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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 cereal products for infants and young children

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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

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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 immensely 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
State General Laboratory - Environmental and other food contamination & natural toxins	Cyprus
University of Chemistry and Technology Prague, Dept. of Food Analysis and Nutrition	Czech Republic
Czech Agriculture and Food Inspection Authority (CAFIA)	Czech Republic
National Food Institute, Technical University of Denmark	Denmark
Finnish Food Safety Authority Evira Research and Laboratory Department Chemistry and Toxicology Research Unit	Finland
Laboratoire SCL de Rennes Mycotoxins analysis	France
Amt für Verbraucherschutz, Chemische- und Lebensmitteluntersuchung	Germany
Landesuntersuchungsamt Rheinland-Pfalz	Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt, Fachbereich Lebensmittelsicherheit	Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, LM Zentrale Analytik	Germany
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei	Germany
Gesellschaft für Bioanalytik mbH	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
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Erlangen	Germany
Institut Kirchhoff Berlin GmbH, R&D Management	Germany
Eurofins Sofia GmbH	Germany
GALAB Laboratories GmbH	Germany
Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Cereals	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
Department of Food Science, University of Parma	Italy
Istituto zooprofilattico sperimentale della Lombardia e Dell'Emilia, Food Chemistry	Italy
Laboratoire National de Santé - Depart. Food Control	Luxembourg

Institute for Hygiene and Veterinary Public Health - Depart Residues and Contaminants	Romania
National Lab. of Health, Environment and Food (NLZOH), Dep. for Chemical Analysis	Slovenia
IRTA - Chemical Food Safety	Spain
National Center for Food Spanish Consumer, Food Safety and Nutrition Agency	Spain
CNTA	Spain
RIKILT - Wageningen UR	The Netherlands
Nofa Lab	The Netherlands
Edinburgh Scientific Services	United Kingdom
Fera Science Ltd., Food Chemistry Dept.	United Kingdom
Staffordshire Scientific Services	United Kingdom
Public Analyst Scientific Services Limited	United Kingdom

Abstract

Tropane alkaloids (TAs) are plant toxins that occur mainly in *Datura*, *Atropa* and *Hyoscyamus* sp, belonging to the Solanaceae family, besides a variety of other families such as Erythroxylaceae, Brassicaceae, Proteaceae, Euphorbiaceae, Rhizophoraceae, Convolvulaceae and Cruciferae. The TAs occur in all parts of the plants and botanical impurities have been found in a range of crops due to accidental contamination during harvesting. The intoxication via the food leads to anticholinergic effects (e.g. blurred vision, pupil dilation, dry mouth, vomiting, muscle spasms, tachycardia, etc.), culminating in severe intoxications and death.

The EFSA CONTAM Panel established a group Acute Reference Dose (ARfD) of 0.016 µg/kg body weight (b.w.) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. Infants and young children are the most exposed age classes as they consume a higher amount of cereal-based products per body weight. EFSA estimated that the dietary exposure of toddlers could be up to seven times the group ARfD.

Recently, two European legislation acts were published in this field: Commission Recommendation (EU) 2015/976, recommending the monitoring of tropane alkaloids in certain food categories, and Commission Regulation (EU) 2016/239, enforcing maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children.

The EURL-Mycotoxins organised a proficiency test (PT) concerning the determination of atropine and scopolamine in cereal-based baby food, aiming to underpin and assess the measurement capability of Member States' (MS) laboratories. Particular focus was given to levels relevant for enforcement of legislation. Two samples were distributed to the participants: one sample labelled "C" – biscuits for infants containing approx. 1.2 µg/kg of atropine and 0.2 µg/kg of scopolamine, and one sample labelled "E" – cereals for porridge containing approx. 7.4 µg/kg of atropine and 1.0 µg/kg of scopolamine.

Forty-eight datasets from 18 EU MS laboratories were received. Overall, 81 % of the z-scores were in the range of -2 to 2 and 90 % were in the range of -3 to 3. For the lowest TA level (sample C) still 75 % of z-scores fell into an acceptable range ($|z| \leq 2$), while the mass fraction of scopolamine was far below the target level of 1 µg/kg. In line with this observation, the vast majority of reported LOQs were below 1.0 µg/kg. The methodologies used by the participants can be clustered into three groups: the method supplied by the EURL; the RIKILT SOP A1070 and methods based on QuEChERS. The instrumental determination was by LC-MS/MS, with one exception (GC-MS). The recoveries reported by the participants were close to 95 %. No statistically significant dependence of the z-scores on the analytical methodology was observed.

These results support the assumption that atropine and scopolamine can be reliably determined at the maximum levels proposed by the EU to ensure the protection of infants and young children's health using state-of-the-art analytical instrumentation.

1. Introduction

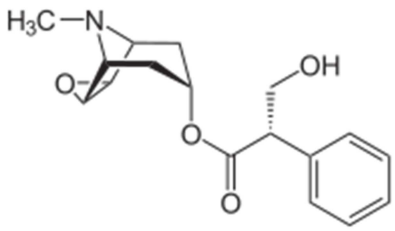
Tropane alkaloids (TAs) are secondary metabolites naturally occurring in plants of several families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae, amongst others [**1**].

Datura stramonium, also known as Jimson weed or thorn apple, is widely distributed in temperate and tropical regions. 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*) and henbane (*Hyoscyamus niger*) as well as mandrake (*Mandragora officinarum*). The TA class contains over 200 compounds, but the most common ones are atropine and scopolamine.

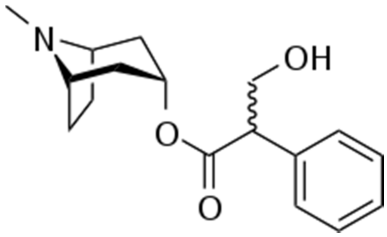
The consumption of small quantities of parts from these plants has caused severe intoxication, including deaths in young children [**1**]. As a result of the anticholinergic activity of the tropane alkaloids, the following symptoms may be observed: blurred vision, pupil dilation, dry mouth, vomiting, clouded consciousness, muscle spasms, low body temperature, hallucinations, tachycardia, and ultimately death.

Tropane alkaloids occur in all parts of the plants and the content (up to 0.5 % in *Datura spp* and 1 % in *Atropa spp*) is such that a small portion (few mg of plant material per kg of goods) is enough to contaminate that product at a level of a few µg/kg.

The most studied TAs, which are biologically active are (-)-hyoscyamine and (-)-scopolamine enantiomers. During sample preparation, (-)-hyoscyamine racemizes to (+)-hyoscyamine and the mixture is called atropine. Due to analytical constraints, it is not always possible to distinguish between the enantiomers of hyoscyamine; therefore, atropine (and scopolamine) are usually determined. Their structures can be found below.



Scopolamine



Atropine

A survey conducted in The Netherlands in 2011, 2012 and 2014 investigating the presence of TAs in cereal-based food for infants and young children resulted in average TA levels of 4.6, 4.4 and 0.5 µg/kg, respectively, with maximum levels of 80.8, 57.6 and 3.9 µg/kg. The ARfD established by EFSA (0.016 µg/kg body weight (b.w.)) would actually have been exceeded for 8 of the 93 products sampled in 2011 and 2012 [**2**].

Taking into consideration the available scientific evidence, the European Commission has published a recommendation to Member States (Commission Recommendation (EU) 2015/976) to monitor the presence of tropane alkaloids in food, in particular: cereals and cereal-derived products, gluten-free products, food supplements, teas and herbal infusions and, legume vegetables (without pods), pulses and oilseeds and derived products. The limit of quantification of the analytical method for determination of TAs in cereal-based foods for infants and young children should be preferably below 1 µg/kg [**3**].

A proficiency test (PT) was organised by the EURL-Mycotoxins to underpin and assess the measurement capacity of laboratories in the Member States (MS) concerning the Commission Regulation (EU) 2016/239, enforcing maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children [**4**]. Laboratories that didn't

have a method already implemented for the determination of atropine and scopolamine in the relevant matrices were offered the possibility to request a suitable method description. The laboratories were required to analyse atropine and scopolamine in two cereal-derived products for infants and young children in the range of 0.2-7.4 µg/kg.

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 proficiency tests for the benefit of the NRLs. In view of the recent and foreseen EU legislation on tropane alkaloids in food at the time of planning this study [**3, 4**], the EURL-Mycotoxins organised a proficiency test on the determination of tropane alkaloids (atropine and scopolamine) in cereals and cereal products (in the range of 0.2-7.4 µg/kg). The proficiency test was addressed to the EU Member States' competent laboratories (designated by the national competent authority) plus expert laboratories from industry and academia. Participation was free of charge. Fifty-two laboratories from 18 MS registered for the PT.

The EURL-Mycotoxins performed the planning, execution and assessment of the measurement results on the basis of the requirements laid down in ISO/IEC 17043:2010 [**6**]. Participant's results were evaluated using the ProLab software package (Quodata, Dresden, DE). The team who organized this PT is an ISO/IEC 17043:2010 accredited PT provider [**7**].

3. Confidentiality

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

4. Time frame

The PT was announced on the EURL-Mycotoxins webpage [**7**] on 29 September 2015 and through the EU CIRCABC database on 04 October 2015. Registration for this PT was initially open until 06 November 2015 and then extended until 06 January 2016 (**Annex 9.1**). The participants were given six weeks after dispatch of the samples (18 and 19 January 2016) for sending their results along with a questionnaire duly filled. The deadline for reporting the results was 02 March 2016.

5. Materials

5.1. Preparation

Two different cereal-derived products were purchased in the local retail market: biscuits for infants (Sample C) and cereals for the preparation of porridge (Sample E). These materials were milled with a Retsch ZM 200 centrifugal mill (Retsch GmbH, Haan, Germany) to pass a 2 mm sieve. The materials were spiked with suitable amounts of a *Datura stramonium* (stems) extract in methanol to resemble, as much as possible, a natural contamination. Therefore, the proportion of atropine to scopolamine levels and co-extracted soluble constituents were kept as native as possible. The materials were spiked, then thoroughly homogenised in a tumbler mixer, bottled and stored in the freezer until dispatch. Batches of approximately 3 kg of both materials were prepared, and 15 g portions were packed in amber plastic bottles.

5.2. Homogeneity

For testing the homogeneity of the PT materials, 10 units per material (Samples C and E) were selected randomly. Two independent determinations per bottle were performed using a liquid chromatography-isotope dilution tandem mass spectrometry detection (LC-ID-MS/MS) based method. The 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. The periods of time considered for this study were: 14 days, 28 days and 48 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 assay 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 (≈20 °C, 4 °C and -18 °C for the period between dispatch (t=0) and the deadline for submission of results (t=48 days).

5.4. Distribution

The test materials were dispatched on 18 and 19 January 2016 in polystyrene boxes, containing cooling packs. The samples were mostly received within 24 hours after dispatch. The materials were shipped such that +4 °C was not exceeded.

Each participant received:
a) two test materials for analysis, packed in amber plastic bottles
- Sample C - biscuits for infants
- Sample E - cereals for porridge
b) two amber glass ampoules containing
- Isotope labelled Internal Standard Solution (ISTD mix)
- Tropane Alkaloids Standard solution (TA mix)
c) accompanying letter with instructions on sample handling and reporting (**Annex 9.4**)
d) sample receipt form (**Annex 9.5**) and
e) laboratory specific reporting files with a lab code (by email).

6. Instructions to participants

The laboratories were required to report the mass fractions of atropine and scopolamine (in µg/kg to the nearest 0.01 µg/kg). Then, in the Questionnaire (**Annex 9.6**), participants were asked to mention whether the results **were corrected** for recoveries or **not** and to provide the recoveries figures (in %).

The results were reported by the participants using RingDat software, which is part of the ProLab software. 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 and laboratory details to allow insights on possible individual and general effects observed for discussion at the next EURL/NRL workshop. 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 that the materials were shipped with cooling packs and that upon arrival, the materials needed to be stored at -18 °C until the analysis was performed. Participants were encouraged to perform the analysis as soon as possible.

7. Reference values

The assigned values of the measurands in the test samples were established by Exact-Matching Double Isotope Dilution Mass Spectrometry (EMD-IDMS) at JRC-Geel. This methodology provides the best possible accuracy [**10**].

Table 2: Assigned values and their associated expanded uncertainties for both materials (samples C and E).

Analyte/sample	Assigned value µg/kg	U (k=2) µg/kg
Atropine/sample C	1.16	0.11
Scopolamine/sample C	0.183	0.033
Atropine/sample E	7.44	0.29
Scopolamine/sample E	1.03	0.07

U - expanded uncertainty of the assigned value

8. Evaluation of the results

8.1. General observations

Out of the 52 laboratories that received the PT samples, 48 reported back their results. Four laboratories did not report due to technical problems.

The laboratories were free to use their method of choice. An LC-MS/MS-based SOP for the determination of TAs in cereals was provided to those laboratories that did not have a method beforehand. This was the method developed, validated and used by the EURL Mycotoxins. This method consists of an extraction of the sample with a mixture of MeOH: H_2O: formic acid (39:60:1) by shaking for 1 hour. The extract is analysed by LC-MS/MS with a column containing a pentafluorophenyl stationary phase, and MilliQ water and acetonitrile (both containing 0.1 % formic acid) as mobile phases.

Forty-seven laboratories used an LC-MS/MS technique for the determination of TAs in cereal while one laboratory used GC-MS.

8.2. Scores and evaluation criteria

The individual laboratory performance was assessed in terms of z-scores following the 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 the 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 [**11**] 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 [**12**]. The robust mean and reproducibility standard deviation were computed according to Algorithm A of ISO 13528:2015, and are given just for information purposes [**8**]. Z-scores were calculated for scopolamine and atropine considering as target concentrations the values assigned by EMD-IDMS. The target values were in good agreement with the consensus values (robust mean).

81.2 % of the results reported by the participants obtained acceptable z-scores, $|z| \leq 2$.

10.3 % of the results (16 results) fell into the unacceptable performance range, $|z| \geq 3$ (Figure 1).

