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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

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Acknowledgements

The organisers of the study would like to thank the colleagues involved in the project for their support. The laboratories that participated in this exercise, listed in **Table 1**, are also sincerely acknowledged.

Table 1 - Participating laboratories

Department	Country
Department for Pesticide and Food Analytics (PLMA)	Austria
ANALYTEC® Labor für Lebensmitteluntersuchung und Umweltanalytik	Austria
CODA-CERVA-NRL Mycotoxins, O.D. Chemical Safety of the Food chain, Toxins and Natural components	Belgium
Euroinspekt-Croatiakontrola	Croatia
Andrija Stampar Teaching Institute of Public Health, Department of Environmental Protection and Health Ecology	Croatia
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, LM Zentrale Analytik	Germany
Gesellschaft für Bioanalytik mbH	Germany
Landesuntersuchungsamt Rheinland-Pfalz	Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt, Fachbereich Lebensmittelsicherheit	Germany
Lower Saxony State Office for Consumer Protection and Food Safety	Germany
Chemical and Veterinary Analytical Institute, Muensterland-Emscher-Lippe (CVUA-MEL)	Germany
Eurofins WEJ Contaminant GmbH	Germany
PhytoLab GmbH & Co KG	Germany
SGS, Department of Chromatography	Germany
Quality Systems International, AOII	Germany
Landesbetrieb Hessisches Landeslabor (LHL), Standort Kassel	Germany
Chemisches und Veterinäruntersuchungsamt Rhein Ruhr Wupper	Germany
Federal Institute for Risk Assessment, Unit Contaminants (FG82)	Germany
Landeslabor Berlin-Brandenburg	Germany
Thueringer Landesamt für Verbraucherschutz; Abt.4 Dezernat 45	Germany
LEON Institute of Applied Analytics and Research GmbH	Germany
Institut Kirchhoff Berlin GmbH, R&D Management	Germany
Fa. Teekanne GmbH & Co.KG, Labor für QS	Germany
General Chemical State Laboratory of Greece, A' Chemical Service of Athens	Greece
Public Analyst's Laboratory Dublin	Ireland
Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna (ISZLER) - Chemical Department - Bologna	Italy
Veterinary Public Health and Food Safety - Istituto Superiore di Sanità	Italy
Laboratoire National de Santé - Depart. Food Control	Luxembourg
IRTA - Chemical Food Safety	Spain
National Center for Food Spanish Consumer, Food Safety and Nutrition Agency	Spain
RIKILT - Wageningen UR	The Netherlands
Nofa Lab B.V	The Netherlands
Fera Science Ltd., Food Chemistry Dept.	United Kingdom

Abstract

Tropane alkaloids (TAs) are toxins found in a wide variety of plant species growing in mild climates. The most well-known are *Datura*, *Atropa* and *Hyoscyamus* sp., belonging to the Solanaceae family. The TAs family comprises more than 200 compounds, of which atropine and scopolamine are the most active producing anticholinergic symptoms (e.g. blurred vision, dry mouth, muscle spasms, tachycardia and death) if ingested in toxic quantities. The presence of botanical impurities (e.g. seeds, leaves and roots) has been reported in a variety of tea and herbal blends, stressing the need to control the quality of these products in the EU market.

The European Union Reference Laboratory (EURL) for Mycotoxins organised a proficiency test (PT) on the determination of TAs (atropine and scopolamine) in tea and herbal infusions upon request from DG SANTE. The measurand levels were targeted to provide insight on the measurement capabilities of EU Member States' laboratories at concentrations close to the recommended limit of quantification (LOQ) established by the Commission Recommendation 2015/976 (preferably below 5 µg/kg and not higher 10 µg/kg). Additionally, the ratio of atropine to scopolamine was kept as native in the plant materials in some samples.

Three matrices appropriately processed were provided to the participants: black tea, peppermint leaves and fennel seeds. The concentrations of atropine varied from 8.3 to 42.2 µg/kg while those of scopolamine ranged from 1.5 to 20.8 µg/kg. The participants were asked to determine atropine and scopolamine in 6 contaminated samples (2 per matrix) and 3 blank materials spiked by them with a TAs solution of unknown concentration. This setup was also aimed to allow a preliminary assessment of the robustness of the EURL-developed method.

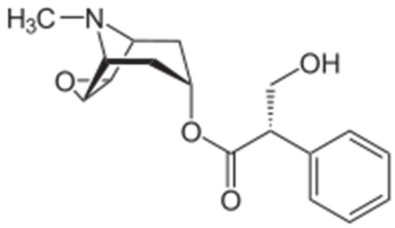
Thirty-three laboratories from 11 Member States joined the PT, with a very significant participation from Germany. The performance of the laboratories was assessed using z-scores with regard to the assigned values obtained by exact matching double isotope dilution mass spectrometry (EMD-IDMS), in line with the ISO 13528:2015. In all cases, the consensus values derived from the participants' data were within the range of the assigned values, considering the respective confidence intervals. On average, eighty-seven percent of the z-scores for atropine and 84 % for scopolamine fell in the acceptable range ($|z| \leq 2$). The success rate varied from 83 to 94 % for atropine and from 67 to 94 % for scopolamine, across the distributed matrices and concentration levels. The robust standard deviations of the reported results for both TAs were in good agreement with the target standard deviation (22 %).

The results of this PT indicate that EU Member States' laboratories can determine atropine and scopolamine reliably in tea and herbal infusions at levels relevant to the current legislation (Commission Recommendation 2015/976).

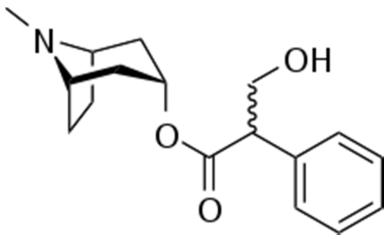
1. Introduction

Plant toxins have been recognised 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]. *Datura stramonium*, also known as Jimson weed or thorn apple, is widely distributed in temperate and tropical zones of the world. Seeds of this plant have been found as impurities in important agricultural crops such as linseed, soybean, millet, sunflower and buckwheat and products thereof. Other well-known TA-containing plants are the deadly nightshade (*Atropa belladonna*), henbane (*Hyoscyamus niger*) and mandrake (*Mandragora officinarum*). The consumption of small quantities of parts of these plants has caused severe intoxication, including deaths in young children [2]. As a result of the anticholinergic activity of the TAs, the following intoxication symptoms may be observed: blurred vision, pupil dilation, dry mouth, vomiting, clouded consciousness, muscle spasms, low body temperature, hallucinations, tachycardia, and ultimately death [1,2].

Among the over 200 TAs known, the most studied and biologically active are (-)-hyoscyamine and (-)-scopolamine enantiomers. Due to analytical limitations, it is not always possible to distinguish between the enantiomers of hyoscyamine; therefore the racemate (atropine) is usually determined [1-3]. Their structures can be found below.



Scopolamine



Atropine

Over the past decades, several TA intoxications from the consumption of contaminated herbal teas; e.g. burdock (*Arctium*) root tea, nettle (*Urtica*) tea, comfrey (*Symphytum*) tea and Paraguay (*Ilex paraguariensis*)) tea, were reported [3]. The most often reported route of ingestion by humans is through tea (contaminated or mislabelled), although ingesting seeds or other plant parts and smoking dried leaves are also common. In 2013 in the Netherlands, four persons were hospitalized after developing typical signs of anticholinergic poisoning within 2 hours after drinking tea prepared from marshmallow (*Althaea officinalis*) root that was contaminated with *A. belladonna* root. In a survey carried out in the Israeli market, out of 8 different herbal teas investigated, atropine and scopolamine occurred in 80 % of the peppermint samples with mean values of 171 µg/kg (range: 20–208 µg/kg) and 81 µg/kg (range: 14–171 µg/kg), respectively. Although the concentrations per tea bag were below the recommended acute reference dose, frequent consumption of highly contaminated peppermint teas for long periods of time might expose humans to hazardous adverse effects [3].

In 2015, the European Commission published a Recommendation to the Member States (2015/976) to monitor the presence of tropane alkaloids in food commodities, among them: food supplements, teas and herbal infusions [4].

A PT was organised by the EURL-Mycotoxins to underpin and assess the measurement capability of Member States' laboratories concerning the determination of atropine and scopolamine in tea and herbal infusions. Laboratories that didn't have a method already implemented for this determination were offered the possibility to request a suitable method description. The concentrations of atropine and scopolamine were planned to resemble a natural contamination, in part of the samples.

2. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [5], one of the core duties of the EURL is to organise PTs for the benefit of the National Reference Laboratories (NRLs). Given the Recommendation 2015/976 [4] from the European Commission and the envisaged future legislation setting maximum limits, the EURL-Mycotoxins organised on the request of DG SANTE a proficiency test on the determination of tropane alkaloids (atropine and scopolamine) in tea and herbal infusions. The target concentration was set at the LOQ requirement established in the above mentioned Recommendation (10 µg/kg) and the levels covered the range from 1.5 to 42 µg/kg, respecting the tropane alkaloids' natural proportion.
This proficiency test was addressed to the EU Member States' competent laboratories (designated by the competent national authority) and expert laboratories. Participation was free of charge and not mandatory. Forty-two laboratories from 13 Member States registered for the PT.

The EURL for Mycotoxins performed the planning, execution and assessment of the measurement results based on the requirements laid down in the legislation and followed the administrative and logistic procedures of the ISO/IEC 17043:2010 [6]. The team who organized this PT is an ISO/IEC 17043:2010 accredited PT provider [7].

3. Confidentiality

Confidentiality of the identity of participants and their results towards third parties is guaranteed.

4. Time frame

The PT was announced on the EURL-Mycotoxins' webpage [7] and by direct mailing to NRLs, Official Control Laboratories (OCLs) and expert laboratories on 26 and 27 October 2015. The registration for this PT was open until 06 January 2016 (**Annex 9.1**). The samples were dispatched between 22 and 23 February 2016 and the participants were given six weeks to analyse the samples and to report the results along with the questionnaire duly filled. The deadline for reporting the results was 04 April 2016.

5. Material

5.1 Preparation

Three different teas and herbal products (black Assam tea, peppermint leaves and fennel seeds) were kindly supplied by the german association THIE - tea & herbal infusions Europe. These materials were milled to pass a 2 mm sieve using a Retsch ZM200 mill (Retsch GmbH, Haan, Germany). The acquired materials were shown to be blank. In order to resemble a natural contamination, three materials were spiked with suitable amounts of *Datura stramonium* (stems and seeds) extracts in methanol. In another three materials, the concentration of scopolamine was raised to approximate the proportion that can be found in other plant species and to provide various combinations of the analytes over the PT items. Batches of approximately 4 kg of the three teas and herbs were spiked with the respective methanol extracts following an in-house procedure[1], each one at low and high contamination levels. Then, the materials were

[1] The procedure used was based on the dilution of the methanol extracts in t-butylmethylether, which then was used to bedew the material. This allowed obtaining a rather homogeneous moistened mix, which was then allowed to evaporate prior further processing.

thoroughly homogenised, packed in amber plastic bottles in 25 g portions and stored in the freezer until dispatch. The participants were also provided with blank matrices for method optimization and to prepare spiked samples.

5.2 Homogeneity

For homogeneity testing 10 units per material (2 black teas, 2 peppermint and 2 fennel materials) were selected randomly. Two independent determinations were performed per bottle using a liquid chromatography-isotope dilution tandem mass spectrometry detection (LC-ID-MS/MS) based method. Homogeneity was evaluated according to the ISO 13528:2015 standard [8]. The materials proved to be adequately homogeneous (**Annex 9.2**).

5.3 Stability

The stability study was conducted following an isochronous experimental design [9]; -70 °C was chosen as the reference temperature for sample storage. Stability was assessed at the following test temperatures: room temperature (≈20 °C), 4 °C and -18 °C. The periods of time considered in this study were: 14, 25 and 49 days. The stability was evaluated according to the requirements of the ISO 13528:2015 [8]. A linear regression was drawn for each tested temperature over the duration of the PT, and the significance of the slope departure from zero at 95 % confidence level was verified (**Annex 9.3**). The materials proved to be adequately stable at room temperature, 4 °C and 18 °C for the period between dispatch (t=0) and the submission date of the last results (t=49 days). An exception was noted for atropine in fennel at room temperature, which underwent a decrease in concentration of 18.6 % after 49 days. Nevertheless, shipment of the PT items was carried out under cooling conditions and the participants were instructed to store the PT items at -18 °C until analysis; therefore, this finding is not expected to have any impact on the participants' performance.

5.4 Distribution

The test materials were dispatched in polystyrene boxes, containing cooling packs, on 22 and 23 February 2016. The samples were mostly received within 24 hours after dispatch.

Each participant received:
a) nine test materials for analysis, packed in amber plastic bottles
- Samples B001-100 and B101-200 – black tea
- Samples P001-100 and P101-200 – peppermint leaves
- Samples F001-100 and F101-200 – fennel seeds
- Peppermint blank, Black tea blank and Fennel seeds blank

b) five amber glass ampoules containing
- Isotope labelled internal standard solution (ISTD mix)
- Tropane Alkaloids standard solution (TA mix)
- Spiking solutions specific for black tea, peppermint leaves and fennel seeds

c) accompanying letter with instructions on sample handling and reporting (**Annex 9.4**)
d) a sample receipt form (**Annex 9.5**) and
e) laboratory specific reporting files with a lab code (by email).

The materials were shipped such that 4 °C was not exceeded. Upon arrival, storage was required to be at -18 °C until analysis.

6. Instructions to participants

The scope of the PT and the instructions for sample handling and reporting was communicated to the participants via an accompanying letter (**Annex 9.4**). The laboratories were required to report the concentrations of atropine and scopolamine (in µg/kg), as it was standard practice in their laboratory. Then, in the Questionnaire (**Annex 9.6**), participants were asked to mention whether the results **were corrected for recoveries or not** and provide the recoveries figures (in %).

The results were reported by the participants using RingDat software, which is part of the ProLab software [10]. Laboratory specific files generated by the ProLab software were sent to each laboratory by email. A specific questionnaire was also included. The questionnaire was intended to provide further information on method-related aspects and laboratory details to allow insights on potential individual and general trends observed in the results for possible follow-up procedures. Method-related details and performance parameters such as chromatographic conditions, MRM transitions, S/N ratio of peak signals (as peak-to-peak, instead of RMS) and LOQs were requested.

Participants received information on the required storage conditions and were encouraged to perform the analysis as soon as possible, to allow enough time for data treatment and to get acquainted with the reporting software.

7. Reference values and their uncertainties

The assigned values of the analytes in the test samples and their uncertainties were established by Exact-Matching Double Isotope Dilution Mass Spectrometry (EMD-IDMS) at JRC-Geel (Table 2). This methodology is considered to provide the highest degree of accuracy of the assigned values [11].

Table 2 - Assigned values of the analytes and their associated expanded uncertainties in the tea and herbal infusion test items.

Sample	Analyte	Assigned value (µg/kg)	U (k=2) (µg/kg)
Black tea 001-100	Atropine	16.9	0.7
	Scopolamine	2.3	0.3
Black tea 101-200	Atropine	8.3	0.3
	Scopolamine	9.5	0.5
Peppermint 001-100	Atropine	9.5	0.3
	Scopolamine	1.5	0.1
Peppermint 101-200	Atropine	21.2	0.5
	Scopolamine	2.5	0.2
Fennel 001-100	Atropine	42.2	1.8
	Scopolamine	13.4	0.4
Fennel 101-200	Atropine	18.8	0.3
	Scopolamine	20.8	0.9

U - expanded uncertainty of the assigned value

The spiking solutions for the 3 matrices all had the same concentration. Following the spiking protocol mentioned in the accompanying letter (**Annex 9.4**), the resulting concentrations were: scopolamine – 14.6 µg/kg and atropine – 14.4 µg/kg.

8. Evaluation of results

8.1 General observations

Out of the 42 laboratories that received the PT samples, 33 reported back their results. Nine laboratories declined to send their results either due to a change of interest or time constraints. Eleven laboratories were NRLs for Mycotoxins, and 22 were expert laboratories.

The laboratories were free to use their method of choice. An LC-MS/MS standard operating procedure (SOP) suitable for the determination of TAs in cereals was provided to laboratories that placed a request. This SOP could be used to analyse tea and herbal infusions after minor amendments. The method provided was developed, validated and used by the EURL for Mycotoxins.

Only liquid chromatography coupled with mass spectrometric detection methods were used by the participants for the determination of the two TAs in tea and herbal infusions.

This PT was organised in a way to resemble also a layout of a collaborative method validation study. Nine test items were supplied to the participants, and seven laboratories entirely followed the EURL-provided SOP.

8.2 Scores and evaluation criteria

Individual laboratory performance was assessed in terms of z-scores in accordance with ISO 13528:2015 [8].

$$z = \frac{x_{lab} - X_{ref}}{\sigma_p} \qquad \textbf{Equation 1.}$$

where:

x_{lab} is the measurement result reported by a participant
X_{ref} is the reference value (assigned value)
σ_p is the standard deviation for proficiency assessment (target standard deviation)

σ_p was calculated using the Horwitz equation, modified by Thompson [12] for analyte concentrations <120 µg/kg:

- for analyte concentration <120 µg/kg

$$\sigma_p = 0.22 \cdot c \qquad \textbf{Equation 2.}$$

where:
c = concentration of the measurand (assigned value, $X_{ref,}$) expressed as a dimensionless mass ratio, e.g. 1 µg/kg = 10^{-9}, 1 mg/kg = 10^{-6}

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

$\|z\| \leq 2$	acceptable result
$2 < \|z\| < 3$	questionable result
$\|z\| \geq 3$	unacceptable result

8.3 Laboratory results and scoring

The statistical evaluation of the results was performed using the ProLab software [10]. The robust mean and the reproducibility standard deviation were computed according to Algorithm A of ISO 13528:2015, and are given just for information purposes [8]. Z-

scoring was calculated for scopolamine and atropine using the values assigned by EMD-IDMS instead of the consensus values (robust mean).

85.9 % of the results reported by the participants obtained acceptable z-scores (|z|≤ 2) whereas 6.7 % of the results fell into the unacceptable range with |z|≥ 3 (Figure 1)

Figure 1 - Distribution of all z-scores across measurands/samples/laboratories.

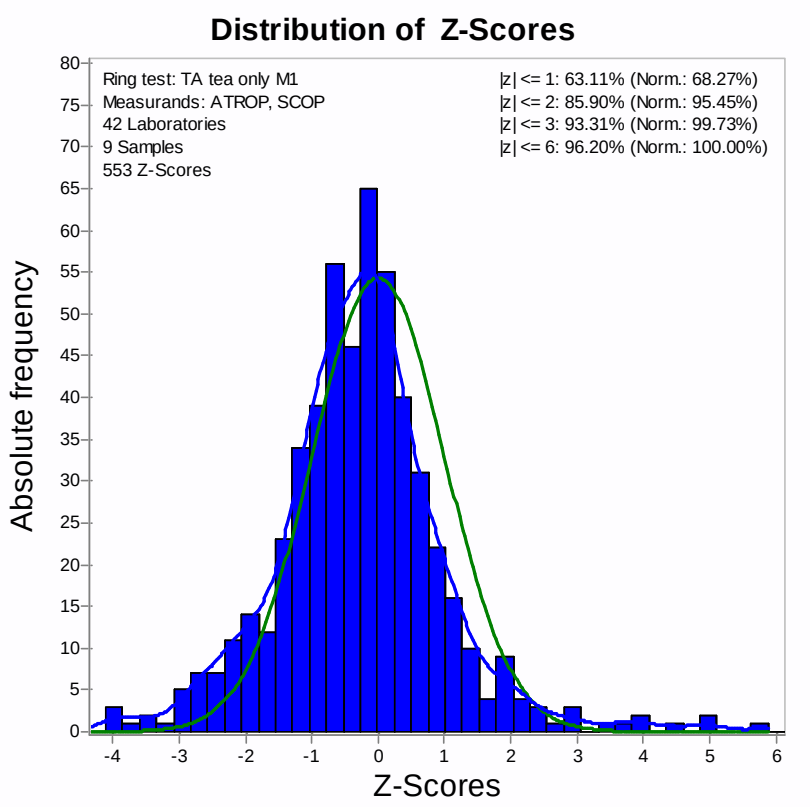
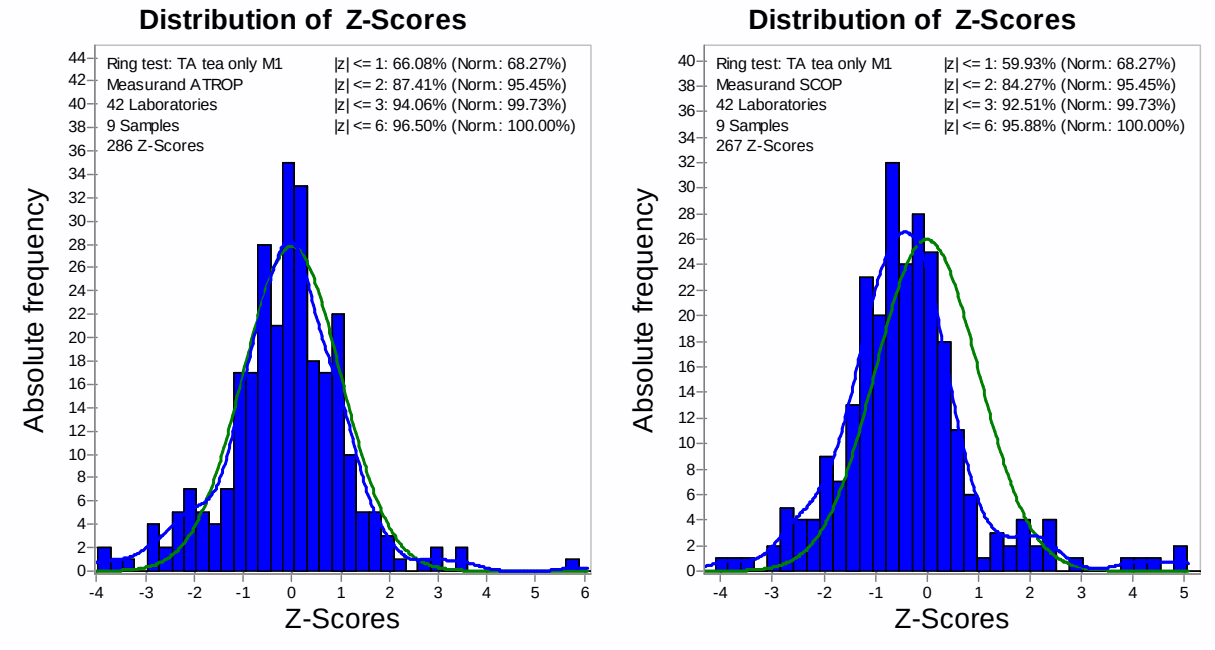


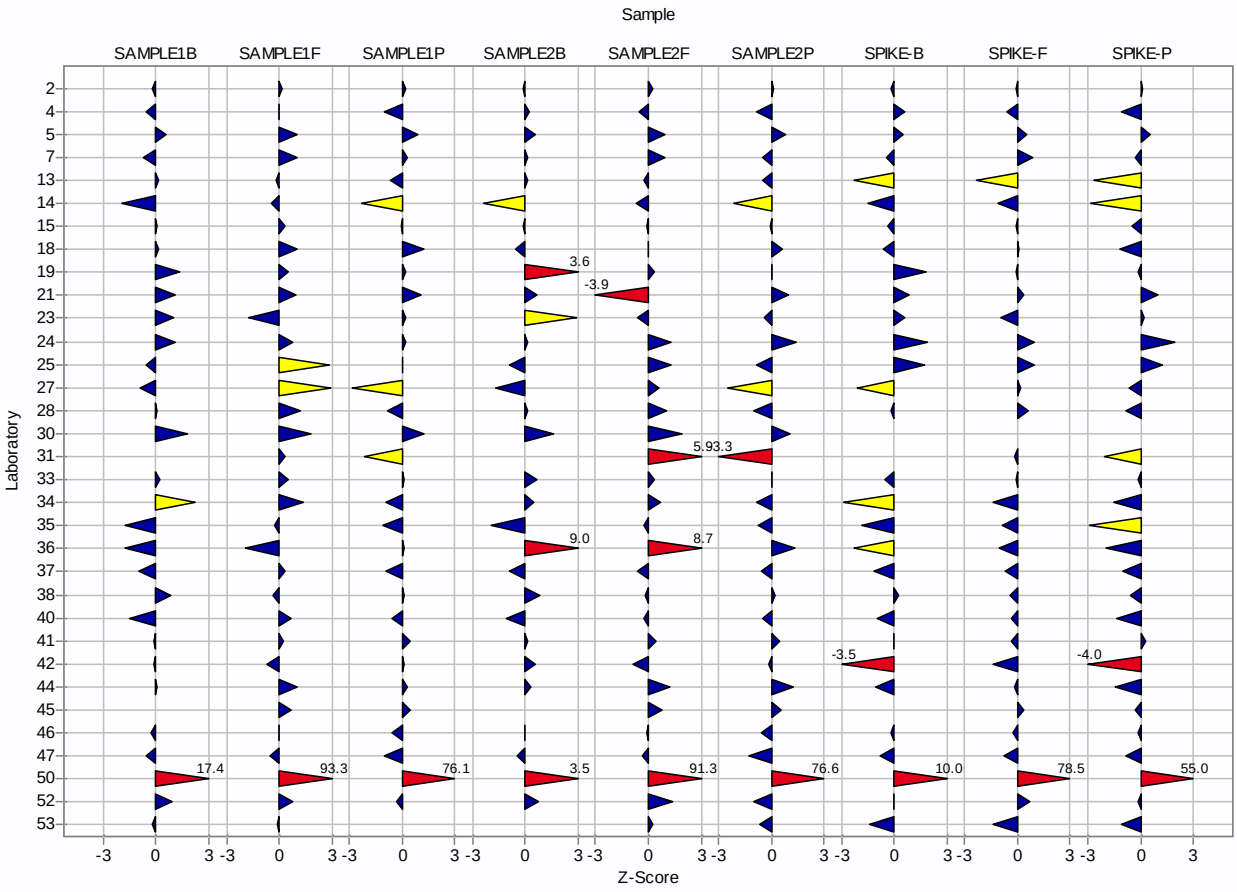
Figure 2 - Distribution of the z-scores for atropine (left) and scopolamine (right) across samples/laboratories.



The breakdown of the z-scores by analyte (Figure 2) shows that the laboratories' performance for atropine was slightly better than for scopolamine. This finding might be explained by the fact that scopolamine concentrations were in general lower or much lower than atropine, rendering the determination more demanding.

Figures 3 and 4 provide an overview of the individual z-scores assigned to the results of atropine and scopolamine, respectively, in the tea and herbal infusion test materials. The longer the triangles, the larger were the differences to the assigned values. Blue triangles represent z-scores in the acceptable range, yellow triangles in the questionable range and red triangles in the unacceptable performance range. The corresponding scores are shown next to the triangles.

