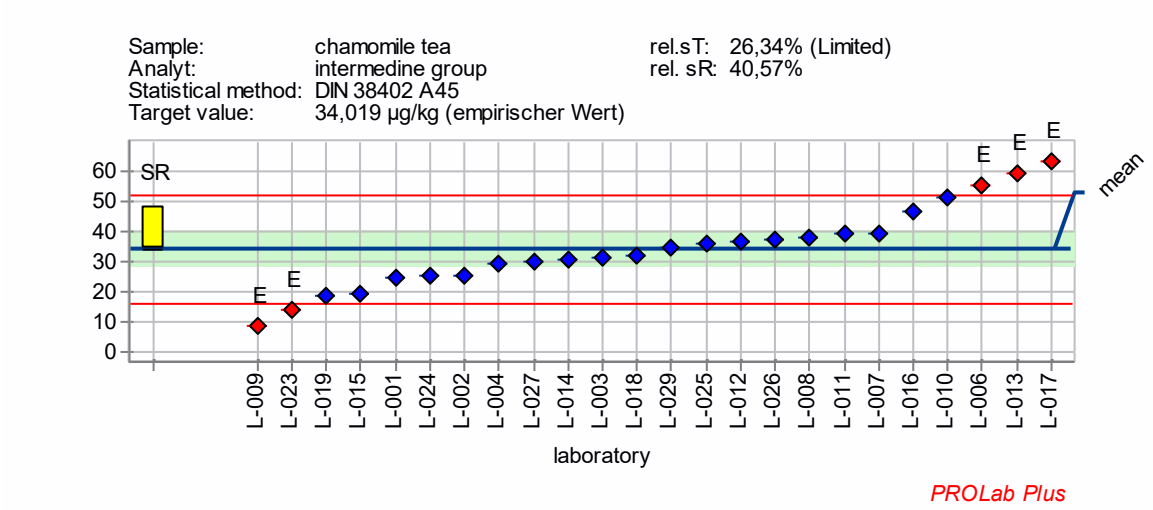

Figure 22: Laboratory results and limits of tolerance for chamomile tea, intermedine group

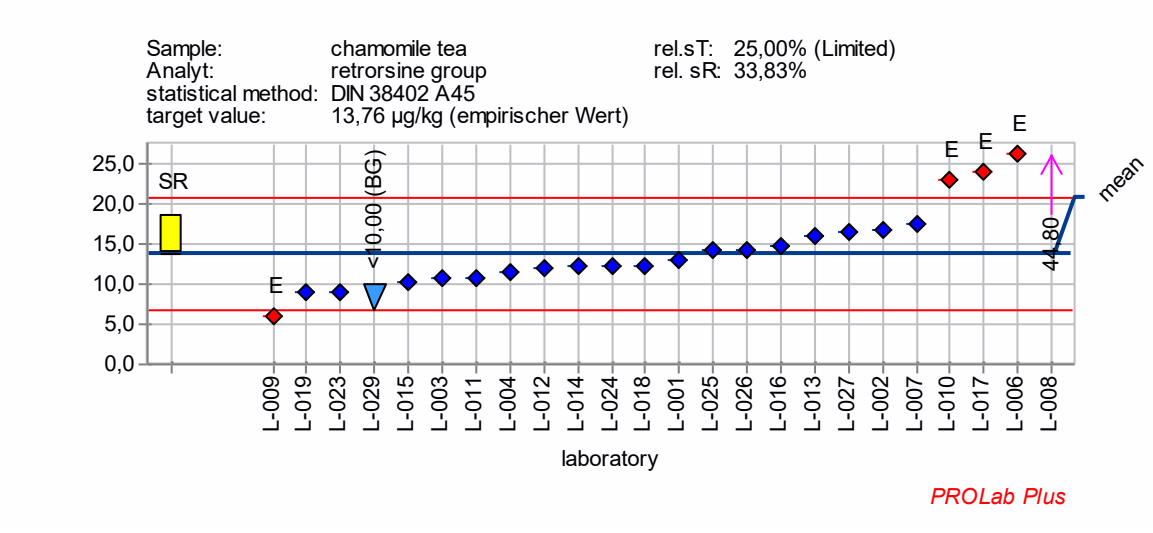

Figure 23: Laboratory results and limits of tolerance for chamomile tea, retrorsine group

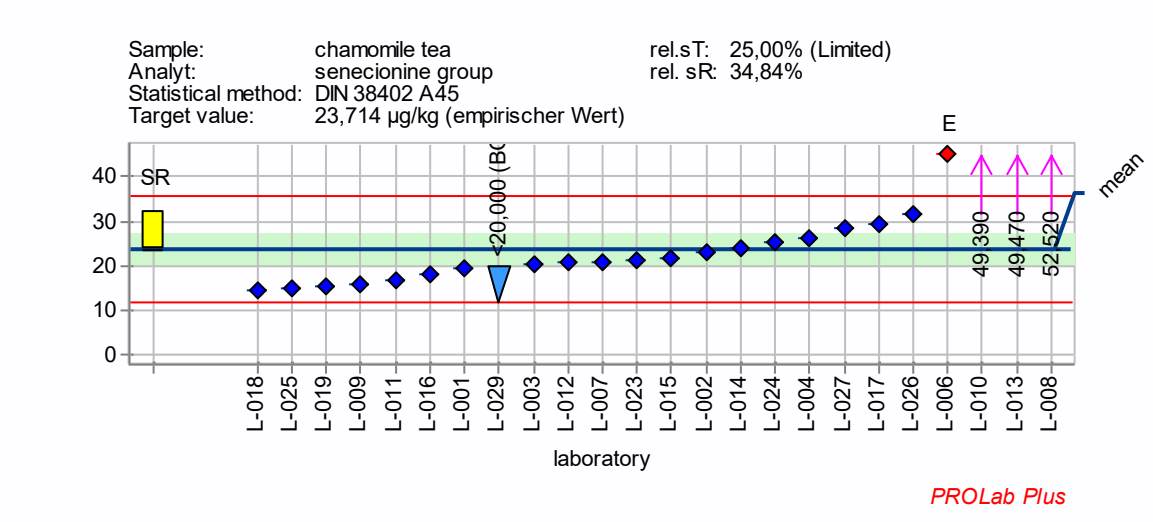

Figure 24: Laboratory results and limits of tolerance for chamomile tea, senecionine group

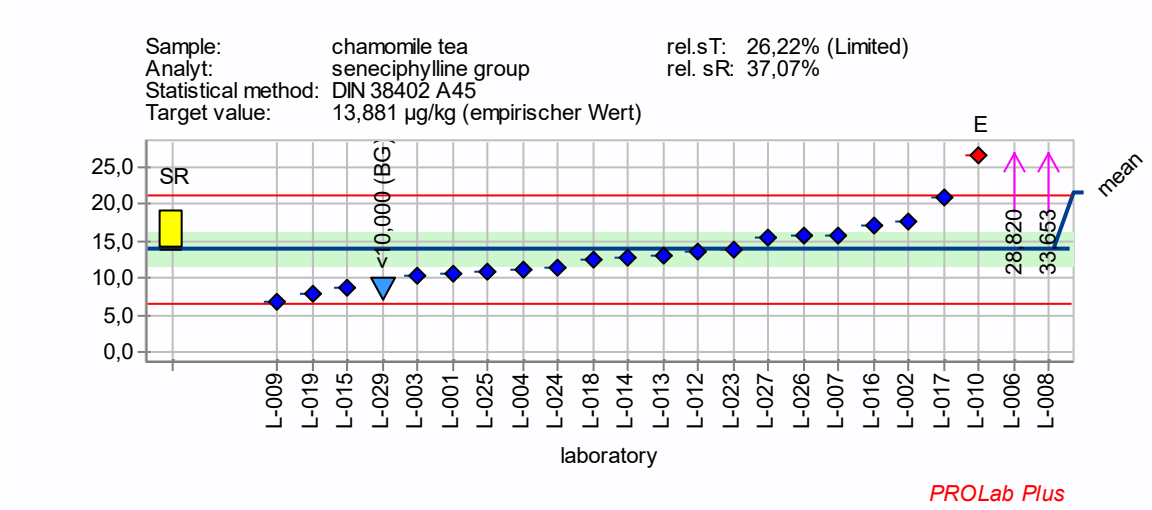

Figure 25: Laboratory results and limits of tolerance for chamomile tea, seneciphylline group