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

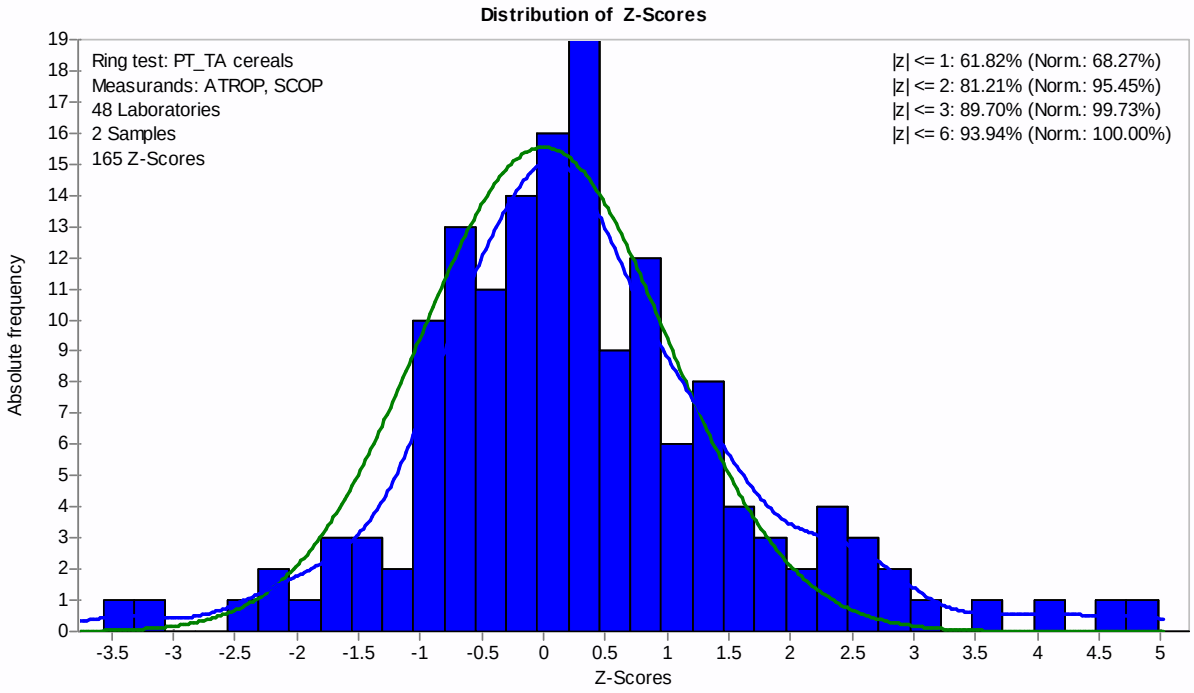
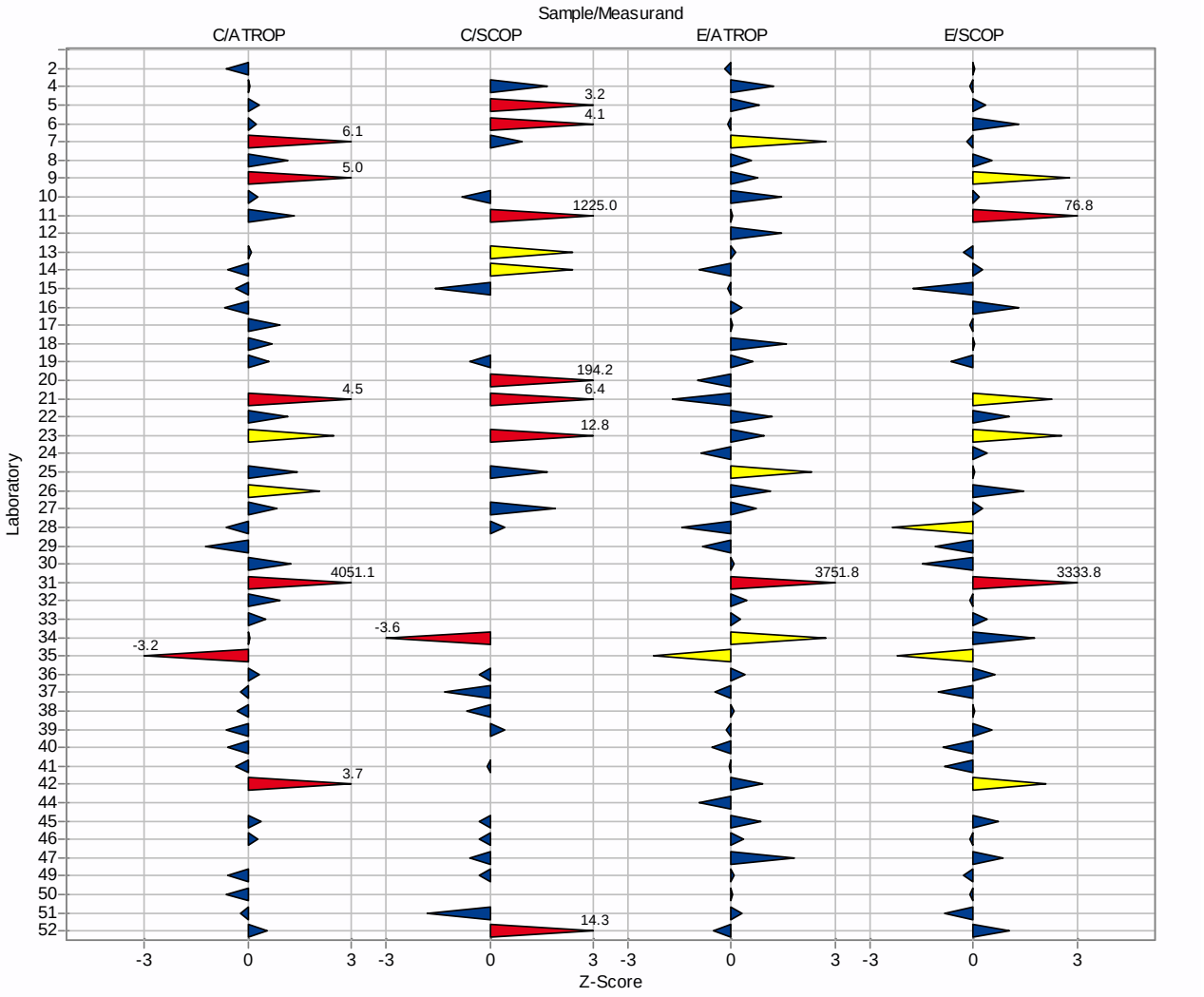


Figure 2 provides an overview of the individual z-scores assigned to the results submitted by the laboratories for atropine and scopolamine in the two cereal test materials. The longer the triangles, the larger were the differences to the assigned values. Yellow triangles represent z-scores in the questionable range and red triangles in the unacceptable performance range. The corresponding scores are shown next to the triangles**.**

Figure 2 - Distribution of z-scores based on the assigned values for each combination of sample (C and E) and measurand (atropine-ATROP and scopolamine-SCOP).



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

Table 3: Analytical results and respective z-scores for TAs in samples C and E.
(Colour code: yellow – questionable, red – unacceptable)

	Sample C				Sample E			
Lab.	**ATROP µg/kg**	**Z score**	**SCOP µg/kg**	**Z score**	**ATROP µg/kg**	**Z score**	**SCOP µg/kg**	**Z score**
2	1	-0.6	< 0.30		7.12	-0.2	1.04	0.1
4	1.18	0.1	0.25	1.7	9.46	1.2	1	-0.1
5	1.24	0.3	0.31	3.2	8.77	0.8	1.11	0.4
6	1.22	0.2	0.35	>4	7.23	-0.1	1.32	1.3
7	2.71	>4	0.22	0.9	11.88	2.7	0.98	-0.2
8	1.46	1.2	< 0.50		8.35	0.6	1.15	0.5
9	2.43	>4	< 1.00		8.65	0.7	1.66	2.8
10	1.23	0.3	0.15	-0.8	9.82	1.5	1.07	0.2

11	1.5	1.3	49.5	>4	7.5	0.04	18.4	>4
12	< 5.00		< 5.00		9.8	1.4	< 5.00	
13	1.19	0.1	0.28	2.4	7.6	0.1	0.96	-0.3
14	1.01	-0.6	0.28	2.4	5.93	-0.9	1.09	0.3
15	1.07	-0.4	0.12	-1.6	7.28	-0.1	0.63	-1.8
16	0.99	-0.7	< 0.60		7.94	0.3	1.32	1.3
17	1.4	0.9	< 1.00		7.49	0.03	1	-0.1
18	1.34	0.7	< 0.30		10.04	1.6	1.03	0
19	1.32	0.6	0.16	-0.6	8.49	0.6	0.88	-0.7
20	< 2.00		8	>4	5.8	-1	< 2.00	
21	2.32	>4	0.44	>4	4.67	-1.7	1.54	2.3
22	1.46	1.2	< 1.00		9.39	1.2	1.26	1
23	1.8	2.5	0.7	>4	9	1	1.6	2.5
24	not tested		not tested		6	-0.9	1.12	0.4
25	1.53	1.4	0.25	1.7	11.25	2.3	1.04	0.1
26	1.69	2.1	< 0.20		9.32	1.1	1.36	1.5
27	1.37	0.8	0.26	1.9	8.58	0.7	1.09	0.3
28	1	-0.6	0.2	0.4	5.1	-1.4	0.5	-2.3
29	0.85	-1.2	< 0.20		6.03	-0.9	0.78	-1.1
30	1.48	1.3	not tested		7.55	0.1	0.69	-1.5
31	1035	>4	< 100.00		6150	>4	755	>4
32	1.4	0.9	not tested		8.17	0.4	1	-0.1
33	1.29	0.5	< 0.50		7.86	0.3	1.12	0.4
34	1.18	0.1	0.04	-3.6	11.9	2.7	1.43	1.8
35	0.35	-3.2	< 0.14		3.72	-2.3	0.53	-2.2
36	1.24	0.3	0.17	-0.3	8.11	0.4	1.17	0.6
37	1.11	-0.2	0.13	-1.3	6.68	-0.5	0.8	-1
38	1.08	-0.3	0.16	-0.7	7.54	0.1	1.04	0
39	1	-0.6	0.2	0.4	7.22	-0.1	1.15	0.5
40	1.01	-0.6	< 0.41		6.5	-0.6	0.83	-0.9
41	1.07	-0.4	0.18	-0.1	7.33	-0.1	0.84	-0.8
42	2.1	3.7	not tested		8.9	0.9	1.5	2.1
44	< 1.00		< 1.00		5.9	-0.9	< 1.00	
45	1.26	0.4	0.17	-0.3	8.84	0.9	1.19	0.7
46	1.23	0.3	0.17	-0.3	8.02	0.4	1	-0.1
47	1.16	0	0.16	-0.6	10.42	1.8	1.22	0.8
49	1.01	-0.6	0.17	-0.3	7.57	0.1	0.96	-0.3
50	1	-0.6	not tested		7.5	0.04	1	-0.1
51	1.11	-0.2	0.11	-1.8	7.9	0.3	0.84	-0.8
52	1.3	0.5	0.76	>4	6.61	-0.5	1.26	1

The results are written as reported by the laboratories.

The graphical representations of the distribution of the results (µg/kg) for each combination of measurand/sample are given in Figure 3. 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 3 - sigmoidal distribution of the individual laboratory values as reported for atropine and scopolamine in samples C and E.

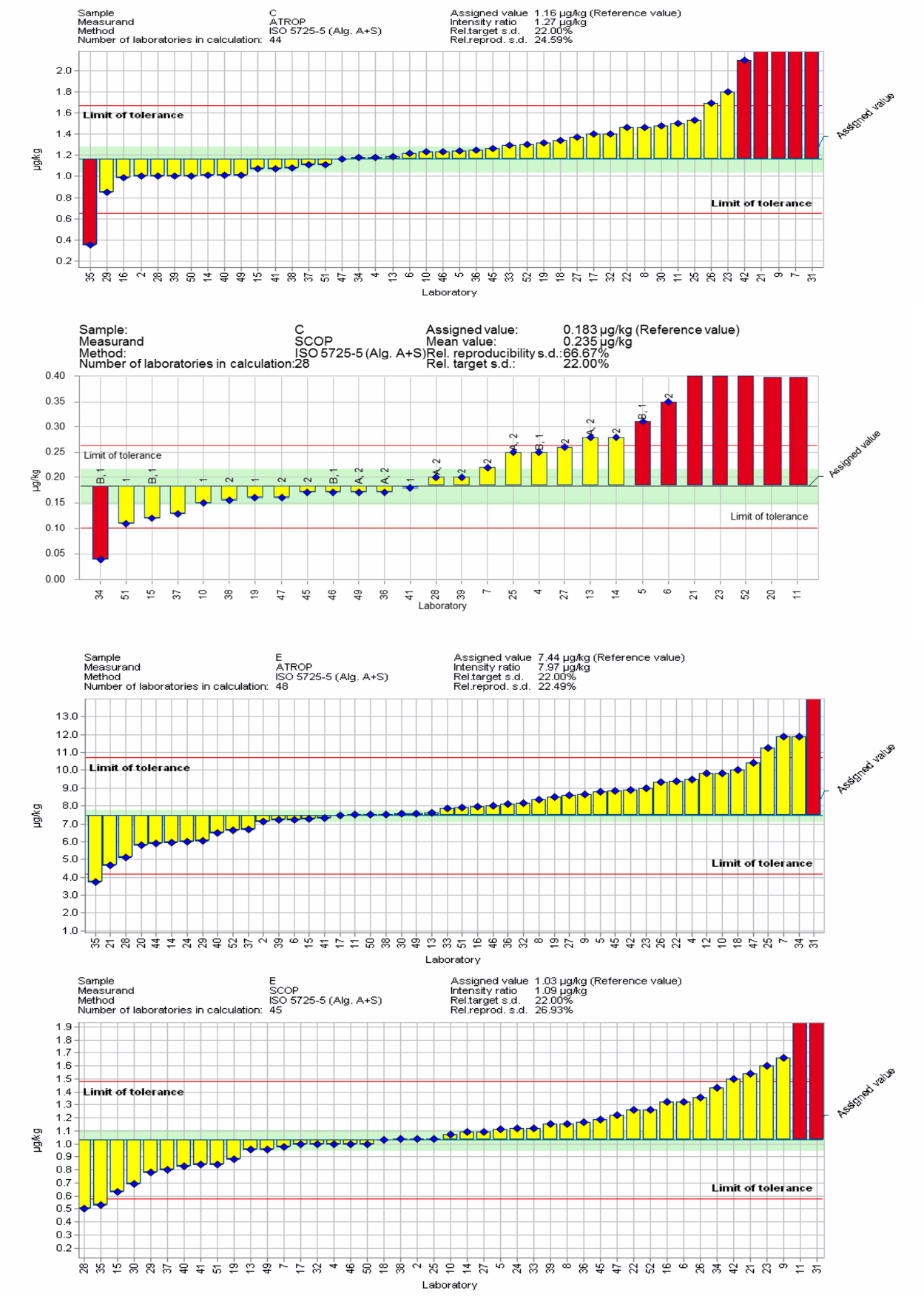
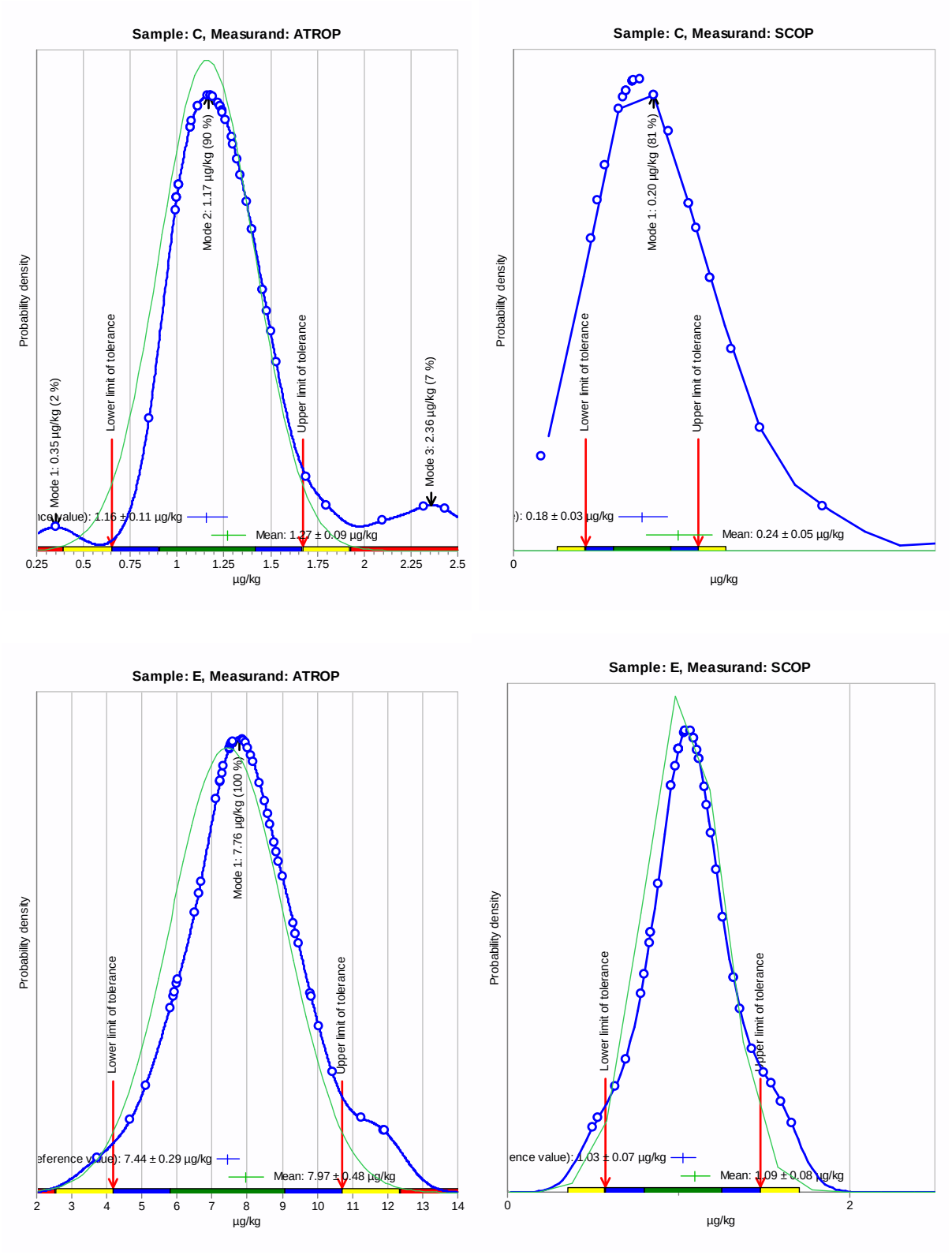