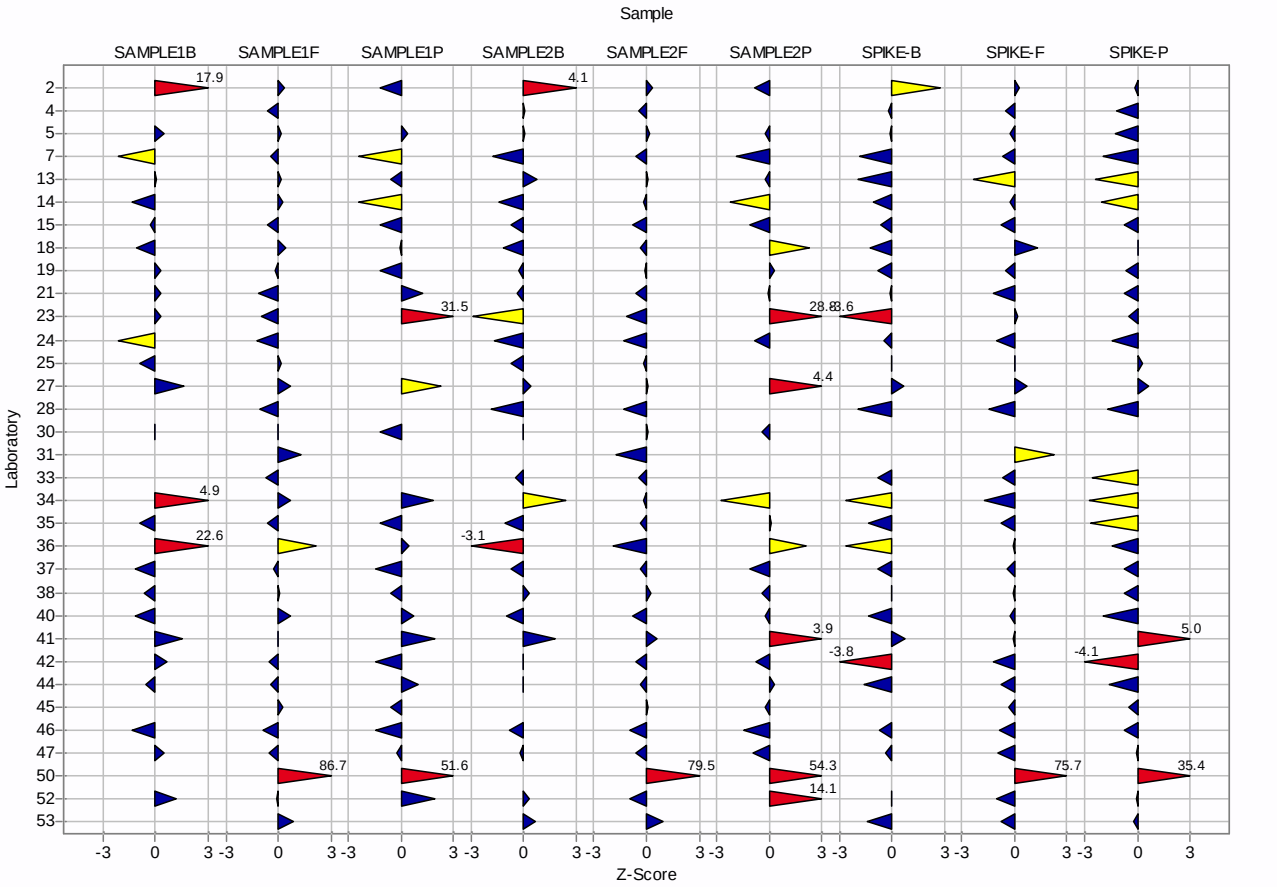
Figure 3 – Individual laboratory z-scores for atropine across the 9 test items. B - black tea, F - fennel and P – peppermint.



The numerical values of the calculated z-scores are compiled in Tables 3 and 4. All z-scores in the questionable performance range are shown with a yellow background, while z-scores indicating unacceptable performance are presented a with a light-red background. This mode of presentation allows for easy distinction between the two performance ranges, even on black-and-white prints.

The graphical representations of the sigmoidal distribution of the results (µg/kg) for each combination of measurand/sample are given in Figure 5. Reported results are shown as bars. The green line corresponds to Xref; the green shadow covers the boundary of the reference interval (Xref ± u_{ref}), and the red lines mark the boundary of the target interval (Xref ± 2σ). Yellow bars represent results with |z-score| <3 while red bars represent unacceptable results.

Figure 4 – Individual laboratory z-scores for scopolamine across the 9 test items. B - black tea, F - fennel and P – peppermint.



Annex 9.7 shows the kernel density plots drawn for atropine and scopolamine across the nine test items. The confidence intervals of the robust means calculated from the participants' results overlap with the confidence intervals of the assigned values for all the analytes and matrix combinations. For the spiked samples, no uncertainty was calculated for the target value, as the spiking was done by the participants. The dispersion of the results approximates a Gaussian distribution (in green). The major mode is close to the assigned (reference) value and the robust mean calculated from the results of the participants.

The robust standard deviations of the reported results for both TAs are also in good agreement with the target standard deviation (22 %). The HORRAT values are generally in the range from 0.7 to 1.4, with two exceptions (1.8 and 2.2 for low concentrations of scopolamine). Summaries of the statistical evaluation of the results for atropine and scopolamine in the test items are presented in Tables 5 and 6. The robust standard deviations ranged from 18.7 to 32.9 % for atropine and from 15.8 to 46.4 % for scopolamine. The highest standard deviation for atropine was observed in spiked black tea at the 14.4 µg/kg level while the highest standard deviation for scopolamine was observed in peppermint contaminated at the low level (2.5 µg/kg).

The above evidence supports the assumption that the measurement of atropine and scopolamine in tea and herbs is sufficiently reliable in terms of precision and bias within the participants population. It can therefore be assumed that the methods available allow monitoring of tropane alkaloids at the target level of 10 µg/kg and even below for each alkaloid.

Table 3 - Analytical results and z-scores for atropine in the nine test items. B - black tea, F - fennel and P – peppermint. Colour code: yellow – questionable, red – unacceptable

Samples	SAMPLE1B	z-score	SAMPLE1F	z-score	SAMPLE1P	z-score	SAMPLE2B	z-score	SAMPLE2F	z-score	SAMPLE2P	z-score	SPIKE-B	z-score	SPIKE-F	z-score	SPIKE-P	z-score
Lab/Unit	µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg	
2	15.97	-0.2	44.15	0.2	9.92	0.2	8.13	-0.1	19.87	0.2	21.66	0.1	13.90	-0.2	14.15	-0.1	14.81	0.1
4	14.88	-0.5	42.22	0.0	7.41	-1.0	8.68	0.2	16.65	-0.5	17.40	-0.8	16.27	0.6	12.58	-0.6	10.86	-1.1
5	19.10	0.6	51.60	1.0	11.30	0.9	9.30	0.5	22.70	0.9	25.10	0.8	15.90	0.5	16.20	0.6	16.20	0.6
7	14.30	-0.7	51.60	1.0	10.10	0.3	8.50	0.1	22.90	1.0	18.80	-0.5	13.10	-0.4	17.30	0.9	13.60	-0.3
13	17.40	0.1	40.50	-0.2	8.00	-0.7	8.50	0.1	17.70	-0.3	18.80	-0.5	7.10	-2.3	7.10	-2.3	5.90	-2.7
14	9.50	-2.0	38.50	-0.4	4.70	-2.3	4.00	-2.4	16.10	-0.7	11.30	-2.1	9.60	-1.5	11.00	-1.1	5.50	-2.8
15	17.10	0.1	45.50	0.4	9.30	-0.1	8.10	-0.1	18.40	-0.1	20.90	-0.1	13.40	-0.3	14.20	-0.1	13.00	-0.5
18	17.29	0.1	51.90	1.0	12.04	1.2	7.23	-0.6	18.83	0.0	23.99	0.6	12.31	-0.7	14.82	0.1	10.83	-1.1
19	22.00	1.4	46.70	0.5	9.80	0.2	14.90	3.6	20.20	0.3	21.40	0.0	20.20	1.8	14.10	-0.1	14.10	-0.1
21	21.00	1.1	50.70	0.9	11.70	1.1	9.60	0.7	2.80	-3.9	25.80	1.0	17.10	0.8	15.60	0.4	17.60	1.0
23	20.70	1.0	26.10	-1.7	9.90	0.2	13.70	2.9	16.30	-0.6	19.10	-0.4	16.30	0.6	11.50	-0.9	15.10	0.2
24	20.94	1.1	49.56	0.8	9.86	0.2	8.52	0.1	24.10	1.3	27.71	1.4	20.46	1.9	17.48	1.0	20.55	1.9
25	14.90	-0.5	68.60	2.8	9.50	0.0	6.60	-0.9	24.40	1.3	17.20	-0.9	19.80	1.7	17.60	1.0	18.50	1.3
27	13.64	-0.9	69.24	2.9	3.56	-2.8	5.26	-1.7	21.39	0.6	9.54	-2.5	7.70	-2.1	15.07	0.2	12.40	-0.6
28	17.00	0.0	53.60	1.2	7.70	-0.8	8.50	0.1	23.30	1.1	16.30	-1.0	13.80	-0.2	16.40	0.6	11.90	-0.8
30	23.45	1.8	58.99	1.8	11.98	1.2	11.26	1.6	26.68	1.9	26.18	1.1	not tested		not tested		not tested	
31	not tested		45.64	0.4	4.98	-2.2	not tested		43.20	>4	5.86	-3.3	not tested		13.96	-0.2	7.82	-2.1
33	17.80	0.3	46.70	0.5	9.70	0.1	9.60	0.7	20.10	0.3	21.20	0.0	12.80	-0.5	14.20	-0.1	14.00	-0.1
34	25.20	2.2	55.10	1.4	7.48	-1.0	9.28	0.5	21.60	0.7	17.40	-0.8	5.20	-2.9	10.15	-1.4	9.55	-1.5
35	10.30	-1.8	40.10	-0.2	7.20	-1.1	4.70	-2.0	17.60	-0.3	17.60	-0.8	8.50	-1.9	11.80	-0.8	5.30	-2.9
36	10.32	-1.8	24.54	-1.9	9.73	0.1	24.84	>4	54.82	>4	27.20	1.3	7.08	-2.3	11.16	-1.0	8.17	-2.0
37	13.20	-1.0	45.40	0.3	7.50	-0.9	6.70	-0.9	16.30	-0.6	18.30	-0.6	10.70	-1.2	12.40	-0.6	11.30	-1.0
38	20.04	0.9	39.18	-0.3	9.60	0.1	9.92	0.9	18.20	-0.2	22.29	0.2	15.17	0.2	13.21	-0.4	12.58	-0.6
40	11.11	-1.6	48.30	0.7	8.23	-0.6	6.36	-1.1	17.93	-0.2	19.04	-0.5	11.28	-1.0	13.31	-0.4	10.18	-1.3
41	16.50	-0.1	44.49	0.2	10.45	0.5	8.62	0.2	20.78	0.5	23.26	0.4	14.38	0.0	13.34	-0.3	15.43	0.3
42	16.30	-0.2	35.90	-0.7	9.60	0.1	9.40	0.6	15.40	-0.8	20.50	-0.1	3.20	-3.5	10.10	-1.4	1.80	-4.0
44	17.00	0.0	52.00	1.1	10.00	0.3	8.90	0.3	24.00	1.2	27.00	1.3	11.00	-1.1	14.00	-0.1	10.00	-1.4
45	not tested		48.60	0.7	10.50	0.5	not tested		22.20	0.8	23.80	0.6	not tested		15.50	0.3	13.40	-0.3
46	15.80	-0.3	42.50	0.0	8.30	-0.6	8.20	-0.1	18.40	-0.1	18.50	-0.6	13.70	-0.2	13.70	-0.2	14.00	-0.1
47	14.80	-0.6	37.50	-0.5	7.40	-1.0	7.50	-0.4	17.50	-0.3	15.40	-1.2	12.00	-0.8	12.00	-0.8	11.90	-0.8
50	81.30	>4	908.90	>4	167.90	>4	14.80	3.5	397.40	>4	377.70	>4	46.30	>4	263.70	>4	189.30	>4
52	20.30	0.9	49.59	0.8	8.79	-0.3	9.67	0.7	24.65	1.4	16.47	-1.0	14.30	0.0	16.56	0.7	13.90	-0.2
53	16.00	-0.2	41.00	-0.1	< 10.00		< 10.00		20.00	0.3	18.00	-0.7	10.00	-1.4	10.00	-1.4	11.00	-1.1

Table 4 - Analytical results and z-scores for scopolamine in the nine test items. B - black tea, F - fennel and P – peppermint. Colour code: yellow – questionable, red – unacceptable

Samples	SAMPLE1 B	z-score	SAMPLE1F	z-score	SAMPLE1P	z-score	SAMPLE2 B	z-score	SAMPLE2F	z-score	SAMPLE2P	z-score	SPIKE-B	z-score	SPIKE-F	z-score	SPIKE-P	z-score
Lab/Unit	µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg		µg/kg	
2	11.15	>4	14.43	0.3	1.10	-1.2	18.07	>4	22.53	0.4	2.07	-0.8	23.54	2.8	15.39	0.3	14.20	-0.1
4	< 2.00		11.70	-0.6	< 2.00		9.65	0.1	18.84	-0.4	< 5.00		13.86	-0.2	12.99	-0.5	10.87	-1.2
5	2.50	0.5	14.00	0.2	1.60	0.3	9.60	0.0	21.40	0.1	2.40	-0.2	14.30	-0.1	13.80	-0.2	10.50	-1.3
7	1.20	-2.1	12.20	-0.4	0.70	-2.4	5.90	-1.7	18.00	-0.6	1.50	-1.8	8.60	-1.9	12.50	-0.6	8.40	-1.9
13	2.30	0.1	13.90	0.2	1.30	-0.6	11.10	0.7	21.10	0.1	2.40	-0.2	8.30	-2.0	7.20	-2.3	6.90	-2.4
14	1.60	-1.3	14.10	0.2	0.70	-2.4	6.60	-1.4	19.90	-0.2	1.30	-2.2	11.20	-1.1	13.70	-0.3	8.10	-2.0
15	2.10	-0.3	11.70	-0.6	1.10	-1.2	8.00	-0.7	17.20	-0.8	1.90	-1.1	12.60	-0.6	12.20	-0.7	12.20	-0.7
18	1.74	-1.0	14.74	0.5	1.47	-0.1	7.14	-1.1	19.22	-0.3	3.80	2.3	10.67	-1.2	18.84	1.3	14.74	0.1
19	2.40	0.3	12.80	-0.2	1.10	-1.2	8.90	-0.3	20.20	-0.1	2.70	0.3	12.10	-0.8	12.80	-0.6	12.60	-0.6
21	2.40	0.3	10.00	-1.2	1.90	1.3	8.70	-0.4	17.80	-0.6	2.50	0.0	14.30	-0.1	10.80	-1.2	12.20	-0.7
23	2.40	0.3	10.50	-1.0	11.80	>4	3.50	-2.9	15.60	-1.1	18.50	>4	3.09	-3.6	15.10	0.2	13.00	-0.5
24	1.22	-2.1	9.76	-1.2	not tested		6.07	-1.7	14.69	-1.3	2.04	-0.9	13.11	-0.5	11.14	-1.1	9.87	-1.5
25	1.80	-0.9	14.00	0.2	not tested		8.00	-0.7	19.80	-0.2	not tested		14.60	0.0	14.70	0.0	15.60	0.3
27	3.04	1.6	15.56	0.7	2.25	2.3	10.41	0.4	20.99	0.1	4.97	>4	16.79	0.7	16.75	0.7	16.59	0.6
28	< 5.00		10.20	-1.1	< 5.00		5.60	-1.9	14.80	-1.3	< 5.00		8.30	-2.0	9.90	-1.5	9.00	-1.7
30	2.24	0.0	13.51	0.0	1.09	-1.2	9.47	0.0	21.00	0.1	2.29	-0.4	not tested		not tested		not tested	
31	not tested		17.20	1.3	< 0.20		not tested		12.72	-1.8	< 0.20		not tested		21.90	2.3	not tested	
33	< 4.00		11.40	-0.7	< 2.00		8.60	-0.4	18.70	-0.4	< 2.00		12.00	-0.8	12.40	-0.7	6.40	-2.5
34	4.70	>4	15.50	0.7	2.09	1.8	14.50	2.4	19.80	-0.2	1.02	-2.7	6.13	-2.6	9.10	-1.7	5.93	-2.7
35	1.80	-0.9	11.60	-0.6	1.10	-1.2	7.20	-1.1	19.10	-0.4	2.60	0.1	10.40	-1.3	12.20	-0.7	6.20	-2.6
36	13.48	>4	19.84	2.2	1.64	0.5	3.09	-3.1	11.98	-1.9	3.67	2.1	6.12	-2.6	14.35	-0.1	9.86	-1.5
37	1.70	-1.1	12.70	-0.2	1.00	-1.5	8.00	-0.7	19.20	-0.3	1.90	-1.1	12.00	-0.8	13.30	-0.4	12.20	-0.7
38	1.92	-0.7	13.67	0.1	1.30	-0.6	10.17	0.3	21.95	0.3	2.32	-0.4	14.54	0.0	14.32	-0.1	12.14	-0.8
40	1.66	-1.2	15.43	0.7	1.72	0.7	7.42	-1.0	17.16	-0.8	2.39	-0.2	10.20	-1.4	13.78	-0.2	8.27	-2.0
41	3.00	1.5	13.47	0.0	2.12	1.9	13.35	1.8	23.46	0.6	4.67	3.9	16.99	0.8	14.21	-0.1	30.75	>4
42	2.60	0.7	11.90	-0.5	1.00	-1.5	9.50	0.0	17.80	-0.6	2.10	-0.8	2.40	-3.8	10.60	-1.2	1.40	<-4
44	2.00	-0.5	12.00	-0.5	1.80	0.9	9.40	-0.1	19.00	-0.4	2.70	0.3	9.50	-1.6	12.00	-0.8	9.50	-1.6
45	not tested		14.20	0.3	1.30	-0.6	not tested		21.10	0.1	2.40	-0.2	not tested		13.40	-0.4	13.00	-0.5
46	1.60	-1.3	10.80	-0.9	1.00	-1.5	7.80	-0.8	16.40	-1.0	1.70	-1.5	12.40	-0.7	11.80	-0.9	12.10	-0.8
47	2.50	0.5	11.80	-0.5	1.40	-0.3	9.10	-0.2	18.00	-0.6	2.00	-0.9	13.30	-0.4	11.40	-1.0	14.50	0.0
50	not tested		269.10	>4	18.40	>4	not tested		383.80	>4	32.60	>4	not tested		257.10	>4	128.00	>4
52	2.83	1.1	13.21	-0.1	2.11	1.9	10.29	0.4	16.20	-1.0	10.31	>4	14.36	-0.1	11.19	-1.1	14.53	0.0
53	< 5.00		16.00	0.9	< 5.00		11.00	0.7	25.00	0.9	< 5.00		10.00	-1.4	12.00	-0.8	14.00	-0.2

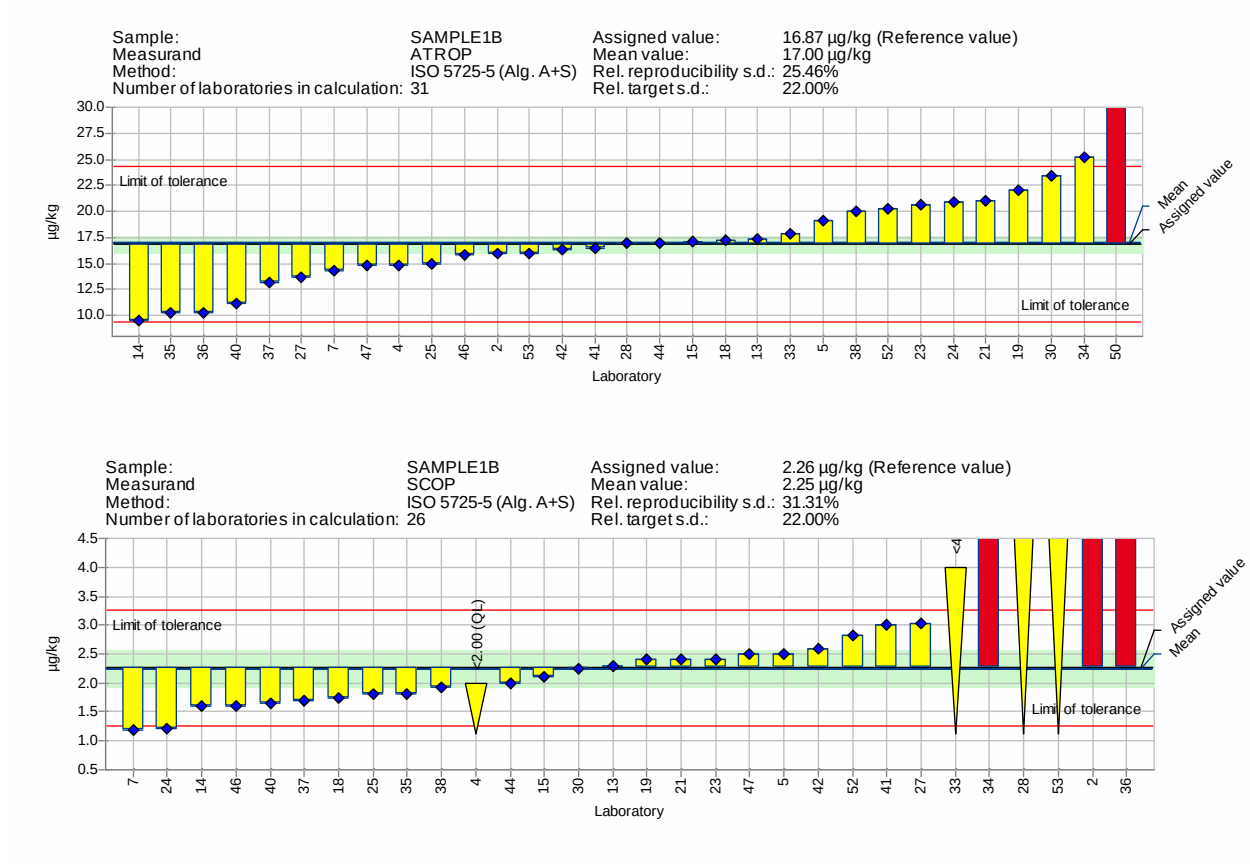
Table 5 - Summary statistics of the results for atropine in the nine test items. B - black tea, F - fennel and P – peppermint.

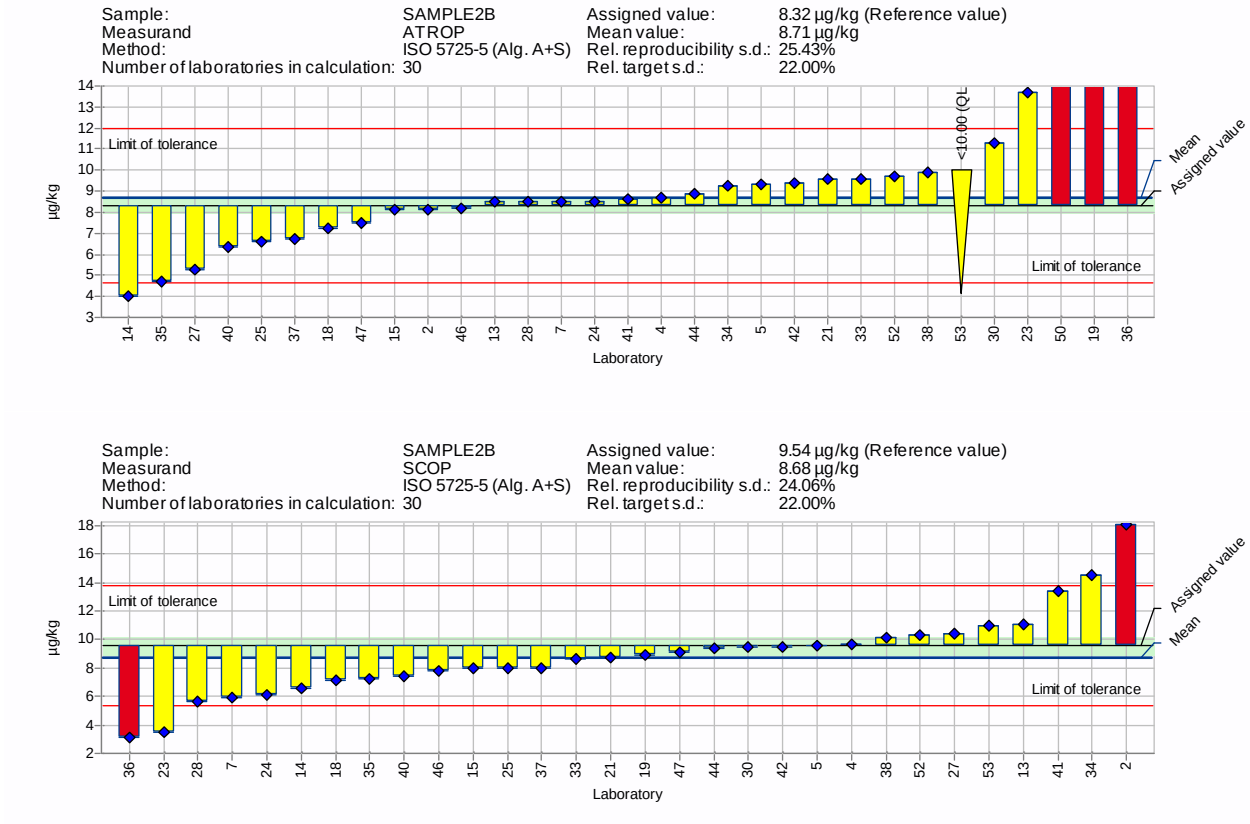
	Units	SAMPLE1B	SAMPLE1F	SAMPLE1P	SAMPLE2B	SAMPLE2F	SAMPLE2P	SPIKE-B	SPIKE-F	SPIKE-P
No. of laboratories that submitted results		31	33	33	31	33	33	30	32	32
No. of participants (according to design)		42	42	42	42	42	42	42	42	42
Assigned (reference) value	µg/kg	16.9	42.2	9.5	8.3	18.8	21.2	14.4	14.4	14.4
Uncertainty of the assigned value (k=2)	µg/kg	0.7	1.8	0.3	0.3	0.3	0.5			
Mean (robust)	µg/kg	17.0	46.8	9.2	8.7	20.6	20.4	13.0	13.8	12.3
Target s.d.	µg/kg	3.7	9.3	2.1	1.8	4.1	4.7	3.2	3.2	3.2
Reproducibility s.d.	µg/kg	4.3	8.2	1.9	2.1	4.1	4.9	4.8	2.7	4.2
Rel. SDPA	%	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Rel. reproducibility s.d. (robust)	%	25.5	19.3	20.4	25.4	21.7	23.2	32.9	18.7	28.7

Table 6 - Summary statistics of the results for scopolamine in the nine test items. B - black tea, F - fennel and P – peppermint.