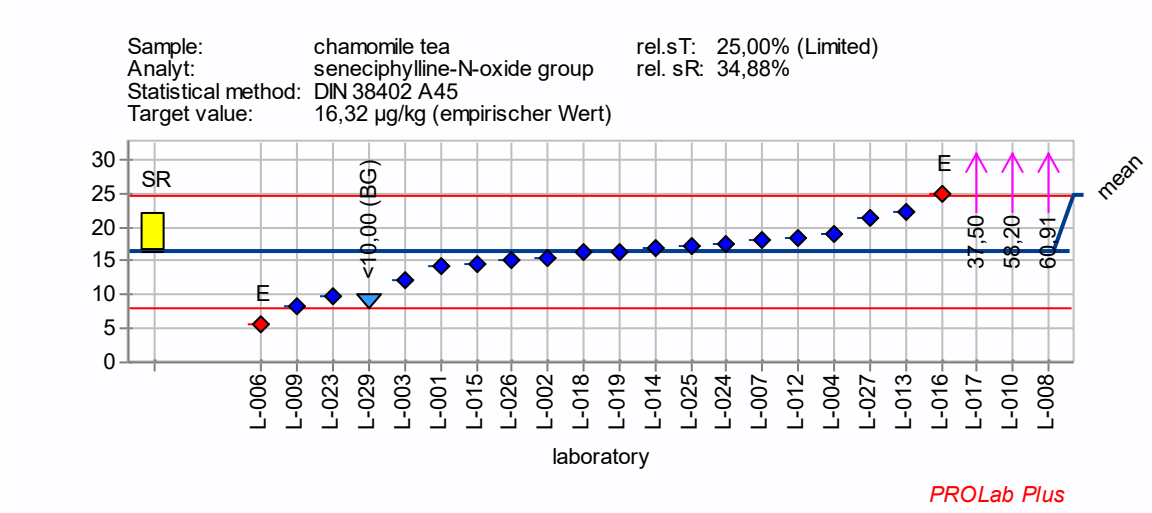

Figure 26: Laboratory results and limits of tolerance for chamomile tea, seneciphylline-*N*-oxide group

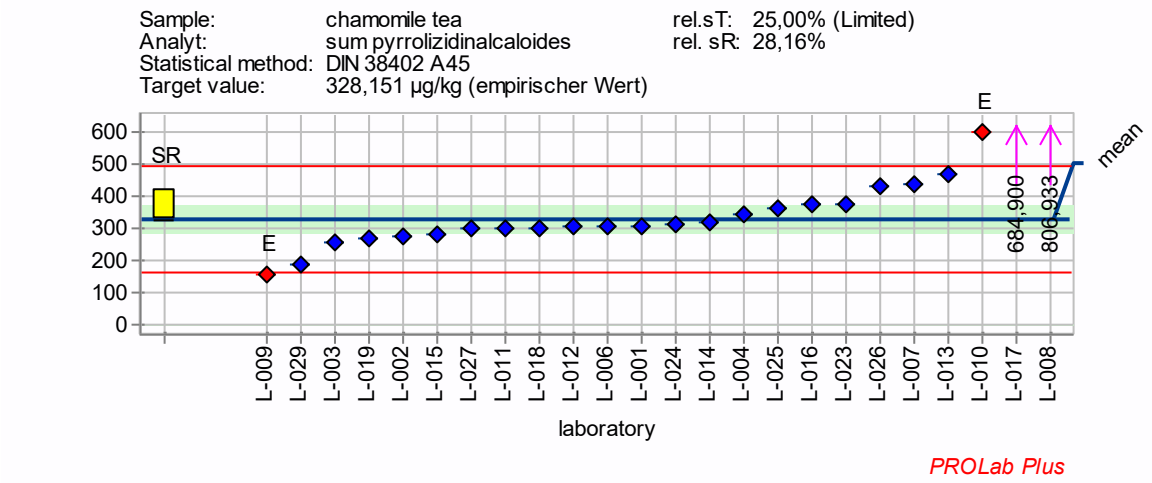

Figure 27: Laboratory results and limits of tolerance for chamomile tea, sum pyrrolizidinalcaloides

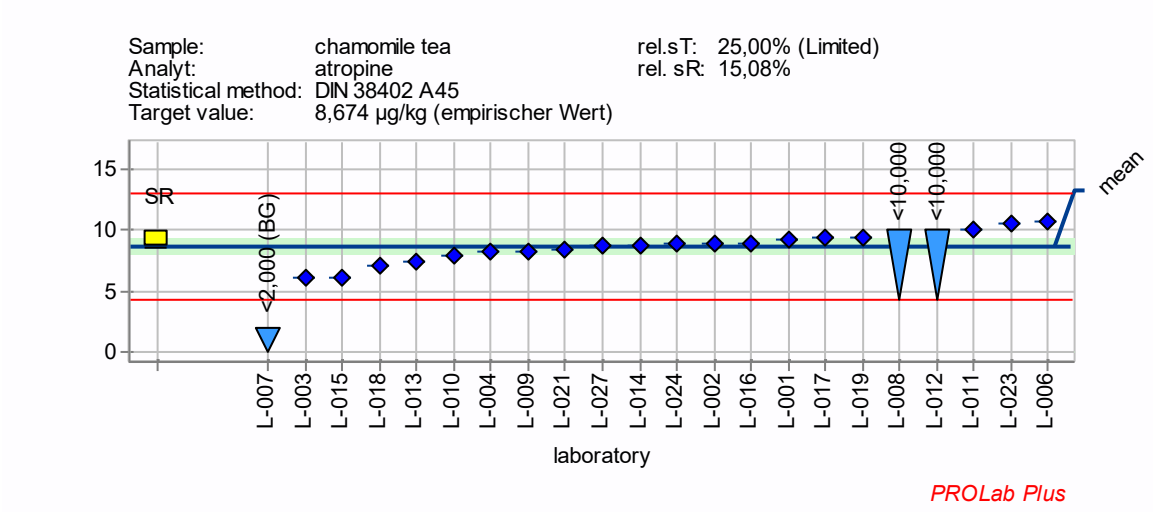

Figure 28: Laboratory results and limits of tolerance for chamomile tea, atropine

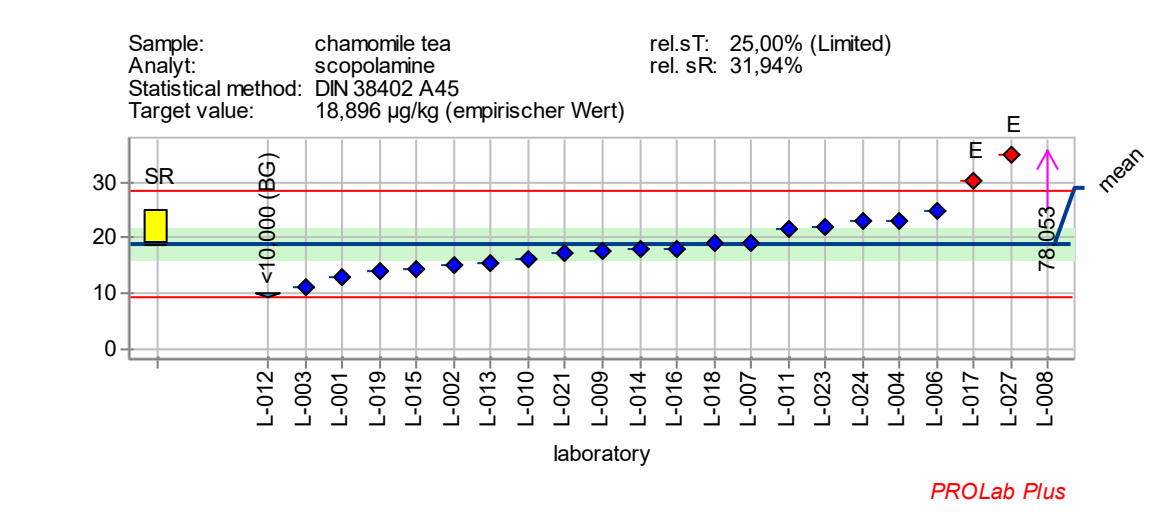

Figure 29: Laboratory results and limits of tolerance for chamomile tea, scopolamine