Figure 4 - Kernel density plots of the reported values for atropine (ATROP) and scopolamine (SCOP) in samples C and E.



It should be noted that the confidence intervals of the assigned values always overlap with the confidence intervals of the robust means calculated from the participants' results (Kernel density plot). In particular, a very good match is observed between the target values assigned by IDMS and the main mode of the kernel distribution. This is due to the fact that the robust mean is still influenced by extreme values outside the z-score limit on the higher end; see red flagged bars in Figure 3.

The robust standard deviations of the reported results for both TAs in the cereal test materials are also in good agreement with the target standard deviations, except for the low level of scopolamine in sample C, close to the LOQ (see Table 4).

As it could be seen from the Kernel density plots, the dispersion of the results approximate a Gaussian distribution. The major mode is close to the assigned (reference) value and the robust mean calculated from the results of the participants. This supports the conclusion that the measurement of atropine and scopolamine in cereal samples follows a normal distribution.

Table 4: Summary of the statistical evaluation of the results for scopolamine and atropine in the test samples.

	Units	Scopolamine/ sample C	Scopolamine/ sample E	Atropine/ sample C	Atropine/ sample E
No. of laboratories that submitted results		43	48	47	48
No. of participants (according to design)		48	48	48	48
Assigned (Reference) value	µg/kg	0.183	1.03	1.16	7.44
Uncertainty of the assigned value (k=2)	µg/kg	0.033	0.07	0.11	0.29
Mean (robust)	µg/kg	0.24	1.09	1.27	7.97
Target s.d.	µg/kg	0.04	0.23	0.26	1.64
Reproducibility s.d.	µg/kg	0.12	0.28	0.29	1.67
Rel. SDPA	%	22.0	22.0	22.0	22.0
Rel. reproducibility s.d. (robust)	%	66.7	26.9	24.6	22.5

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 food products.

The questionnaire will be discussed in 3 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 1-4, 31-35 and question 30 of **Annex 9.6**.

2) the second section will deal with the outcome of the Yes/No answers concerning analytical aspects: questions 13-14, 20-21, 24-25, 27-29 and 36 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 cereal products: questions 5-12, 15-19, 22-23, 26 and 37 of **Annex 9.6**.

9.1. Experience and organisational aspects

In Table 5, the number of responses received and the percentage of Yes/No answers regarding the experience of the participants and general organisational matters are compiled. Around 60 % of the participants declared to have prior experience in the analysis of TAs (Q.1). A large majority of these were just capable of determining atropine and scopolamine and the most common matrices were cereals and baby food products. The majority of laboratories had less than two years' experience in this field, except 2 cases (>5 or 10 years). Two laboratories are capable of analysing a range of TAs, up to 20, which may include: acetylscopolamine, anisodamine, anisodine, apoatropine, aposcopolamine, atropine, convolamine, convolidine, convolvine, fillalbin, homatropine, hydroxymethylatropine, littorine, noratropine, norscopolamine, phenylacetoxytropane and scopolamine. There were also five laboratories that analyse atropine and scopolamine in tea and herbs.

Table 5: Response to the questions related to the experience of the participants on the determination of tropane alkaloids in cereals and organisational aspects of the PT

	Q.1	Q.3	Q.13	Q.20	Q.24	Q.25	Q.28	Q.31	Q.32	Q.34	Q.35
Response - NO											
Nr.	19	17	23	17	0	2	39	2	8	25	3
%	*40*	*36*	*49*	*36*	*0*	*4*	*83*	*4*	*17*	*63*	*7*
Response - YES											
Nr.	28	30	24	30	47	43	8	45	39	15	41
%	*60*	*64*	*51*	*64*	*100*	*96*	*17*	*96*	*83*	*38*	*93*

When inquired whether they were capable of analysing other plant toxins (Q.3), 64 % answered positively; however 8 laboratories answered wrongly indicating mycotoxins. The plant toxins of major concern, in addition to the tropane alkaloids, were: pyrrolizidine, opium and quinolizidine alkaloids, following this ranking. Overall, 16 laboratories declared to be able to analyse pyrrolizidine alkaloids, while 7 can do ergot alkaloids analysis. This demonstrates that a wide range of these toxins can already be determined in food products, mainly through multi-toxin LC-MS/MS methods.

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.31), the length of time they spent for issuing the results (Q.33) and whether the sample amount was sufficient for their needs (Q.32). Ninety-six percent of the participants did not find any limitation in the allocated time for reporting back the results (6 weeks). In fact, about 38 % of the participants could perform the analysis of the samples, treat the data and issue the results in **2 days** while 12 % did it in just **1 day**. Forty-eight percent of the participants indicated that they needed **more** than 2 days. The major limitation may have been related to technical problems with the instruments, as LC-MS/MS equipments require frequent maintenance and/or repair.

Eighty-three percent of the participants found the amount of sample dispatched (15 g) enough for performing the analysis (Q.32).

The major complaints were related to the process of results reporting, more precisely, the questionnaire filling, using the RingDat software (Q.34). Thirty-eight per cent of the participants experienced problems, which were timely communicated to us, and deserved our highest priority. As it is a relatively new software solution, the participants

were informed to acquaint themselves with it in advance. Below is a list of the remarks received:

- It was not possible to save all the data filled in the fields to answer the questions. Esp. Q 10, 11 and 12.
- It crashed once, something on the system resources in German was reported
- Difficulties to download ringdat, breakdown
- While saving data for the first time, a number of messages in German appeared. Even Task Manager was not able to stop application (another series of messages). But after restarting the computer everything was fine, and all data were saved.
- It kept crashing / locking me out and I lost entries several times so had to repeat the reporting process!
- The .LA2 file was impossible to open.
- Could not open file: 32.LA2, filling in result form is much easier, as multiple people can log on, Download for result submission is often complicated, as administration rights for computer usually belong to IT department
- Some data would not save (ion details + CID etc)
- At first input of results, the program resources were overloaded and the program crashed. The already inputted data was lost
- System was shut down during data entry
- Software very instable; during the input repeated crashs
- Sometimes no free text available (e.g. 24, 27). Once a choice has been made it can't be deleted.
- The software often crashes immediately.
- System is very unstable; it crashed several times during use
- Too long the overall procedure for reporting back the results. The error messages are not in english

Overall, 93 % of the participants found the instructions (**Annex 9.4**) appropriate and sufficiently explanatory (Q.35).

The participants were informed about this PT through different routes, sometimes cumulatively (Q.30). According to the table below, most participants knew about the PT by direct invitation through mailings from the European Commission CIRCABC database or by the NRLs contact.

Information source about the PT TAs in cereal products	%
Through the EURL Mycotoxins website	16
During the EURL workshop for the NRLs on mycotoxins	11
By invitation from the European Commission communication office	30
By the NRL in your country	20
By professional associates in your sector	13
Other	11

9.2. Analytical aspects

When asked whether the analytical method used for analysing TAs in the PT samples was validated (Q.13, Table 4), about 50 % of the participants replied that they have not collected validation data. This finding might be explained by the fact that many laboratories have implemented the method just before the proficiency test, and therefore didn't have enough time to validate the method properly.

Of those who validated the method, the **recoveries and the LOQs** were estimated by 78 % of the laboratories while the **precision, linearity and LOD** were estimated by just 65 % of the laboratories. Only one laboratory stated to have estimated the measurement uncertainties. This figure of merit was not asked for this PT.

As isotope-labelled internal standards for atropine and scopolamine are commercially available, the participants were asked whether they used this strategy (isotope dilution MS) for quantification (Q.20). Sixty-four percent of the participants answered positively. The majority of them (91 %) added the internal standards **before the extraction** while 9 % added the internal standards **after the extraction** (Q.21). The first approach provides more benefits as the internal standards can correct the results simultaneously for the losses during the extraction step and also compensate the matrix effects during MS analysis.

All the participants have checked the integration of the chromatographic peaks (Q.24) while 96 % also checked the goodness of fit of the calibration curve in the region where the signal of the samples is interpolated (Q.25).

Additionally, the participants were asked to indicate whether the results reported were **corrected for recoveries** or **not corrected for recoveries**, following their normal routine procedures (Q.26). Forty-three per cent answered that they had corrected the results for recoveries while 55 % declared that they didn't. Nevertheless, the answer to this question has to be analysed in relation with Q.21. Whenever the participants stated that the internal standards were added to the sample before the extraction and given that an internal calibration was used, the obtained results were automatically corrected for the recoveries without any further calculation. In light of this, the results that have been reported without correction for recoveries might be just 21 %.

Regarding the satisfaction of the participants with the structure of the PT, 83 % declared that they didn't experience any major difficulties analysing the distributed samples (Q.28). Those who experienced problems reported issues related to the matters mentioned in the table below (copied from the questionnaire). On average, the analyst responsible for conducting the PT had >7 years of experience.

- Low levels of atropin and scopolamin in the samples (<0.1µg/kg)
- The samples lumped during the first extraction step
- Sensitivity of the instrument
- Purchase of standards but the main difficulty was the extraction of samples. It seems that the used one is not appropriate enough
- Very limited amount of sample (not enough to perform e.g. a final standard addition approach)/ without further clean-up LOQ of 1 µg/kg is difficult to reach / Around 15g of sample is not enough to get good and correct results. It should be at least 200g or even more
- Sensitivity of the instrument
- Not stable sensitivity of the instrument
- Too close to LOD/LOQ

The mentioned remarks related to the low levels of TAs in the samples vs. insufficient sensitivity of the instruments affected just a limited number of participants. Eight participants would have preferred to receive a higher amount of sample.

Below is a compilation of the general comments (Q.36), received from the participants including both analytical information and reporting improvement opportunities.

- See email text because here it is not possible to submit all the data, because we were not able to save it. After saving it was deleted automatically by the ringdat software.
- As our RR% were calculated in raw buckwheat by spiking, we didn't apply any recovery correction to the dispatched samples. We did not use isotopic std, since the solutions were slightly opaque after preparation (possible interferences?).
- We used standard addition for quantification purposes. Our own atropin and scopolamin standard solution have much lower response values for the same concentrations (we prepared the

solutions freshly)
- Results reported are inherently corrected for the isotopically labelled internal standard used during the analysis. Recovery values reported are for information only, and were from samples spiked at 10ug/kg analysed at the same time to provide additional information on method performance.
- A blank from the same matrix would have been useful
- Question No 24: Approach for calibration: We use the procedural standard calibration which automatically corrects for recovery losses as well as matrix effects
- A blank for the matrix-matched calibration and recovery experiments would have been really helpful
- We did the matrix calibration with an uncontaminated rye flour, because we did not know which matrix exactly you sent to us. We didn´t want to use the contaminated material for spiking. Our reported results were the mean value of four.
- For the small amounts of sample C we did a standard addition to calculate contents
- Please make the form printable!
- In our method matrix matched calibration is only used to check the linearity and sensitivity of the system. Actual quantitation is performed by standard addition to the sample (in this case 25 ug/kg)
- Sample E064 seems to contain a small amount of anisodamine, aprox. 0.74 µg/kg; Recovery in this PT was calculated by means of the internal standard.

9.3. Methods' overview

Along with the analytical results, the participants in this PT also submitted a compilation of some figures of merit and a description of core methodological features. In **Annex 9.7.1,** the reported limits of quantification (LOQs), recoveries (%), matrix suppression (MatrixSup, %) and retention times (RT, min) for both atropine and scopolamine are shown. As it can be seen in the histograms below (Figure 5), a vast majority of the reported LOQs fall below 1 µg/kg, therefore, the state-of-the-art instrumentation used provided sufficient sensitivity to yield signals for quantification. The majority of the applied methods resort to a simple "dilute and shoot" approach (**Annex 9.7.2**, Question 7).

On average, the participants reported recoveries close to 95 % for both atropine and scopolamine, though with a bigger dispersion in the latter case (Figure 6). Given the diversity of extraction methods applied (Question 6: shaking, QuEChERS, different solvent compositions), these figures fall within an acceptable range. However, it is unknown whether they are relative recoveries, absolute recoveries or a combination of both.

As far as the matrix effects (matrix suppression) are concerned, they span a wide range. However, no objective interpretation can be made as, apparently, the participants reported their values in different units.

An overall evaluation of the analytical methodologies employed (**Annex 9.7.2**) indicated that 10 laboratories applied the EURL-developed method, as they did not have any previous method implemented. Five laboratories applied the RIKILT SOP A1070 while an additional three laboratories applied the method described in Adamse, P; van Egmond H.P. (2010): Report 2010.011, which follows a similar principle. Four laboratories declared that they used a QuEChERS clean-up while five others followed the reference: Jandric *et al.,* Food Additives and Contaminants 28 (9) (2011) 1205-1219, which describes also a QuEChERS-related clean-up. Eighteen 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 resorted to LC-MS/MS for separation and detection, except one laboratory that used GC-MS after derivatisation of the analytes with BSTFA/TMCS. An evaluation of the performance of the laboratories did not reveal any dependence on the methodology used, with statistical significance.