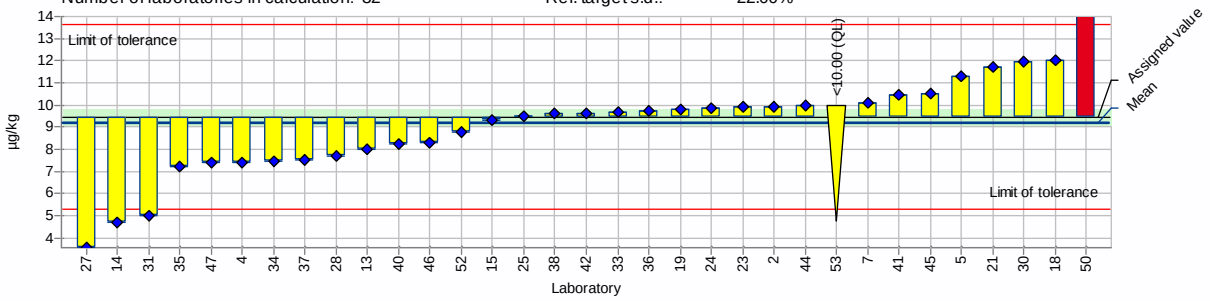
	Units	SAMPLE1B	SAMPLE1F	SAMPLE1P	SAMPLE2B	SAMPLE2F	SAMPLE2P	SPIKE-B	SPIKE-F	SPIKE-P
No. of laboratories that submitted results		30	33	31	30	33	32	29	32	31
No. of participants (according to design)		42	42	42	42	42	42	42	42	42
Assigned (reference) value	µg/kg	2.3	13.4	1.5	9.5	20.8	2.5	14.6	14.6	14.6
Uncertainty of the assigned value (k=2)	µg/kg	0.3	0.4	0.1	0.5	0.9	0.2			
Mean (robust)	µg/kg	2.3	13.2	1.5	8.7	19.0	2.7	11.6	13.0	11.4
Target s.d.	µg/kg	0.5	3.0	0.3	2.1	4.6	0.6	3.2	3.2	3.2
Reproducibility s.d.	µg/kg	0.7	2.3	0.6	2.3	3.0	1.2	3.7	2.3	3.8
Rel. SDPA	%	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Rel. reproducibility s.d. (robust)	%	31.3	16.8	38.4	24.1	14.4	46.5	25.7	15.7	26.5

Figure 5 – sigmoidal plots of individual laboratory results reported for atropine (ATROP) and scopolamine (SCOP) in the test items. B - black tea, F - fennel and P – peppermint.

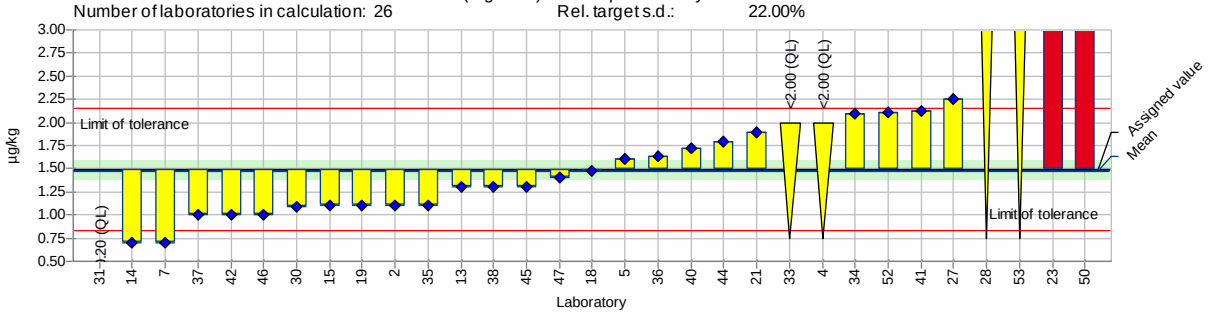




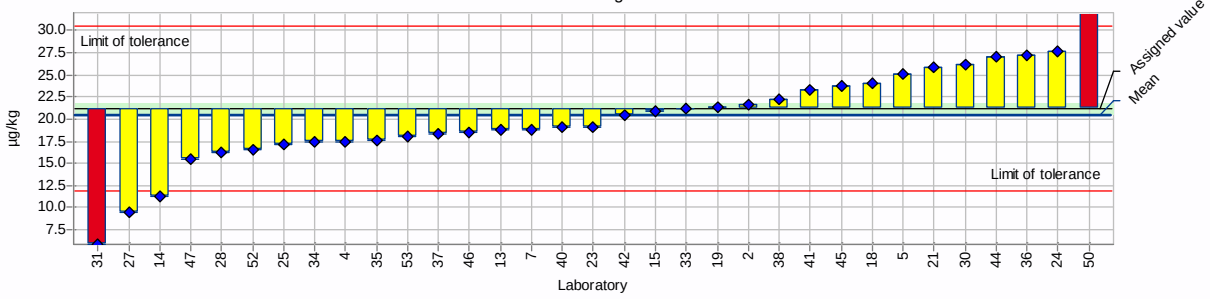
Sample:	SAMPLE1P	Assigned value:	9.46 µg/kg (Reference value)
Measurand	ATROP	Mean value:	9.19 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	20.35%
Number of laboratories in calculation:	32	Rel. target s.d.:	22.00%



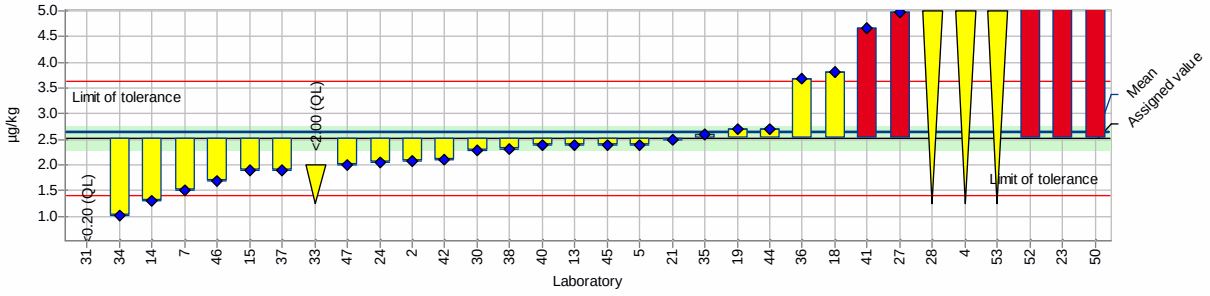
Sample:	SAMPLE1P	Assigned value:	1.49 µg/kg (Reference value)
Measurand	SCOP	Mean value:	1.48 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	38.44%
Number of laboratories in calculation:	26	Rel. target s.d.:	22.00%



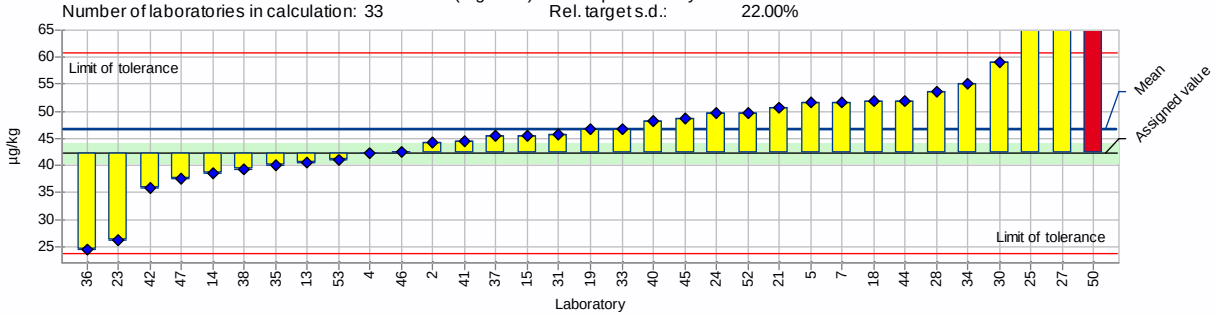
Sample:	SAMPLE2P	Assigned value:	21.17 µg/kg (Reference value)
Measurand	ATROP	Mean value:	20.40 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	23.21%
Number of laboratories in calculation:	33	Rel. target s.d.:	22.00%



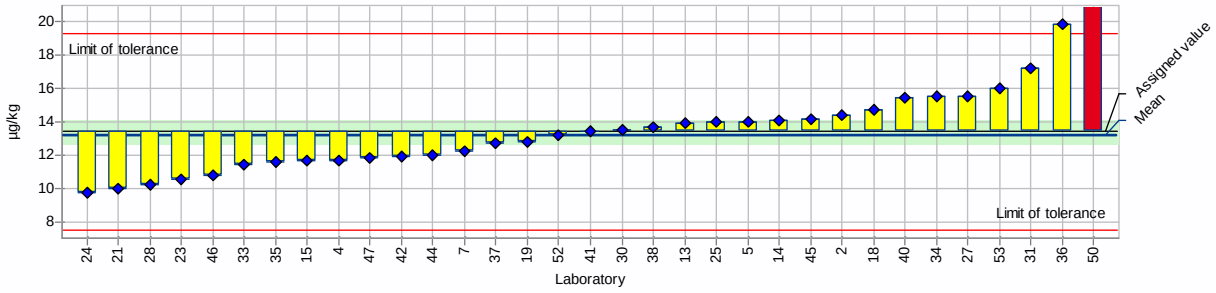
Sample:	SAMPLE2P	Assigned value:	2.52 µg/kg (Reference value)
Measurand	SCOP	Mean value:	2.66 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	46.45%
Number of laboratories in calculation:	27	Rel. target s.d.:	22.00%



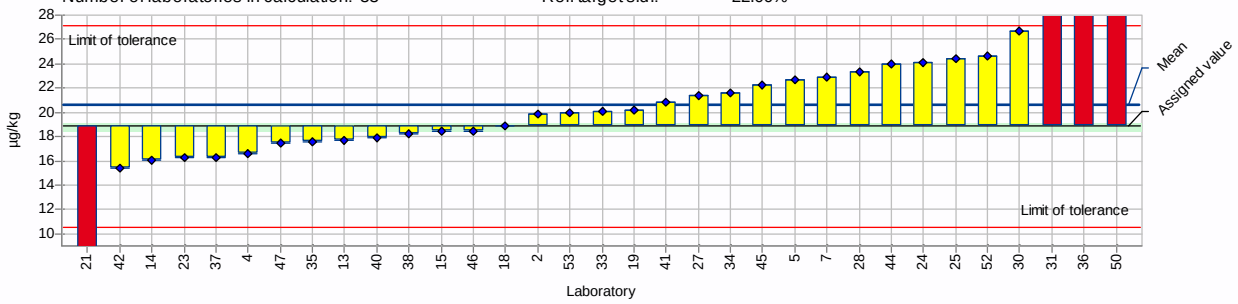
Sample:	SAMPLE1F	Assigned value:	42.23 µg/kg (Reference value)
Measurand	ATROP	Mean value:	46.78 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	19.30%
Number of laboratories in calculation:	33	Rel. target s.d.:	22.00%



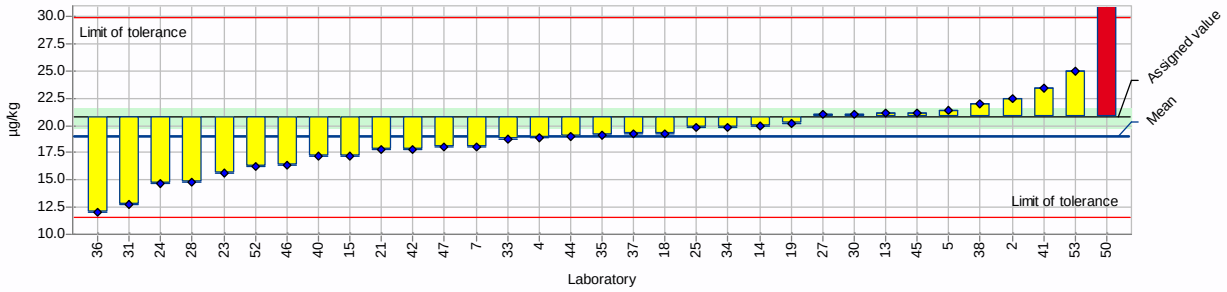
Sample:	SAMPLE1F	Assigned value:	13.41 µg/kg (Reference value)
Measurand	SCOP	Mean value:	13.23 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	16.84%
Number of laboratories in calculation:	33	Rel. target s.d.:	22.00%



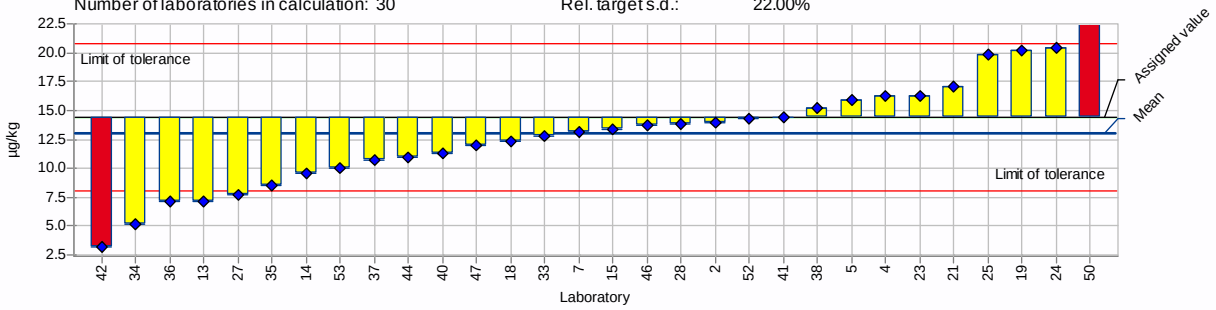
Sample:	SAMPLE2F	Assigned value:	18.84 µg/kg (Reference value)
Measurand	ATROP	Mean value:	20.57 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	21.72%
Number of laboratories in calculation:	33	Rel. target s.d.:	22.00%



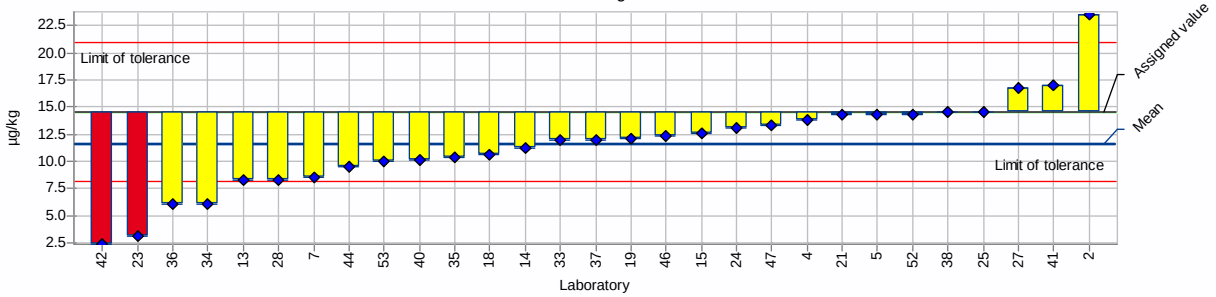
Sample:	SAMPLE2F	Assigned value:	20.75 µg/kg (Reference value)
Measurand	SCOP	Mean value:	19.00 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	14.37%
Number of laboratories in calculation:	33	Rel. target s.d.:	22.00%



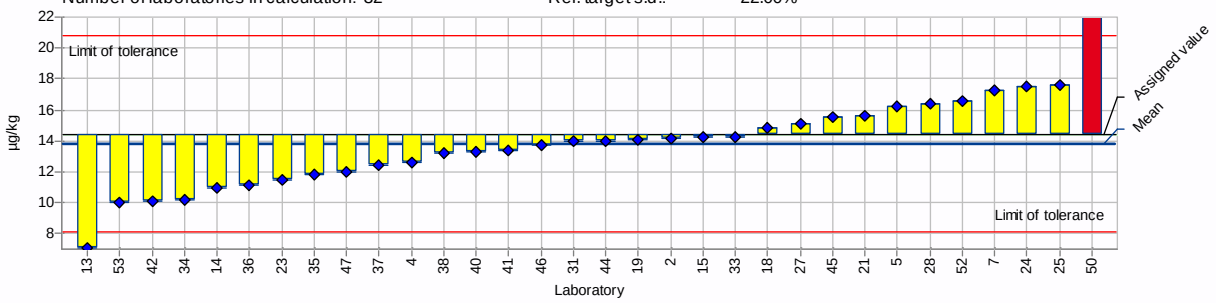
Sample:	SPIKE-B	Assigned value:	14.44 µg/kg (Reference value)
Measurand	ATROP	Mean value:	12.97 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	32.93%
Number of laboratories in calculation:	30	Rel. target s.d.:	22.00%



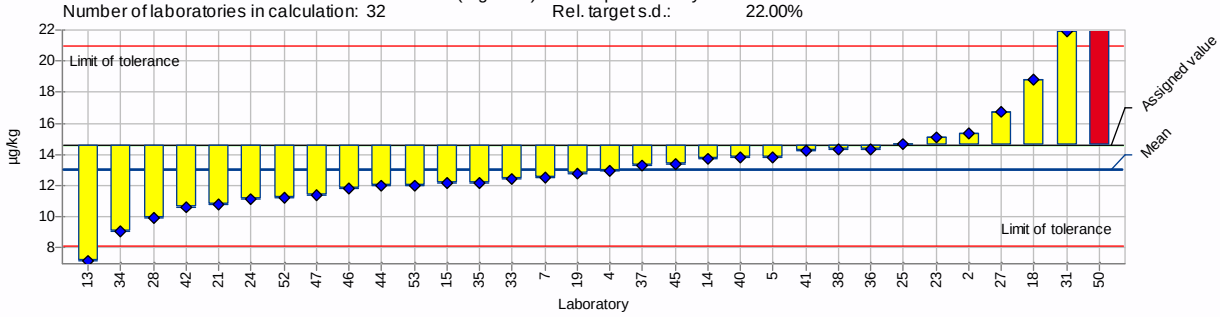
Sample:	SPIKE-B	Assigned value:	14.57 µg/kg (Reference value)
Measurand	SCOP	Mean value:	11.58 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	25.70%
Number of laboratories in calculation:	29	Rel. target s.d.:	22.00%

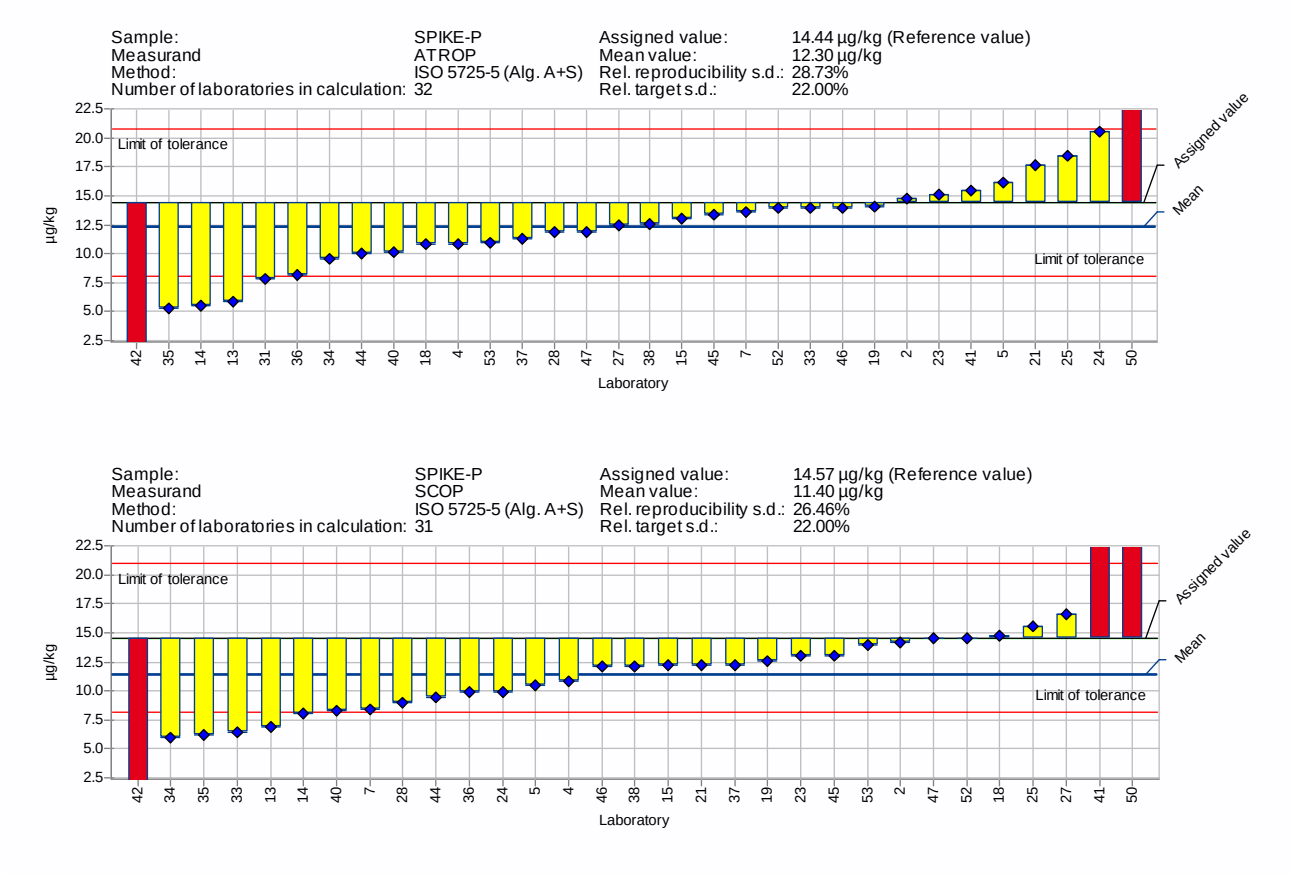


Sample:	SPIKE-F	Assigned value:	14.44 µg/kg (Reference value)
Measurand	ATROP	Mean value:	13.78 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	18.69%
Number of laboratories in calculation:	32	Rel. target s.d.:	22.00%



Sample:	SPIKE-F	Assigned value:	14.57 µg/kg (Reference value)
Measurand	SCOP	Mean value:	13.03 µg/kg
Method:	ISO 5725-5 (Alg. A+S)	Rel. reproducibility s.d.:	15.67%
Number of laboratories in calculation:	32	Rel. target s.d.:	22.00%





9. Evaluation of the questionnaire

The questionnaire distributed to the participants has provided very useful information concerning the approaches and capabilities of the participating laboratories on the determination of tropane alkaloids in tea and herbal infusions.

The questionnaire will be discussed in three sections:

1) the first section will present the outcome of the Yes/No answers regarding the previous experience of the participants and general organisational matters: questions 2-5 and 32-37 of **Annex 9.6**.

2) the second section will deal with the outcome of the Yes/No answers concerning analytical aspects: questions 14-15, 21-22, 26-27, 29-31 and 38 of **Annex 9.6**.

3) the third section will give a more extensive overview of the analytical conditions used by the participants for the determination of tropane alkaloids in tea and herbal products: questions 6-13, 16-20, 24-25 and 28 of **Annex 9.6**.

9.1. Experience and organisational aspects

In Table 7, the number of responses received and the percentage of Yes/No answers regarding the experience of the participants and general organisational matters are compiled. Sixty-seven percent of the participants declared to have prior experience in the analysis of TAs (Q.2). Among them, a vast majority was capable of determining only atropine and scopolamine and the most common matrices were cereals, cereal products (flour, bread, pasta), baby food formulas and animal feeds (Q.3). Five laboratories also declared to analyse TAs in tea and herbal tea. One laboratory extended its analytical scope to about 20 tropane alkaloids, and another could analyse 24 compounds, not specifying which. The experience of the laboratories on the analysis of TAs is relatively

limited. Most have less than two years of experience, with one laboratory mentioning five years of experience. The same number of laboratories (67 %) indicated that they could analyse other plant toxins (Q.4). Fourteen laboratories stated that they can analyse pyrrolizidine alkaloids while three can analyse opium alkaloids and glycoalkaloids. Six laboratories declared to be able to analyse ergot alkaloids, although these fall under the mycotoxin category.

Regarding the satisfaction with the organisational aspects of the PT, the participants were asked to express their opinion on whether the time for reporting the results was adequate (Q.33), the time they spent for analysing the samples, treat the data and issuing the results (Q.35) and whether the amount of test items was sufficient for their needs (Q.34). Eighty-eight percent of the participants found the time for reporting the results (about 6 weeks) as appropriate. Almost all the participants required two or more days to finalise the analytical work. Ninety-four percent of the participants found the amount of sample dispatched (25 g) enough for performing the analysis (Q.34).

Table 7 - Response to the questions related to the experience of the participants on the determination of tropane alkaloids in tea and organisational aspects of the PT

	Q.2	**Q.4**	**Q.14**	**Q.21**	**Q.26**	**Q.27**	**Q.30**	**Q.33**	**Q.34**	**Q.36**	**Q.37**
Response - NO											
Nr.	11	11	19	10	0	2	22	4	2	21	2
%	*33*	*33*	*59*	*30*	*0*	*6*	*71*	*13*	*6*	*78*	*7*
Response - YES											
Nr.	22	22	13	23	33	29	9	28	30	6	25
%	*67*	*67*	*41*	*70*	*100*	*94*	*29*	*88*	*94*	*22*	*93*

Although the participants were asked to submit a great deal of data (both analytical results and answers to the questionnaire) the experience with the RingDat software was positive (Q.34). Seventy-eight percent didn't experience any shortcoming. Still, some comments were received which are compiled below:

- It was not possible to save all the data filled in the fields to answer the questions 11, 12, 13
- Firewall problems
- software very unstable; during the input repeated crashes
- I prefer online forms. Execution of exe-files for non-admins does not always work.
- This time, no problems
- Several crashes when changing window size
- Too long the overall procedure for reporting back the results. The error messages are not in English

About 93 % of the participants found the instructions for performing the PT (**Annex 9.6**) adequate (Q.37). One participant commented that "the spiking part was not obvious to understand immediately" while another noted that the method that was supplied to some laboratories in the past was targeted for the determination of TAs in cereals and not in tea and herbs. Support to analytical issues was always provided to laboratories that requested it. The participants were informed about this PT through different routes, eventually cumulative (Q.32). According to the table below, most participants were informed by direct invitation through the mail from the European Commission CIRCABC database. Many of them were also notified by their respective NRLs or got to know about the PT during the annual EURL Mycotoxins workshop.

Information source about the PT TAs in tea and herbal infusions	%
Through the EURL Mycotoxins website	13
During the EURL workshop for the NRLs on mycotoxins	18
By invitation from the European Commission communication office	32
By the NRL in your country	18
By professional associates in your sector	8
Other	11

9.2. Analytical aspects

The participants in the PT were asked whether the analytical method used for analysing TAs in tea and herbal tea was validated (Q.14, Table 7). About 59 % of the participants replied that they did not perform any assays in that regard. It is important to note that many laboratories have implemented the method just prior to participating in the PT and, therefore, they didn't have enough time to validate the method. Among those who performed the method validation, 80 % included the parameters: precision (mainly repeatability), linearity, LOD and recovery while 90 % also estimated the LOQ. None of them estimated the measurement uncertainty.