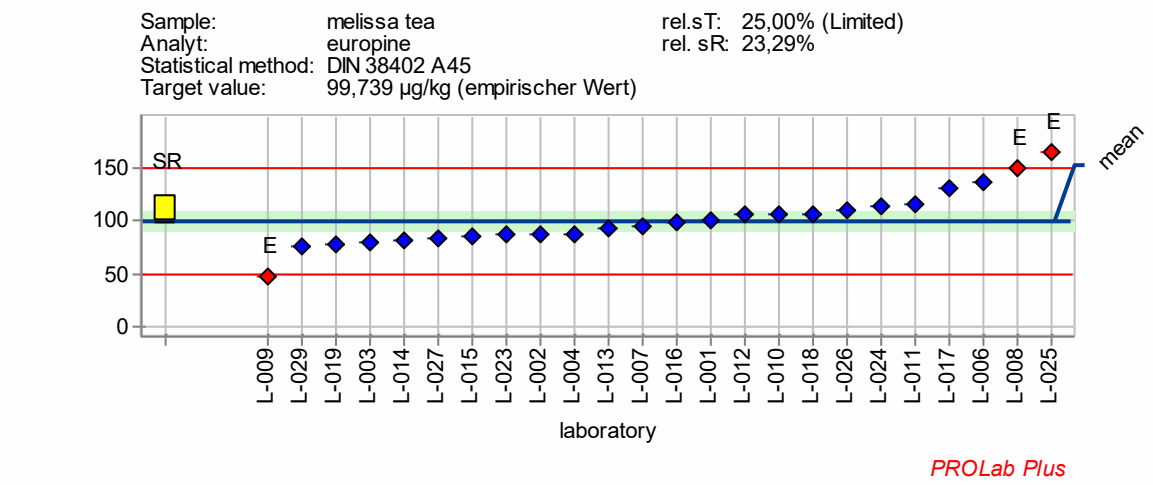

Figure 30: Laboratory results and limits of tolerance for melissa tea, europine

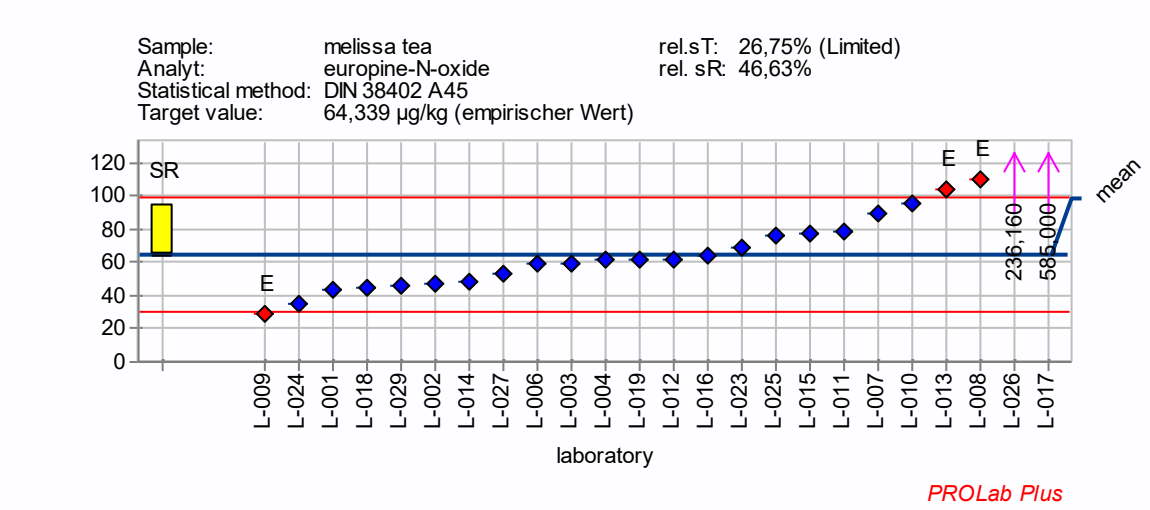

Figure 31: Laboratory results and limits of tolerance for melissa tea, europine-*N*-oxide

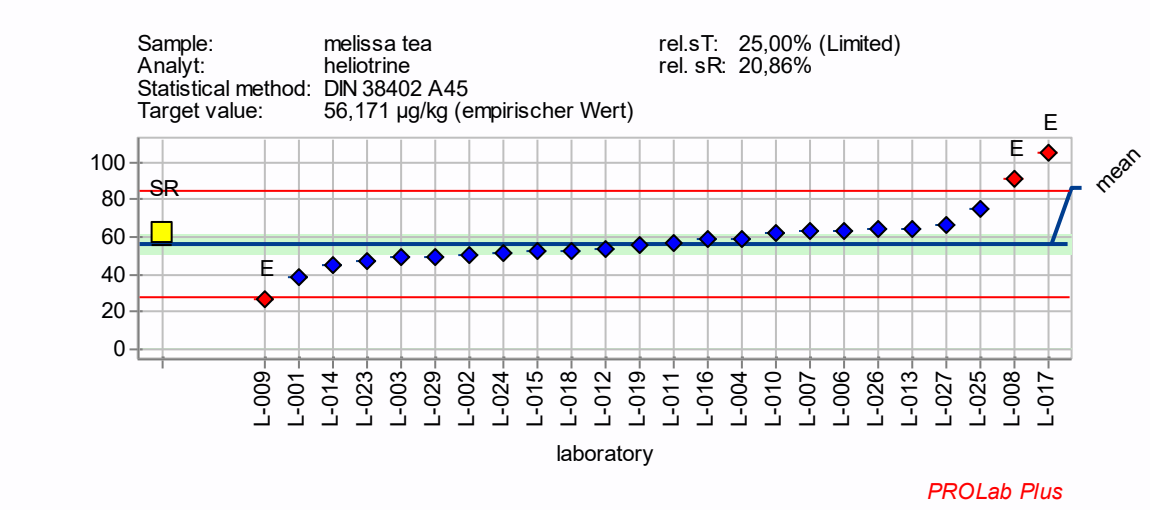

Figure 32: Laboratory results and limits of tolerance for melissa tea, heliotrine

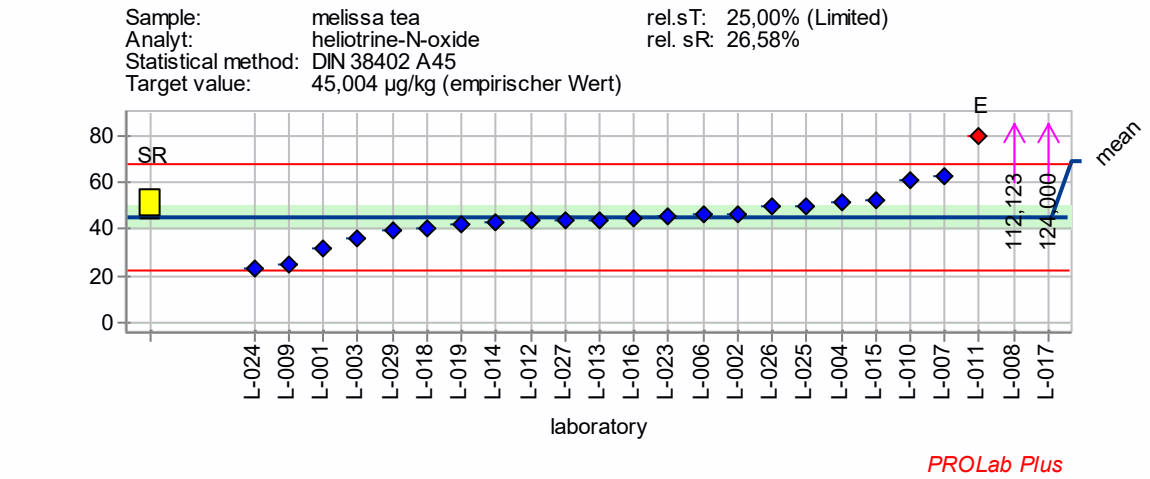

Figure 33: Laboratory results and limits of tolerance for melissa tea, heliotrine-*N*-oxide

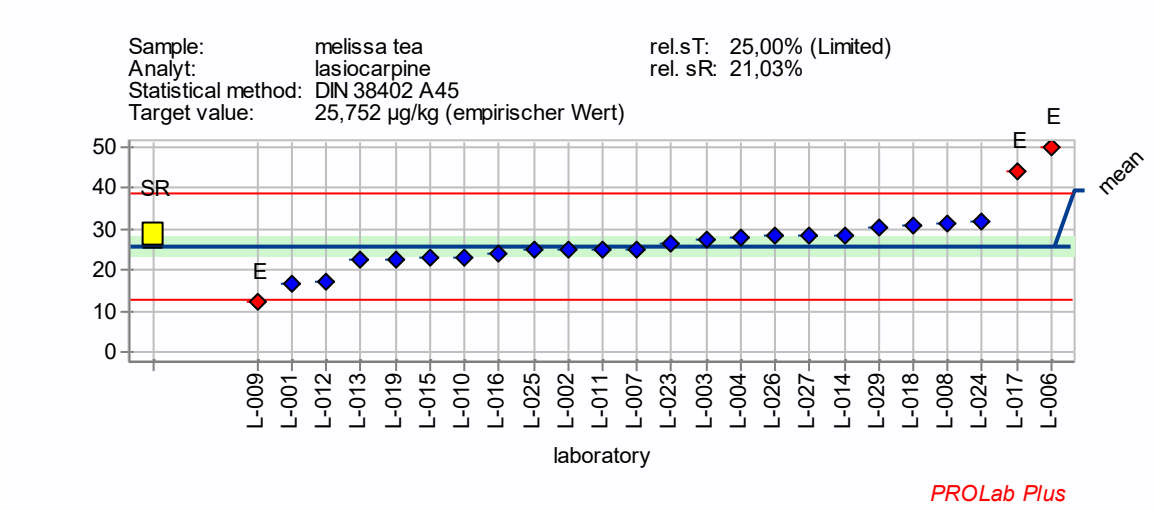

Figure 34: Laboratory results and limits of tolerance for melissa tea, lasiocarpine