Figure 5 – Histograms of the methods' LOQs for atropine and scopolamine in cereal samples

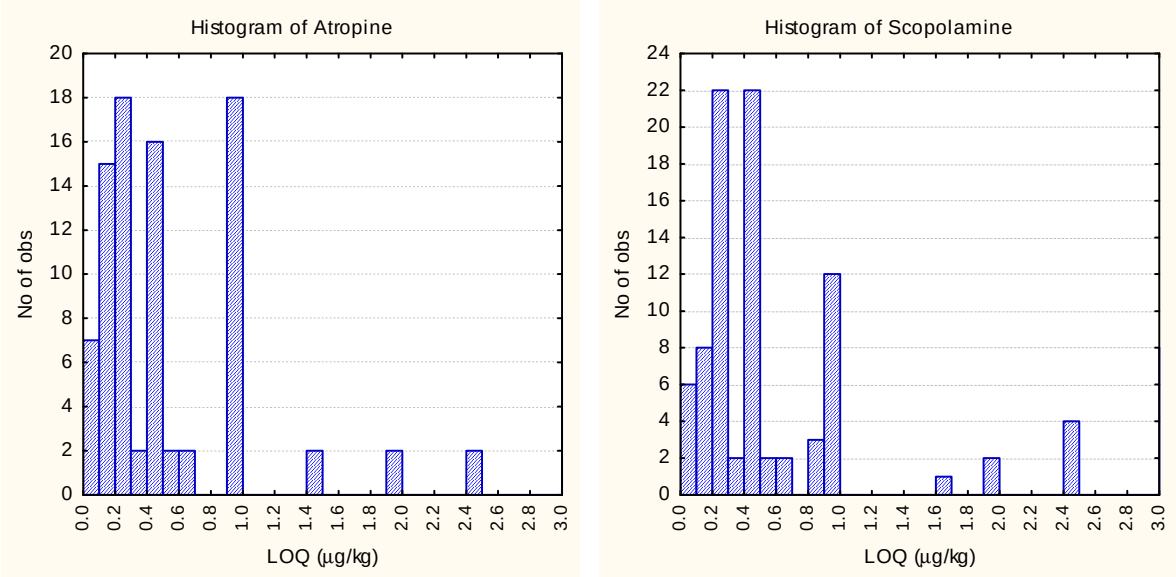
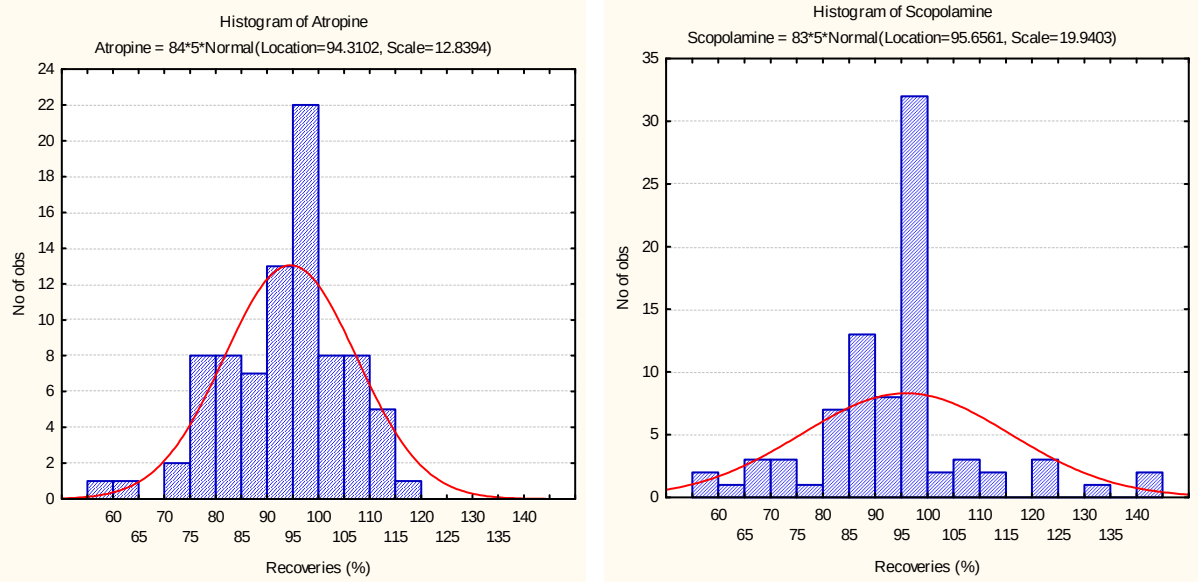


Figure 6 – Histograms of methods' recoveries (%) for atropine and scopolamine in cereal samples.



10. Conclusions

The EURL-Mycotoxins organised a proficiency test on the determination of tropane alkaloids (atropine and scopolamine) in cereal-based baby food upon the DG SANTE request. Both the sample matrices and measurand levels were targeted to provide insight on the measurement capabilities of EU Member States' laboratories concerning the implementation of recently published legislation in this field (maximum limits of atropine and scopolamine of 1.0 µg/kg, Commission Regulation (EU) 2016/239).

Fifty-two laboratories registered for this PT, of which 48 participants from 18 EU Member States submitted their results. Twenty-one participants were from Germany, four from Italy and three from Spain, with the remaining countries having one or two representatives.

Overall, 81 % of the z-scores were in the range of [-2 to 2] and 90 % were in the range of [-3 to 3]. At the lowest TA level (sample C), 75 % of z-scores fell within an acceptable range ($|z| \leq 2$), while this figure improved to 86 % at the highest level (sample E). For atropine, 86 % of the results were in the acceptable range ($|z| \leq 2$) while for scopolamine 75 % were in the same range in the two cereal samples. This does not necessarily mean that scopolamine entails a more difficult analysis as the concentration levels were constantly lower than atropine.

The majority of reported LOQs fell below 1 µg/kg. The extraction conditions used by the participants can be clustered in 3 groups: method supplied by the EURL; RIKILT SOP A1070 and QuEChERS with final determination of the analytes by LC-MS/MS, with one single exception (GC-MS). The recoveries reported by the participants were close to 95 %. No statistically significant dependence of the z-scores on the analytical methodology applied neither on the source of the standards was observed.

These results support the assumption that atropine and scopolamine can be reliably determined at the levels regulated by the Commission Regulation (EU) 2016/239, using the state-of-the-art analytical instrumentation. A good overall performance in the PT was observed although some laboratories have just implemented the method prior to the PT. The outcome of this PT should help the laboratories to consolidate and improve their analytical competence where needed.

References

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URL: https://www.efsa.europa.eu/en/efsajournal/pub/3386

[**2**] Patrick P.J. Mulder, Diana P.K.H. Pereboom-de Fauw, Ron L.A.P. Hoogenboom, Joyce de Stoppelaar and Monique de Nijs, Tropane and ergot alkaloids in grain-based products for infants and young children in the Netherlands in 2011–2014, Food Additives & Contaminants: Part B, 8(4) (2015) 284–290

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[**4**] European Commission. 2016. Commission Regulation (EU) 2016/239, amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children. Official Journal of the European Union. L 45/3.
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[**6**] ISO/IEC 17043:2010 - Conformity assessment - General requirements for proficiency testing

[**7**] JRC Geel. EURL for mycotoxins. Inter-laboratory comparisons. Available from:
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[**11**] Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst 125:385-386.
URL: http://pubs.rsc.org/en/content/articlepdf/2000/an/b000282h

[**12**] Software for PT programs and collaborative studies, ProLab;
URL: http://quodata.de/en/software/for-interlaboratory-tests.html

List of abbreviations and definitions

ACN	Acetonitrile
EURL	European Union Reference Laboratory
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
EMD-IDMS	Exact matching double 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
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.

On behalf of the European Union Reference Laboratory for Mycotoxins (EU-RL 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 in the range of 0.5-20 µg/kg.

The proficiency test (PT) is open to all competent laboratories and expert laboratories. The dispatch of approx. 2-3 samples is expected by mid-November 2015. Participants will have 6 weeks from the dispatch date to report back the results.

Participation is free of charge. Confidentiality of results is guaranteed.

The background for this PT is to underpin the measurement capacity of laboratories concerning the upcoming EU legislation on tropane alkaloids in food by April 2016. Laboratories that do not have a method for atropine and scopolamine in cereal based products can be supplied with a suitable method description upon request.

In case of interest, please fill in your contact details below. The deadline for registration is the 06th of November 2015.

Thank you in advance for your consideration.

EURL Mycotoxins Operating Manager

* Status

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

1

* Department

* Address

* City

* Zip Code

* Country

* Contact person

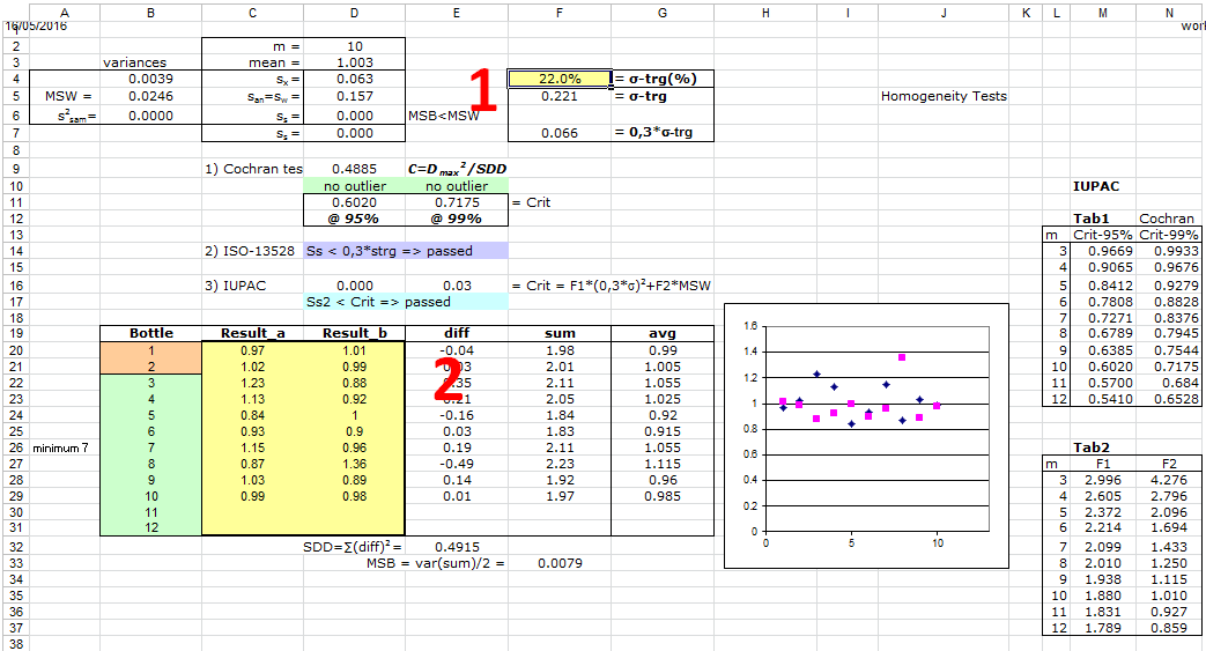
* Telephone number

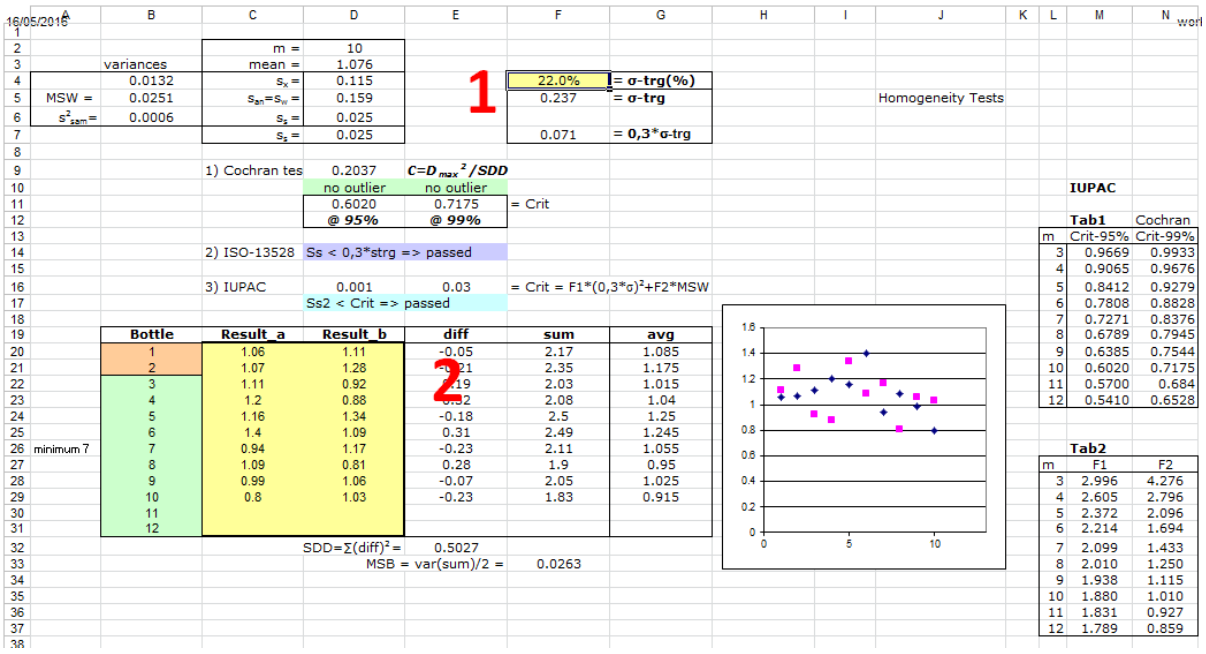
* E-mail address

2

9.2. Homogeneity test

Homogeneity according to ISO 13528:2015 [8]	Sample C	Sample E
	Atropine	Scopolamine
Mean	1.003	1.076
$\hat{\sigma}$	0.221 (22 %)	0.237 (22 %)
0.3 $\hat{\sigma}$ (critical value)	0.066	0.071
S_X (standard deviation of sample averages)	0.063	0.115
S_W (within-sample standard deviation)	0.157	0.159
S_S (between-sample standard deviation)	0.000	0.025
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**





9.3. Stability study

Stability study – Sample C

	Atropine				Scopolamine			
T (ºC)	Slope	Lower 95 % *	Upper 95 % *	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
-18	0.00037	-0.00171	0.00245	**YES**	0.00011	-0.00068	0.00089	**YES**
4	0.00073	-0.00172	0.00319	**YES**	-0.00016	-0.00088	0.00055	**YES**
20	0.00124	-0.00238	0.00486	**YES**	0.00004	-0.00083	0.00092	**YES**

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

Stability study – Sample E

	Atropine				Scopolamine			
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
-18	-0.00117	-0.01928	0.01693	**YES**	-0.00014	-0.00338	0.00310	**YES**
4	-0.00794	-0.02685	0.01098	**YES**	0.00013	-0.00232	0.00259	**YES**
20	0.00850	-0.00231	0.01932	**YES**	0.00027	-0.00079	0.00133	**YES**

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)355208 - 22/01/2016

Geel, 20th of January 2016

2016 PROFICIENCY TESTING FOR ALL COMPETENT LABORATORIES AND EXPERT LABORATORIES REGARDING THE DETERMINATION OF TROPANE ALKALOIDS IN CEREAL-BASED FOOD PRODUCTS

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.).