Isotope-labelled internal standards for atropine and scopolamine are commercially available and were supplied to the participants along with the test items. Seventy percent of the participants answered that they use isotope dilution MS for quantification (Q.21). The majority of them (70 %) added the internal standards **before the extraction**, 3 % added the internal standards **after the extraction** and 21 % responded as "Non applicable" (Q.22). The first approach provides more benefits as the internal standards can correct the results simultaneously for the biases (e.g. loss) during the extraction step and compensate for the matrix effects that occur during MS analysis.

All the participants have checked the integration of the chromatographic peaks (Q.26) while 94% also assessed the goodness of fit of the calibration curve in the region relevant for the quantification of the samples (Q.27). Additionally, the participants were asked to indicate whether they reported the results **corrected for recoveries** or **not corrected for recoveries** (Q.28). About 74 % of the participants stated that they did not correct the results for the recoveries. Nevertheless, as long as the participants added the internal standards to the samples before the extraction (Q.22) and, assuming they did an internal calibration quantification, then the obtained results were automatically corrected for the recoveries (biases). Taking this information into consideration, only 19 % of the results might have been reported without correction for recoveries.

Regarding the satisfaction of the participants with the experience running the PT, 71% declared that they did not have major difficulties analysing the distributed samples (Q.30). Due to a program bug, it was not possible to compile the type of difficulties the remaining laboratories might have undergone (Q.31). On average, the analyst responsible for conducting the PT had about eight years of experience with LC-MS/MS methods (Q.29).

The participants were given the opportunity to raise general comments about the PT (Q.38). As listed below, the comments concern mainly clarifications related to the analytical protocol that was followed and difficulties posed by the matrices when quantifying scopolamine and atropine. Regarding the latter, it is a general opinion that the black tea sample was more difficult to analyse, sometimes hampering to send results. The analysis of scopolamine was mentioned twice as especially troublesome in these complex matrices.

- it was not possible to submit all the data. After saving, it was deleted automatically.
- we used standard addition for quantification purposes
- For the sample F012, 1g instead of 2g was weighted, in order to achieve measurements inside the region of the calibration curve. Thus, the reported values for S/N correspond to 1g of sample diluted with 29 mL extraction solvent.
- The tea-matrix is difficult for Scopolamine, because there are a few ghost peaks.
- We used the procedural standard calibration which automatically corrects for recovery losses as well as matrix effects
- we had huge problems with black tea and weren't able to report results
- S/N ratio: processing with MassLynx Software; Peak to Peak; number of blank (Sample P, B, F spike) was not given: we called it "1" for P, "2" for B, "3" for F
- The secondary ion for the Internal Std was poor or non-existent for some matrices in particular the fennel.
- We could not achieve repeatable and reproducible results for black tea because of matrix interferences. So we could not report results for black tea. Because we have to enter numbers in the form we increased the limits of detection and quantification and reported 0 as the amount for all samples. We did not determine the recoveries. In the form we entered 0 in all cases. We could not find sample codes on the blank samples used for spiking. So we entered 000 as sample code in the cases of spiked samples.
- Samples were analysed on two occasions: Black and Fennel tea on 27/03/2016, Peppermint tea on 31/03/2016
- Matrix suppression could not be calculated because ISTD was added at the beginning of the Sample prep. Analysis of the black tea sample was more difficult, compared to other samples. They were very extract rich and at times difficult to pass over SPE column. Resulting chromatograms were analysable, nonetheless
- In Black Tea, it was not possible to quantify Scopolamine (due to matrix effects)

9.3. Methods' overview

Along with the analytical results, the participants in this PT also submitted a compilation of some validation figures of merit and a description of core methodological features. In **Annex 9.8.1,** the reported limits of quantification (LOQs), recoveries (%), matrix suppression (MatrixSup, %) and retention times (RT, min) for both atropine and scopolamine are shown. The figures reported for Sample1 B, F and P, were taken as representative for the matrices black tea, fennel and peppermint, respectively. As it can be seen in Figure 6, the vast majority of the reported LOQs for atropine and scopolamine fell below 5.0 µg/kg, with a significant number being also below 1.0 µg/kg. The methodologies employed relied mostly on a fairly simple sample preparation, mainly "dilute and shoot" (**Annex 9.8.2**, Question 8).

The average recoveries considering the 3 matrices were 88 % for atropine and 91 % for scopolamine (see Figure 7), but with significant dispersion among the participants. Given the diversity of extraction methods applied (Question 7: shaking, QuEChERS, different solvent compositions and pH from acidic to alkaline), these figures fall within an acceptable range. Regarding the matrix effects, ionization suppression was mostly observed for both atropine and scopolamine with the instrumental response covering a range from about 20 to 80 %.

An overall overview of the analytical methodologies employed (**Annex 9.8.2**) indicates that nine laboratories applied the EURL-developed method. However, two laboratories deviated slightly, using an analytical column other than the recommended (pentafluorophenyl stationary phase). Five laboratories applied the RIKILT SOP A1070 or the method described in Adamse, P; van Egmond H.P. (2010): Report 2010.011, which follows similar principles. Two laboratories followed the reference: Jandric *et al.,* Food Additives and Contaminants 28 (9) (2011) 1205-1219, which is a QuEChERS-derived method and two other laboratories adopted the BfR-PA-Tee-2.0/2014 method. Fifteen laboratories stated that they used either an in-house developed method or the reference did not allow grouping them in any of the previous categories. All the laboratories used LC-MS/MS for separation and detection. The two most used methods (EURL and RIKILT)

generated equivalent results (not statistically different at a significance level of 0.05). Likewise, the results obtained using the calibration standards supplied by the EURL and those existing in the laboratories were not statistically different.

Figure 6 – Histograms of the methods' LOQs for atropine and scopolamine in tea and herbal tea samples

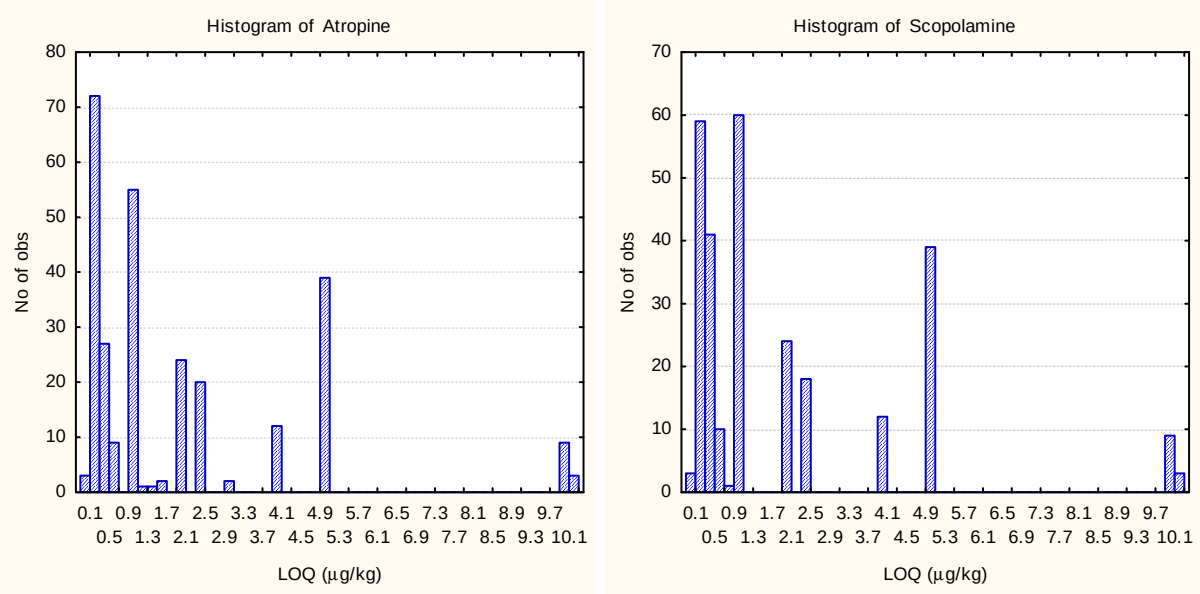
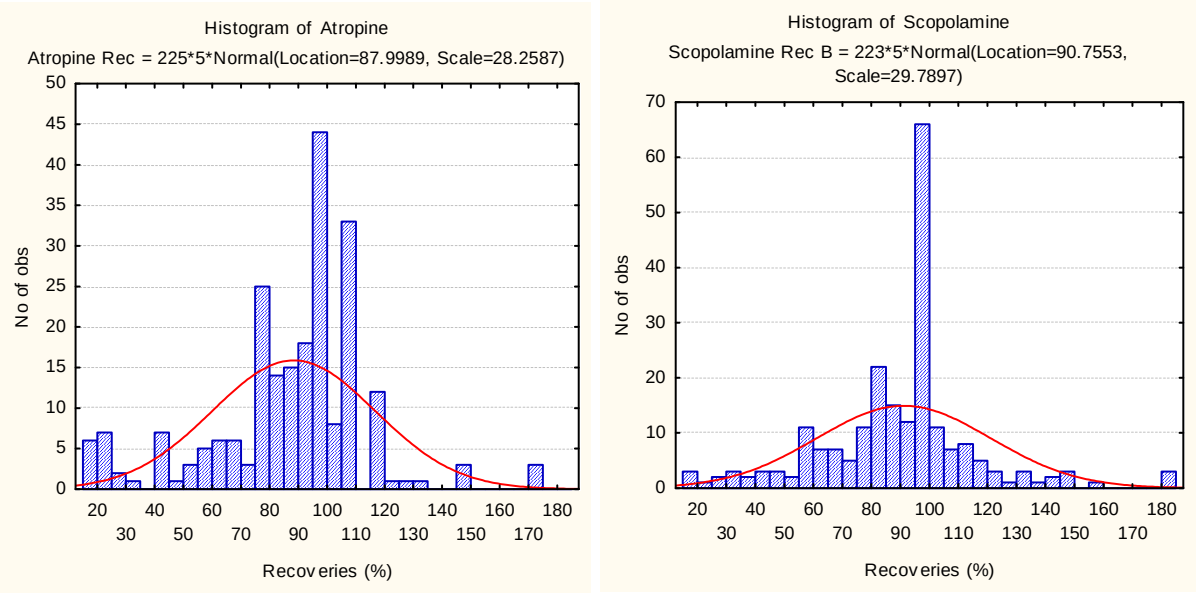


Figure 7 – Histograms of methods' recoveries (%) for atropine and scopolamine in tea and herbal tea samples.



10. Conclusions

On request of DG SANTE, the EURL for Mycotoxins organised a PT aiming to assess the measurement capability of EU Member States' laboratories regarding the determination of tropane alkaloids (atropine and scopolamine) in tea and herbal infusions.

Forty-two laboratories registered for this PT, of which 33 participants representing 11 EU Member States submitted their results. More than half of the participants were German laboratories.

Overall, more than 85 % of the z-scores were in the range of [-2,2], and more than 93 % fell within the range of [-3,3]. For atropine, 87 % of the results fell within the acceptable range ($|z| \leq 2$) while for scopolamine, 84 % of the results were in this range. The performance of the laboratories was, therefore, comparable for both analytes, despite the fact that scopolamine was generally present in much lower concentrations than atropine (lowest level 1.5 µg/kg).

A matrix-wise evaluation of the z-scores for atropine indicated that the success rate ($|z| \leq 2$) was similar in black tea and peppermint (around 85 %) but higher in fennel (91 %). For scopolamine, the rate of acceptable z-scores was the lowest in peppermint (75 %) followed by black tea (83 %) and fennel (94 %). The lowest rate of acceptable z-scores for atropine (83 %) was observed in black tea, which also contained the lowest concentration (8.3 µg/kg). The lowest rate of acceptable z-scores for scopolamine (67 %) was observed in a peppermint sample containing 2.5 µg/kg while other samples with similar concentration (2.3 and 1.5 µg/kg) originated around 81 % acceptable z-scores.

A vast majority of reported LOQs were below 5.0 µg/kg, some of them being even below 1.0 µg/kg. All laboratories used LC-MS/MS in their determinations. Nine participants followed the analytical protocol supplied by the EURL, while two of them used a different analytical column than the recommended one. Five participants used the original or adapted RIKILT SOP A1070. No significantly different results (at the 95 % confidence level) were generated by the two most applied SOPs neither by the use of standards of different origins (supplied by the EURL or the laboratories' standards).

The overall experience of the participants expressed in the questionnaire was very positive, including the organisational, technical and reporting aspects.

The results of the PT support the conclusion that atropine and scopolamine can be reliably determined in tea and herbal infusions at the quantification levels set up in the EU Recommendation 2015/976. A variety of analytical protocols has shown to be adequate for the determination of tropane alkaloids in tea. The laboratories achieved a highly satisfactory performance despite their somewhat short experience in the field. Some had implemented their methods just prior to the PT and underwent limited validation.

References

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[8] ISO 13528:2015; Statistical methods for use in proficiency testing by interlaboratory comparisons.

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List of abbreviations and definitions

EURL	European Union Reference Laboratory
IDMS	Isotope Dilution Mass Spectrometry
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MS	Member States
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PT	Proficiency Test
SOP	Standard operating procedure
SDPA	Standard deviation for proficiency assessment
s.d.	Standard deviation
TA	Tropane alkaloids

9. Annexes

9.1 Opening of registration

PT EU-RL Tropane Alkaloids

Fields marked with * are mandatory.

European Union Reference Laboratory
Mycotoxins

On behalf of the European Union Reference Laboratory for Mycotoxins (EURL Mycotoxins), I have the pleasure to announce the opening for registration to the inter-laboratory comparison/proficiency test on the determination of tropane alkaloids (atropine and scopolamine) in cereals and cereal products as well as herbal infusions and tea. The range set for cereal products is 0.5-20 µg/kg, while the range for tea and herbal infusions is of similar magnitude.

The proficiency test (PT) is open to all laboratories with analytical experience in the field. The participation is free of charge, however the participants number is limited with regards to the test materials available by the organiser. Participation will be granted on a "first come, first serve" basis. The dispatch of samples is expected in the second half of January 2016. Participants will have 6 weeks from the dispatch date to report results.

Confidentiality of results is guaranteed.

In case of interest, please fill in your contact details below. The deadline for registration is the 15th of November 2015.

Thank you in advance for your consideration.

EURL Mycotoxins Operating Manager

*

Which products is your laboratory willing to analyse?

- ☐ Cereal and cereal products
- ☐ Tea and herbal infusions
- ☐ Both

*

Status

- ☐ Official Control Laboratory
- ☐ Official Control Laboratory assigned by the Competent Authority
- ☐ Expert Laboratory with interest in this field

*

Department

*Address

*

City

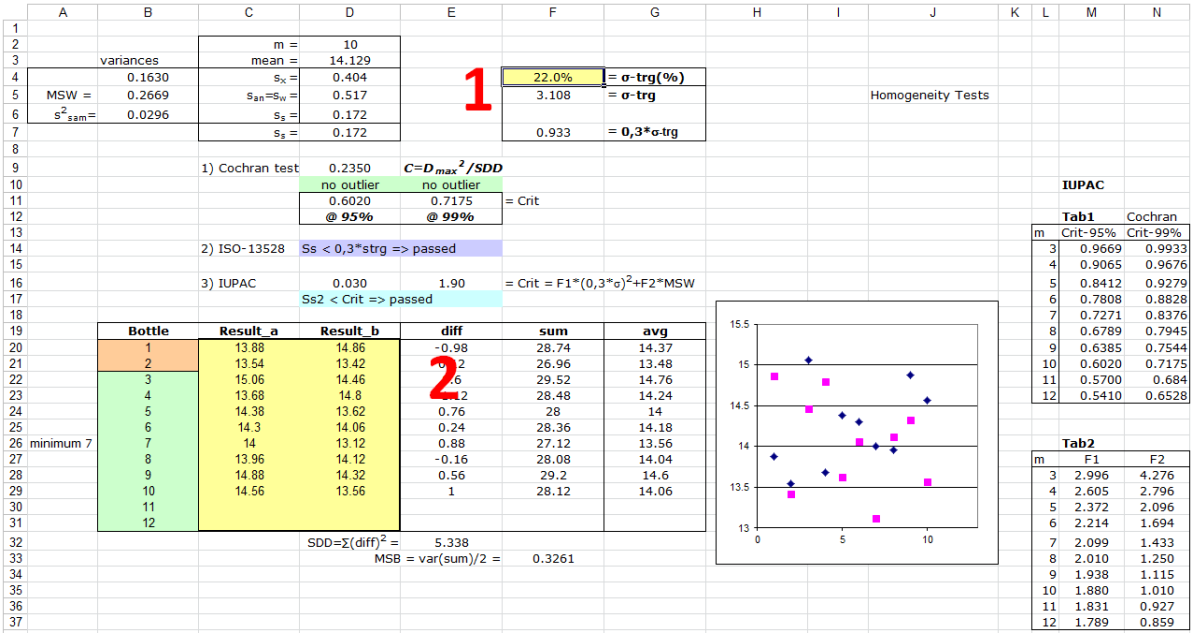
*

Zip Code

*Country

9.2 Homogeneity test

Homogeneity according to ISO 13528:2015	Black tea B001-100	Black tea B001-100	Black tea B101-200	Black tea B101-200
	Atropine	Scopolamine	Atropine	Scopolamine
Mean	14.1	1.77	6.79	7.44
$\hat{\sigma}$	3.11 (22 %)	0.39 (22 %)	1.49 (22 %)	1.64 (22 %)
0.3 $\hat{\sigma}$ (critical value)	0.933	0.117	0.448	0.491
S_X (standard deviation of sample averages)	0.404	0.178	0.371	0.416
S_W (within-sample standard deviation)	0.517	0.255	0.223	0.342
S_S (between-sample standard deviation)	0.172	0.000	0.332	0.339
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**



	A	B	C	D	E	F	G
2			m =	10			
3		variances	mean =	14.129			
4		0.1630	s_X =	0.404		22.0%	= σ-trg(%)
5	MSW =	0.2669	$s_{an}=s_W$ =	0.517		3.108	= σ-trg
6	s^2_{sam}=	0.0296	s_s =	0.172			
7			s_s =	0.172		0.933	= 0,3*σ-trg
9			1) Cochran test	0.2350	$C=D_{max}{}^2/SDD$		
10				no outlier	no outlier		
11				0.6020	0.7175	= Crit	
12				@ 95%	@ 99%		
14			2) ISO-13528	Ss < 0,3*strg => passed			
16			3) IUPAC	0.030	1.90	= Crit = F1*(0,3*σ)²+F2*MSW	
17				Ss2 < Crit => passed			

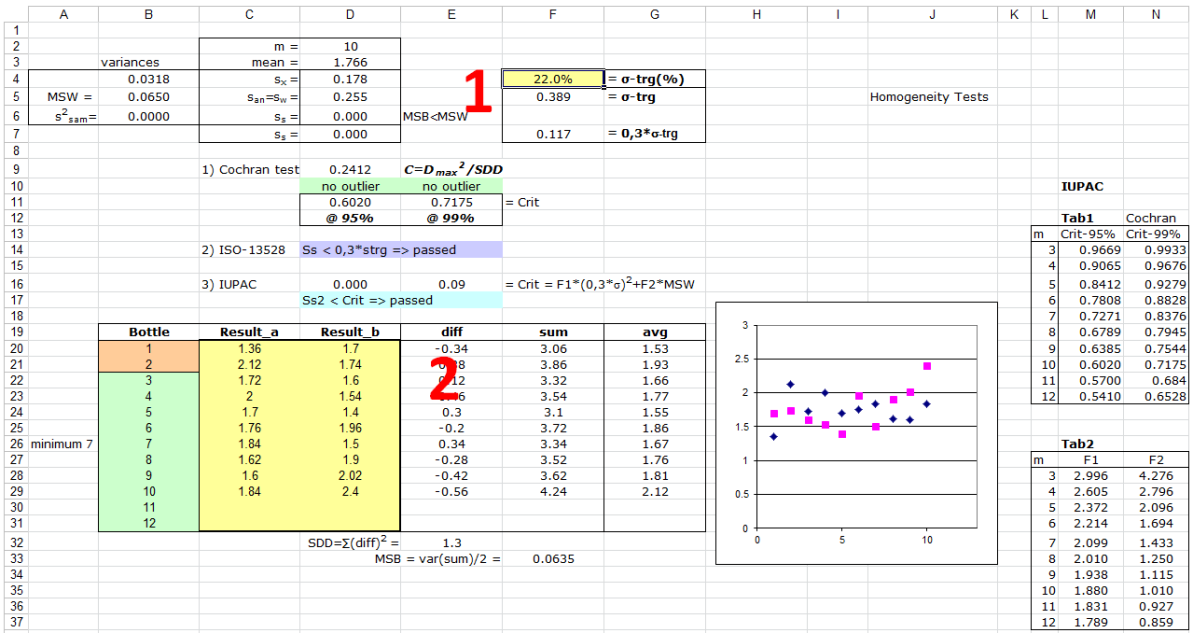
	Bottle	Result_a	Result_b	diff	sum	avg
19						
20	1	13.88	14.86	-0.98	28.74	14.37
21	2	13.54	13.42	0.12	26.96	13.48
22	3	15.06	14.46	0.6	29.52	14.76
23	4	13.68	14.8	-1.12	28.48	14.24
24	5	14.38	13.62	0.76	28	14
25	6	14.3	14.06	0.24	28.36	14.18
26	minimum 7	14	13.12	0.88	27.12	13.56
27	8	13.96	14.12	-0.16	28.08	14.04
28	9	14.88	14.32	0.56	29.2	14.6
29	10	14.56	13.56	1	28.12	14.06
30	11					
31	12					
32			SDD=Σ(diff)² =	5.338		
33			MSB = var(sum)/2 =	0.3261		

Homogeneity Tests

IUPAC

Tab1	Cochran	
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2		
m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859



	A	B	C	D	E	F	G
2			m =	10			
3		variances	mean =	1.766			
4		0.0318	s_X =	0.178		22.0%	= σ-trg(%)
5	MSW =	0.0650	$s_{an}=s_W$ =	0.255		0.389	= σ-trg
6	s^2_{sam}=	0.0000	s_s =	0.000	MSB<MSW		
7			s_s =	0.000		0.117	= 0,3*σ-trg
9			1) Cochran test	0.2412	$C=D_{max}{}^2/SDD$		
10				no outlier	no outlier		
11				0.6020	0.7175	= Crit	
12				@ 95%	@ 99%		
14			2) ISO-13528	Ss < 0,3*strg => passed			
16			3) IUPAC	0.000	0.09	= Crit = F1*(0,3*σ)²+F2*MSW	
17				Ss2 < Crit => passed			

	Bottle	Result_a	Result_b	diff	sum	avg
19						
20	1	1.36	1.7	-0.34	3.06	1.53
21	2	2.12	1.74	0.38	3.86	1.93
22	3	1.72	1.6	0.12	3.32	1.66
23	4	2	1.54	0.46	3.54	1.77
24	5	1.7	1.4	0.3	3.1	1.55
25	6	1.76	1.96	-0.2	3.72	1.86
26	minimum 7	1.84	1.5	0.34	3.34	1.67
27	8	1.62	1.9	-0.28	3.52	1.76
28	9	1.6	2.02	-0.42	3.62	1.81
29	10	1.84	2.4	-0.56	4.24	2.12
30	11					
31	12					
32			SDD=Σ(diff)² =	1.3		
33			MSB = var(sum)/2 =	0.0635		

Homogeneity Tests

IUPAC

Tab1	Cochran	
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2		
m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

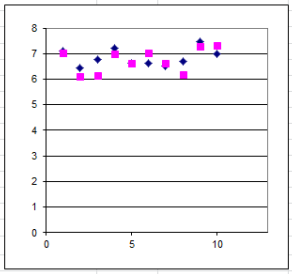
			m =	10										
	variances		mean =	6.790										
	0.1378		s_x =	0.371		22.0%	= σ-trg(%)							
MSW =	0.0545		s_{an}=s_w =	0.233	1	1.494	= σ-trg		Homogeneity Tests					
s^2_{sam}=	0.1105		s_s =	0.332										
			s_s =	0.332		0.448	= 0,3*σ-trg							
		1) Cochran test		0.3528	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.111	0.43	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	7.1	7.04	0.06	14.14	7.07
2	6.44	6.1	0.34	12.54	6.27
3	6.76	6.14	0.62	12.9	6.45
4	7.22	6.98	0.24	14.2	7.1
5	6.64	6.64	0	13.28	6.64
6	6.62	7.02	-0.4	13.64	6.82
minimum 7	6.52	6.62	-0.1	13.14	6.57
8	6.68	6.2	0.48	12.88	6.44
9	7.46	7.3	0.16	14.76	7.38
10	7	7.32	-0.32	14.32	7.16
11					
12					

2

SDD=Σ(diff)² = 1.0896

MSB = var(sum)/2 = 0.2755



IUPAC

	Tab1	Cochran
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

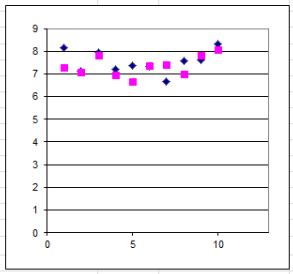
			m =	10										
	variances		mean =	7.440										
	0.1733		s_x =	0.416		22.0%	= σ-trg(%)							
MSW =	0.1169		s_{an}=s_w =	0.342	1	1.637	= σ-trg		Homogeneity Tests					
s^2_{sam}=	0.1148		s_s =	0.339										
			s_s =	0.339		0.491	= 0,3*σ-trg							
		1) Cochran test		0.3313	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.115	0.57	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	8.16	7.28	0.88	15.44	7.72
2	7.12	7.06	0.06	14.18	7.09
3	7.94	7.84	0.1	15.78	7.89
4	7.22	6.96	0.26	14.18	7.09
5	7.36	6.68	0.68	14.04	7.02
6	7.32	7.36	-0.04	14.68	7.34
minimum 7	6.66	7.42	-0.76	14.08	7.04
8	7.56	7	0.56	14.56	7.28
9	7.62	7.84	-0.22	15.46	7.73
10	8.34	8.06	0.28	16.4	8.2
11					
12					