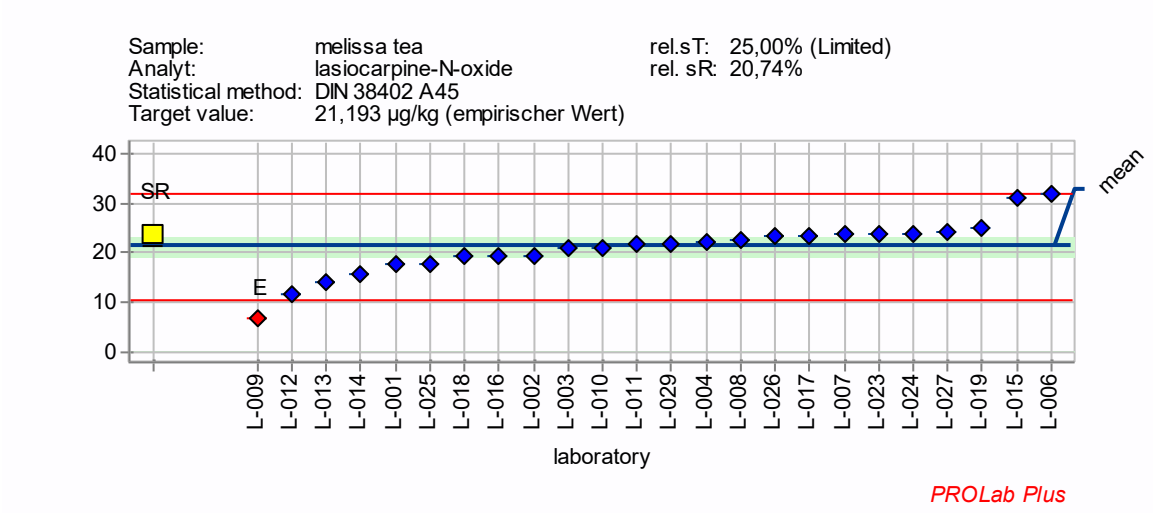

Figure 35: Laboratory results and limits of tolerance for melissa tea, lasiocarpine-*N*-oxide

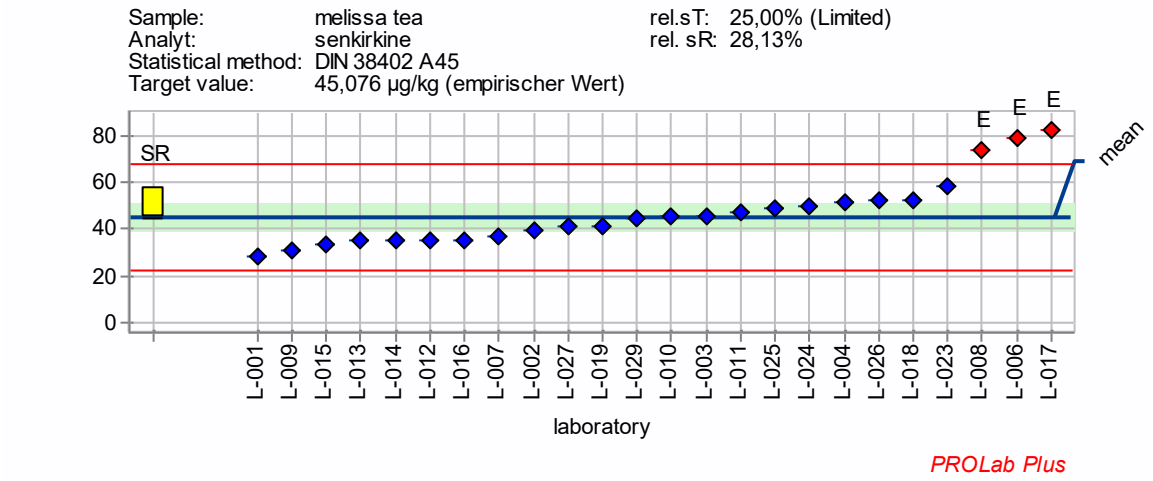

Figure 36: Laboratory results and limits of tolerance for melissa tea, senkirkine

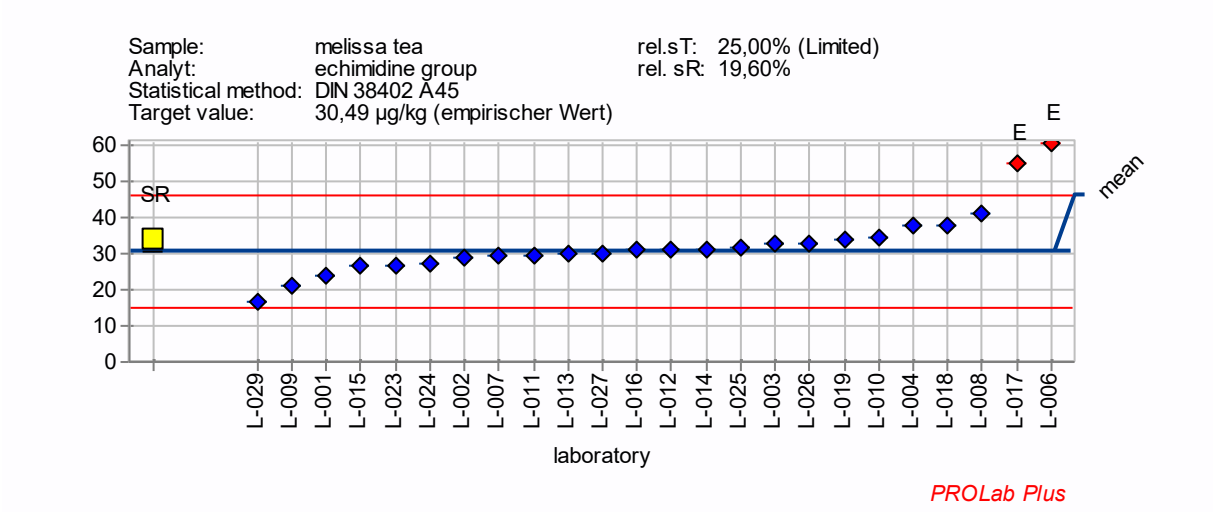

Figure 37: Laboratory results and limits of tolerance for melissa tea, echimidine group

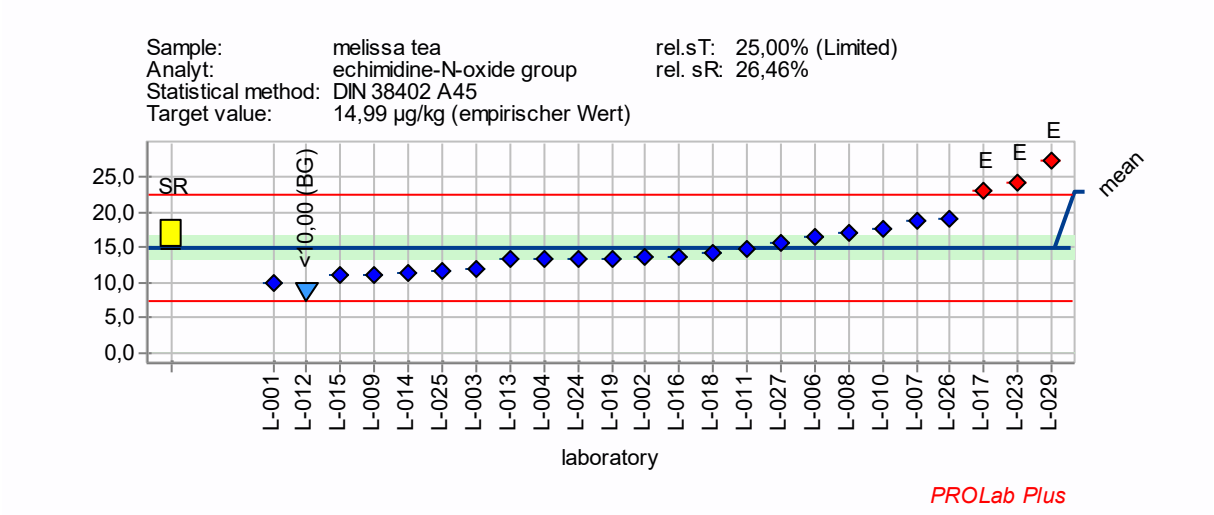

Figure 38: Laboratory results and limits of tolerance for melissa tea, echimidine-*N*-oxide group