The 2016 EURL PT on Tropane Alkaloids aims to assess the content of two contaminated test samples (Sample C# and Sample E#) on atropine and scopolamine. You will be asked to report their **concentration in µg kg^{-1}**, as it is normal practice in your laboratory. 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 the 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 (analytes and internal standards) 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 standards to reach room temperature and homogenise them.

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.

Reporting the results

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 will receive by email two files for reporting the results. You should follow the instructions below:

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., C058);
- 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.

Please report the samples results in µg kg^{-1}, to the closest 0.01 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.

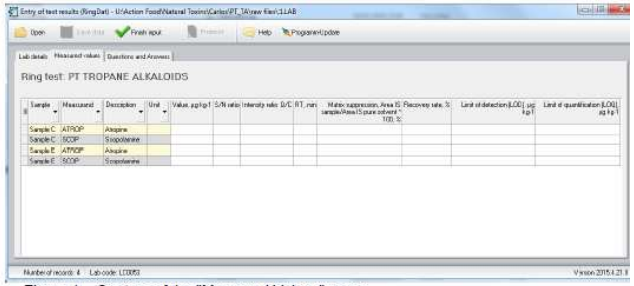


Figure 1 – Capture of the "Measured Values" page

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

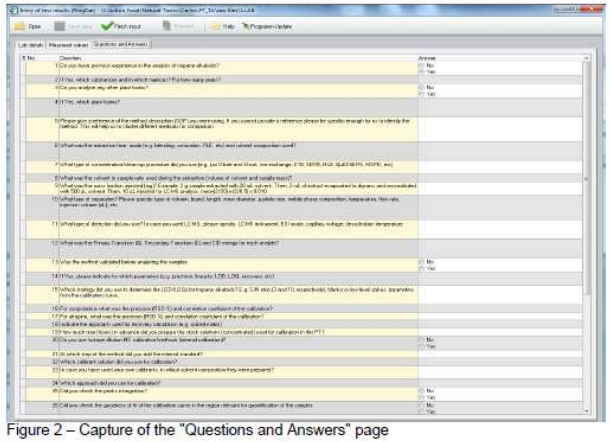


Figure 2 – Capture of the "Questions and Answers" page

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.

Deadline for reporting the PT results is the 02nd of March 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 with the analysis and success!

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

With kind regards,

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

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

9.5. Acknowledgement of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 12th of January 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	
All items have been received undamaged	YES / NO
If NO, please list damaged items:	

Contents of the parcel:

a) Two test materials for analysis packed in ambar bottles:
- Sample C
- Sample E

b) Two ambar glass ampoules
- Isotope labelled Internal Standard solution (ISTD mix)
- Tropane Alkaloids Standard solution (TA mix)

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:

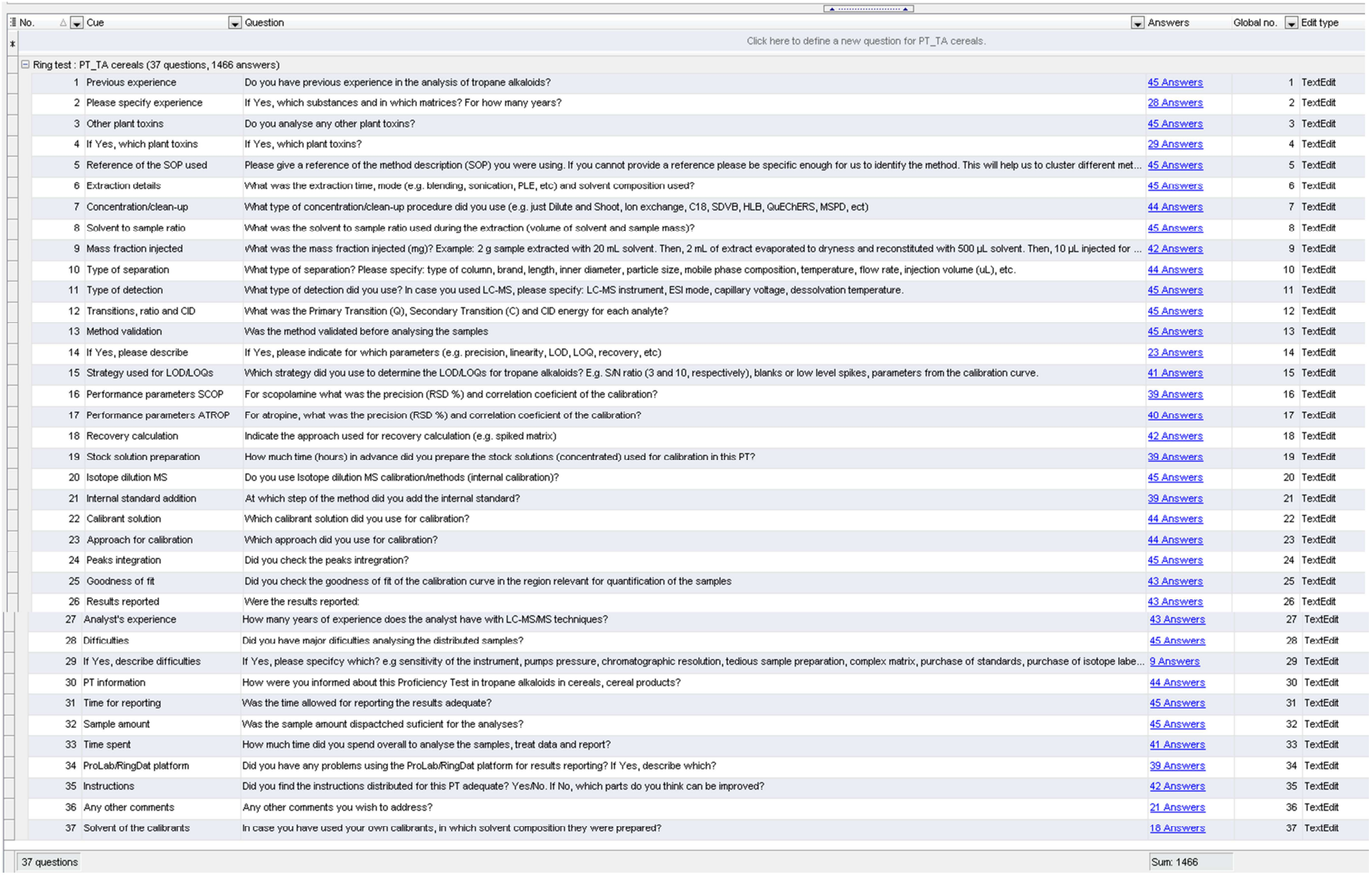
Carlos GONÇALVES

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



No.	Cue	Question	Answers	Global no.	Edit type
*		Click here to define a new question for PT_TA cereals.			
	Ring test : PT_TA cereals (37 questions, 1466 answers)				
1	Previous experience	Do you have previous experience in the analysis of tropane alkaloids?	45 Answers	1	TextEdit
2	Please specify experience	If Yes, which substances and in which matrices? For how many years?	28 Answers	2	TextEdit
3	Other plant toxins	Do you analyse any other plant toxins?	45 Answers	3	TextEdit
4	If Yes, which plant toxins	If Yes, which plant toxins?	29 Answers	4	TextEdit
5	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. This will help us to cluster different met...	45 Answers	5	TextEdit
6	Extraction details	What was the extraction time, mode (e.g. blending, sonication, PLE, etc) and solvent composition used?	45 Answers	6	TextEdit
7	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)	44 Answers	7	TextEdit
8	Solvent to sample ratio	What was the solvent to sample ratio used during the extraction (volume of solvent and sample mass)?	45 Answers	8	TextEdit
9	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 500 µL solvent. Then, 10 µL injected for ...	42 Answers	9	TextEdit
10	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 volume (uL), etc.	44 Answers	10	TextEdit
11	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.	45 Answers	11	TextEdit
12	Transitions, ratio and CID	What was the Primary Transition (Q), Secondary Transition (C) and CID energy for each analyte?	45 Answers	12	TextEdit
13	Method validation	Was the method validated before analysing the samples	45 Answers	13	TextEdit
14	If Yes, please describe	If Yes, please indicate for which parameters (e.g. precision, linearity, LOD, LOQ, recovery, etc)	23 Answers	14	TextEdit
15	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 calibration curve.	41 Answers	15	TextEdit
16	Performance parameters SCOP	For scopolamine what was the precision (RSD %) and correlation coeficient of the calibration?	39 Answers	16	TextEdit
17	Performance parameters ATROP	For atropine, what was the precision (RSD %) and correlation coeficient of the calibration?	40 Answers	17	TextEdit
18	Recovery calculation	Indicate the approach used for recovery calculation (e.g. spiked matrix)	42 Answers	18	TextEdit
19	Stock solution preparation	How much time (hours) in advance did you prepare the stock solutions (concentrated) used for calibration in this PT?	39 Answers	19	TextEdit
20	Isotope dilution MS	Do you use Isotope dilution MS calibration/methods (internal calibration)?	45 Answers	20	TextEdit
21	Internal standard addition	At which step of the method did you add the internal standard?	39 Answers	21	TextEdit
22	Calibrant solution	Which calibrant solution did you use for calibration?	44 Answers	22	TextEdit
23	Approach for calibration	Which approach did you use for calibration?	44 Answers	23	TextEdit
24	Peaks integration	Did you check the peaks intregration?	45 Answers	24	TextEdit
25	Goodness of fit	Did you check the goodness of fit of the calibration curve in the region relevant for quantification of the samples	43 Answers	25	TextEdit
26	Results reported	Were the results reported:	43 Answers	26	TextEdit
27	Analyst's experience	How many years of experience does the analyst have with LC-MS/MS techniques?	43 Answers	27	TextEdit
28	Difficulties	Did you have major dificulties analysing the distributed samples?	45 Answers	28	TextEdit
29	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 of standards, purchase of isotope labe...	9 Answers	29	TextEdit
30	PT information	How were you informed about this Proficiency Test in tropane alkaloids in cereals, cereal products?	44 Answers	30	TextEdit
31	Time for reporting	Was the time allowed for reporting the results adequate?	45 Answers	31	TextEdit
32	Sample amount	Was the sample amount dispactched suficient for the analyses?	45 Answers	32	TextEdit
33	Time spent	How much time did you spend overall to analyse the samples, treat data and report?	41 Answers	33	TextEdit
34	ProLab/RingDat platform	Did you have any problems using the ProLab/RingDat platform for results reporting? If Yes, describe which?	39 Answers	34	TextEdit
35	Instructions	Did you find the instructions distributed for this PT adequate? Yes/No. If No, which parts do you think can be improved?	42 Answers	35	TextEdit
36	Any other comments	Any other comments you wish to address?	21 Answers	36	TextEdit
37	Solvent of the calibrants	In case you have used your own calibrants, in which solvent composition they were prepared?	18 Answers	37	TextEdit
37 questions			Sum: 1466		

9.7. Experimental details

9.7.1. 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	Sample C	0.3	0.9	100	100	100		5.78	4.78
	Sample E	0.3	0.9	100	100	100		5.78	4.77
4	Sample C	0.2	0.2			60.8	54.8	6.78	6.42
	Sample E	0.2	0.2			48.6	42.9	6.78	6.42
5	Sample C	0.25	0.25	101	100	1.04	1.01	10.4	7.4
	Sample E	0.25	0.25	101	100	1.36	1.25	10.4	7.4
6	Sample C	0.5	0.5	91	66	96	85	2.95	2.48
	Sample E	0.5	0.5	93	69	97	87	2.95	2.48
7	Sample C	2.5	2.5	110	93			2.4	2.2
	Sample E	2.5	2.5	110	93			2.4	2.2
8	Sample C	0.5	0.5	95	115	45		6.4	
	Sample E	0.5	0.5	95	115	35	35	6.4	5.3
9	Sample C	1	0.3	104	98			7.95	5.25
	Sample E	1	0.3	104	98			7.95	5.26
10	Sample C			108	94				
	Sample E			108	94				
11	Sample C	1.5	0.5	98	103			15.25	15.95
	Sample E	1.5	0.5	92	94			15.25	15.95
12	Sample C	5	5	100	100			2.91	2.97
	Sample E	5	5	100	100			2.91	2.97
13	Sample C	0.5	0.5	91.2	86.6	95	65	6.7	5.6
	Sample E	0.5	0.5	91.2	86.6	95	65	6.7	5.6
14	Sample C	0.2	0.3	80	85	59	68	6.81	2.69
	Sample E	0.2	0.3	80	85	45	79	6.82	2.69
15	Sample C	0.1	0.1	95.2	75.3				
	Sample E	0.1	0.1	95.7	67.3				
16	Sample C	0.6	0.6	113	121	72	65	5.72	4.68
	Sample E	0.6	0.6	117	131	54	43	5.67	4.63
17	Sample C	1	1	106	94			1.22	1.09
	Sample E	1	1	100	99			1.22	1.09
18	Sample C	0.3	0.3	94	82	81.2	77.9	8	6.85
	Sample E	0.3	0.3	78.5	95.5	71.8	59	7.93	6.8
19	Sample C	0.2	0.2	90	90	145	121	8.9	6.8
	Sample E	0.02	0.2	90	90	145	121	8.9	6.8
20	Sample C	5	5	79	98	35	12	0.98	0.79
	Sample E	5	5	78.9	98	35	12	0.98	0.79
21	Sample C	0.31	0.44						
	Sample E	0.31	0.44						
22	Sample C	1	1	82	82			1.57	1.33
	Sample E	1	1	82	82			1.57	1.33
23	Sample C	0.5	0.5	84	63	102	120	2.95	2.76
	Sample E	0.5	0.5	79	75	109	104	2.95	2.76
24	Sample C	0.28	1.62	108	210				
	Sample E	0.17	0.86	88	165			9.57	8.51
25	Sample C	0.17	0.33	62	89	96	52	6.4	5.3
	Sample E	0.17	0.33	36	106	69	35	6.3	5.2
26	Sample C	0.3	0.2	85	85			5.44	4.68
	Sample E	0.2	0.2	85	85			5.43	4.68
27	Sample C	0.5	0.5	86.6	96.5			4.03	4.04
	Sample E	0.5	0.5	83.5	94.8			4.19	4.2
28	Sample C	0.3	0.3	100	100	1	0.6	9.3	7.9
	Sample E	0.3	0.3	100	100	0.6	0.4	9.3	7.9
29	Sample C	0.7	0.7	80	86			5.1	4.7
	Sample E	0.7	0.7	80	86			5.1	4.7
30	Sample C	1	0.5	100	100			3.56	