2

SDD=Σ(diff)² = 2.3376

MSB = var(sum)/2 = 0.3466



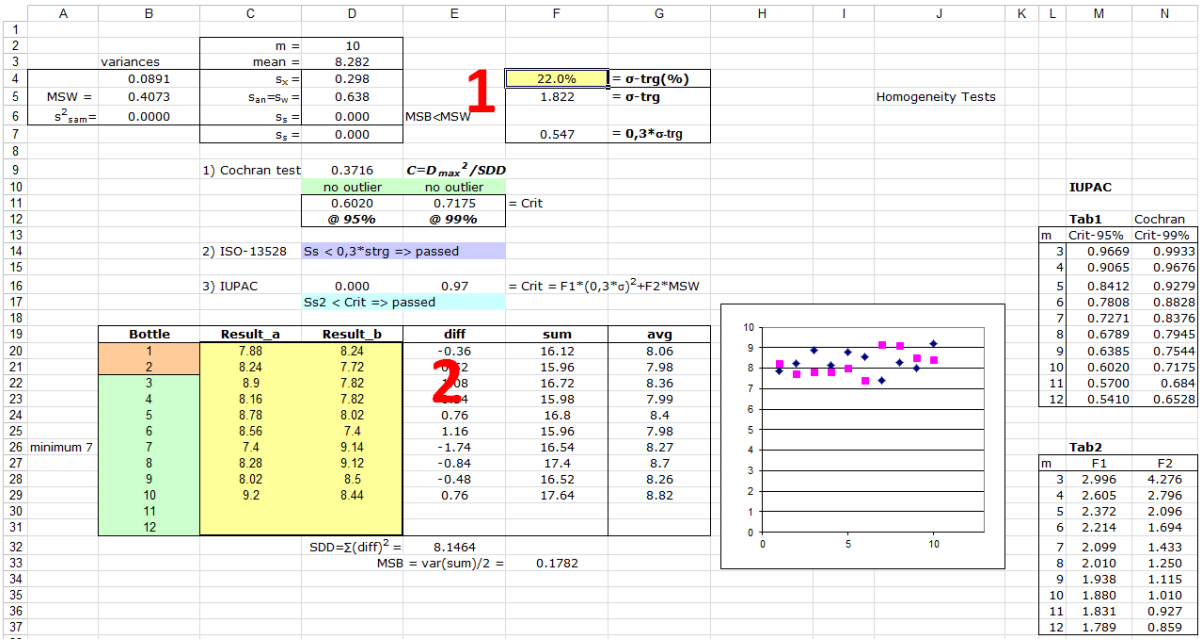
IUPAC

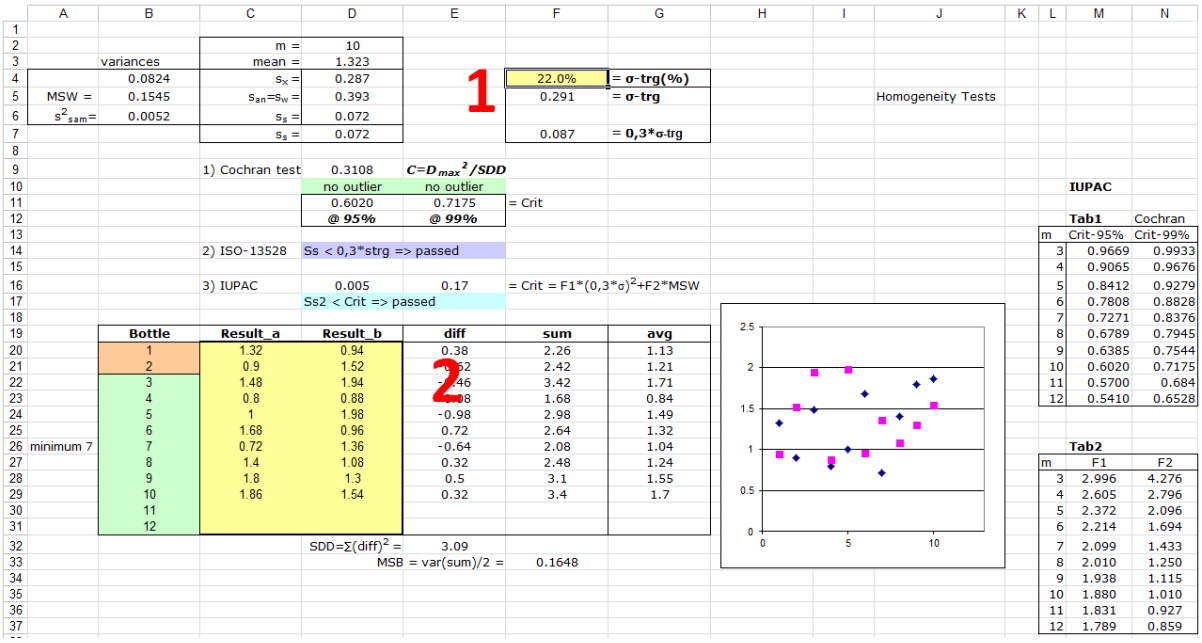
	Tab1	Cochran
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

Homogeneity according to ISO 13528:2015	Peppermint P001-100	Peppermint P001-100	Peppermint P101-200	Peppermint P101-200
	Atropine	Scopolamine	Atropine	Scopolamine
Mean	8.28	1.32	18.24	2.22
$\hat{\sigma}$	1.82 (22 %)	0.29 (22 %)	4.01 (22 %)	0.49 (22 %)
0.3 $\hat{\sigma}$ (critical value)	0.547	0.087	1.204	0.147
S_X (standard deviation of sample averages)	0.289	0.287	0.672	0.345
S_W (within-sample standard deviation)	0.638	0.393	0.731	0.480
S_S (between-sample standard deviation)	0.000	0.072	0.429	0.060
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**





			m =	10											
	variances		mean =	18.238											
	0.4514		s_x =	0.672		1	22.0%	= σ-trg(%)							
MSW =	0.5342		s_{an}=s_w =	0.731			4.012	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.1843		s_s =	0.429											
			s_s =	0.429			1.204	= 0,3*σ-trg							
		1) Cochran test		0.4271	$C=D_{max}^2/SDD$										
				no outlier	no outlier										
				0.6020	0.7175	= Crit									
				@ 95%	@ 99%										
		2) ISO-13528		Ss < 0,3*strg => passed											
		3) IUPAC		0.184	3.26	= Crit = F1*(0,3*σ)^2+F2*MSW									
				Ss2 < Crit => passed											

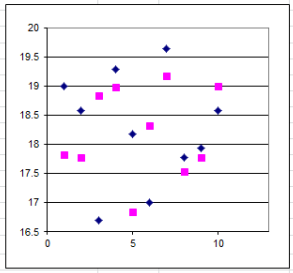
Bottle	Result_a	Result_b	diff	sum	avg
1	19	17.82	1.18	36.82	18.41
2	18.58	17.78	2	36.36	18.18
3	16.704	18.84	-2.136	35.544	17.772
4	19.28	18.98	0.3	38.26	19.13
5	18.18	16.84	1.34	35.02	17.51
6	17.004	18.32	-1.316	35.324	17.662
7	19.64	19.18	0.46	38.82	19.41
8	17.78	17.54	0.24	35.32	17.66
9	17.94	17.78	0.16	35.72	17.86
10	18.58	19	-0.42	37.58	18.79
11					
12					

minimum 7

SDD=Σ(diff)^2 = 10.683552

MSB = var(sum)/2 = 0.9028

2



IUPAC

	Tab1	Cochran
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

			m =	10											
	variances		mean =	2.224											
	0.1190		s_x =	0.345		1	22.0%	= σ-trg(%)							
MSW =	0.2309		s_{an}=s_w =	0.480			0.489	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0036		s_s =	0.060											
			s_s =	0.060			0.147	= 0,3*σ-trg							
		1) Cochran test		0.4124	$C=D_{max}^2/SDD$										
				no outlier	no outlier										
				0.6020	0.7175	= Crit									
				@ 95%	@ 99%										
		2) ISO-13528		Ss < 0,3*strg => passed											
		3) IUPAC		0.004	0.27	= Crit = F1*(0,3*σ)^2+F2*MSW									
				Ss2 < Crit => passed											

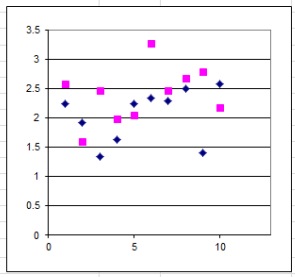
Bottle	Result_a	Result_b	diff	sum	avg
1	2.24	2.58	-0.34	4.82	2.41
2	1.92	1.6	0.32	3.52	1.76
3	1.34	2.46	-1.12	3.8	1.9
4	1.62	1.98	-0.36	3.6	1.8
5	2.24	2.04	0.2	4.28	2.14
6	2.34	3.26	-0.92	5.6	2.8
7	2.28	2.46	-0.18	4.74	2.37
8	2.5	2.68	-0.18	5.18	2.59
9	1.4	2.78	-1.38	4.18	2.09
10	2.58	2.18	0.4	4.76	2.38
11					
12					

minimum 7

SDD=Σ(diff)^2 = 4.6176

MSB = var(sum)/2 = 0.2380

2



IUPAC

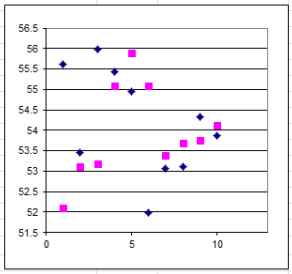
	Tab1	Cochran
m	Crit-95%	Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

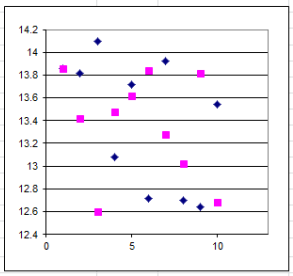
m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

Homogeneity according to ISO 13528:2015	Fennel F001-100	Fennel F001-100	Fennel F101-200	Fennel F101-200
	Atropine	Scopolamine	Atropine	Scopolamine
Mean	54.1	13.4	24.2	21.0
$\hat{\sigma}$	11.9 (22 %)	2.94 (22 %)	5.32 (22 %)	4.62 (22 %)
0.3 $\hat{\sigma}$ (critical value)	3.568	0.883	1.596	1.387
S_X (standard deviation of sample averages)	0.793	0.299	0.740	0.576
S_W (within-sample standard deviation)	1.263	0.569	0.847	0.629
S_S (between-sample standard deviation)	0.000	0.000	0.434	0.366
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2			m =	10										
3		variances	mean =	54.063										
4		0.6290	s_X =	0.793		22.0%	= σ-trg(%)							
5	MSW =	1.5950	s_{an}=s_W =	1.263	1	11.894	= σ-trg			Homogeneity Tests				
6	s^2_{sam}=	0.0000	s_S =	0.000	MSB<MSW									
7			s_S =	0.000		3.568	= 0,3*σ-trg							
8														
9			1) Cochran test	0.3884	$C=D_{max}^2/SDD$									
10				no outlier	no outlier								IUPAC	
11				0.6020	0.7175	= Crit							Tab1	Cochran
12				@ 95%	@ 99%							m	Crit-95%	Crit-99%
13												3	0.9669	0.9933
14			2) ISO-13528	Ss < 0,3*strg => passed								4	0.9065	0.9676
15												5	0.8412	0.9279
16			3) IUPAC	0.000	25.55	= Crit = F1*(0,3*σ)^2+F2*MSW						6	0.7808	0.8828
17				Ss2 < Crit => passed								7	0.7271	0.8376
18												8	0.6789	0.7945
19		Bottle	Result_a	Result_b	diff	sum	avg					9	0.6385	0.7544
20		1	55.62	52.1	3.52	107.72	53.86					10	0.6020	0.7175
21		2	53.46	53.12	0.34	106.58	53.29					11	0.5700	0.684
22		3	55.98	53.18	2.8	109.16	54.58					12	0.5410	0.6528
23		4	55.44	55.1	0.34	110.54	55.27							
24		5	54.96	55.9	-0.94	110.86	55.43						Tab2	
25		6	51.98	55.1	-3.12	107.08	53.54					m	F1	F2
26	minimum 7	7	53.06	53.38	-0.32	106.44	53.22					3	2.996	4.276
27		8	53.12	53.68	-0.56	106.8	53.4					4	2.605	2.796
28		9	54.34	53.76	0.58	108.1	54.05					5	2.372	2.096
29		10	53.86	54.12	-0.26	107.98	53.99					6	2.214	1.694
30		11										7	2.099	1.433
31		12										8	2.010	1.250
32				SDD=Σ(diff)^2 =	31.8996							9	1.938	1.115
33				MSB = var(sum)/2 =	1.2580							10	1.880	1.010
34												11	1.831	0.927
35												12	1.789	0.859
36														
37														



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2			m =	10										
3		variances	mean =	13.386										
4		0.0896	s_X =	0.299		22.0%	= σ-trg(%)							
5	MSW =	0.3239	s_{an}=s_W =	0.569	1	2.945	= σ-trg			Homogeneity Tests				
6	s^2_{sam}=	0.0000	s_S =	0.000	MSB<MSW									
7			s_S =	0.000		0.883	= 0,3*σ-trg							
8														
9			1) Cochran test	0.3473	$C=D_{max}^2/SDD$									
10				no outlier	no outlier								IUPAC	
11				0.6020	0.7175	= Crit							Tab1	Cochran
12				@ 95%	@ 99%							m	Crit-95%	Crit-99%
13												3	0.9669	0.9933
14			2) ISO-13528	Ss < 0,3*strg => passed								4	0.9065	0.9676
15												5	0.8412	0.9279
16			3) IUPAC	0.000	1.79	= Crit = F1*(0,3*σ)^2+F2*MSW						6	0.7808	0.8828
17				Ss2 < Crit => passed								7	0.7271	0.8376
18												8	0.6789	0.7945
19		Bottle	Result_a	Result_b	diff	sum	avg					9	0.6385	0.7544
20		1	13.86	13.86	0	27.72	13.86					10	0.6020	0.7175
21		2	13.82	13.42	0.4	27.24	13.62					11	0.5700	0.684
22		3	14.1	12.6	1.5	26.7	13.35					12	0.5410	0.6528
23		4	13.08	13.48	-0.4	26.56	13.28							
24		5	13.72	13.62	0.1	27.34	13.67						Tab2	
25		6	12.72	13.84	-1.12	26.56	13.28					m	F1	F2
26	minimum 7	7	13.92	13.28	0.64	27.2	13.6					3	2.996	4.276
27		8	12.7	13.02	-0.32	25.72	12.86					4	2.605	2.796
28		9	12.64	13.82	-1.18	26.46	13.23					5	2.372	2.096
29		10	13.54	12.68	0.86	26.22	13.11					6	2.214	1.694
30		11										7	2.099	1.433
31		12										8	2.010	1.250
32				SDD=Σ(diff)^2 =	6.4784							9	1.938	1.115
33				MSB = var(sum)/2 =	0.1793							10	1.880	1.010
34												11	1.831	0.927
35												12	1.789	0.859
36														
37														



			m =	10										
	variances		mean =	24.177										
	0.5470		s_x =	0.740		22.0%	= σ-trg(%)							
MSW =	0.7170		s_{an}=s_w =	0.847	1	5.319	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.1885		s_s =	0.434										
			s_s =	0.434		1.596	= 0,3*σ-trg							
		1) Cochran test		0.4714	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.189	5.51	= Crit = F1*(0,3*σ)^2+F2*MSW								
				Ss2 < Crit => passed										

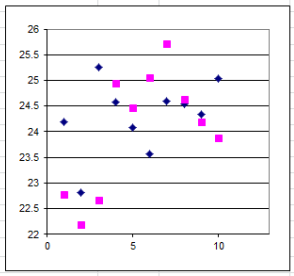
Bottle	Result_a	Result_b	diff	sum	avg
1	24.2	22.78	1.42	46.98	23.49
2	22.82	22.18	0.64	45	22.5
3	25.26	22.66	2.6	47.92	23.96
4	24.58	24.94	-0.36	49.52	24.76
5	24.08	24.46	-0.38	48.54	24.27
6	23.56	25.06	-1.5	48.62	24.31
7	24.6	25.72	-1.12	50.32	25.16
8	24.54	24.64	-0.1	49.18	24.59
9	24.34	24.2	0.14	48.54	24.27
10	25.04	23.88	1.16	48.92	24.46
11					
12					

minimum 7

2

SDD=Σ(diff)^2 = 14.3396

MSB = var(sum)/2 = 1.0940



IUPAC

m	Tab1 Crit-95%	Cochran Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

			m =	10										
	variances		mean =	21.017										
	0.3319		s_x =	0.576		22.0%	= σ-trg(%)							
MSW =	0.3952		s_{an}=s_w =	0.629	1	4.624	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.1343		s_s =	0.366										
			s_s =	0.366		1.387	= 0,3*σ-trg							
		1) Cochran test		0.3079	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.134	4.02	= Crit = F1*(0,3*σ)^2+F2*MSW								
				Ss2 < Crit => passed										

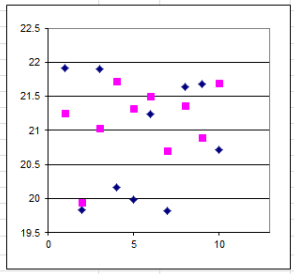
Bottle	Result_a	Result_b	diff	sum	avg
1	21.92	21.26	0.66	43.18	21.59
2	19.84	19.94	-0.1	39.78	19.89
3	21.9	21.04	0.86	42.94	21.47
4	20.16	21.72	-1.56	41.88	20.94
5	19.98	21.32	-1.34	41.3	20.65
6	21.24	21.5	-0.26	42.74	21.37
7	19.82	20.7	-0.88	40.52	20.26
8	21.64	21.36	0.28	43	21.5
9	21.68	20.9	0.78	42.58	21.29
10	20.72	21.7	-0.98	42.42	21.21
11					
12					

minimum 7

2

SDD=Σ(diff)^2 = 7.9036

MSB = var(sum)/2 = 0.6638



IUPAC

m	Tab1 Crit-95%	Cochran Crit-99%
3	0.9669	0.9933
4	0.9065	0.9676
5	0.8412	0.9279
6	0.7808	0.8828
7	0.7271	0.8376
8	0.6789	0.7945
9	0.6385	0.7544
10	0.6020	0.7175
11	0.5700	0.684
12	0.5410	0.6528

Tab2

m	F1	F2
3	2.996	4.276
4	2.605	2.796
5	2.372	2.096
6	2.214	1.694
7	2.099	1.433
8	2.010	1.250
9	1.938	1.115
10	1.880	1.010
11	1.831	0.927
12	1.789	0.859

9.3 Stability study

Sample - Black tea B101-200

	Scopolamine				Atropine			
T (ºC)	Slope	Lower 95 % *	Upper 95 % *	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
-18	-0.00815	-0.02352	0.00722	**YES**	-0.00293	-0.01297	0.00711	**YES**
4	-0.00193	-0.01904	0.01518	**YES**	0.00351	-0.01957	0.02660	**YES**
20	-0.00426	-0.02207	0.01356	**YES**	0.00215	-0.01234	0.01665	**YES**

* Upper and lower intervals of the regression slope at 95 % confidence level.

Sample – Peppermint P101-200

	Scopolamine				Atropine			
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
-18	0.00167	-0.01292	0.01626	**YES**	-0.01143	-0.04052	0.01767	**YES**
4	-0.00266	-0.01592	0.01059	**YES**	0.00318	-0.04145	0.04781	**YES**
20	0.00185	-0.00768	0.01139	**YES**	-0.01804	-0.06513	0.02904	**YES**

Sample – Fennel F101-200

	Scopolamine				Atropine			
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
-18	0.00906	-0.02618	0.04430	**YES**	0.00793	-0.02935	0.04521	**YES**
4	0.00639	-0.01918	0.03197	**YES**	-0.01848	-0.04055	0.00359	**YES**
20	-0.01946	-0.04355	0.00463	**YES**	-0.08208	-0.11278	-0.05138	**NO**

9.4 Accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Ref. ARES(2016)975941 - 25/02/2016

Geel, 22nd of February 2016

2016 PROFICIENCY TESTING FOR ALL COMPETENT LABORATORIES AND EXPERT LABORATORIES REGARDING THE DETERMINATION OF TROPANE ALKALOIDS IN TEA AND HERBAL INFUSIONS

Dear Participant,

Please read the following information carefully before starting any analysis. If doubts remain, do not hesitate to contact us either by phone or e-mail (see details at end of this doc.).

Please confirm the receipt of the parcel by e-mail immediately upon arrival, by using the "**Materials Receipt Form**" that is enclosed. If some test material is damaged, please request new material immediately.

The materials are shipped cooled. After receipt transfer the samples immediately to -18°C until the analysis is performed. Begin the analysis as soon as possible.

The 2016 EURL PT on Tropane Alkaloids (Tea and Herbal Infusions) aims to assess the content of six contaminated test samples (2 samples P#, 2 samples B# and 2 samples F#) on atropine and scopolamine. Additionally, you will receive 3 blank samples (1 per matrix: peppermint, black tea and fennel seeds) to be spiked with the corresponding spiking solutions.

You will be asked to report their **concentration in µg kg^{-1}**. Then, in the Questionnaire please **mention whether the results WERE CORRECTED for recoveries OR NOT and provide the recoveries in the "Measured values" table (in %)**.

Additional information (analytical and instrumental details) will be asked to enable us to interpret methodological trends and therefore allow the deepest insight in laboratory independent method-related aspects. As the presence of tropane alkaloids in food is expected to be regulated in the European Union shortly, we count with your cooperation.

The standard solutions provided (containing tropane alkaloids - TA mix, and their isotopologues - ISTD mix) can be used at your discretion if they fit well the procedure that you have already implemented. Before starting the analysis please allow the samples and standard solutions to reach room temperature. Please shake the solutions and homogenise the test materials with a spatula, as segregation might have occurred during transport.

For the spiking experiments (3 matrices), please do the following:
a) weigh your sample intake (e.g: 2 g of Black Tea BLANK)
b) add 100 µL of the spiking solution per each gram weighed (in this case, 200 µL Spiking solution Black Tea)
c) mix the sample and let stand open at least overnight allowing the solution to evaporate
d) analyse according to your protocol

IMPORTANT: before spiking, please ensure that the blank samples are free of interferences under your analytical conditions, or otherwise the results have to be compensated accordingly.

All solutions were prepared in a mixture of ACN:H_2O 50:50 + 0.1% Formic acid

Reporting the results and Questionnaire

Data generated by the participants will be collected by using the software RingDat, supplementary to ProLab software, that has been used for professional data handling and statistical analyses of interlaboratory tests results. You should have received two files attached to this email for reporting the results. The instructions on how to use the software RingDat can be found in Annex at the end of this document.

Please report the samples results in µg kg^{-1}, to the closest 0.1 µg kg^{-1}. Please provide also additional method details and performance parameters as requested in the relevant Table and the Questionnaire. E.g. S/N ratio of peak signals (as peak-to-peak, instead of RMS), LOQs, MRM transitions, chromatographic conditions, etc.

The deadline for reporting the PT results is the 04th of April 2016.

Given the tight time schedule that we are obliged to comply with, **an extension of the deadline for reporting the results cannot be granted**

In case you need assistance, please do not hesitate to contact us as soon as possible.

Good luck and most of all, success with the analysis!

With kind regards,

Carlos Gonçalves
(on behalf of the Operating Manager of the EU-RL Mycotoxins)

Tel: +32-14-571823 / Fax: +32-14-571 783
E-mail: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

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

Annex: Instructions for reporting the results using RingDat.

1. Download a simple data entry program (called RingDat) free from the QuoData web page using following link: http://quodata.de/ringdat_en.php
User: *ringdat*
Password: *prolabdata*

2. Save the two lab specific files with the extension **"*.Lab"** and **"*.LA2"**, generated by the ProLab software and provided to each individual laboratory (personal files attached to this email) to the same folder as RingData.exe.

3. Start the RingDat.exe program and open **"*.LAB"** file for reporting the results. A table will appear with cells for every measurand/sample combination

- the name of each laboratory and the samples are codified by the software, so that each participant will receive samples with unique codified numbers (i.e., F058);
- in the column "Sample Code" please enter just the number of the sample as the corresponding letter (B, P or F) is already assigned in the column "Sample name"
- The **"*.LA2"** file contains information about the participant – laboratory name and laboratory code;
- The **"*.LAB"** file is unique to each laboratory (personal) and contains information about the samples and measurand that have to be analysed and reported.
- The first tab contains detailed information for the laboratory
- The second tab contains a table for entering the results.
- The third tab contains a general questionnaire.

4. Fill in the results table (Measured values) with your data. Please find below some captures of the RingDat pages that have been configured for this PT.

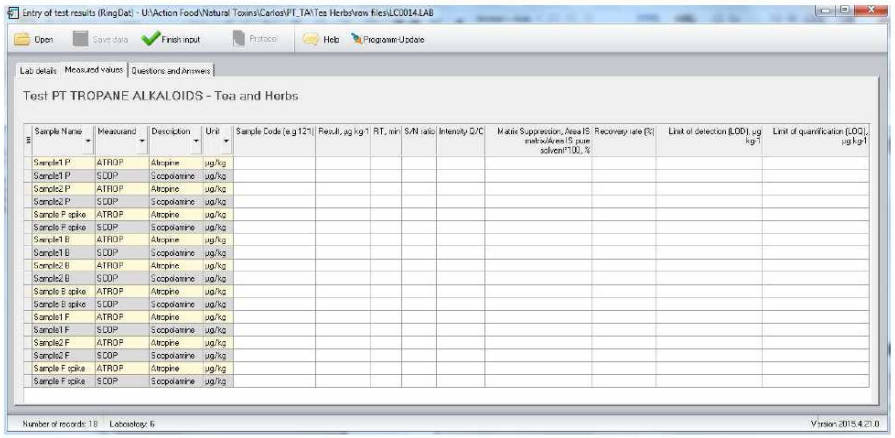


Figure 1 – Capture of the "Measured Values" page

5. Afterwards, please fill in the questionnaire on the next tab.

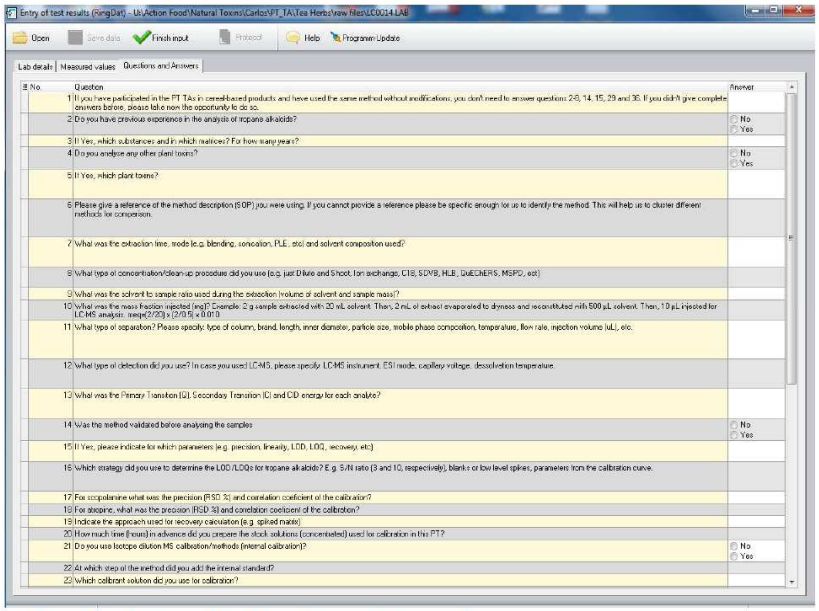


Figure 2 – Capture of the "Questions and Answers" page

6. After finishing the input, Save the file using the button on the top menu of the window. You can change the inputs after saving the file as long as you haven't pushed "Finish input" button. At the end finalise the data entry by pressing the "Finish input" button.