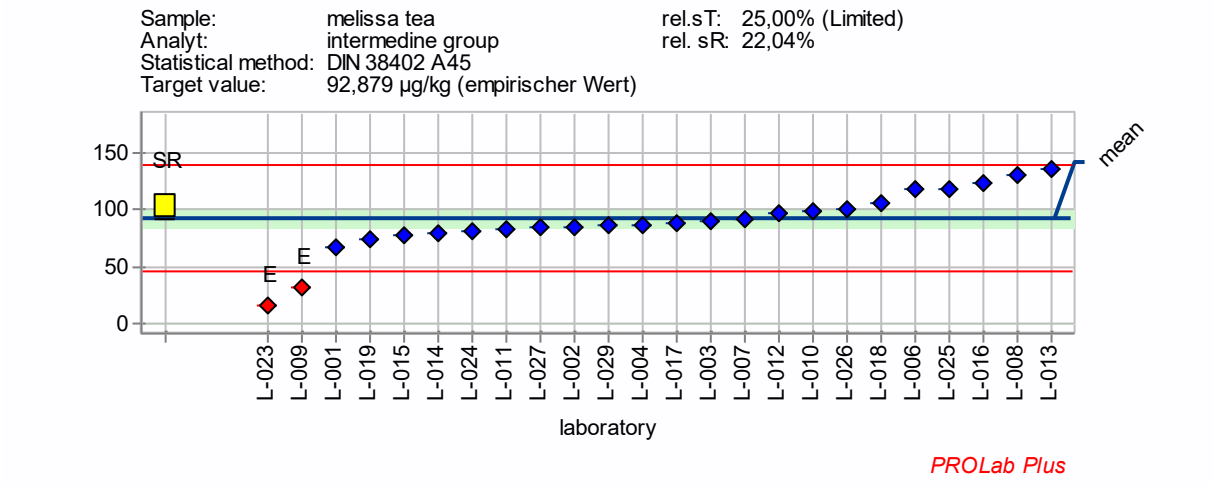

Figure 39: Laboratory results and limits of tolerance for melissa tea, intermedine group

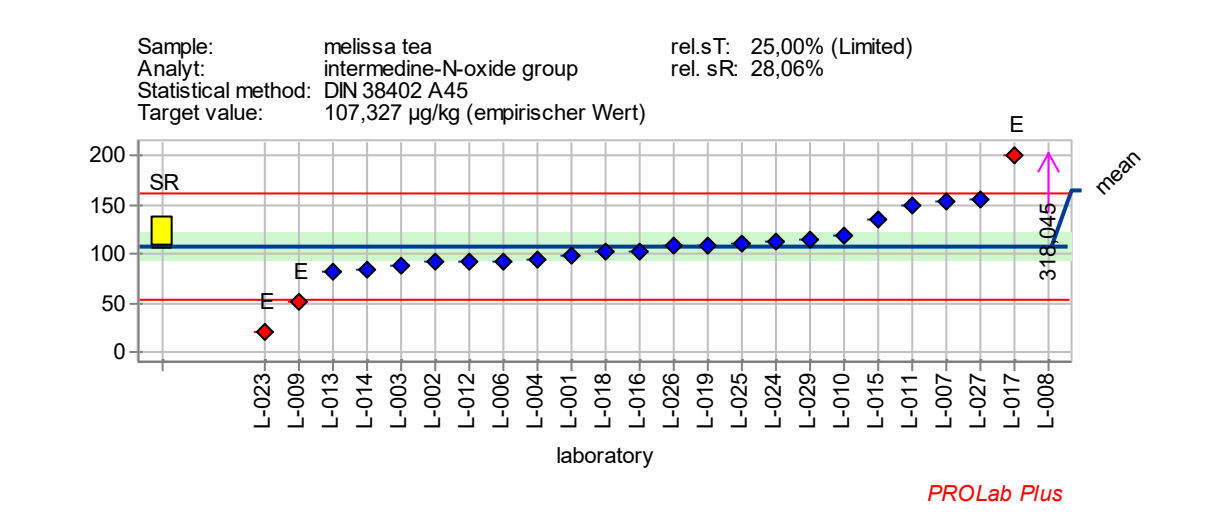

Figure 40: Laboratory results and limits of tolerance for melissa tea, intermedine-*N*-oxide group

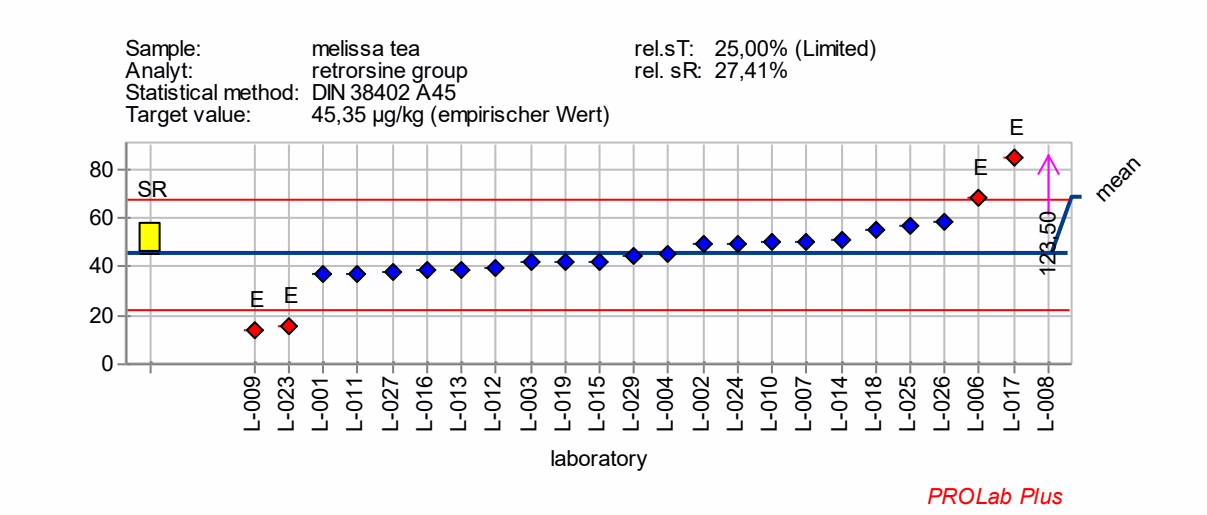

Figure 41: Laboratory results and limits of tolerance for melissa tea, retrorsine group

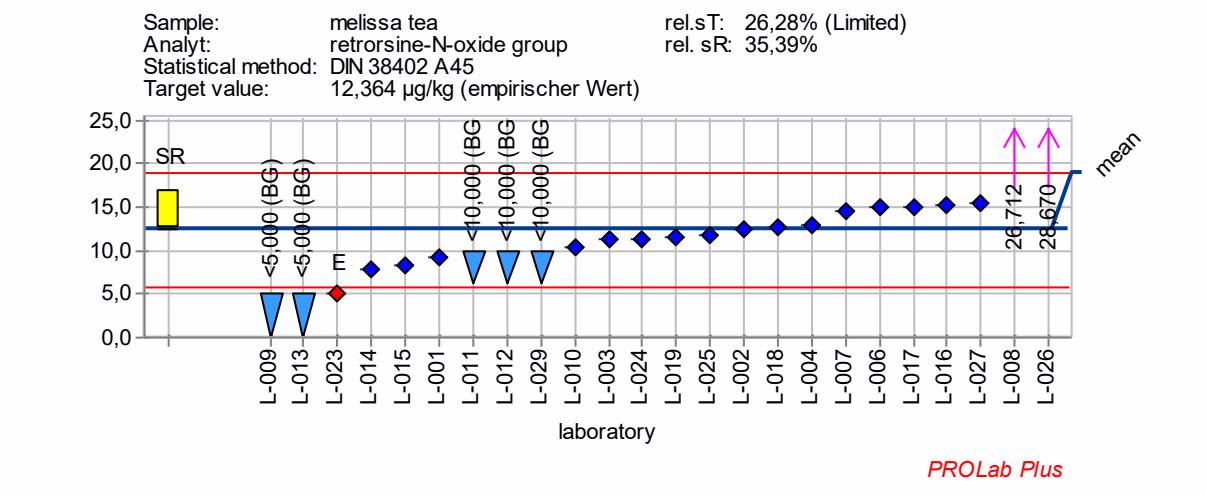

Figure 42: Laboratory results and limits of tolerance for melissa tea, retrorsine-*N*-oxide group

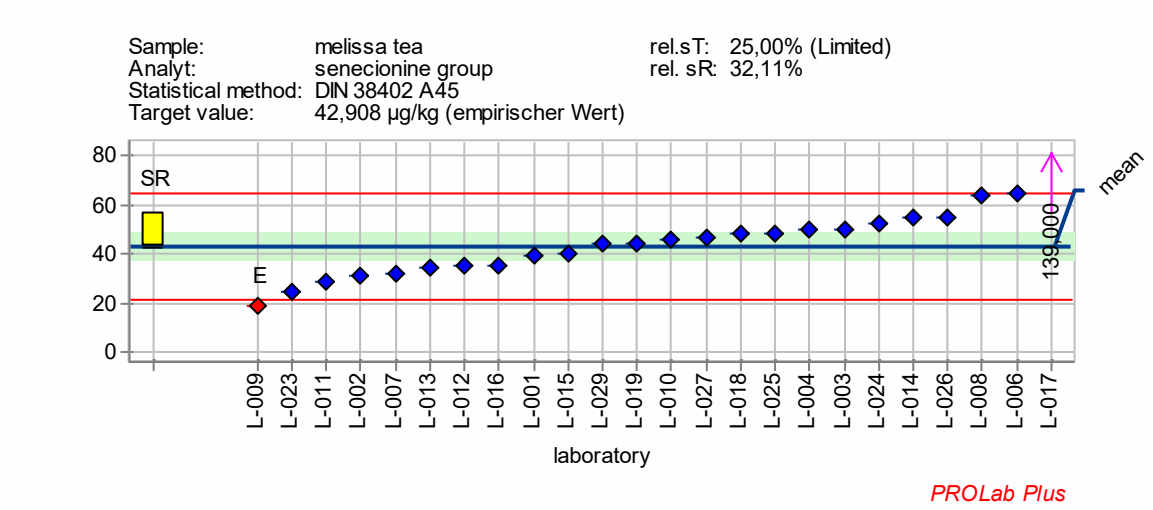

Figure 43: Laboratory results and limits of tolerance for melissa tea, senecionine group