	Sample E	1	0.5	100	100			3.58	2.92
31	Sample C	100	200	113.8	121.3	123	107	4.4	2.6
	Sample E	100	200	113.8	121.3	137	127	4.4	2.6
32	Sample C	1	1	72	58			3.83	3.73
	Sample E	1	1	72	58			3.83	3.72
33	Sample C	1	1	105	97	110	102	4.82	2.49
	Sample E	1	1	105	97	111	102	4.82	2.49
34	Sample C	2	2	113	110			2.71	1.94
	Sample E	2	2	104	106			2.71	1.94
35	Sample C	0.25	0.25			-	-	7.05	3.69
	Sample E	0.25	0.25			-	-	7.06	3.71
36	Sample C	0.3	0.3	100	100	11.86	8.34	4.31	3.46
	Sample E	0.3	0.3	100	100	12.41	8.3	4.28	3.42
37	Sample C	0.1	0.1	100	100			7.02	4.68
	Sample E	0.1	0.1	100	100			7.02	4.68
38	Sample C	1	2.5	94.18	97.88	93.1	47.8	3.17	1.64
	Sample E	1	2.5	94.18	97.88	93.1	47.8	3.16	1.63
39	Sample C	0.2	0.3	97	99	84	84	6.12	4.84
	Sample E	0.2	0.3	97	98	75	65	6.14	4.87
40	Sample C	0.12	0.41	104				7	6.36
	Sample E	0.12	0.41	114.3	96			7	6.36
41	Sample C	0.2	0.2	109	101	47	55	2.37	2.01
	Sample E	0.2	0.2	107	89	66	69	2.35	2.01
42	Sample C	5	5	99	97	0	0	6.43	5.68
	Sample E	5	5	99	97	0	0	6.43	5.68
44	Sample C	1	1						
	Sample E	1	1						
45	Sample C	0.3	0.3					10.2	8.7
	Sample E	0.3	0.3					10.2	8.7
46	Sample C	0.5	0.25	85.1	87.5			7.99	7.25
	Sample E	0.5	0.25	91.9	90.4			7.99	7.25
47	Sample C	0.1	0.1			27.7	31	6.48	5.39
	Sample E	0.1	0.1			77.64	76.95	6.48	5.39
49	Sample C	0.26	0.27	88.5	98.3	59	86	3.65	2.84
	Sample E	0.26	0.27	88.5	98.3	45	60	3.65	2.84
50	Sample C	1	1	91	88	91	88	4.1	3.6
	Sample E	1	1	91	88	91	88	4.1	3.6
51	Sample C	0.5	0.5	83	89	207	63	5.9	4.76
	Sample E	0.5	0.5	98	74	165	81	6.13	4.92
52	Sample C	0.5	0.5	83	89			5.9	4.76
	Sample E	0.5	0.5	98	74			6.13	4.92

Atrop – atropine; Scop - scopolamine

9.7.2. Analytical conditions

Lab	Q.5 Reference of the SOP used	Q.6 Extraction details	Q.7 Concentration /clean-up	Q.8 Solvent to sample ratio	Q.9 Mass fraction injected
2	Jakabová et al. Journal of Chromatography A, 1232 (2012) 295– 301	1 min. vortex, 20 min sonication in: 300 mL MeOH + 200 mL H2O + 0.5 mL FormAc	Filtration; Dilute and Shoot	1 g in 20 mL	0.0001
4	Sample preparation procedure for the analysis of tropane alkaloides in food and feed by LC-MS/MS (RIKILT SOP A 1070)	extraction time: 30 min / 0,4 % formic acid in methanol / water (69:40)	2 ml of the supernatant centrifuged with ultrafilter tubes (40kDa)	2 g sample extracted with 20 ml sol. 0.4 % formic acid in MeOH/water 60:40 (v/v)	10 µl filtrate injected for LC-MS/MS analysis
5	Adamse, P.; Egmond, H.P. van; Noordam, M.Y.; Mulder, P.P.J.; Nijs, M. de, Quality Assurance and Safety of Crops & Foods 6 (1) (2014) 15 - 24	stir 45 min, pH9, Ammonium carbonate /Acetonitrile 16/ 84	Bondesil PSA 40 µm	5 g to 25 ml extraction solvent	5g / 25ml / 1ml evaporate to dryness / reconstitute in 5ml/ inject 10µl

6	QuEChERS	5 min, blending with Geno/Grinder. Water (0.5 % acetic acid) and acetonitrile.	QuEChERS EMR	S/S=4. 2.5 g sample extracted with 10 mL solvent. 2 µL injected for LC-MS analysis.	meq =0.0005
7	Detection of ergot and tropane alkaloids by LC-MS/MS	modified quechers / 4 g of sample / 30 mL ACN/H2O + 2.1 mmol/L ammonium carbonate (84/16, v/v) / 45 min rotate overhead / Add salts MgSO4 (4g) /NaCl (1g) / Centrifuge at 10,000 rpm 5 min / 2 mL of supernatant through a 0.2 um PTFE syringe filter / Standard addition with 5 µl of 100 ppb	modifed quechers	4 g of sample 30 ml of solvent (with 25.2 mL of organic solvent)= 6.3	meq= 4 g of sample 30 ml of solvent (with 25.2 mL of organic solvent)* 1 µl injection
8	RIKILT SOP A1070	30 min blending, extraction solvent: 0,4 % Formic acid in Methanol/Water (6/4)	No concentration/ clean-up procedure	40 mL to 4g sample, i.e. solvent to Sample ratio =10	meq: (40/4) x 0.002 = 0.0002
9	The method is based on LC-MS/MS detection	Blending extraction; solvent: water:methanol 2:3 v/v followed by centrifugation at 4000 rpm	Dilution and shoot. The supernatant was diluted 10 times, for a total dilution 1:50 w/v	1:5 w/v	meq = 0.0008
10	This is our in-house method.	SLE		2g sample and 20 mL solvent	
11	Based upon a paper by Akira Namera (Springer-Verlag Berlin 2005	Mix with NT 20 Kieselguhr and extract with 3x5mL of dichloromethane. Evaporate to dryness derivatise with BSTFA/TMCS	Evaporate to dryness at 50°C and derivatise with BSTFA/TMCS	15mL of dichloromethane to 0.5 g of sample	10-100 ng (nanogram)
12	Stefan Kittlaus, Julia Schimanke, Günther Kempe, Karl Speer Journal of Chromatography A, 1283 (2013) 98-109	30 min ACN:water	dilute and shoot	20 mL to 2.5 g sample	0.0625
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	(2/20)*0.020 = 0.002
14	extraction with 0.05M H2SO4, centrifugation, supernatant pH adjustment to 9-10 with NH4OH, extraction with ethylacetate, EtOAc evaporation, dissolution, high-speed centrifugation, injection	extraction by agitation (vortex 10s, overhead 15min) and sonication 15 min, 0.05M H2SO4	pH adjustment to 9-10, LLE with EtOAc, evaporation of the EtoAc, dissolution and centrifugation	20 mL to 2 g	0.002
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	4 g sample in 40 ml extraction solvent	4g/40ml, 10ml for SPE, made up in 0.5ml = 2g/ml. Injection 2ul meq=0.004 g
16	Draft method which was sent from JRC.	Vortex few seconds, shaking 1 hour. Solvent methanol/water/formic acid 39:60:1	Dilute and shoot	10	0.001
17	QuEChERS	30 min	QuEChERS	5/2	0.72
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)x 0.010
19	Jandric et al., Food Additives and Contaminants Vol 28 (9), 2011, 1205-1219	5 Minutes, centrifugation	C18	10/2	
20	Jandric et al., Food Additives and Contaminants 28 (9) (2011) 1205-1219		just dilute and inject	2 g sample/20 mL solvent	0.2
21					

22	In house developped method	60 min by shaking with a solution of ACN:H20=3:2	dilute and shoot	5:1	0.2 mg
23	Mol et. al, Food Additives and Contaminants 28(10) (2011) 1405-1423	shaking	dilute and shoot	100	0.0000125
24	Prüfmethode "Bestimmung von Tropanalkaloiden in Lebensmitteln mittels UPLC-MS/MS", Hessisches Landeslabor, Seite 1-5, M 3.3.3.806.01	30 min shaked, solvent: 60 % methanol + 40 % water + 0.4 % formic acid	no concentration, no cleanup, PVDF-syringe-filtered	1.0 g sample + 25 ml solvent	5 µl
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)x 0.010
26	principle: tropanic and ergots alcaloids are extracted by buffer at pH=9 and purification with dispersive phase (Bondesil PSA) then the extracts are filtered and injected for analysis by LC-MS.	Time extraction : 30 min., extraction solid-liquid with mechanical rotation and centrifugation (4000 g). Solvent composition: acetonitrile-ammonium carbonate (84/16 v/v)	Purification with Bondesil PSA and dilution 1/2 with ammonium carbonate sol. (200 mg/L)	Sample mass : 5 g and volume solvent extraction: 25 mL	=2 mg injected (5/25 x1/2x0.020)
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 0.5% Formic acid in water:acetonitrile. 50:50 (v:v)	QuEChERS (MgSO4+NaCl), dSPE (MgSO4+PSA)	4 (10 mL:2.5 g)	10
28	EURL method	2g with methanol/water/formic acid 39:60:1 / shaker for 1 hour / centrifuge and inject	just dilute	10	0,002
29	Modified after Jandric et al., FAC, 28 (2011)1205-19.	extraction time: 10 min, vortex/shaking; water, 0,5% HCOOH/ACN (1/3)	QuEChERS	20 mL/5 g	5 mg
30	For this proficiency test we used the Method from the EURL Mycotoxins. Before we used QuEChERS	60 minutes on a shaker	no	2 g on 20 ml extraction solvent	0.001
31	Deutsche Lebensmittelrundschau, Oktober 2015 page 418	30 minutes (methanol/water 60/40%)	filtration Chromafil Xtra PA 0.45 um	10	7.6 mg
32	QuEChERS: Extraction for dry samples (extract 1) as Wheatflour: §64 LFGB L 00.00 - 115; EN 15662	a: 4 g of sample + 10 ml water, blend with vortex mixer, let soak for 5 min / b: add 4 ml ACN + Internal Standard (Pririmicarb-D6) / c: shake vigorously, 15 min overhead shaker, 30 min freezer, add first salt composite / d: centrifuge for 5 min / e: use 1 ml of the ACN-phase / f: dilute extract: 1+1 with MeOH, inject for LC-MSMS analysis	QuEChERS	1:1	meq=(4/4)*(1/2)*0.001 = 0.0005
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, solvent volume: 20 ml	0.2 mg
34	Adamse, P; H. P. Van Egmond (2010): Tropane alkaloids in food, RIKILT - Institute of Fodd 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 0.010 = 5 mg
35	2.5g homogenized sample; add 25 ml extraction-solvent; blending for 30 min.; centrifugation for 10 min. 4400 rpm; Filtration with syringe filter 0.2µm; dilution 1/5 with water; 5µl injection to LC-MS/MS	Blending for 30 Min.; extraction-solvent: MeOH 600ml + Water 400ml + Formid acid 4 ml	Filtration and dilute	2.5 g/25 mL	2.5 g/25 mL; Dilution 1/5; Inj. 5µl = 0.1 mg
36	Analysed as per method supplied	As per method supplied	As per method supplied	As per method supplied	As per method supplied
37	acid extraction, SPE, drying of sample, reconstitution and LC-MS/MS	30 min, 0.05 M H2SO4, sonication	SPE	40 ml solvent for 2 g sample mass	

38	in house method: Hessisches Landeslabor / "Bestimmung von Tropanalkaloiden in Lebensmitteln mittels UPLC-MS/MS" (M 3.3.3.806.01)	shaking 30 min methanol/water 60:40 with 0.4% formic acid	dilute and shoot	1.25 g sample in 12.5 mL solvent	0.2 mg
39	EURL method	1 hour shaking, methanol/water/formic acid (39/60/1, v/v/v)	no concentration or cleanup	20 mL solvent 2g sample mass	1 mg
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	2 gram sample and 20 mL solvent	meq=(2/20)x 0.002
42	In house method (own development)	60% methanol with 0.4% formic acid, 45 min, sonication	dilute and shoot	2 g 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 µl
45	Food Additives and Contaminants, 28(9) (2011), 1205-1219	2 min, shaking, ACN/water 75/25 with 0.5% formic acid	LLE with SupelQUE, Order No. 55227-U, Sigma Aldrich	20 mL / 2.5 g	1.33 mg
46	RIKILT SOP A1070 Determination of ergot and tropane alkaloids in animal feed by LC-MS/MS. Special application for cereal-based foods	30 min extraction (head-over-head) with methanol/water/formic acid 60/40/0.4	Clean up by ultrafiltration over 30 kD UF filter.	2 g sample with 20 mL extraction solvent	5 ul injection: (2/20)x0.005 = 0.5 ug
47	In-house developed method	15 min extraction by sonication in methanol : acetonitrile 1:1 [v:v]	SPE: ion exchange (SCX)	1 g sample 10 mL solvent	meq = (1/10)*(5/1) * 0.010 = 5 mg
49	EURL Draft SOP	Shake 1h, Extraction solvent MeOH:H2O:Formic Acid (39:60:1)	Dilute and shoot	10	0.001mg
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 with 25 ml solvent	0.5 mg
51	Method was validated in-house	30 min. shaking with extraction solvent: methanol/water/formic acid sol. (60/40/0,4 % HCOOH)	Dilute and shoot	Volume of solvent: 10 ml Sample mass: 1 g	meq=0.0002
52	EURL method - Determination of tropane alkaloids in cereals and cereal products by LC-MS/MS	1h, shaker, methanol/water/formic acid (39:60:1, v/v)	dilute and shoot	10 (2 g in 20 mL)	

Lab	Q.10 Type of separation	Q.11 Type of detecton	Q.12 Transitions, ratio and CID	Q.22 Calibrant	Q.23 *
2	ZORBAX Extend C18 4.6x100 mm, 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: 304.2->156.1; 304.2-->138.1	Our own	MMC
4	Waters, ACQUITY UPLC BEH C18; 150 x 2.1 mm; 1.7 µm / A: 0.1 % formic acid in water B: 0.1 %formic acid in acetonitrile / column temp.: 30 °C / flow rate: 0.200 ml / min / inj. vol.: 10 µl	Agilent 6460 QQQ; ESI positive / Capillary voltage: 3000 V	Atropine: 290 < 124 CE 30 eV / 290 < 93 CE 30 eV Atropine-D3: 293 < 127 CE 20 eV / 293 < 96 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	Phenomenex. Gemini, C18, 150 mm x 2.0 mm, 5 µm, Ammonium carbonate 400 mg/l pH 10.5, Acetonitrile, gradient flow 200 µl/min, 30°C, 10 µl	AB Sciex 5500, ESI positive, 5500 V, 550°C	Atrop: Q 290 C 124 CID 31 V Atrop D3 Q 293 C 127 CID 33V Scop Q 304 C 127 CID 27	Our own	SPS
6	Column: Zorbax Eclipse Plus C18 (2.1x100 mm; 1.8 µm) ; Mobile phase:	HPLC-Agilent 1290 INFINITY and MS-6495	Atropine 1= 290.2>124.2 (30V) Atropine 2: 290.2>93.1 (40V)	Supplied along	SPS