7. Send both the **"*.LAB"** and **"*.LA"** files back to us by e-mail to our functional mail box - JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

8. Should you want **to correct some of your entries after finishing the input, you must use the original *.LAB file** downloaded from the email and introduce all the information again.

9.5 Acknowledgement of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Geel, 19th of February 2016

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Address:
Member State:

NOTE: STORE ALL MATERIALS IN A FREEZER AT -18 °C!

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

Date of receipt	
Samples' numbers (e.g. F023, P118, etc)	
All items have been received undamaged	YES / NO
If NO, please list damaged items:	

Contents of the parcel:

a) **Nine** test materials for analysis packed in ambar bottles:
- 2 Samples B#, 2 Samples P# and 2 Samples F#
- Peppermint Blank, Black tea Blank and Fennel seeds Blank

b) **Five** ambar glass ampoules
- Isotope labelled Internal Standard solution (ISTD mix)
- Tropane Alkaloids Standard solution (TA mix)
- Spiking sol. Peppermint / Spiking sol. Black tea / Spiking sol. Fennel seeds

c) A bag containing the following documents:
- This materials **receipt** form
- Copy of **instructions**

Your Signature / Stamp here:

Please sign this completed form and e-mail it to:

Carlos GONÇALVES

E-mail: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

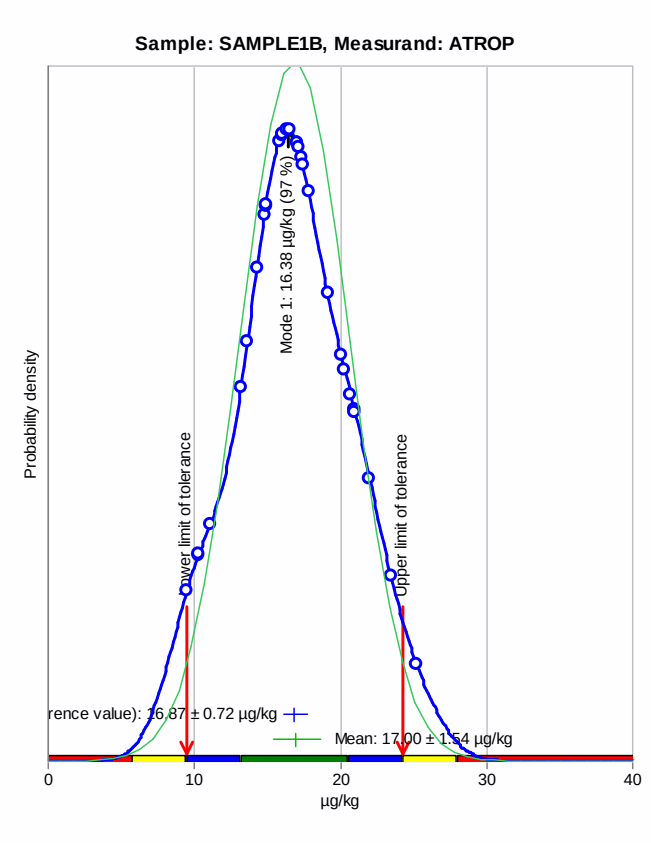
E-mail: jrc-irmm-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

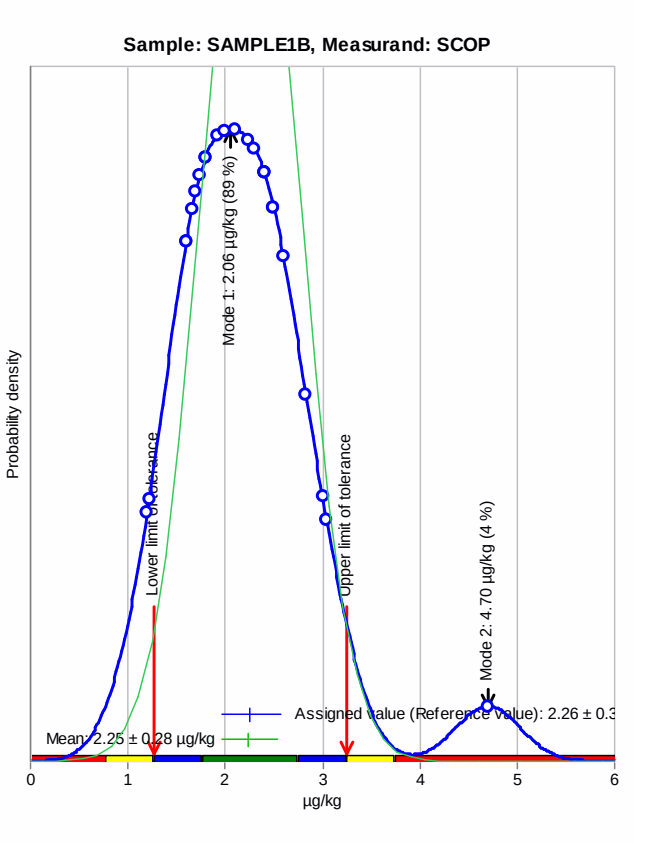
9.6 Questionnaire

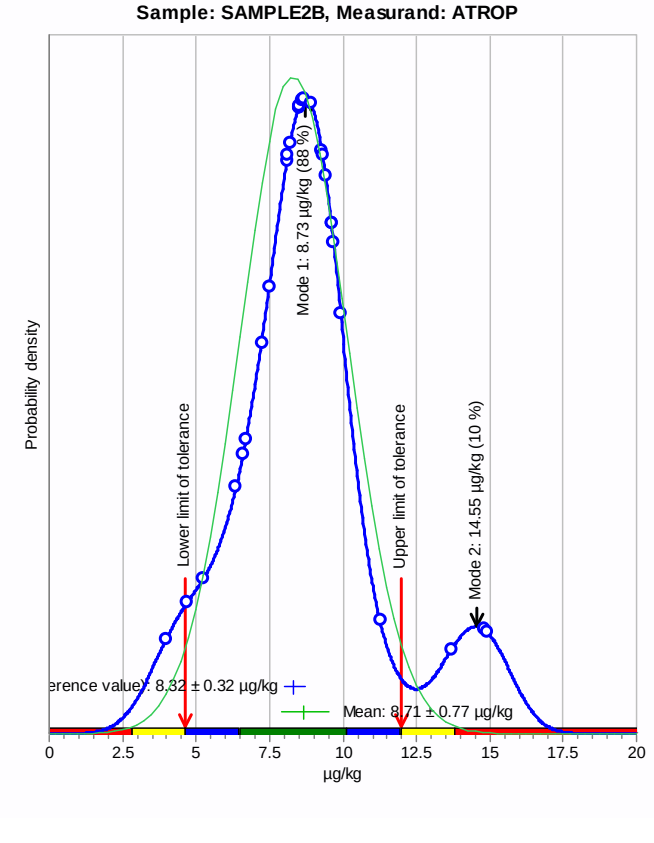
Ring test : PT TROPANE ALKALOIDS - Tea and Herbs (38 questions, 862 answers)

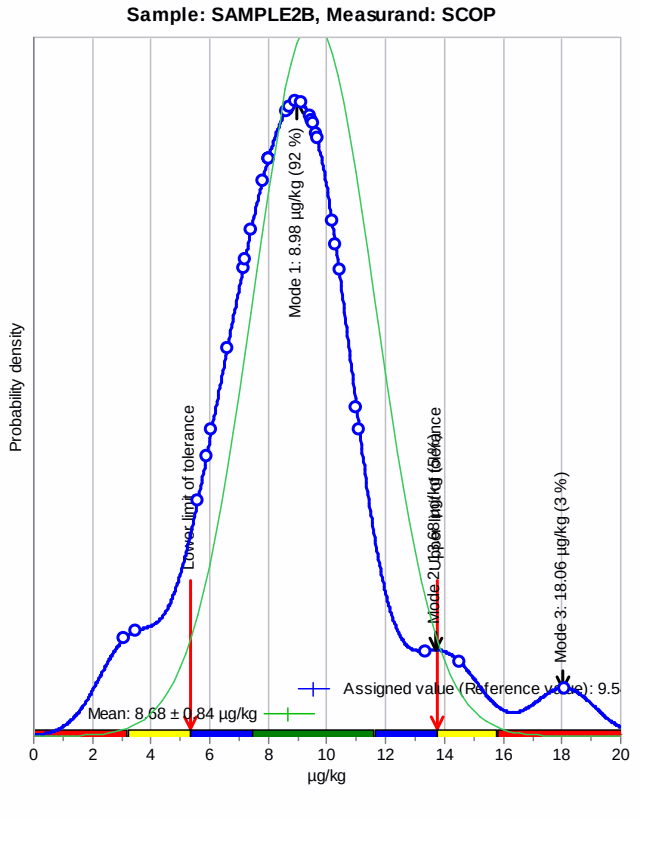
1	Information	If you have participated in the PT TAs in cereal-based products and have used the same method without modifications, you don't need to answer questions 2-8, 14, 1...	10	10 Answers	TextEdit
2	Previous experience	Do you have previous experience in the analysis of tropane alkaloids?	11	12 Answers	TextEdit
3	Please specify experience	If Yes, which substances and in which matrices? For how many years?	12	7 Answers	TextEdit
4	Other plant toxins	Do you analyse any other plant toxins?	13	13 Answers	TextEdit
5	If Yes, which plant toxins	If Yes, which plant toxins?	14	8 Answers	TextEdit
6	Reference of the SOP used	Please give a reference of the method description (SOP) you were using. If you cannot provide a reference please be specific enough for us to identify the method. T...	15	10 Answers	TextEdit
7	Extraction details	What was the extraction time, mode (e.g. blending, sonication, PLE, etc) and solvent composition used?	16	9 Answers	TextEdit
8	Concentration/clean-up	What type of concentration/clean-up procedure did you use (e.g. just Dilute and Shoot, Ion exchange, C18, SDVB, HLB, QuEChERS, MSPD, ect)	17	10 Answers	TextEdit
9	Solvent to sample ratio	What was the solvent to sample ratio used during the extraction (volume of solvent and sample mass)?	18	31 Answers	TextEdit
10	Mass fraction injected	What was the mass fraction injected (mg)? Example: 2 g sample extracted with 20 mL solvent. Then, 2 mL of extract evaporated to dryness and reconstituted with 50...	19	29 Answers	TextEdit
11	Type of separation	What type of separation? Please specify: type of column, brand, length, inner diameter, particle size, mobile phase composition, temperature, flow rate, injection volum...	20	32 Answers	TextEdit
12	Type of detection	What type of detection did you use? In case you used LC-MS, please specify: LC-MS instrument, ESI mode, capillary voltage, dessolvation temperature.	21	32 Answers	TextEdit
13	Transitions, ratio and CID	What was the Primary Transition (Q), Secondary Transition (C) and CID energy for each analyte?	22	32 Answers	TextEdit
14	Method validation	Was the method validated before analysing the samples	23	22 Answers	TextEdit
15	If Yes, please describe	If Yes, please indicate for which parameters (e.g. precision, linearity, LOD, LOQ, recovery, etc)	24	9 Answers	TextEdit
16	Strategy used for LOD/LOQs	Which strategy did you use to determine the LOD/LOQs for tropane alkaloids? E.g. S/N ratio (3 and 10, respectively), blanks or low level spikes, parameters from the c...	25	30 Answers	TextEdit
17	Performance parameters SCOP	For scopolamine what was the precision (RSD %) and correlation coeficient of the calibration?	26	28 Answers	TextEdit
18	Performance parameters ATROP	For atropine, what was the precision (RSD %) and correlation coeficient of the calibration?	27	28 Answers	TextEdit
19	Recovery calculation	Indicate the approach used for recovery calculation (e.g. spiked matrix)	28	30 Answers	TextEdit
20	Stock solution preparation	How much time (hours) in advance did you prepare the stock solutions (concentrated) used for calibration in this PT?	29	22 Answers	TextEdit
21	Isotope dilution MS	Do you use Isotope dilution MS calibration/methods (internal calibration)?	30	32 Answers	TextEdit
22	Internal standard addition	At which step of the method did you add the internal standard?	31	30 Answers	TextEdit
23	Calibrant solution	Which calibrant solution did you use for calibration?	32	29 Answers	TextEdit
24	Solvent of the calibrants	In case you have used your own calibrants, in which solvent composition they were prepared? Which conc. do you measure for the calibration standard we delivere...	7	15 Answers	Memo
25	Approach for calibration	Which approach did you use for calibration?	33	29 Answers	TextEdit
26	Peaks integration	Did you check the peaks intregration?	34	32 Answers	TextEdit
27	Goodness of fit	Did you check the goodness of fit of the calibration curve in the region relevant for quantification of the samples	35	30 Answers	TextEdit
28	Results reported	Were the results reported:	36	30 Answers	TextEdit
29	Analyst's experience	How many years of experience does the analyst have with LC-MS/MS techniques?	1	17 Answers	TextEdit
30	Difficulties	Did you have major dificulties analysing the distributed samples?	3	30 Answers	RadioGroup
31	If Yes, describe difficulties	If Yes, please specifcy which? e.g sensitivity of the instrument, pumps pressure, chromatographic resolution, tedious sample preparation, complex matrix, purchase o...	37		TextEdit
32	PT information	How were you informed about this Proficiency Test in tropane alkaloids in cereals, cereal products?	38	29 Answers	TextEdit
33	Time for reporting	Was the time allowed for reporting the results adequate?	8	31 Answers	RadioGroup
34	Sample amount	Was the sample amount dispactched suficient for the analyses?	6	31 Answers	RadioGroup
35	Time spent	How much time did you spend overall to analyse the samples, treat data and report?	9	27 Answers	ComboBox
36	ProLab/RingDat platform	Did you have any problems using the ProLab/RingDat platform for results reporting? If Yes, describe which?	5	19 Answers	Memo
37	Instructions	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	4	26 Answers	Memo
38	Any other comments	Any other comments you wish to address?	2	21 Answers	Memo

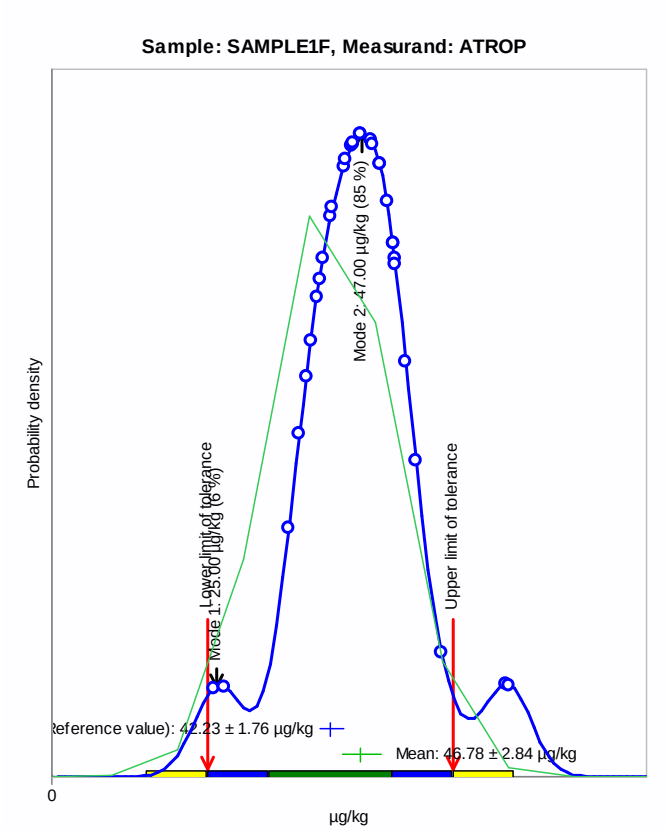
9.7 Kernel density plots

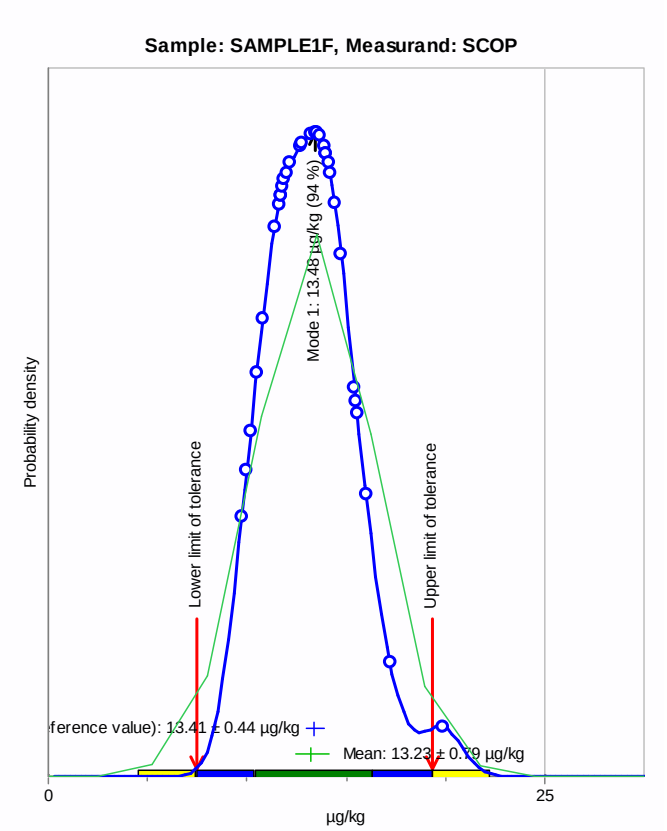


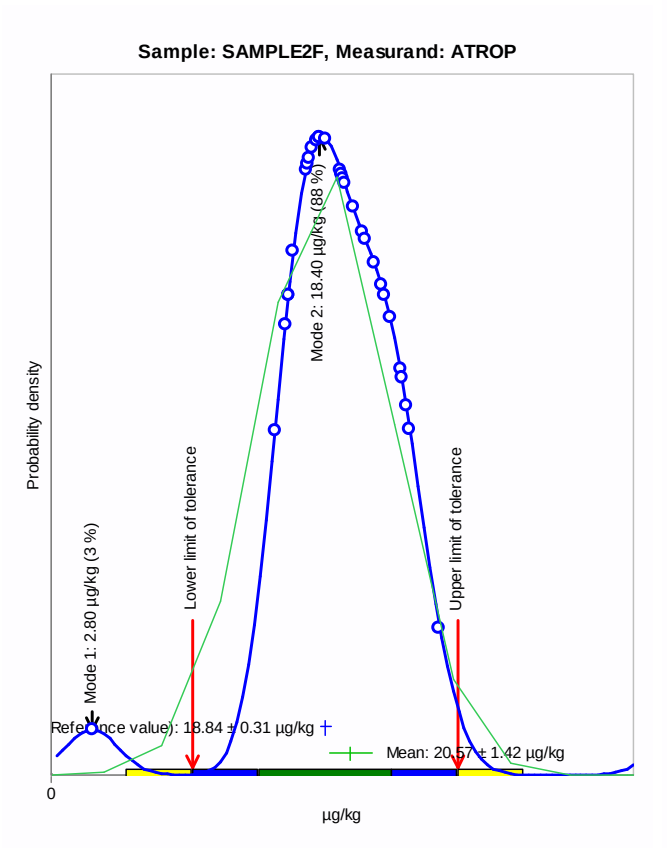


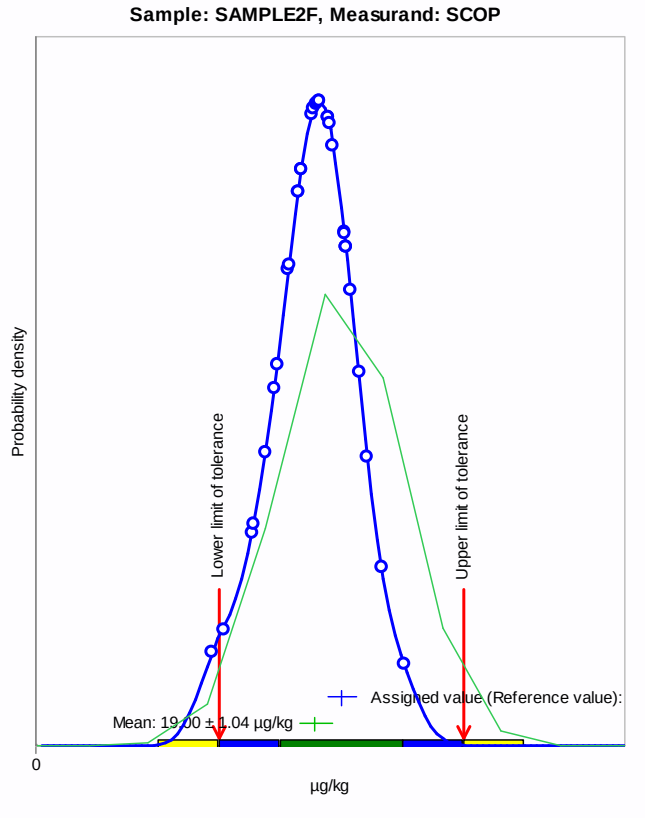


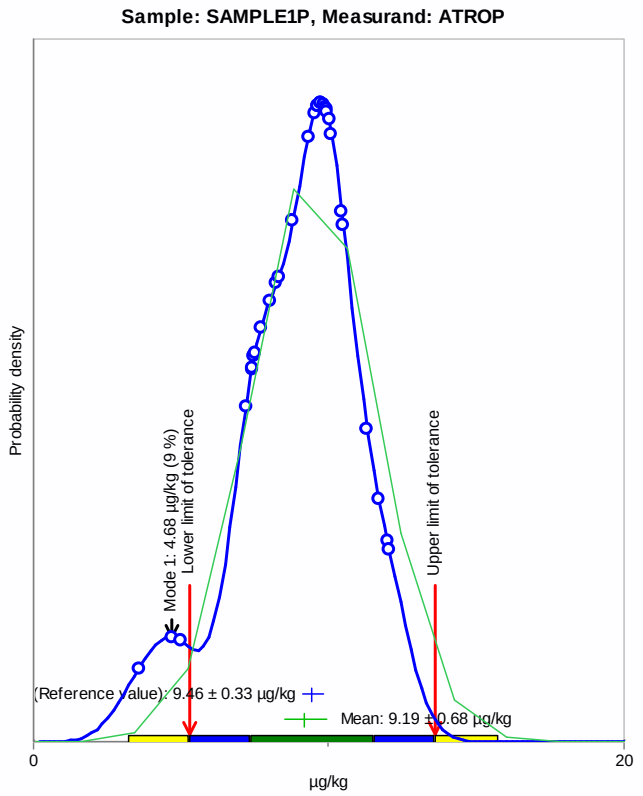


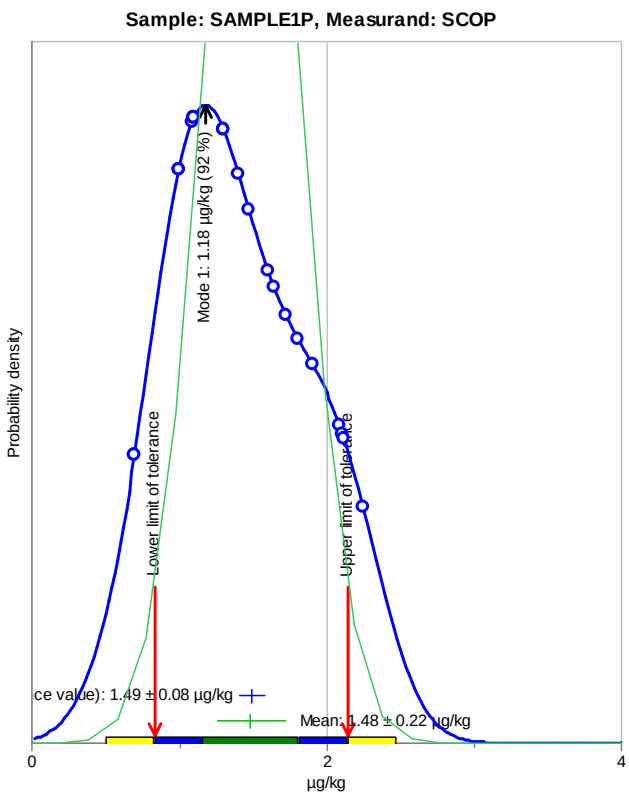

Sample: SAMPLE1F, Measurand: ATROP
Probability density
Mode 2: 47.00 µg/kg (85 %)
Mode 1: 25.00 µg/kg (6 %)
Lower limit of tolerance
Upper limit of tolerance
eference value): 42.23 ± 1.76 µg/kg
Mean: 46.78 ± 2.84 µg/kg
0
µg/kg

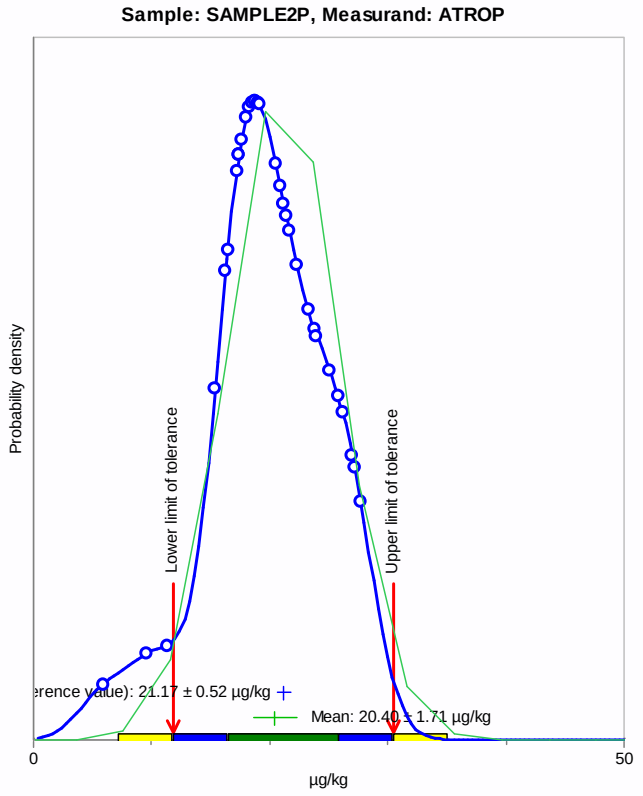

Sample: SAMPLE1F, Measurand: SCOP
Probability density
Mode 1: 13.48 µg/kg (94 %)
Lower limit of tolerance
Upper limit of tolerance
ference value): 13.41 ± 0.44 µg/kg
Mean: 13.23 ± 0.79 µg/kg
0
25
µg/kg