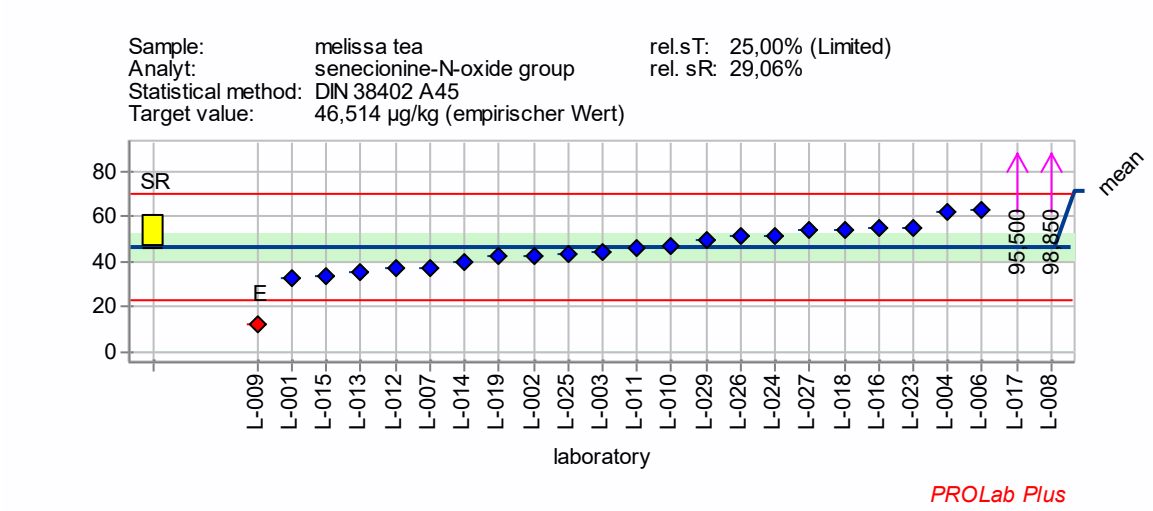

Figure 44: Laboratory results and limits of tolerance for melissa tea, senecionine-*N*-oxide group

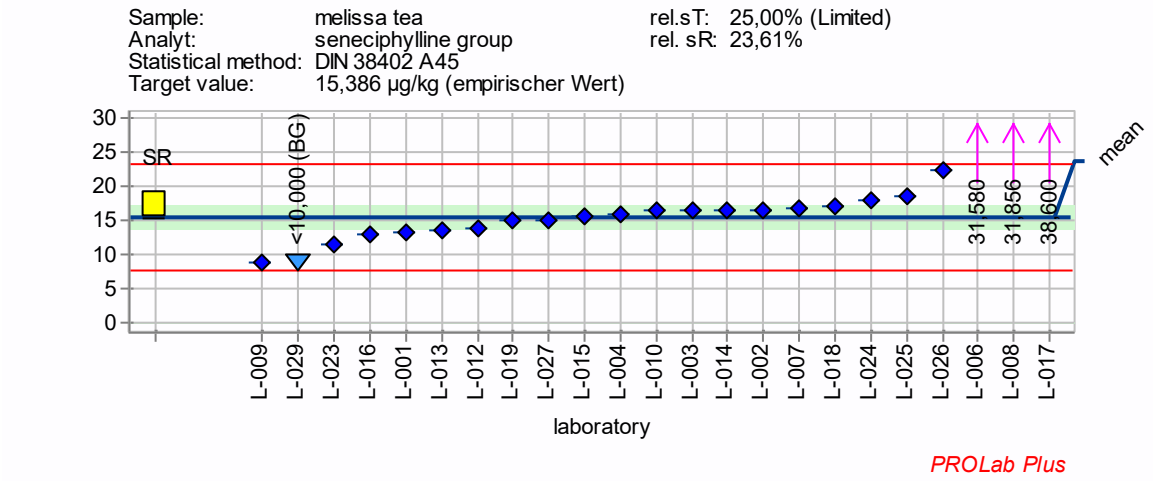

Figure 45: Laboratory results and limits of tolerance for melissa tea, seneciphylline group

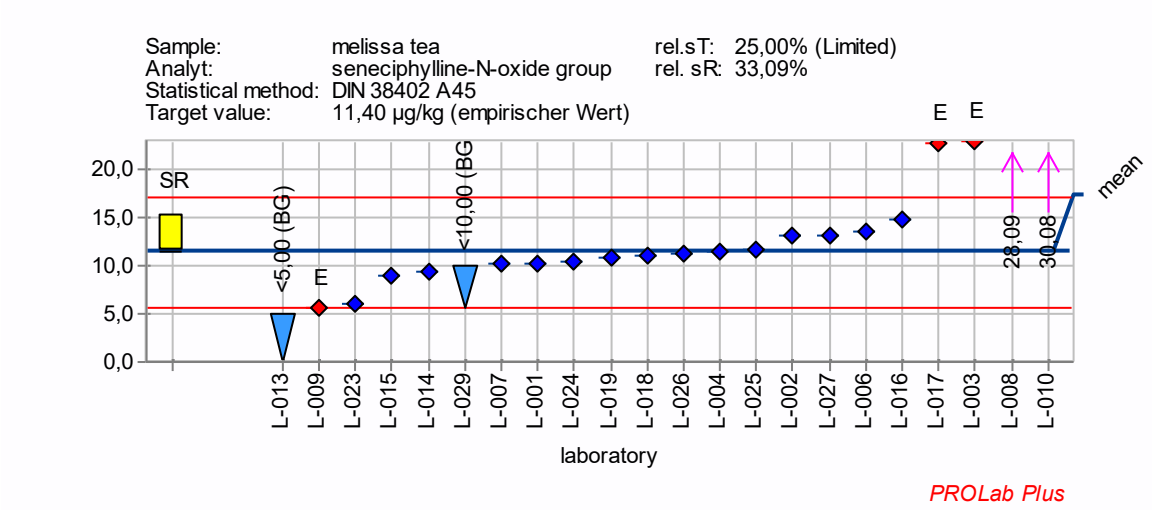

Figure 46: Laboratory results and limits of tolerance for melissa tea, seneciphylline-*N*-oxide group

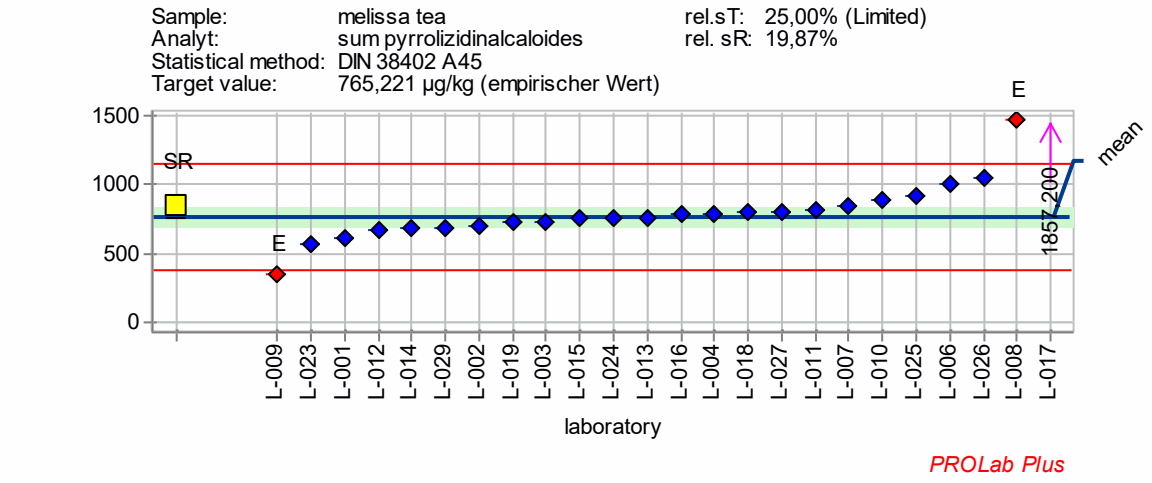

Figure 47: Laboratory results and limits of tolerance for melissa tea, sum pyrrolizidinalcaloides