	Water (5mM Ammonium formate, 0.1% formic acid)/MeOH (5mM Ammonium formate, 0.1% formic acid) / Flow=0.3 ml/min; T= 50ºC ; injection volume: 2µl	Triple Quad, positive mode, 3500 V, T. dessolvation= 350ºC	Scopolamine 1= 304.1>138.1 (30V) Scopolamine 2: 304.1>156.3 (20V)	with the PT samples	
7	Reversed phase: Waters Kinetex EVO C18 column 1.7µm 100 x 2.1 mm / 40°C / 1 µl injection / 0.5 mL/min of mobile phase: ACN-H20 with ammoniak buffer / 40°C	HPLC_MS/MS XEVO TQS WATERS / ESI+ / CV: 1 kv / Desolv temp: 450 °C	Atropine Q124.0 (25V) - C93.0 (30V) Scopolamine Q138.0 (20V) - C156.0 (15V)	Supplied along with the PT samples	SPS
8	Column: Waters XBridge C18 (75mm x 3mm, 2.5um) / Mobile Phase: A: 6mM NH4OH B: 6mM NH4OH in Methanol / Column Temperature: 40C / Flow rate: 0.4 mL/min / Injection Volume: 2uL	LC-MS/MS: Agilent 1290 LC/Agilent 6490 QQQ, ESI+, Capillary Voltage 3000V, Gas Temperature 180C	Atropine: Primary Transition (Q) 290/124, CID 29V, Secondary Transition (C) 290/93, CID 37V, Scopolamine: Primary Transition (Q) 304/138, CID 29V, Secondary Transition (C) 304/156, CID 17	Supplied along with the PT samples	MMC
9	The separation was performed on a RP-C18 Kinetex column (2.6µ, 100A; 100x2.10 mm; Phenomenex, Torrance, CA, USA). Flow rate was 0.35 mL/min, column oven temperature was at 40°C, sample temperature was at 20°C and the injection volume was 4 uL	UHPLC Dionex Ultimate coupled with a triple quadrupole mass spectrometer TSQ Vantage with an ESI interface. Positive ionization mode. Spray voltage 3500 V, capillary temp. 270 °C. Vaporizer temp. 250 °C, sheath gas flow 50 units, auxiliary gas flow 15 units	Multiple reaction monitoring (MRM) with the following transitions: 290 =>93 (CE = 33 eV) and 290=>124 (CE = 25 eV) for atropine 304==>103 (CE = 35 eV), 304 =>138 (CE = 19 eV) and 304 =>156 (CE = 16 eV) for scopolamine.	Supplied along with the PT samples	SPS
10		LC-MS-MS	atropine: 290/93, 290/124 scopolamine: 304/138, 304/156 atropine D3: 293/127	Our own	MMC
11	HP5 Msi 30mx 0.25x0.25	GC-MS	Deuterated atropine		
12	YMC-Pack Diol 2.1 x 100 mm; 5 µm; 120 Å; Phenomenex Synergi Fusion -RP 2 x 100 mm; 2.5 µm; 100 Å	LC-LC-MSMS (2-dimensional) ESI pos	Atropine 290/124 +290/93 Scopolamine 305/138+305/156	Our own	MMC
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; 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; delta EMV 600	Scoplolamin 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 and precolumn / mobile phase (gradient): starting 10% (5% ACN 95% 10mM ammonium actetate) end to 80% (95% ACN 5% 10mM ammonium actetate) in 10 min, 30°C, 0.3mL/min, 10µL	LC-MS/MS (API 4000 QTrap), ESI+, Declustering potential: 76V (atropine), 51V (scopolamine); cell exit potential 6V (atropine and scopolamine),	Atropine: Q 290.1- 124.1 C 290.1 – 93.1, collision energy 35eV Scopolamine: Q 304.1 – 138.2 C 304.1 – 156.2, collision energy 31eV	Supplied along with the PT samples	
15	Column: Waters UPLC BEH C18 150 x 2.1 mm, 1.7 µm. Column temperature: 50 °C. Mobile phase A: 6.65 mM ammonia in water, Mobile phase B: 1.30 mM ammonia in acetonitrile, Flow: 0.4 ml/min, Injection volume: 2 µl, Total run time: 15 min, Gradient (linear): 100 % A for 2 min and then goes to 60 % B at 12 min. Then stepped up to 99 % B for a 3 min, equilibrate for 5 min. A: 10 mM ammonium carbonate in water at pH 10, B: acetonitrile.	Waters Acquity UPLC with Waters Xevo TQ-S ESI+, capillary voltage 2 kV and desolvation temperature 500 oC	Atropine 290>124, CE 20eV, 290>93 CE = 25 eV Scopolomine 304>138, CE 20eV, 304>103 CE = 30 eV	Our own	SPS
16	Column: Ascentis Express F5, Supelco, 100mm x 2.1 mm, 2.7 µm Mobile phase: A 0.1% Formic acid in water, B 0.1% Formic acid in acetonitrile 0 min 10% B -> 1 min 20% B -> 10 min 90% B Flow rate: 0.3 ml/min Temperature: 40 °C Injection volume: 10 µl	LC-MS/MS: Shimadzu LCMS-8050 / ESI+ / Interface voltage: 4 kV / Desolvation temperature: 150 °C / Interface temperature: 300 °C	Atropine: Q 290.00 ->124.20, CID 24 / C 290.00 -> 93.05, CID 30 D5-atropine: Q 295.00 ->124.15, CID 25 / C 295.00 -> 93.05, CID 30 Scopolamine: Q 304.10 ->138.15, CID 21 / C 304.10 -> 156,10, CID 17 13C D3-scopolamine: Q 308.10 -	Supplied along with the PT samples	MMC

			>142.20, CID 21 / C 308.10 -> 160,15, CID 18		
17	Acquity UPLC BEH Phenyl; 2.1X100 mm; 1,7µm 50 ° C; 0.4ml/min; 2µl	LC-MS/MS; ESI: 550°C; 0.3CV	Atropine: 290.12 >124.7; 290.12 >93.08 Scopolamine: 304.11>138.06 ; 304.11>156.07	Supplied along with the PT samples	SPS
18	F5 column, 10cm x 2.1mm, 2.7um particle size, mob. phase water+0.1%FA and MeOH+0.1%FA gradient, temp. 40oC, flow rate 0.3mL/min, inj. vol. 10uL	LC-MSMS, Thermo Finnigan TSQ Quantum, ESI+, Spray Voltage 3400V, Cap. Temp. 325ºC	Atropine Q:124.2 (22V) C:93.1 (31V) Scopolamine Q:156.18 (16V) C:138.14 (22V)	Supplied along with the PT samples	SPS
19	n.A.	LC-MS/MS Applied Biosystems, API 5500	290/128; 304/138	Our own	SPS
20	Acguity BEH C18 , 1,7 um, 2.1 x 50 mm	Waters UPLC-MSMS, ESI+, capillary 2.5 kV, desolvation temperature 450°C, source 120°C	Atropine 290/124 (20 V) Scopolamine 304/156 (35 V) 304/136 (35 V)	Our own	SPS
21	Waters UPLC BEH C18 100 x 1 mm, 1.7 µm Mobile phase A: 6,65 mM ammonium hydroxyde : H2O Mobile phase B: 1.3 mM ammonium hydroxyde : ACN Flow: 0.15 ml/min. (gradient elution between A&B) / Injection volume: 2 µl.	Waters Acquity UPLC-TQD, Source temp.: 135ºC / Desolvation gas temp.: 400ºC / Desolvation gas flow: 400 L/h / Cone gas flow: 150 L/h.	Atropine 289.9> 124.1 > 91.0 AtropineDd3 293.2> 127.0 > 93.0 Scopolamine 303.9 >138.1 > 156.0 Scopolamine-d3 307.2 >141.0 > 159.0		
22	Column ZORBAX SB-C18 (50 mm, ID 2.1 mm, particle size 1.8 micron). Phase A: 0.1% formic acid in H2O. Phase B: 0.1% formic acid in acetonitrile. Temperature 40°C. Flow rate 0.4 mL/min. Injection volume 10 uL. Gradient elution - from 95% A to 95% B in 5 min, followed by a 1 min isocratic step at 95% B. The column was reconditioned at 95% A for 1.5 min.	LC-MS/MS (LC Agilent 1290; MS/MS detector Agilent 6430). ESI+. Capillary voltage 4000 V. Desolvation temp. 300°C. Gas temperature 300°C. Gas flow 10 L/min. Nebulizer 35 psi	Scopolamine: Q (304.16->156.1, collision energy 9V), C (304.16->138.1, collision energy 13V). Atropine: Q (290.18->124.1, collision energy 21V), C (290.18->103.1, collision energy 40V)	Supplied along with the PT samples	SPS
23	RP18plus, Macherey-Nagel Nucleoshell, 100mm x 2 mm, 2.7 µm; 30 °C, 0.3 ml/min, 5 µl	Agilent 6490, ESI, 4000,	Atropine 290.2/124.1 (CE 28V), Atropine 290.2/77 (CE 60 V) Atropine 290.2/93 (CE 37 V) Scopolamine 304.2/ 103 (CE 49 V), Scopolamine 304.2/138 (CE 21 V), Scopolamine 304.2/156 (CE 17 V)	Supplied along with the PT samples	SPS
24	Gemini C18, 3µ, 100x3mm / mobile phase: A: 0.1 % Formic acid in Water, B: 0.1 % Formic acid in Methanol / Temp.: 40 °C; Flow: 0.2 ml/min	LC-MS/MS, ABSCIEX 5500 QTRAP, Esi positive, Ion Spray Voltage: 4000 V; Temp.: 500 °C	Atropine: Precursor 290, Transition 1: 124; Transition 2: 93; CE: 33 / 39 Scopolamine: Precursor 304, Transition 1: 156; Transition 2: 103; CE: 23 / 55	Supplied along with the PT samples	SPS
25	Ascentis Express F5 100mmx2.1mm 2.7 µm; water/acetonitrile with 0.1 % formic acid; 40 °C; 0.3 ml/min; 10 µL injection volume	LC-MS, ABSciex 5500 QTrap, ESI positive, IS 2500 V, TEM 200 °C	Atropine: Q 290 ->124 CE 33 V, C 290 -> 93 CE 39 V Scopolamine: Q 304 ->138 CE 29 V, C 304 -> 156 CE 25 V	Supplied along with the PT samples	SPS
26	X Bridge Column C18, 150 mm x 3 mm, 5 µm / Mobile phase: ACN/Ammonium carbonate aq. (from 10/90 to 80/20) / Column temp.: 40°C / Flow rate: 0.4 mL/min / volume inj.: 20 µL	Waters Xevo TQMS Acquity HPLC; ESI+; capillary voltage: 3,0 kV Dessolvatation temp.: 500°C	Atropine Q:290>92.9 CID:32 - C:290>124.1 CID:28 Scopolamine Q:304.1>130.03 CID:22 - C:304.1>156.1 CID: 16 Secondary transition: atropine 124.09, Scopolamine: 156.07	Our own	MMC
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 temp.: 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 µm particle size / Column 40 °C, 20 µL inject, flow rate 0.250 mL / Mobile fase A: ultrapure water 0.1% formic acid / Mobile fase B: ACN 0.1% formic acid	LC-MS/MS Quattro Ultima PT Waters / ESI + / capillary voltage 3500 V, dessolvation temperature: 350 °C	Atropine: 290.1>124.1 290.1>93.1 Scopolamine 304.1>138.0 304.1>156.0	Supplied along with the PT samples	SPS
29	Kinetex 2,6 u Biphenyl, 150x2.1 mm,	LC/MS, Micromass	Atropin: 290.2 > 124.3 (Q);	Supplied	MMC

	MP-A: water 0.1% HCOOH; MP-B: 47.5% MeOH, 47.5%ACN, 5%water, 0.1% HCOOH, 35 ^{0}C, 0.1 ml/min, 10uL.	Ultima, ESI+, 3.5 kV; 400 ^{o}C,	290.2 > 93.2 (C), 25V Scopolamine: 304.3 > 138.1 (Q); 304.3 > 156.1 (C), 20V	along with the PT samples	
30	Supelco Ascentis Express F5 / 10cm x 2.1mm, 2.7µm / mobile phase A: 98% Water + 2% Acetonitrile + 0,1% formic acid / mobile phase B: 100% Acetonitrile + 0,1% formic acid	LC-MS/MS (API 4000 QTRAP, Siex) / ESI pos; CUR 30, CAD medium, Ion spray voltage 5500, TEM 500, GS1 40, GS2 60	Scopolamine Q 304.0 / 138.0 DP 71 CE 27 / C 304.0 / 155.9 DP 71 CE 23 Atropine: Q 290.0 / 124.0 DP 126 CE 33 / C 290.0 / 93.0 DP 126 CE 41	Our own	MMC
31	BEH C18 50 mm 1.7 um	Waters Acquity TQD	Atropine: 290.1>93.1; 290.1>124.1 Scopolamine: 304>138.1; 304>156.0	Supplied along with the PT samples	SPS
32	Kinetex C18 (Phenomenex), 2.1*100mm, 2.6µm, 100A; A: H2O+5mM NH4-Formiate, B: MeOH+5mM NH4-Formiate; Flow: 0.4 ml/min, 20°C, Injection: 1µl	Agilent 1290-6495; LC-MSMS Triplequad, ESI pos, D EMV(+): 400V, Capillary Voltage: 3500 V, Gas Temp: 120°C, Sheath Gas: 375°C	Atropine: 290.3-124.1 (CE:24, CAV:1); 250.3-103.1 (CE:51, CAV:1) Scopolamine: 304.2-156.2 (CE:19, CAV:1); 304.2-138.1 (CE: 28, CAV:0); 304.2-103 (CE: 51, CAV:1)	Our own	MMC
33	Waters Acquity BEH C18 1.7 µm, 2.1 x 50 mm / mobile phase A: 0.1 % formic acid in water / mobile phase B: 0.1 % formic acid in methanol / flow rate: 0.3 ml/min / temperature: 40 °C / injection volume: 10 µl	LC-MSMS: Waters Acquity Xevo TQD, ESI+	Scopolamine: 304 -> 138 (Q); 304 -> 103 (C) Atropine: 290 -> 124 (Q); 290 -> 93 (C)	Our own	SPS
34	UPLC C18, 1.7 µm, 2.1 x 50 mm precolumn, mobile phase: A: 0.1 % formic acid/ in water; B: 0.1 % formic acid in MeOH, temp.: 40 °C, flow: 0.3 ml/min, injection volume: 10 µl	LC-MS/MS, ES+, capillary voltage 0,3 kV, dessolvation temp.: 550°C	Atropine: 290.25 -> 93; 290.25-> 124.1; Scopolamine: 304.2->156.1; 304.2->138.1	Our own	MMC
35	Kinetex C18 2.6µ 100A; 100 mm id x 2.1 mm; particle size: 2.6 µm; Eluent A: Water + 0.1% Formic acid; Eluent B: MeOH + 0.1% Formic acid; Gradient from 10% B at 0 Min. to 90% B at 10 Min; Temperature 35°C; Injection volume: 5µl	Sciex QTrap5500; ESI pos.; 5500 Volts; 650°C	Atropine: 290.078 -> 124.0; CE 33 ; 290.078 -> 93.0; CE 39 Scopolamine: 304.062 -> 138.1; CE 27 ; 304.062 -> 156.1; CE 23	Supplied along with the PT samples	MMC
36	As per method supplied. Gradient time reduced to 8 mins as we used UPLC. (All time intervals in method supplied divided by 2)	Quattro Ultima	As per method supplied CID varied slightly	Supplied along with the PT samples	SPS
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 1 µl, flow rate 0.5 ml/min	LC-MS/MS, ESI mode (neg.)	Atropine: Q 290.2 > 124 (CE = 21); C 290.2 > 93 (CE = 29) Scopolamine: Q 304.2 > 156 (CE = 9); C 304.2 > 138 (CE = 21)		MMC
38	Agilent Zorbax Eclipse Plus C18 RRHD 2.1x100 mm 1.8 µm methanol/water 20:80 with 0.1% formic acid	Agilent 6460 Triple Quadrupole, ESI+, 300°C Gas temperature	Atropine Prim 290.2 > 124.0 Sec 290.2 > 93.0 / Atr.-d5 295.3>124.1 Scopolamine Prim 304.1>138.1 Sec. 304.1>156.1 / Scop d3,C13 308.2>142.0	Supplied along with the PT samples	MMC
39	Phenomenex 5u Luna Phenyl Hexyl 150*2mm, Eluent A: 0.1% formic acid in water, Eluent B:Methanol, Temperature 40°C, Injection Volume 10 µL / Gradient: 1min - 10% B, 2min - 10% B, 10min - 80% B, 12min - 80% B, 13 min - 10%B, 15min - 10% B, Temperature: 40°C, Injection Volume 10 µL	LC MS: AB Sciex API 4000 QTrap, Scheduled MRM ESI+, Capillary Voltage 3500, Temperature 600 ^{o}C	Atropine: Quantifier: 290/124.1 CE 38, Qualifier: 290/93.1 CE 75 Scopolamine Quantifier: 304.17/138.2 CE 35, Qualifier 304.17/156.2 CE 23	Supplied along with the PT samples	SPS
40	XBridge C18, 5µm, 3.0 x 150 mm, Waters; mobile phase:: water / acetonitrile, 6 mM NH4OH; 40ºC: 0.4 ml/min; 10µl	LC-MS/MS; Waters TQ; ES+; 2 kV; 400 ºC	Atropine: 290.16 > 124.24 (25 eV); 290.16 > 93.17 (25 eV); Scopolamine: 304.21 > 138.25 (30 eV); 304.21 > 156.25 (20 eV)	Supplied along with the PT samples	SPS
41	Column: acquity UPLC HSS T3 1.8 um, 2.1*100 mm / mobile phase Eluent A 0.02% formic acid in water, Eluent B 0.02% formic acid in acetonitrile. Flow rate 0.3 ml/min. Injection volume 2 uL. Temperature 40°C.	LC-MS-MS, ESI+, capillary voltage 1.05 KV, dessolvation temperature 600°C	Atropine:290.1 to 124.1 / CID 23eV ; 290.1 to 93.1 / CID 28 eV Scopolamine: 304.1 to 138 / CID 20 eV ; 304.1 to 156 / CID 16 eV	Our own	SPS