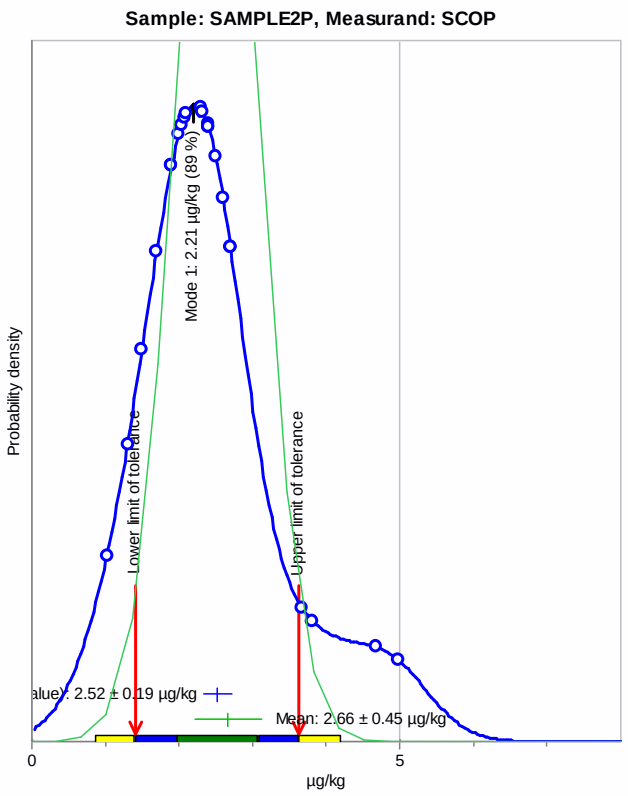

Sample: SAMPLE2F, Measurand: ATROP
Probability density
Mode 2: 18.40 µg/kg (88 %)
Mode 1: 2.80 µg/kg (3 %)
Lower limit of tolerance
Upper limit of tolerance
Reference value): 18.84 ± 0.31 µg/kg
Mean: 20.57 ± 1.42 µg/kg
0
µg/kg

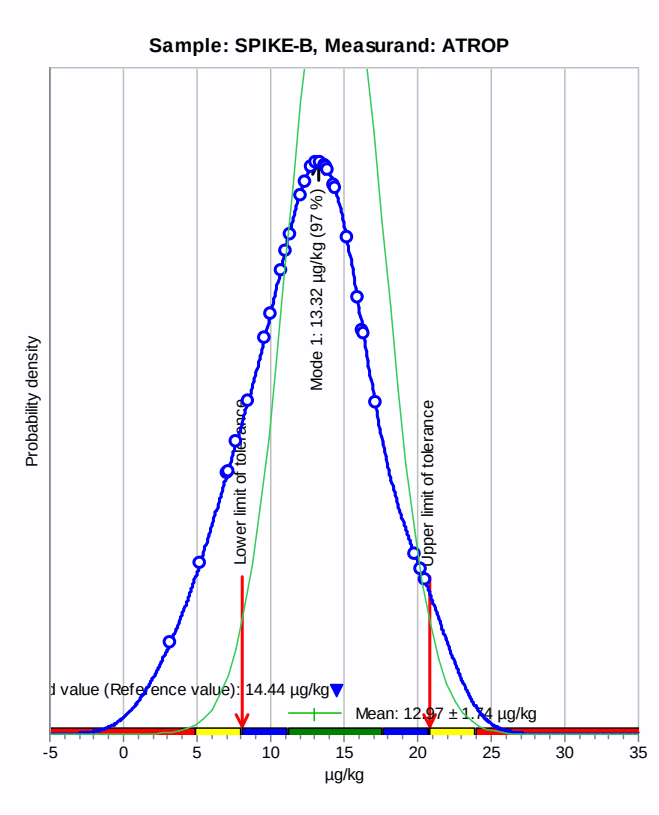

Sample: SAMPLE2F, Measurand: SCOP
Probability density
Lower limit of tolerance
Upper limit of tolerance
Assigned value (Reference value):
Mean: 19.00 ± 1.04 µg/kg
0
µg/kg

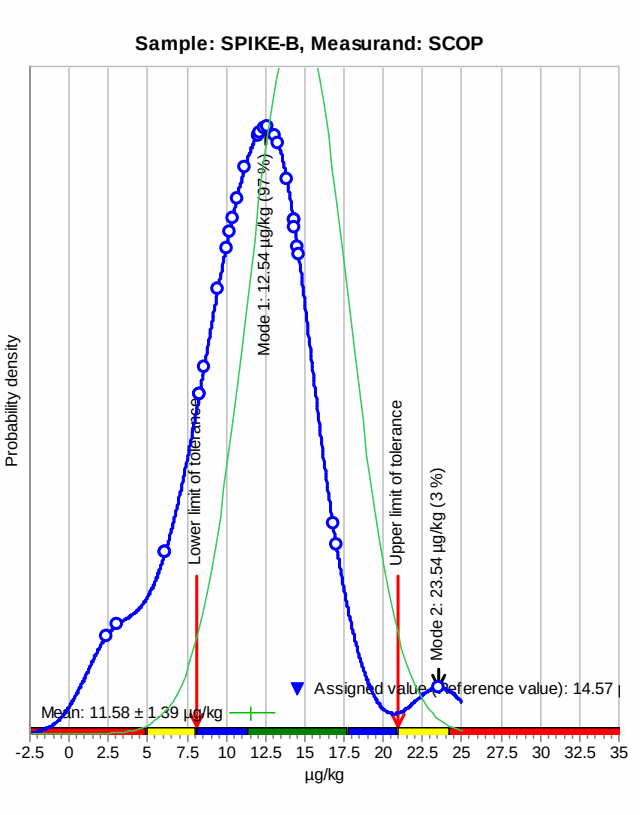

Sample: SAMPLE1P, Measurand: ATROP
Probability density
Mode 1: 4.68 µg/kg (9 %)
Lower limit of tolerance
Upper limit of tolerance
(Reference value): 9.46 ± 0.33 µg/kg
Mean: 9.19 ± 0.68 µg/kg
0
20
µg/kg

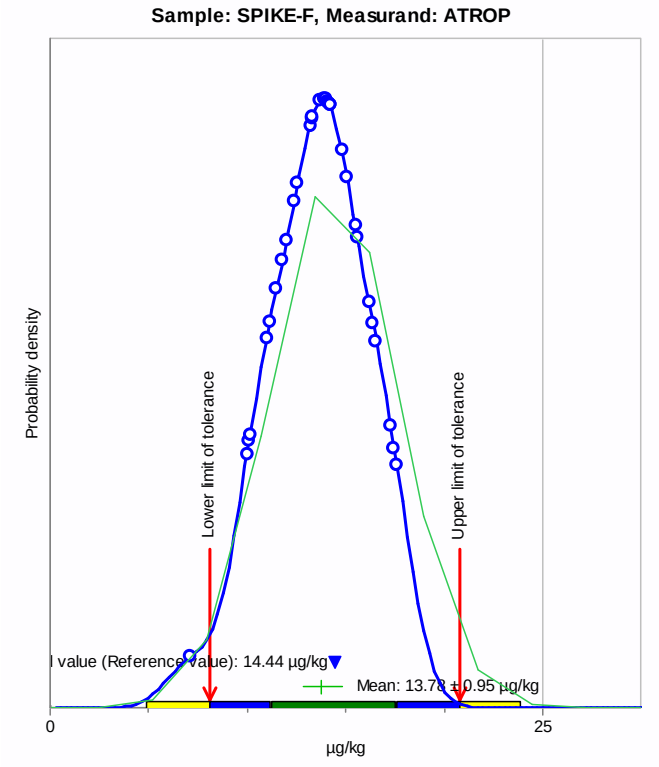

Sample: SAMPLE1P, Measurand: SCOP
Probability density
Mode 1: 1.18 µg/kg (92 %)
Lower limit of tolerance
Upper limit of tolerance
ce value): 1.49 ± 0.08 µg/kg
Mean: 1.48 ± 0.22 µg/kg
0
2
4
µg/kg

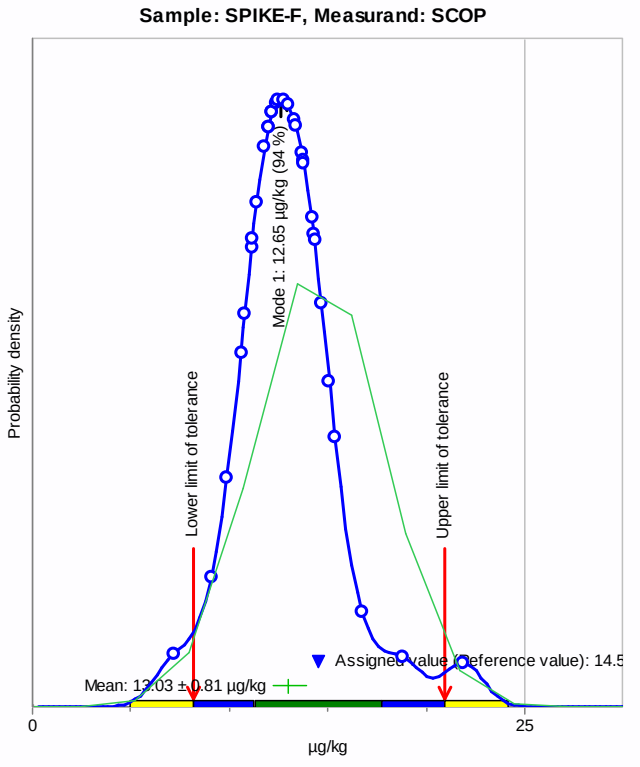

Sample: SAMPLE2P, Measurand: ATROP
Probability density
Lower limit of tolerance
Upper limit of tolerance
erence value): 21.17 ± 0.52 µg/kg
Mean: 20.40 ± 1.71 µg/kg
0
50
µg/kg

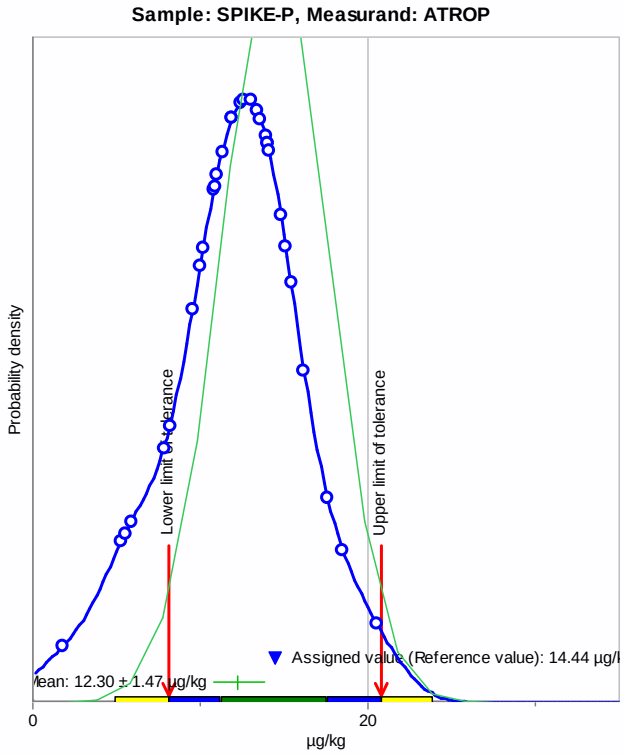

Sample: SAMPLE2P, Measurand: SCOP
Probability density
Mode 1: 2.21 µg/kg (89 %)
Lower limit of tolerance
Upper limit of tolerance
alue): 2.52 ± 0.19 µg/kg
Mean: 2.66 ± 0.45 µg/kg
0
5
µg/kg

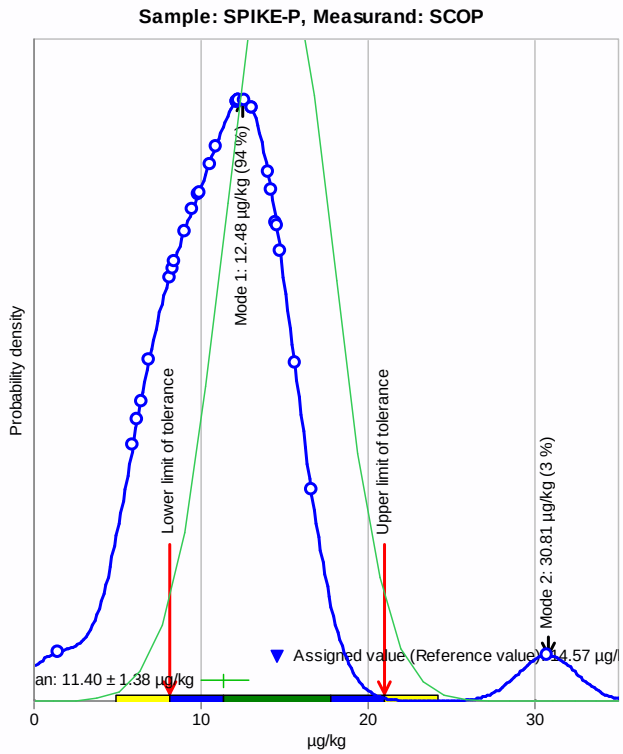

Sample: SPIKE-B, Measurand: ATROP
Probability density
Mode 1: 13.32 µg/kg (97 %)
Lower limit of tolerance
Upper limit of tolerance
d value (Reference value): 14.44 µg/kg
Mean: 12.97 ± 1.74 µg/kg
-5 0 5 10 15 20 25 30 35
µg/kg


Sample: SPIKE-B, Measurand: SCOP
Probability density
Mode 1: 12.54 µg/kg (97 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 23.54 µg/kg (3 %)
Assigned value (Reference value): 14.57
Mean: 11.58 ± 1.39 µg/kg
-2.5 0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 25 27.5 30 32.5 35
µg/kg


Sample: SPIKE-F, Measurand: ATROP
Probability density
Lower limit of tolerance
Upper limit of tolerance
l value (Reference value): 14.44 µg/kg
Mean: 13.78 ± 0.95 µg/kg
0 25
µg/kg


Sample: SPIKE-F, Measurand: SCOP
Probability density
Mode 1: 12.65 µg/kg (94 %)
Lower limit of tolerance
Upper limit of tolerance
Assigned value (Reference value): 14.5
Mean: 13.03 ± 0.81 µg/kg
0 25
µg/kg


Sample: SPIKE-P, Measurand: ATROP
Probability density
Lower limit of tolerance
Upper limit of tolerance
Assigned value (Reference value): 14.44 µg/
lean: 12.30 ± 1.47 µg/kg
0
20
µg/kg


Sample: SPIKE-P, Measurand: SCOP
Probability density
Mode 1: 12.48 µg/kg (94 %)
Lower limit of tolerance
Upper limit of tolerance
Mode 2: 30.81 µg/kg (3 %)
Assigned value (Reference value): 14.57 µg/
an: 11.40 ± 1.38 µg/kg
0
10
20
30
µg/kg

9.8. Experimental details

9.8.1. Method performance characteristics

Lab	Sample	LOQ (µg/kg) Atrop	LOQ (µg/kg) Scop	Rec (%) Atrop	Rec (%) Scop	MatrixSup (%) Atrop	MatrixSup (%) Scop	RT (min) Atrop	RT (min) Scop
2	Sample1 B	5.0	5.0	100	100	0.23	0.23	5.80	4.78
	Sample1 F	1.0	1.0	100	100	0.8	0.8	5.80	4.78
	Sample1 P	1.0	1.0	100	100	0.64	0.64	5.80	4.78
4	Sample1 B	5.0	5.0			5.08	6.86	6.92	6.54
	Sample1 F	5.0	5.0			22.21	11.31	6.92	6.54
	Sample1 P	5.0	5.0			5.72	16.2	6.92	6.54
5	Sample1 B	2.5	2.5	106	97	1.01	0.95	10.40	7.40
	Sample1 F	2.5	2.5	106	97	1.11	1.08	10.40	7.40
	Sample1 P	2.5	2.5	106	97	0.8	0.52	10.40	7.40
7	Sample1 B	2.5	2.5	110	93			2.50	2.20
	Sample1 F	2.5	2.5	110	93			2.50	2.20
	Sample1 P	2.5	2.5	110	93			2.50	2.20
13	Sample1 B	0.5	0.5	119.9	111.7	46.8	32.2	7.30	6.00
	Sample1 F	0.5	0.5	100.8	92	46	25.9	7.30	6.00
	Sample1 P	0.5	1.0	94.9	98	13.9	4.9	7.20	5.90
14	Sample1 B	0.3	1.0	80	85	22	33	6.90	2.70
	Sample1 F	0.2	0.5	80	85	43	61	6.90	2.70
	Sample1 P	0.5	0.5	80	85	18	36	6.90	2.70
15	Sample1 B	0.2	0.2	23	30	23	30	8.27	7.94
	Sample1 F	0.2	0.2	69	70	69	70	8.27	7.95
	Sample1 P	0.2	0.2	88	53	88	53	8.27	7.95
18	Sample1 B	0.3	0.3	105.7	107.2	39.8	26	7.97	6.85
	Sample1 F	0.3	0.3	84	83.4	58.7	50.7	8.04	6.91
	Sample1 P	0.3	0.3	90.3	79.1	26.3	15.11	8.00	6.88
19	Sample1 B	0.5	0.5	100	100	4.1	1.8	9.00	7.00
	Sample1 F	0.5	0.5	100	100	36.6	46.5	9.00	7.00
	Sample1 P	0.5	0.5	100	100	25.8	35.6	9.00	7.00
21	Sample1 B							8.61	6.65
	Sample1 F							8.63	6.65
	Sample1 P							8.61	6.65
23	Sample1 B	5.0	5.0	100	100	80	80	2.96	2.76
	Sample1 F	5.0	5.0	120	120	60	60	2.96	2.76
	Sample1 P	5.0	5.0	80	80	60	60	2.96	2.76
24	Sample1 B	1.3	0.4	79	52	0.54	0.56	8.32	4.29
	Sample1 F	1.6	0.4	130	76	0.48	0.50	8.42	4.36
	Sample1 P	1.6	0.8	117	64	0.42	0.44	8.31	
25	Sample1 B	0.1	0.3	64	117	36	24	6.30	5.30
	Sample1 F	0.1	0.3	21	112	34	27	6.20	5.20
	Sample1 P	0.1	0.3	20		24		6.20	
27	Sample1 B			71.2	108.4			3.72	3.89
	Sample1 F			74.6	100.1			4.33	4.47
	Sample1 P			134.8	88			4.09	4.16
28	Sample1 B	5.0	5.0	80	60	0.7	0.80	8.30	7.10
	Sample1 F	5.0	5.0	100	60	1	1.20	8.30	7.10
	Sample1 P	5.0	5.0	80	70	0.9	0.70	8.30	7.10
30	Sample1 B	1.0	1.0	100	100			2.99	2.46
	Sample1 F	1.0	1.0	100	100			2.94	2.42
	Sample1 P	1.0	1.0	100	100			2.94	2.37
31	Sample1 B	0.1	0.2					4.40	2.80
	Sample1 F	0.1	0.2	78	149.8	47.9	47.8	4.40	2.80
	Sample1 P	0.1	0.2	44.2	72.1	82	78	4.40	2.80
33	Sample1 B	4.0	4.0			65.3	91.9	4.73	2.36
	Sample1 F	4.0	4.0			104.3	111.3	4.71	2.35
	Sample1 P	4.0	4.0	90.1	78.1	91.7	159.1	4.71	2.27
34	Sample1 B	2.0	2.0	44	69	49	66	2.63	1.84
	Sample1 F	2.0	2.0	57	69	61	67	2.63	1.84
	Sample1 P	2.0	2.0	57	145	63	140	2.63	1.84
35	Sample1 B	1.0	1.0					7.06	3.75
	Sample1 F	1.0	1.0					7.06	3.74
	Sample1 P	1.0	1.0					7.08	3.77
36	Sample1 B	0.6	0.6	52.83	40.82	81	44.6	4.28	3.47
	Sample1 F	0.6	0.6	79.23	97.36	61.27	80.72	4.2	3.42

	Sample1 P	0.6	0.6	61.56	60.5	50.17	20.15	4.21	3.42
37	Sample1 B	0.1	0.1	100	100			7.03	4.69
	Sample1 F	0.1	0.1	100	100			7.02	4.69
	Sample1 P	0.1	0.1	100	100			7.03	4.69
38	Sample1 B	4.0	4.0	175	111	13	37	4.37	3.56
	Sample1 F	2.0	2.0	150	134	43	52	4.37	3.56
	Sample1 P	2.0	2.0	118	104	31	46	4.37	3.56
40	Sample1 B	0.1	0.4	75.4	56.9	51.9	41.3	8.16	6.40
	Sample1 F	0.1	0.4	83.4	80.3	90.6	50.6	7.89	6.41
	Sample1 P	0.1	0.4	69	80.7	50.5	24.6	7.88	6.44
41	Sample1 B	0.2	0.2	97	128	78.3	77.5	2.38	2.03
	Sample1 F	0.2	0.2	93	123	68.8	83.3	2.36	2.02
	Sample1 P	0.2	0.2	121	235	81.5	87.1	2.39	2.04
42	Sample1 B	2.0	2.0	82	87			6.32	5.70
	Sample1 F	2.0	2.0	105	104			6.30	5.69
	Sample1 P	2.0	2.0	90	96			6.31	5.71
44	Sample1 B	1.0	1.0						
	Sample1 F	1.0	1.0						
	Sample1 P	1.0	1.0						
45	Sample1 B	300	300						
	Sample1 F	1.0	1.0			74	77	10.10	8.70
	Sample1 P	1.0	1.0			74	82	10.10	8.70
46	Sample1 B	0.5	0.5			24.1	25.6	8.84	8.10
	Sample1 F	0.3	0.3			32.2	42.4	8.84	8.09
	Sample1 P	0.5	0.5			39.7	26.9	8.67	8.06
47	Sample1 B	1.0	1.0	7.6	9.6			6.57	5.46
	Sample1 F	1.0	1.0	88.4	63.3			6.55	5.45
	Sample1 P	1.0	1.0	29.1	37.8			6.54	5.65
50	Sample1 B	5.0	5.0	88	88	57	58	4.10	3.60
	Sample1 F	5.0	5.0	88	88	53	64	4.10	3.60
	Sample1 P	5.0	5.0	88	88	51	82	4.10	3.60
52	Sample1 B	1.0	1.0	116	96	51	54	5.05	4.06
	Sample1 F	1.0	1.0	108	85	76	84	5.06	4.08
	Sample1 P	1.0	1.0	96	103	40	49	5.05	4.04
53	Sample1 B	10	10	108	96			9.50	7.00
	Sample1 F	10	10	108	96			9.50	7.00
	Sample1 P	10	10	108	96		0.23	9.50	7.00

Atrop – atropine; Scop - scopolamine

9.8.2. Analytical conditions

Lab	Q.6 Reference of the SOP used	Q.7 Extraction details	Q.8 Concentration /clean-up	Q.9 Solvent to sample ratio	Q.10 Mass fraction injected
2	Journal of Chromatography A Determination of tropane alkaloids atropine and scopolamine by liquid chromatography- mass spectrometry in plant organs of Datura species	1 min. vortex, 20 min sonication in: 300 mL MeOH + 200 mL H2O + 0.5 mL FormAc	Filtration; Dilute and Shoot	1g in 20 ml	0.0001
4	Sample preparation procedure for the analysis of tropane alkaloids in food and feed by LC-MS/MS	extraction with 0.4 % formic acid in methanol/water 60/40, v/v	only centrifugation and filtration	1 g sample extracted with 20 ml extraction solvent	10 µl filtrate from extract derived from no. 9 injected for LC-MS/MS analysis
5	Adamse, P.; Egmond, H.P. van; Noordam, M.Y.; Mulder, P.P.J.; Nijs, W.C.M. de, Tropane alkaloids in food: poisoning incidents, Quality Assurance and Safety of Crops & Foods 6 (2014)1. p. 15 - 24.	stir 45 min, pH 9, Ammonium carbonate / acetonitrile 16/ 84	Bondesil PSA 40 µm	25/5	2.5 g / 25 ml / 1ml evaporated to dryness / reconstituted 5 / injected 10 µl
7	Detection of ergot and tropane alkaloids by LC-MS/MS	extraction could be done in about 2h, modified quechers / 4g (+/- 0,02 g) of sample / 30 ml Acetonitrile/ H2O + 2.1 mmol/L ammonium	modified quechers	4 g of sample 30 ml of solvent(with 25.2 mL organic solvent) =6.3	meq= 4 g of sample 30 ml of solvent (with 25.2 mL of organic solvent)*

		carbonate (84/16, v/v) / 45 min rotate overhead / Add salts MgSO4 (4g)/NaCl (1g) / Centrifuge for 10,000 rpm for 5 min / 2 mL of supernatant through a 0.2 um PTFE syringe filter / standard addition with 5 microl of 100 ppb			1 microl injection
13	Draft protocol given by EURL after registration for PT	Solvent: methanol/water/formic acid 39:60:1; "head over head" extraction for 1 hour	dilute and shoot	10	0.002
14	extraction from matrix by 0.05M H2SO4, centrifugation, supernatant pH adjustment to 9-10 with ammonia, extraction with ethyl acetate, EtOAC evaporation, dissolution, centrifugation, injection	extraction by agitation (vortex 10s, overhead 15min) and sonication 15min, 0.05M H2SO4	pH adjustment to 9-10, liquid-liquid extraction with ethyl acetate, evaporation of the EtoAc phase, dissolution and high-speed centrifugation,	20 ml to 2 g	1 mg
15	RIKILT SOP for TAs in cereals and cereal products, EFSA project	30 minutes shaking. Extraction solvent methanol/water/formic acid solution (75/25/0.4%)	SPE clean-up using Strata X-C cartridges	4g/40ml	4g/40ml, 5ml for SPE, made up in 0.5ml = 1g/ml. Injection 2ul, meq= 0.002g (2mg)
18	SOP provided by the EC Joint Research Centre, IRMM, EURL Mycotoxins	Extraction time: 1hour shaking, 1 min vortex, methanol/water/formic acid 39/60/1	extract, dilute and shoot. No clean up, just filter with 0.45um membrane	2g of sample, 20 mL extraction solvent	meq=(2/20)x0.010
19	Jandric et al.: Development of a liquid chromatography-tandem mass spectrometric method for the determination of tropane alkaloids and glycoalkaloids in crops. Food Additives and Contaminants Vol 28 (9), 2011, 1205-1219 European Food Safety Authority (EFSA): Scientific Opinion on Tropane alkaloids in food and feed. EFSA Journal 2013;11(10):3386	5 Minutes, Centrifugation	non	1/3.25	
21	TAs are extracted from solid matrices with an acidic aqueous/methanolic solution. The extracts are purified and concentrated by means of solid phase extraction on a polymeric strong cation exchange cartridge. Tea infusions are purified and concentrated by means of solid phase extraction on a polymeric strong cation exchange cartridge. The purified extract is analysed by LC-MS/MS.	The test sample size is 4 g. IS is added and 40 ml of extraction solution (methanol/water/formic acid solution (75/25/0.4%) are added. Extraction is carried out for 30 min on a rotary tumbler and the samples are centrifuged for 15 min at 3500 rpm.	SPE with Strata X-C	10:1	2.4 mg
23	FAC Vol 28, No. 10, October 2011, 1405-1423 Screening of plant toxins in food, feed and botanicals using full-scan high resolution mass spectrometry, Mol et. al	shaking	dilute and shoot	100	0.0000125
24	Ergot alkaloids in Feed by HPLC-MS/MS, BfR Berlin	30 minutes shaking, solvent-mixture: 90 % Methanol + 10 % Water + 0.4 % formic acid	concentration of spike: 0.5 ng/ml; clean-up: SPE Strata-X	20 ml solvent to 0.5 g sample	0.5 g sample extracted with 20 ml solvent, then 2 ml over SPE Strata-X, evaporated to dryness,