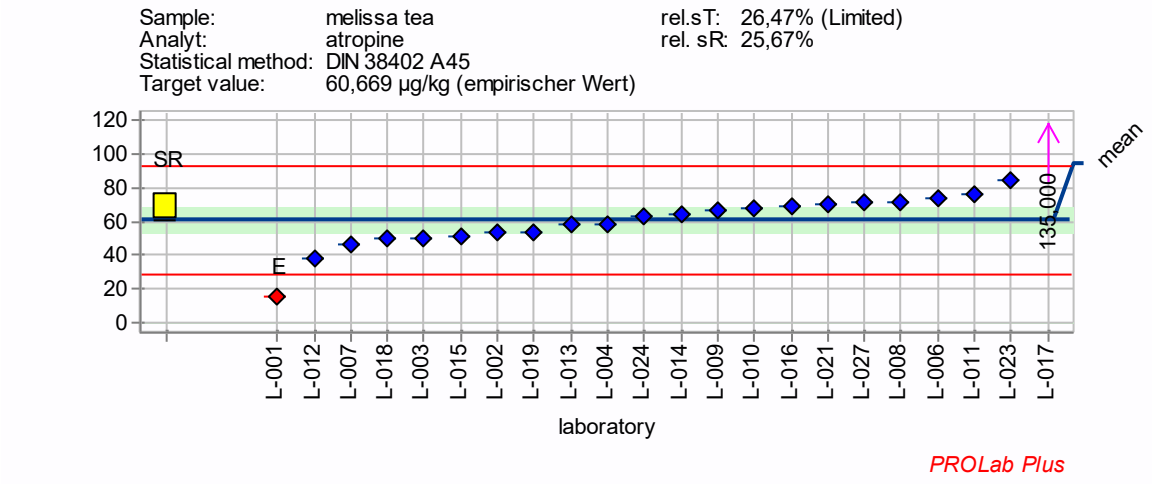

Figure 48: Laboratory results and limits of tolerance for melissa tea, atropine

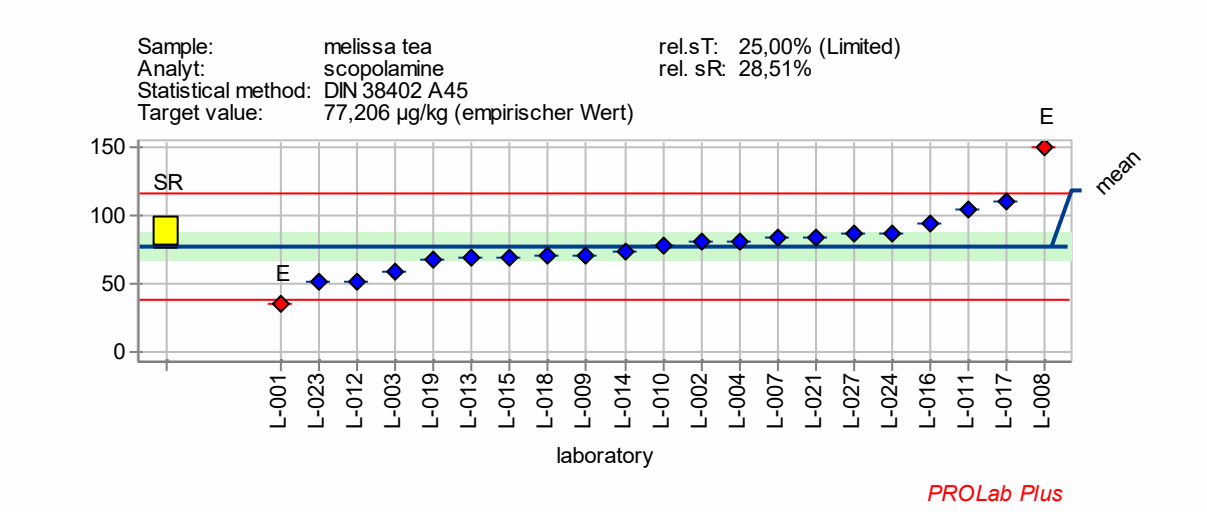

Figure 49: Laboratory results and limits of tolerance for melissa tea, scopolamine

The following information can be taken from the graphs:

rel.sT:	relative target standard deviation (25 % or $RSD_{s'soll}$)
rel.sR:	relative reproducibility standard deviation
sR:	reproducibility standard deviation (yellow bar)
red line:	lower and upper tolerance limit (robust mean +/- 2*sT)
blue line:	robust mean
light green marked area:	confidence range of mean value
blue square:	value of the laboratory within the tolerance limit
red arrow with number:	measured value of the laboratory can no longer be displayed in the diagram area, measured value of the laboratory outside the tolerance limits
E /red square:	value of the laboratory without the tolerance limit
blue triangles:	indication of the laboratory "< BG" [< (indication of BG)] or "< NG" <<(indication of NG)], blunt end of the triangle indicates the position of the BG or NG (if the blunt end of the triangle is at the upper edge of the diagram, the BG or NG can no longer be shown in the diagram)

11.4 Reported limits of quantification

Table 18: Limits of quantification reported by laboratories [µg/kg]

		AT	SCO	Em_G	EmN_G	Eu	EuN	Ht	HtN	Im_G	ImN_G	Lc	LcN	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	Sk
L-001	melissa	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
L-002	melissa	1	1	0.5	0.5	1	1	0.5	0.5	1	1	0.5	0.5	3	5	1	2	2	2	0.5
	chamomile	1	1	0.5	0.5	1	1	0.5	0.5	1	1	0.5	0.5	3	5	1	2	2	2	0.5
L-003	melissa	5	5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	chamomile	5	5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
L-004	melissa	1	1	2.5	5	2.5	2.5	2.5	2.5	1	2.5	1	1	2.5	5	5	2.5	2.5	5	2.5
	chamomile	1	1	2.5	5	2.5	2.5	2.5	2.5	1	2.5	1	1	2.5	5	5	2.5	2.5	5	2.5
L-006	melissa	0.2	0.2	5	10	5	5	5	10	5	5	5	10	10	10	15	5	5	5	5
	chamomile	0.2	0.2	5	10	5	5	5	10	5	5	5	10	10	10	15	5	5	5	5
L-007	melissa	2	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile	2	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
L-008	melissa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	chamomile	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
L-009	melissa	0.5	0.5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile	0.5	0.5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
L-010	melissa	7.5	7.5	15	15	7.5	7.5	3.8	7.5	7.5	15	7.5	7.5	15	10	15	15	15	15	7.5
	chamomile	7.5	7.5	15	15	7.5	7.5	3.8	7.5	7.5	15	7.5	7.5	15	10	15	15	15	15	7.5
L-011	melissa	0.5	0.5	2	2	1	1	1	2	8	10	1	1	6	10	6	6			1
	chamomile	0.5	0.5	2	2	1	1	1	2	8	10	1	1	6	10	6	6			1
L-012	melissa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	chamomile	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
L-013	melissa	1	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile	1	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Continuation Table 18: Limits of quantification reported by laboratories [µg/kg

		AT	SCO	Em_G	EmN_G	Eu	EuN	Ht	HtN	Im_G	ImN_G	Lc	LcN	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	Sk
L-014	melissa	2	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile	2	2	5	5	5	5	5	5	5	5	5	5	6	10	5	5	7	5	5
L-015	melissa	2	2	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	chamomile	2	2	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-016	melissa	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	chamomile	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
L-017	melissa	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	10	5	5
	chamomile	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	10	5	5
L-018	melissa	5	5	2	2	1	1	1	2	2	2	1	2	2	2	5	2	5	2	1
	chamomile	5	5	2	2	1	1	1	2	2	2	1	2	2	2	5	2	5	2	1
L-019	melissa	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	chamomile	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
L-021	melissa	2	2																	
	chamomile	2	2																	
L-023	melissa	0.1	0.4	0.4	0.2	9.3	4.4	0.7	0.3	2.7	0.4	0.3	0.2	2.5	0.6	2	0.7	1.8	0.4	0.1
	chamomile	0.1	0.9	0.4	0.9	4.3	2.4	3.7	0.6	1.1	0.2	0.5	0.9	3.4	2.4	3.8	0.6	2	0.8	0.2
L-024	melissa	0.5	1	5	10	5	5	5	5	5	5	5	10	5	10	10	10	5	10	5
	chamomile	0.5	1	5	10	5	5	5	10	5	5	5	10	10	10	10	10	10	10	5
L-025	melissa	n.a.	n.a.	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	chamomile	n.a.	n.a.	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-026	melissa			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	chamomile			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
L-027	melissa	1	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	chamomile	1	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
L-029	melissa	-	-	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	chamomile	-	-	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

11.5 Information on methods

Table 19: Overview on product ions used as quantifier by participants for PA and TA analyses (number in the table correspond to the number of laboratories)

m/z	AT	SCO	Em_G	EmN_G	Eu	EuN	Ht	HtN	Im_G	ImN_G	Lc	LcN	Re_G	ReN_G	Sc_G	ScN_G	Sp_G	SpN_G	Sk
93	2																		
94							1	1	5			2	1	11		3	3	9	
103		3																	
111								1											
118														9		15		7	
119																1			
120			22				1				18	1	15		10	4	16	5	
124	19																		0
136												1		3				1	
138		14						2	8	1								1	
138					19		19						6		14		2		
150																			1
156		4									1								
156					1		2		10										
168																			22
172						21		19		22		1							
220			1																
254				23								18							
254					3														
270						1													
300											1								
306																	2		
308															1	1			
324													1						
328						1													
336											3								