42	50 x 2.1 mm Kinetex C18, 2.6 µm, gradient: 0.2% formic acid in water and methanol, temperature 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	SPS
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: 2.7um; eluent A: 315 mg ammonium formate + 1 mL formic acid + 1 L water; eluent B: 315 mg ammonium formate + 1mL formic acid + 1 L methanol; 40°C; inj. vol. 1 µl	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 phase A: 10 mM ammonium carbonate pH 10.0, mobile phase B: acetonitrile. 400 ul/min, 15 min run time, 50oC, 5 ul injection	Waters Xevo TQ-S LC-MS/MS. pos ESI, cap V: 3.0kV, cone V: 30V, Desolvation gas T: 600oC, cone T: 150oC, collission gas: Argon, 4.2x10-3 mbar	Atropine: Q = 290.2>124.0, CE: 20eV, C = 290.2>93.0, CE: 25eV / Scopolamine: Q = 304.2>138.0, CE: 20eV; C = 304.2>103.0, CE: 35eV	Our own	MMC
47	Waters Acquity BEH 150 x 2.1 mm, 1.7 µm / Mobile phase A: 5mM ammonium formiate in water + 0,1% formic acid /Mobile phase B: 5mM ammonium formiate in methanol + 0,1% formic acid / Temp.: 40°C / Flowrate: 0.3 ml/min /injection vol: 10µL	LC-MS² AbSciex 5500 in ESI+ mode / Capillary voltage 5000V / Temp 300°C / IS: 4500V / Source Temp: 300°C	Q(Atropine) = 290.2 / 124 CE = 33eV C(Atropine) = 290.2 / 93.0 CE = 49 eV Q(Scopolamine) = 304.2 / 138.1 CE = 29 eV C(Scopolamine) = 304.2 / 156.1 CE = 23 eV CID = CE in AbSciex MS²	Supplied along with the PT samples	SPS
49	Ascentis Expess F5, 10cmx2.1mm, 2.7um / H2O+0.1% Formic acid, ACN+0.1% Formic acid gradient. / 40ºC, 0.3ml/min, 10uL injection volumn.	LC-MS/MS, Waters Xevo TQ-S, ESI mode, capilary voltage: 3.5kV, Dessolvation T:280C	Atropine : Q: 290.16 > 124.10 CID: 24eV , C: 290.16 > 93.05 , CID: 28eV Scopolamine : Q: 304.16 > 138.10 CID: 22eV , C: 304.16 > 156.10 , CID: 15eV	Supplied along with the PT samples	SPS
50	Waters Xselect HSS T3, 2.5 µm, 2.1 x 100 mm, Methanol, Water 1 % Formic Acid, 350 µl/min, 35 °C, 5 µl	LC-MS/MS, SCIEX QTRAP 5500, ESI pos, 400 °C, 5500 V	Scopolamine 304 >138.2; DP 66 V, CE 27 V 304> 156. DP 66 V, CE 23 V Atropine 290 > 124.1 DP 96 V, CE 33 V; 290 > 92.9 DP 96 V, CE 39 V	Supplied along with the PT samples	SPS
51	Column - Acquity HSS T3 C18 (100 x 2.1 mm , 1.8 µm), Waters; Mobile phase A: 0.1 % HCOOH + 5 mM ammonium formate in water; Mobile phase B: 0.1 % HCOOH + 5 mM ammonium formate in methanol; Column temperature: 40ºC; Flow: 0.4 ml/min.; Injection volume: 2 µl	Agilent 6495 Triple Quad LC/MS, ESI+, coupled with a Agilent 1290 Infinity II UPLC. Gas Temp: 200ºC; Gas Flow: 12 L/min; Nebulizer: 20 psi; Sheath Gas Temp: 400ºC; Sheath Gas Flow: 11 L/min; Capillary: 3500 V; Nozzle Voltage: 500 V; iFunnel parameters: High Pressure RF: 200 V, Low Pressure RF: 100 V	Atropine Q: 290 ->124 (CE 24); C: 290->91 (CE 48), 290->93 (CE 36); Scopolamine Q: 304->138 (CE 20); C: 304->103 (CE 44), 304->156 (CE 16)	Our own	MMC
52	Supelco ascentis express F5. 10cmX2.1mm, 2.7um	LC-MS. Waters quattro premier, ESI+, 3.5KV, 280°C	Atropine: Q=290.1-124.1 (CID 22eV); C=290.1- 93.1 (CID 24eV)	Supplied along with the PT samples	SPS

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

Lab	Q.15 Strategy used for LOD/LOQs estimation	Q.16 Performance parameters SCOP	Q.17 Performance parameters ATROP	Q.18 Recovery calculation	Q.26 Results reported
2	Low level spiking	R^2=0.99985549	R2=0.99996418	spiking matrix	NOT CORRECTED for recoveries
4	S/N 3:1 qualifier for LOD, 10:1 S/N LOQ qualifier	R^2 = 0.99942611	R^2 = 0.99973123	internal standard	NOT CORRECTED for recoveries
5	DIN 32645	RSD 13 %, r=0.99960	RSD 20 %, r=0.99995	spiked matrix	NOT CORRECTED for recoveries
6	Low level spikes	RSD (average)=10	RSD (average)=19 %;	Recovery calculation	CORRECTED for

		%; r^2=0.998	r^2=0.9925	of the isotope-labelled internal standard concentration spiked before extraction	recoveries
7	Lowest validated level = LOQ	R^2=0.998 / (conc-RSD% n=3 days): 2.5 ppb-4.31%/ 50 ppb-5.66%/150 ppb-11.65%	R^2 =0.988 (conc-RSD% n=3 days) 2.5 ppb-19.90%/ 50 pbb-8.19%/150 ppb-11.50%	spiked matrix during validation of the method	NOT CORRECTED for recoveries
8	S/N ratio (3 and 10 resp.) from the lowest calibration point	precision RSD 8%, correlation coefficient 0.999	precision RSD 2%, correlation coefficient 0.999	spiked matrix	NOT CORRECTED for recoveries
9	LOD and LOQ were determined by analysing blanks at low level spikes.	RSD%: 5.2%; correlation coefficient 0.995	RSD%: 1.8%; correlation coefficient 0.997	Buckwheat matrix spiked at 5 ppb. RR% was 104% for atropine and 98% for scopolamine	NOT CORRECTED for recoveries
10					CORRECTED for recoveries
11	Low level spikes and reagent blank results	Insufficient data at this time. r^2 0.999997	Insufficient data at this time. r^2 0.999260	Spiked sample in presence of deuterated atropine	
12	10			spiked sample	CORRECTED for recoveries
13		R^2 = 0.9969	R^2 = 0.9989	spiked blank sample, whole analytical procedure	CORRECTED for recoveries
14	LOD S/N ratio 3 / LOQ S/N ratio 10	RSD% 1-8%, standard addition curve: 0.9992	RSD% 1-8%, standard addition curve: 0.9991	spiked matrix	NOT CORRECTED for recoveries
15	LOQ - lowest calibration standard LOD - comparison of S/N in solvent std, then adjusted for background / baseline in matrix	RSD%= 4-14% at 1ug/kg, r =>0.95	RSD%= 2-18% at 1ug/kg, r=>0.95		CORRECTED for recoveries
16	LOD S/N 3 LOQ S/N 10	r=0.9992, r^2=0.9985	r= 0.9998, r^2=0.9995	spiked sample matrix	CORRECTED for recoveries
17					CORRECTED for recoveries
18	Parameters from the calibration curve	R^2=0.9969	R^2=0.9972	spiked sample	CORRECTED for recoveries
19	Calibration Curve	>0.999	>0.999	n.A.	NOT CORRECTED for recoveries
20	Low level spikes	RSD 7.8%, R^2=0.998	RSD 11.9%, R^2= 0.996	Spiked matrix	NOT CORRECTED for recoveries
21					NOT CORRECTED for recoveries
22	S/N ratio at least 5 for LOQ	coefficient correlation 0.99999	coefficient correlation 0.99999	use of reference materials	CORRECTED for recoveries
23	S/N, linearity with matrix	RSD 11%, r^2 0.995	RSD 2.3%, r^2 0.998	spiked matrix	CORRECTED for recoveries
24	S/N ratio (3 and 10) of spiked samples	r=0.9956	r=0.9888	spiked samples	NOT CORRECTED for recoveries
25	S/N 3 and 10	RSD 1%, correlation coefficient 0.9999	3 %, correlation coefficient 0.9997	spiked matrix	CORRECTED for recoveries
26	S/N ratio	RSD: 4.60% and R: 0.9994	RSD: 3.64% and R: 0.9994	spiked matrix on our own blank babyfood cereals matrix	CORRECTED for recoveries
27				Spiked matrix	CORRECTED for recoveries
28	S/N, blank and low level spike	R^2 0.999	R^2 0.999	spiked matrix	NOT CORRECTED for recoveries
29	LOQ: S/N:10, low levels spikes	RSD%: 14% ; R^2>0.98	RSD%: 7%; R^2>0.98	spiked matrix	NOT CORRECTED for recoveries
30	Low level spike	correlation coefficient 0.999	correlation coefficient 0.999	spiked matrix	CORRECTED for recoveries
31	Estimation; we didn't have the time to determine them exactly	correlation coefficient 0.9996	correlation coefficient 0.9989	spiking of sample E	NOT CORRECTED for recoveries
32	LOQ: acc. to SANTE 11945/2015 (Recovery between 70-120%, Standard deviation < 20%)	RSD: 10.9%, R^2: 0.97	RSD: 8.1%, R^2: 0.96	two levels of spiked matrix at 1.0 and 10 µg/kg	NOT CORRECTED for recoveries

	/ LOD: lowest calibration level				
33	S/N ratio (3 and 10) of spiked blanks	r=0.997896	r=0.999228	spiked blank matrices	NOT CORRECTED for recoveries
34	S/N ratio	RSD 15%; correlation coefficient 0.9988	RSD 3%; correlation coefficient 0.9990	spiked matrix	NOT CORRECTED for recoveries
35	DIN 32645 (Calibration Curve with std.'s in low concentration)	RSD 3.42%; r = 0.9998	RSD 6.27%; r= 0.9998	spiked Matrix	NOT CORRECTED for recoveries
36	LOD taken as lowest std 0.03ug/l & LOQ lowest std multiplied by calculation factor in method supplied	R^2: 0.995428	R^2: 0.997551	No spikes/recovery data	NOT CORRECTED for recoveries
37	Low level spikes			we used spiked matrix	
38	LOD S/N at least 3 LOQ S/N at least 10	corr.coeff. 0.9988	corr.coeff. 0.9996	spike of an uncontaminated rye flour. calculation with a matrix calibration (with ISTD)	NOT CORRECTED for recoveries
39	Estimated from calibration, S/N ratio (3 and 9)	CV (%) = 4.43 r=0.99934	CV (%) = 3.34 r=0.99975	spiked samples	
40	S/N ratio 3/6, respectively.	RSD 8.0%; correlation coefficient 0.998	17.0 %; correlation coefficient 0.999	spiked matrix	CORRECTED for recoveries
41	Blanks used / low spike / S/N ratio	RSD 21 %, correlation coefficient 0.999	RSD 15%, correlation coefficient 0.999	spiked matrix	NOT CORRECTED for recoveries
42	Low level spikes	correlation coefficient: 0.998	correlation coefficient: 0.999	spiked matrix	NOT CORRECTED for recoveries
44	S/N ratio	n.s.	n.s.	n.s.	CORRECTED for recoveries
45	S/N ratio (LOQ: 10; LOD: 3), low level spikes	RSD 9.5%; correlation coefficient 1.0000	RSD 7%; correlation coefficient 0.9997	spiked matrix	NOT CORRECTED for recoveries
46	LOD: S/N= 3 for C-transition; LOQ: S/N= 6 for C transition, rounded to the next higher spiking level (e.g. 0.25, 0.5, 1 ug/kg, etc)	linearity: 0.999	linearity: 0.999	Sample spiked before extraction and sample extract spiked after extraction	CORRECTED for recoveries
47	S/N ratio 3 and 10 for LOD, LOQ resp. in low level spiked samples	r=0.9993 RSD=4.04%	r=0.9988 RSD=9.79%	Matrix Spiked with internal Standard	CORRECTED for recoveries
49	We used low level spikes	RSD%=7.48, r^2= 0.9971	RSD%=2.72 , r^2= 0.9986	spiked matrix (wheat-flour)	NOT CORRECTED for recoveries
50	LOD S/N 10; LOQ: S/N 20	r= 0.999	r= 0.999		NOT CORRECTED for recoveries
51	Parameters from the calibration curve	RSD=2.91%, R^2= 0.9997	RSD=4.94%, R^2= 0.9999)	spiked matrix	CORRECTED for recoveries
52	LOD: S/N 3; LOQ: S/N 6	RSD%<7; R^2=0.9949	RSD%<13; R^2=0.9963	spiked matrix	NOT CORRECTED for recoveries

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