					reconstituted with 2 ml solvent, 5 µl injected for LC-MS analysis
25	IRMM method from EURL "Determination of tropane alkaloids in cereals and cereal products by LC-MS/MS"	shaking in methanol/water/formic acid 39/60/1 for 1 hour	centrifugation, supernatant was used for analysis	10	meq=(2/20)x0.010
27	In house method based on modified QuEChERS procedure. Addition of internal standard was used for detection, quantification and recovery by LC-MS/MS.	Vortex-mixing 3x2minutes, automatic shaker for 2 hours; 10 mL v:v 50:50 0,5% Formic acid in water:acetonitrile.	QuEChERS (MgSO4+NaCl), dSPE (MgSO4+PSA)		
28	EURL method	2g with methanol/water/formic acid 39:60:1 / shaker for 1 hour / centrifuge and inject	just dilute 1:10 compared to TA in cereals	10	0.0001
30	For this proficiency test we used the Method from the EURL Mycotoxins. Before we used Quechers	60 minutes on a shaker	no	20 ml solvent and 2 g Sample	0.001
31	Deutsche Lebensmittelrundschau, Oktober 2015 page 418	30 minutes (methanol/water 60/40%)	filtration Chromafil Xtra PA 0.45 um	10	4
33	ADAMSE, P. u. H.P. VAN EGMOND (2010): Tropane alkaloids in food, RIKILT - Institute of Food Safety, Report 2010.011	methanol/water/formic acid (60+40+0,4) / 30 min shaking	Dilute and Shoot	sample mass: 2 g ; volume of solvent: 20 ml	0.2 mg
34	ADAMSE, P; H. P. VAN EGMOND (2010): Tropane alkaloids in food, RIKILT - Institute of Food Safety, Report 2010.011	extraction time: 30 sec / Ultraturax MeOH (600ml) / H2O (400ml) / formic acid (4ml) / Ultraturax solvent composition: MeOH/H2O	centrifugation / filtration 0.2µm	10	(2.5/25) x (1/5) x 0.1 = 5 mg
35	2.5 g homogenized sample; add 25 ml Extraction solvent; Blending for 30 Min.; Centrifugation for 10 Min. 4400 rpm; Filtration with syringe filter 0.2 µm; Dilution 1/20 with water: 5 µl injection to LC-MS/MS	Blending for 30 Min.; Extraction solvent: MeOH 600 ml + Water 400 ml + Formic acid 4 ml	Filtration and Dilute	2.5 g/25 ml	2.5 g/25 ml; Dilution 1/20; Inj. 5µl = 0.025 mg
36	Analysed as per method supplied	As per method supplied	As per method supplied	2 g/20 ml	(2/20)x (1/0.5) x 0.02
37	acid extraction, SPE, drying of sample, reconstitution, LC-MS/MS	30 min, 0.05 M H2SO4, sonication	SPE	40 ml solvent for 2 g sample mass	
38	Your Method	1h, shaking, Methanol/Water/formic acid 39/60/1	Dilute and Shoot	1g Sample / 10 ml Solvent	2 g sample extracted with 20 mL solvent. Then, 5 µL injected for LC-MS analysis. meq=(2/20) x 0.005
40	RIKILT SOP A1070 (modified)	15 min, blending, methanol / water (60:40) + formic acid (0.4 %)	Dilute and Shoot	4	0.002
41	EURL method provided was used	extraction time 1,5 hours / 60:39:1 water: methanol:formic acid (see EURL method provided)	None	20:2 (solvent: ml : sample g)	meq = (2/20) * 0.002= 0.0002
42	In house method (own development)	60% methanol with 0.4% formic acid, 45 min, sonication	dilute and shoot	2g in 20 mL	0.025 mg
44	BfR-PA-2.0	n.s.	n.s.	n.s.	1g sample, 20 ml solvent, SPE with entire extract, reconst. in 1 mL, Injection of 5 mL
45	http://www.bfr.bund.de/cm/343/bestimmung-von-pyrrolizidinalkaloiden.pdf	15 min sonication (twice); 0,05 M sulfuric acid	C18	10	2
46	SOP for analysis of TAs in dry tea, in house validated (no SOP number)	30 min extraction with methanol/water/formic acid 75/25/0.4 v/v/v	SPE using strong cation exchange (150	2 g sample and 20 ml solvent	2 g extracted with 20 ml solvent, 5 ml

			mg/6 cc)		extract cleaned by SPE, in 500 uL solvent, 2 ul injected. meq = (2/20) x (5/0.5) x 0.002 = 2 mg
47	In-house developed method	15 min extraction by sonication in Methanol : Acetonitrile 1 : 1 [v : v]	SPE: ion exchange (SCX)	10 mL solvent, 1g sample	meq = (1/10)*5/1)*0,0 1 = 5mg
50	Jandric et al.(2011); Food Additives and Contaminants 28 (9), 1205-1219	30 min, shaking, Methanol + Water (60+40) + 0.4 % formic acid	dilution	2.5 g in 25 ml	0.5 mg
52	EURL method - Determination of tropane alkaloids in cereals and cereal products by liquid chromatography-tandem mass spectrometry	1h, shaker, Methanol/Water/Formic acid (39:60:1, v/v)	dilute and shoot	20 mL, 1 g	0.5 mg
53	internal method	30min, shaking/sonication. Solvent: 1/3 acetic acid; 2/3 methanol, filtration	chemical precipitation by polarity gradient, followed by membrane filtration	2 g/20 ml	0.001

Lab	**Q.11 Type of separation**	**Q.12 Type of detecton**	**Q.13 Transitions, ratio and CID**	**Q.23 Calibrant**	**Q.25 ***
2	ZORBAX Extend C18 4,6x100mm, 3,5µm	Agilent 6460 Triple Quad LC/MS; ESI +; Capilary voltage 3000V, dessolvation Temp. 370°C	Atropine: 290,2-->124,1; 290,2-->103 Scopolamine: 304-->138; 304-->156,1	Our own	SPS
4	Waters, Acquity UPLC BEH C18 150x2.1 mm; 1.7 µm. Eluent A: 0,1 % formic acid in water. Eluent B: 0,1 % formic acid in acetonitrile. gradient elution. 30 °C; 0,2 ml/min; 10 ml Inj	Agilent QQQ 6460, ESI positive, Capillary voltage 3000 V, 300 °C	Atropine: 290 > 124 CE 20 eV, 290 > 93 CE 30 eV / Atropine d3 293 > 127 CE 20 eV, 293 > 93 CE 30 eV / Scopolamine 304 > 156 CE 10 eV, 304 > 138 CE 18 eV / Scopolamine d3 307 > 159 CE 10 eV, 307 > 141 CE 18 eV	Our own	SPS
5	cfr. Methode Cereal Products	cfr. Methode Cereal Products	cfr. Methode Cereal Products	Our own	SPS
7	reversed phase: Waters Kinetex EVO C18 column 1.7microm 100 x 2.1 mm 40°C 1 microl injection 0.5 mL/min of mobile phase: ACN and H20 w ith ammoniak buffer . 40°C. 1 microl injection. 0.5 mL/min of mobile phase: ACN and H20 w ith. ammoniak buffer. 1.7ìm 100 x 2.1 mm. 40°C. 1 microl injection. 0.5 mL/min of mobile phase: ACN-H20 w ith. ammoniak buffer	HPLC_MS/MS XEVO TQ-S WATERS ESI+ CV: 1 kv Desolv temp: 450 °C . ESI+. CV: 1 kv. Desolv temp: 450 °C. ESI+. CV: 1 kv. Desolv temp: 450 °C	Atropine Q124.0 (25V) -- C93.0 (30V) Scopolamine Q138.0 (20V) -- C156.0 (15V). Scopolamine Q138.0 (20V) -- C156.0 (15V). Scopolamine Q138.0 (20V) -- C156.0 (15V)	Supplied along with the PT samples	SPS
13	Column: Supelco Ascentis Expres F5, 10 cm x 2,1 mm; 2,7 um MF: A - 0,1% FA/water, B - 0,1% FA/acetonitrile; flow rate: 0,3 ml/min; column temperature: 40 C deg; Injection volume: 20 ul	LC-QQQ Agilent 6460, ESI + JetStream; Gas Temp 325°C; Gas Flow 10 l/min; Nebulizer: 25 psi; Sheath Gas Temp 400°C; Sheath Gas Flow 11 l/min; Capillary Voltage 3000 V; deltaEMV 600	Scopolamin Q: 304,2-->156 (10); q 304,2-->138 (18); Atropin Q 290,3-->124,1 (20); q 290,3-->93,1 (30)	Supplied along with the PT samples	MMC
14	ZIC-Hilic (SeQuant Merck) 150mm*2,1mm*5µm; start 10% (5% ACN+95% ammoniumacetate) 90% (95%ACN+5%ammoniumacetate) gradient mode; 30°C; 0,3 ml/min; 10 µl	LC-MS/MS (API4000QTrap) ESIpositive, DP 76V (atropine), 51V (scopolamine), CE 35eV(atropine) 31eV (scopolamine), CXP 6V	atropine Q 290,1-124,1 C 290,1-93,1	Supplied along with the PT samples	
15	UPLC Acquity Waters Acquity BEH C18 1.8 µm (150 x 2.1 mm).. The gradient is 100 % A for 2 min and then goes to	Waters Acquity UPLC, the MS is a Waters Xevo TQ-S triple quadrupole. The	Primary transition Atropine 290>124, CE=20eV, scopolamine 304>138, CE=20eV, secondary	Our own	SPS

	40 % A 60 % B at 12 min. We then step it up to 99 % B for a 3 min flush and re-equilibrate for 5 min.. A is 10 mM ammonium carbonate in water at pH 10 (adjusted with ammonia) and B is acetonitrile.	capillary voltage was 2 kV and the desolvation temperature was 500 oC.	transitions were 290>93 CE = 25 eV for atropine and 304>103 CE = 30 eV for scopolamine.		
18	F5 column, 10cm x 2.1 mm, 2.7 um particle size, mob. phase water+0.1% FA and MeOH+0.1% FA gradient, temp. 40oC, flow rate 0.3 mL/min, inj. vol. 10 uL	LC-MS/MS, Thermo Finnigan TSQ Quantum, ESI+, Spray Voltage 3400V, Cap. Temp. 325oC	Atropine: Q: 124.2 (22V), C: 93.1 (31V)	Supplied along with the PT samples	SPS
19	Column Oven Agilent 1290 G1316C Agilent 1260 G1312B 0 AutoSampler CTC	see 11	see 11		
21	Waters UPLC BEH C18 (1.0 mm id * 100 mm), 40 ºC, flow 0.150 mL/min, Mobile phase A: 6.65 mM ammonium hydroxide pH 10.0 ; Mobile phase B: acetonitrile . 2 microL inyected	WATERS TQD (MS/MS), ESI positive, cone voltage 30 V, capillary 3 V, desolvation temperature 400 ºC	Atropine 289.9->124.1 (CID 25 V) Scopolamine 303.9 ->138.1 (CID 40 V)	Our own	MMC
23	RP18plus, Macherey-Nagel, Nucleoshell, 100 mm x 2 mm. 2.7 µm. 30°C, 0,3 mL/min, 5 µL	Agilent 6490, ESI +,	Atropin 290.2/124.1 (CE28 V), Atropin 290.2/77 (CE 60 V), Atropin 290.2/93 (CE 37 V); Scopolamin 304.2/103 (CE 49 V), Scopolamin 304.2/138 (CE 21 V), Scopolamin 304.2/156 (	Our own	SPS
24	1. Aqua C18, 3 µm, 50 x 2 mm; 2. Gemini C18, 3 µm, 100 x 3 mm in series connection mobile phase A: 10 mM Ammoniumbicarbonate in water mobile phase B: Acetonitrile temperature: 40 °C flow rate: 200 µl/min inject. vol.: 5 µl	LC-MS instrument: API 5500 QTRAP ESI positiv Ions spray voltage: 5500 V Temperature: 500 °C	Atropine: Q1: 124 CE: 33 Q3: 93 CE: 39 Scopolamine: Q1: 156 CE: 23 Q3: 103 CE: 55 Q3: 103 Atropine-d3: Q1: 127 CE: 33 Q3: 77 CE: 85	Our own	SPS
25	Pentafluorophenyl column, Ascentis Express F5. 100mmx2.1mm 2.7µm; water/acetonitrile with 0.1% formic acid; 40 °C; 0.3 ml/min; 10 µL injection volume	LC-MS, ABSciex 5500 QTrap, ESI positiv, IS 2500 V, TEM 200 °C	atropine: Q 290-->124 CE 33 V, C 290-->93 CE 39 V. scopolamine: Q 304-->156 CE 25 V, C 304-->138 CE 29 V. scopolamine: Q 304 --> 156 CE 25 V, C 304 --> 138 CE 29 V	Supplied along with the PT samples	SPS
27	Column: XBridge Amide, Waters, 150x2,1 mm, particle size 3,5 um, temperature 30 C, flow rate 0,2 mL/min, injection volume 20 uL. Mobile phase: water and acetonitrile.	LC-MS/MS, Thermo Finnigan type TSQ Quantum ULTRA EMR, ESI (+); Spray voltage 4000 V; Dessolvation temperature: 200 C, Capillary temp. 325 C.	290.030>93.100 (31V); 290.030>124.200 (22V); 295.120>93.100 (31V); 295.120>124.170 (24V); 304.100>138.140 (22V); 304.100>156.180 (22V); 304.100>182.200 (20V); 308.100>142.160 (22V); 308.100>160.200 (22V);	Supplied along with the PT samples	SPS
28	Ascentis Express F5 10cm x 2,1 mm 2,7	LC-MS/MS Quattro Ultima Platinum Waters	Atropine Q 124.3 C 93.1 Scopolamine Q 138.0 C 155.9	Supplied along with the PT samples	SPS
30	Supelco Ascentis Express F5; 10cm * 2.1mm, 2,7µm; Water 0,01% Formic Acid, Acetonitrile + 0,01% Formic Acid; 0.300 ml/min; 10µl Inj	LC-MSMS ABSciex 400QTrap; Esi pos; DP 71, CD 27	Scopolamin: 304,007 - 155,9 304,007 - 138,0 Atropin: 290,032 - 124,0 290,032 - 93,0	Our own	MMC
31	Waters BEH C18: 1.7 um; 2.1x50 mm; injection 5 ul; 0.3 ml/min. gradient elution Water/Methanol with 0.1% formic acid	UPLC-MS/MS Waters Acquity TQD	atropine: 290.1-93.1/290.1-124.1. atropine IS: 295.1-93.1/295.1-124.1. scopolamine: 304-138.1/304-156. scopolaine IS: 308-142.1/308-160		
33	Waters Aquity BEH C18 1,7 µm, 2,1 x 50 mm	LC-MSMS: Waters Acquity Xevo TQD, ESI+	Scopolamin: 304 -> 138 (Q); 304 -> 103 (C)	Our own	MMC
34	look PT TA in cereal-based products	look PT TA in cereal-based products	look PT TA in cereal-based products	Our own	MMC
35	Kinetex C18 2.6µ 100A; lenght: 100mm; inner diameter: 2.1mm; particle size: 2.6µm; Eluent A: Water with 0,1% Formic acid; Eluent B: MeOH with 0,1% Formic acid; Gradient from 10% B at 0 Min. to 90% B at 10 Min.; Temperature 35°C;	Sciex QTrap 5500; ESI pos.; 5500 Volts; 650°C	Atropin: 290.078 --> 124.0; CE 33 ; 290.078 --> 93.0; CE 39;. Scopolamin : 304.062 --> 138.1; CE 27; 304.062 --> 156.1; CE 23;	Supplied along with the PT samples	SPS
36	As supplied	ESI: Positive, KV:3.75, Cone:35, Source	Scopolamine:304.3 >138(Q) CID 22 304.3> 156.1(C) CID 55 /	Supplied along with	MMC

		Temp:120, Desolvation Temp:280	Atropine 290.3> 124.2(Q) CID 26 290.3>93.2 CID 21	the PT samples	
37	C18 column, 2.1 x 150 mm, 1.8 micron; mobile phases: H2O (A) and MeOH (B) both containing formic acid and ammonium formiate, injection 1µl, flow rate 0.5 ml/min	LC-MS/MS, ESI mode (pos.)	Atropin: Q 290.2 > 124 (CE = 21); 290.2 > 93 (CE = 29). Scopolamin: Q 304.2 > 156 (CE = 9); C 304.2 > 138 (CE = 21)	MMC	
38	Agilent Zorbax Eclipse Plus C18 RRHD 2,1 x 100mm 1,8µm, Acetonitril/Methanol/Water 1:1+0,1 %FA / Water+0,1% FA 10:90, 40°C, Flow: 0,4µl, Inj: 5µl	Agilent 6460, LC-MS/MS, ESI+, 3000V, 400°C	Scopolamin: Quant: 304.2 > 156.1, Quali: 304.2 > 138.1, 304.2 >103 Atropin: Quant: 290.2 > 124, Quali: 290.2 > 93	Supplied along with the PT samples	MMC
40	XBridge C18, 5µm, 3.0 x 150 mm, Waters; mobile phase: water/acetonitrile, 6 mM NH4OH; 40 °C	LC-MSMS; Waters TQ; ES+; 2 kV; 400 °C	Atropine: 290.16 > 124.24 (25 eV); 290.16 > 93.17 (25 eV); Scopolamine: 304.21 > 138.25; 304.21 > 156.25	Supplied along with the PT samples	SPS
41	Column = Acquity UPLC HSS T3, (1.8 µm, 2.1x 100 mm) Eluens A = 0.02% formic acid in H2O Eluens B= 0.02% fomic acid in acetonitril Temp. 40°C, flow rate = 0,3 ml/min, injection volume = 2.0 µl	LC=MS-MS, ESI+, capillary voltage = 1.05V, dessolvation temperature=600°C	Atropine: 290.1 ->124.1 (23 eV) / 290.1-> 93.1 (28 eV) Scopolamine: 304.1-> 138.0 (20 eV) / 304.1-> 156.0 (16 eV) CID-Energy	Our own	SPS
42	50 x 2,1 mm Kinetex C18, 2,6 µm, gradient, 0.2% formic acid in water and methanol, 25°C, 0.3 mL/min, 10 µL	LC-MS/MS, API 5500 (Sciex), ESI positive	atropine Q 290.0 -> 124.0, CE 33; C 290.0 -> 93.0, CE 45 / scopolamine Q 304.1 -> 138.0, CE 31; 304.1 -> 103.1; CE 50	Our own	MMC
44	n.s.	LC-MS/MS (Sciex API 5500)	n.s.	Our own	SPS
45	Macherey-Nagel, Nucleoshell RP 18plus, 150 x 2 mm, particle diameter: 2,7 µm; eluent a: 315 mg ammonium formate + 1 ml formic acid + 1 l water; eluent b: 315 ammonium formate + 1 ml formic acid + 1 l methanol	LC-MS/MS; 5500 Triple Quad (SCIEX), ESI positive, 4500 V, 550 °C	Atropine: 290-->124 (Q), CID 41V; 290-->93 (C), CID 43V; scopolamine: 304-->138 (Q), CID 31V; 304-->156 (C), CID 23V	Supplied along with the PT samples	SPS
46	Waters UPLC BEH C18 150x2.1 mm, 1.7 um. mobile phaes A: 10 mM ammonium carbonate pH 10.0, mobile phase B: acetonitrile. 400 ul/min, 50°C	Waters Xevo TQ-S LC-MS/MS. Pos ESI, cap V: 3.0 kV, cone: 30 V., desolvation gas: 600°C, cone : 150°C, collission gas: argon, 4.2x10-3 mbar	Atropine: Q = 290.2 > 124.0, CE: 20 eV; C = 290.2 > 93.0, CE: 25 eV. Scopolamine: Q = 304.2 > 138.0, CE: 20 eV; C = 304.2 > 103.0, 35 eV.	Our own	MMC
47	Waters Acquity BEH 150 + 2.1 mm, 1.7 µm	LC-MS² AbSciex 5500 in ESI+ mode	Q(Atropin) = 290,2 / 124 CE = 33eV C(Atropin) = 290.2 / 93.0 CE = 49 eV Q(Scopolamin) = 304.2 / 138.1 CE = 29 eV C(Scopolamin) = 304.2 / 156.1 CE = 23 eV	Supplied along with the PT samples	SPS
50	Water Xselect HSS T3, 2.5 µm 2.1 x 100 mm, Methanol, Water + 1% Formic Acid, Flow Rate 350 µm7min, 5 ml	LC-MS/MS, SCIEX QTrap 5500, Esi pos, 400 °C, 5500 V	Scopalamin: 304>138.2 DP 66V; CE 27 V; 304>156, DP 66 V, CE 23 .Aropin; 290>124.1 DP 96V; CE 33 V; 290>92.9 DP 96 V; CE 39 V	Supplied along with the PT samples	SPS
52	Supelco ascentis express F5 10cmx2.1mm; 2.7um	Waters UPLC-Quattro premier. parameters as reported in the EURL method	Same as reported in the EURL methods	Supplied along with the PT samples	SPS
53	Thermo Hypersil Gold (150 x 2,1 mm, 3 micrometer) eluent A water, eluent B methanol temperature 40°C flow rate 300 mL/min injection volume 10	SCIEX API 400, SCIEX Qtrap5500 ESI + dessolvation temperature 500°C IS 5500	Atropine 290.259/124.200 CE 31 CXP 8 / 290.259/93.000 CE 39 CXP 14 /290.259/91.000 CE 59 CXP 10 / DP 106 EP 10 Scopolamine 304.232/138.100 CE61 CXP10 / 304.232/156.100 CE23 CXP12 / 304.232/103.000 CE55 CXP10 DP61 EP10	Our own	SPS

* Approach for calibration: MMC – matrix-matched calibration / SPS – standards in pure solvent

Lab	Q.16 Strategy used for LOD/LOQs estimation	Q.17 Performance parameters SCOP	Q.18 Performance parameters ATROP	Q.19 Recovery calculation	Q.28 Results reported
2	low level spiking	R2=0,99985549	R2=0,99996418	spiking matrix	NOT CORRECTED for recoveries
4	S/N 3.1 qualifier for LOD; 10 : 1 qualifier for LOQ	R^2 = 0,99885797	R^2 = 0,99918363	isotope labled internal standard	NOT CORRECTED for recoveries
5	cfr. Methode Cereal Products	cfr. Method Cereal Products	cfr. Methode Cereal Products	cfr. Methode Cereal Products	NOT CORRECTED for recoveries
7	the lowest validated level was chosen as an LOQ (2.5 ppb)	R2=0.998 Scopolamine (conc: RSD% day1 to 3) : 2.5 ppb: 4.31/ 50 pbb: 5.66/150 ppb: 11.65	R2 =0.988 Atropine (conc: RSD% day1 to 3) : 2.5ppb: 19.90/ 50 pbb: 8.19/150 ppb: 11.50	based on the in validation spiked cereal samples- a total mean recovery per component was determined which included results of 3 days and 3 concentrations	NOT CORRECTED for recoveries
13		R2=0.997	R2=0.998	spiked blank sample, whole analytical procedure	NOT CORRECTED for recoveries
14	LOD S/N 3	RSD % 1-8 % standard addition curve > 0,98	RSD % 1-8 % standard addition curve > 0,98	spiked matrix	NOT CORRECTED for recoveries
15	LOQ = lowest calibration standard	r2=0.998	r2=0.998	Internal standard added before extraction to carry out inherent recovery correction.	CORRECTED for recoveries
18	parameters from the calibration curve	R2=0.9981	R2=0.9978	Spiked blank matrix	CORRECTED for recoveries
19	calibration curve	RSD <20%, >0,999	RSD <20%, >0,999	0,2-2µg/kg	CORRECTED for recoveries
21	LOD = s/n 3	r=0.998	r=0.998	recovery not calculated for these matrices	NOT CORRECTED for recoveries
23	low level spikes	1.6 %, 0.998	3,18 %, 0.997	spiked matrix	NOT CORRECTED for recoveries
24	S/N ratio (3 and 10) of each sample and spiking each sample	r= 0.9995 (linear regression)	r= 0.9996 (linear regression)	spiked matrix	NOT CORRECTED for recoveries
25	S/N 3 and 10	2 %, 0,9999	3 %, 0,9998	spiked matrix	CORRECTED for recoveries
27				Spiked matrix	CORRECTED for recoveries
28	S/N, blank and low level spike	R2 0,999	R2 0,999	spiked matrix	NOT CORRECTED for recoveries
30	Low Level spike	r=0,99657	r=0,98981	Procedural Standard Calibration	CORRECTED for recoveries
31	estimation from standard curve			determination of ratio of [analyte in solution of spiked sample]/[theoretical concentration calculated from area of spiked solution diluted in mobile phase]	
33	S/N ration (3 and 10) of spiked blanks	r = 0,99836	r = 0,99919	spiked blank matrices	NOT CORRECTED for recoveries
34	S/N ratio	10 %; 0.9973	6 % ; 0.9984	spiked matrix	NOT CORRECTED for recoveries
35	DIN 32645 (Calibration Curve with Std.'s in low Concentration)				NOT CORRECTED for recoveries
36	LOD Taken as lowest std & LOQ lowest std x calculation of method factor	Black tea:0.998765, PM:0.998849, Fennel:0.99775	Black tea:0.996609, PM:0.997278, Fennel:0.999009	As requested 0.2 mls of spiking sol into 2g as requested	CORRECTED for recoveries
37	low level spikes			we used spiked matrix	

38	S/N (3 and 10, Peak-to-Peak)	99,99%, 0,999922	99,99%, 0,999889	Matrix calibration	NOT CORRECTED for recoveries
40	S/N ratio 3/6, respectively	24.6; 0.999	16.4; 0.998	Spiked matrix	NOT CORRECTED for recoveries
41	Blank low level spikes + s/n ratio	RSD = 20%; correlation coeficient =0.9999	RSD= 25%;, correlation coefficient =0.9999	spiked matrix	NOT CORRECTED for recoveries
42	low level spikes	0.998	0.999	spiked matrix	NOT CORRECTED for recoveries
44	S/N ratio	n.s.	n.s.	n.s.	NOT CORRECTED for recoveries
45	S/N ratio (LOQ: 10; LOD: 3), low level spikes	0.9998	0.9999	-	NOT CORRECTED for recoveries
46	LOD: S/N =3 for secondary transition (rounded off) LOQ: S/N = 6 for secondary transition (rounded off)	calibration: 0.998-1.000	calibration: 0.999-1.000	Spiked to blank matrix (3 PT materials supplied)	CORRECTED for recoveries
47	S/N ratio 3 and 10 for LOD, LOQ respectively in low level spiked samples	r = 0.99898 RSD = 5.14%	r = 0.99989 RSD = 4.03%	Matrix spiked with internal standard (SIDA)	NOT CORRECTED for recoveries
50	LOD: S/N 3: LOQ: S/N: 5	r = 0.999	r = 0.999		NOT CORRECTED for recoveries
52	LOD S/N=3; LOQ S/N=6	17	13	spiked matrix	NOT CORRECTED for recoveries
53	S/N ratio	-	-	spiked matrix	NOT CORRECTED for recoveries

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