Table 20: Information on calibration

Lab-code	number of calibration level	range of calibration [ng/mL]	Accuracy of the calibration levels checked	the nature of the weighting	Forced through origin	weighted calibration	Accu-racy toler-ances	Type of calibration
L-001	4	2-160 ng/mL	yes		no	yes	+/- 5%	solution: solvent calibration; chamomile: matrix calibration. melissa: standard addition
L-002	5	ca. 0.1 - 100	yes		yes	yes	+/- 10%	standards in solvent, standard addition
L-003	6	PA: 0.04-10ng/mL TA: 0.01- 10ng/mL	yes		no	yes	+/- 30%	PA chamomile: matrix calibration; melissa : standard in solvent TA: solvent calibration;
L-004	6	0.005 bis 2.75	yes		no	yes	+/- 10%	standards in solvent, standard addition
L-006	8	0.2-10	no		no	no		matrix calibriation., standards in solvent
L-007	6	1-50	yes		no	no	+/- 20%	matrix calibriation, standards in solvent
L-008	TA: 7 PA: 10(extern)	TA: 1-100.0 PA extern: 0.005-10.0	yes	1/x (TA)	no	TA: yes PA: no	+/- 30%	TA: matrix calibriation, PA: standards in solvent, standard addition
L-009	TA 7 Kals; PA 6 Kals	TA: 0.01 - 1.0 µg/L; PA tea: 1 - 100 µg/L; PA LSG: 0.1 - 10 µg/L	no		TA: yes; PA: LSG1/2: yes; tea: no (include)	no (linear)		PA: tea: matrix cali LSG 1/2 standards in solvent, TA: solvent calibration ISTD
L-010	10	depending on analyte: 0.05-5 ng/ml; 0.1-10 ng/ml; 0.2-20 ng/ml	yes	1/x…	no	no	+/- 20%	matrix calibriation
L-011	1	(0.05-0.5)-37.5ng/mL	yes		yes	no	+/- 10%	standards in solvent
L-012	standard addition: 2 point cali; external cali: 5-6 Level		no					Standardaddition; STD Mix and isomeres: external cali
L-013	6	PA: 2.5 - 100; TA: 0.01- 4.0	no		no	yes		PA: Matrix-matched standards; TA: internal standards (D3-Atropin. D3-Scopolamin)
L-014	PA: 6. TA: 7	PA: 0.05-5 ng/ml. TA: 0.02-2 ng/ml	yes/no		no	yes/ no	+/- 15%	matrix calibriation, MMC
L-015	PA: 8 - 50 bzw. 12 - 150 ng/ml TA: 8	PA: 0 - 50 ng/ml. if necessary until 150 ng/ml,first cali level 0.25 ng/ml TA: 0-25 ng/ml, first cali level 1 ng/ml	no		yes	no		standards in solvent
L-016	LSG: 8 level. standard addition: dot. 3 level	standards in solvent: 0.1 - 5 or 5 -150. standard addition: 0,10,50,100	yes		no	yes	+/-25%	LSG1/2: standards in solvent; tea: standard addition
L-017	10	0.05 - 25 ng/mL	yes. correc-tion for accuracy	nein	yes	no	30 % - 140 % Accuracy ok	matrix calibriation
L-018	PA: 8. TA: 6	PA: 0.05 - 15 TA: 0.06 - 2	yes		yes	yes	+/- 20%	standards in solvent
L-019	6	0.1 - 20	yes	1/x…	no	yes	+/- 15%	standards in solvent
L-021	6	0.05 - 5	yes		no	no	+/- 20%	matrix calibriation, standards in solvent, standard addition
L-023	5	1 ng/mL - 50 ng/mL	yes		yes	no	+/- 20%	externe Matrix-Kalibrierung
L-024	6	0.05 - 10	yes		no	yes	+/- 10%	PA: standard addition, TA: standards in solvent
L-025	8	0.5 - 100	no		no	yes		matrix calibriation
L-026	tea: 7-8; LSG1/2: 8	tea: 2.5 - 100 (only EuN: 2.5 - 150); LSG1/2: 2.5 - 150	yes		no	yes	+/- 15%	tea: matrix calibriation, LSG1/2: standards in solvent
L-027	7	PA: 10-300 TA: 1-50	no	---	no	no		tea: matrix calibriation.; LSG1/2: standards in solvent
L-029	5	2.5 - 75 ng/ml (per calibrated individual substance)	no		no	no		standards in solvent. recovery correction by spiking the sample

Table 21: Information on sample preparation and extraction

	PA				TA		
Lab-code	**method "BfR-PA-Tee-2.0/2014"**	**extracted amount**	**extraction agent**	**reconstruction**	**within method "BfR-PA-Tee-2.0/2014"**	**extracted amount**	**Extraction and reconstruction**
L-001	yes				yes		
L-002	modified				modified		
L-003	no	2 g in 25 mL	chamomile: 2%ig HCOOH in H2O; melissa: 2%ige HCOOH in 50%ige MeOH	30 min sonication, centrifugation	no	2 g in 20 mL	H2O:MeOH:HCOOH (200:300:2= V:V:V), 45 min sonification, centrifugation, diloution: 1:40
L-004	no	2 g in 20 mL	ACN:MeOH:H2O= 1:1:1 (v:v:v),	15 min sonication, dilute and shoot	no	2 g in 20 mL	ACN:MeOH:H2O= 1:1:1 (v:v:v), sonification, diloution: 1:40
L-006	yes				yes		
L-007	yes				yes		
L-008	no	2 g in 40 mL	2%ig HCOOH	centrifugation, 10 fold diluted and shoot	no	2 g in 10 mL	QuEChERS-methode , 2 g in 10 mL H2O + 10 ml ACN , QuEChERS Buffer-salt mixture, centrifugation, dilution 1:1 with MeOH
L-009	no	2 g in 40 mL	2%ig HCOOH, 30 min shake, centrifugalise, 5 ml ammonia solution (25%,) pH 10, 15 min shake, centrifuglise	SPE procedure, dissolve 0,5 ml MeOH/H2O (1/9), filtrate	no	1 g in 20 mL	0,4 % HCOOH in MeOH / H_2O (6/4), shake 30 min., centrifugation, filtration
L-010	yes				yes		
L-011	no	2 g in 40 mL			no	2.0 g in 40 mL	extraction solution
L-012	no	2 g	aqueous acidic extraction, dilution		yes / no		aqueous acidic extraction, dilution
L-013	yes				no	2.0 g in 20 mL	Methanol:H2O:HCOOH (60:40:0,4); shake 30 min; centrifugation, membran filtration; 1:5 dilution with H2O
L-014	modified	2 g in 100 mL	0,05 M H2SO4, sonication	SPE 5 ml, 1:10 dilute; Matrix intensity of the test sample 0,01 g/ml)	modified		SPE: ammoniacal washing and ammoniacal- methanolic elution
L-015	modified		0,05 M H2SO4, sonication	10 fold diluted or not diluted	no	2.0 g in 2 x 20 mL (reduced to 0.6 g if outside cali)	0.05M H2SO4, SPE: conditioning MeOH and 0.05 M H2SO4,washed with 0.05 M H2SO4, elution with 1% NH3 in MeOH, dried, reconstitution with 5% MeOH in H2O
L-016	modified				modified		directly measured without processing
L-017	modified	1 g	aqueous buffer solution	SPE C18	yes		
L-018	no		extraction	centrifugation, dilution	no		
L-019	yes				yes		
L-021	no				no	2.5 g in 25 ml	MeOH:H2O:HCOOH (60/40/0,4= v/v/v)) mixing, centrifugation 10 min at 9500U/min, 11 °C , 1:5 dilution, filtration
L-023	no	2 g in 25 ml	0,05 M H2SO4, sonication, shake, centrifugalise	SPE PCX 10 mL, elution with MeOH + 5% NH3, eluate is dried, dissolved in H2O + 0,1 % HCOOH + 5 mmol/L Ammoniumformiate	no		see PA
L-024	no	1 g in 2 x 10 mL	3%ig HCOOH, shake 15 min, sonication at 50°C, filtration	dilution 1:5, filtration	no	1 - 2 g in 25 ml	MeOH:H2O:HCOOH (60/40/0,4= v/v/v)) mixing 30 min, centrifugation, 1:5 dilution, filtration, 3 fold process
L-025	yes*			5 ml on SPE, eluate is dried, dissolved in 0.5 ml			
L-026	yes						
L-027	yes				no	2 g in 10 mL	QuEChERS see AOAC 2007.01 (2 g in 10 mL acetic ACN)
L-029	yes, modified			dissolved with 2 mL eluent A	no		