Proficiency test for tropane alkaloids in food and feed matrices

EURLPT-MP04 (2020)

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Contents

Summary

A proficiency test (PT) for the determination of the tropane alkaloids (TAs) atropine and scopolamine in buckwheat flour and maize flour was organised by the European Union Reference Laboratory for mycotoxins & plant toxins (EURLMP) between August and October 2020. This PT was carried out by Wageningen Food Safety Research (WFSR) in accordance with ISO/IEC 17043 (R013). The measurand levels were targeted to provide insight on the measurement capabilities of the EU Member States' National Reference Laboratories (NRLs) at concentrations corresponding to the levels of TAs in processed cereal-based foods and baby foods for infants and young children, containing millet, sorghum, buckwheat or their derived products as regulated by Commission Regulation (EU) 2016/239. In addition to this food product, a maize sample containing a higher level of TAs was included in this PT since an amendment to the legislation is foreseen.

The participants were asked to quantify atropine and scopolamine in two materials and to report the compounds individually as well as the sum value. The participants performance was assessed as z-score in both materials for the individual TAs (maximum score 4 out of 4) and for the sum of the two TAs in one sample (maximum score 2 out of 2).

Thirty-eight participants, of which 29 NRLs for mycotoxins and/or plant toxins in food and feed (from 22 EU Member States plus Iceland and Norway) and 9 Official Laboratories (8 from 4 EU Member States and Switzerland) participated in the PT.

Two materials, buckwheat flour (material A) and maize flour (material B), were prepared containing atropine and scopolamine. Levels were artificially increased by spiking with atropine and scopolamine standard solutions. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of each material.

For the identification and quantification of atropine and scopolamine 34 participants used liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS), one participant used LC single quadrupole MS, two participants used LC high resolution mass spectrometry (HRMS) and one participant provided no information.

In this PT the robust mean was used as consensus value. The consensus value based on the participants' results was used as the assigned value. The assigned values of atropine and scopolamine in material A were, respectively, 1.15 and 1.16 µg/kg and in material B, respectively, 15.3 and 52.7 µg/kg. Obtained interlaboratory reproducibility (RSD_R) ranged from 14% to 26%. The RSD_R were for all TAs below the target standard deviation, except for scopolamine (26%) in material A. For the sum of TAs (atropine and scopolamine) the RSD_R was 20% and 17% for material A and B, respectively.

The proficiency of the participants was assessed through z-scores, calculated using the assigned values and a relative target standard deviation of 25%. All participants submitted results for atropine and scopolamine. One participant analysed only material A. For both materials (A and B) 87% of the results for atropine and scopolamine were rated with satisfactory z-scores ($|z| \leq 2$), 6% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$. Twenty-six participants achieved optimal performance for both materials by detecting both TAs with the correct quantification and the absence of false negative results. In this PT, two false negatives were reported.

Characteristics of the PT materials and the outcome of this PT are summarised in Table 1.

Table 1 *Summary of proficiency test materials parameters and participants' performance.*

Tropane alkaloid	Matrix	Assigned value (µg/kg)	Uncertainty (µg/kg)	Robust RSD_R[1)] (%)	No of labs reporting		
					Quant. value	<LOQ	FN
Atropine	A	1.15	0.049	19.9	34	4	
	B	15.3	0.439	13.8	36	1	1
Scopolamine	A	1.16	0.063	25.6	35	3	
	B	52.7	2.21	20.1	36	1	1
Sum	A	2.36	0.099	20.1	36	2	
	B	68.4	2.35	16.7	37		

Tropane alkaloid	Matrix	Assigned value (µg/kg)	z-scores[2)]			Labs out of 38 with Acceptable z-score	
			satisfactory (% of z-scores)	questionable (% of z-scores)	unsatisfactory (% of z-scores)	No[3)]	%[3)]
Atropine	A	1.15	88	3	9	30	79
	B	15.3	92	0	8	34	90
Scopolamine	A	1.16	77	17	6	27	71
	B	52.7	92	3	5	34	90
Sum	A	2.36	83	6	11	30	79
	B	68.4	92	3	5	34	90

Matrix: A= Buckwheat flour, B= Maize flour

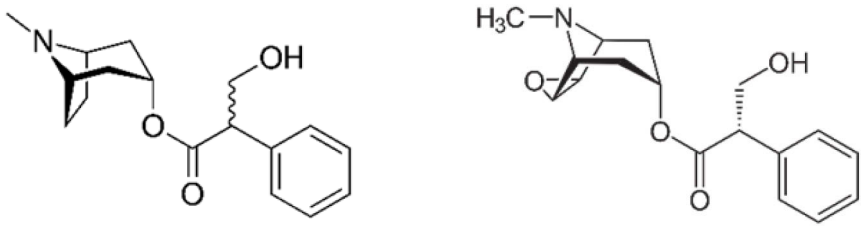
1) robust relative standard deviation (interlaboratory RSD based on participants' results).

2) calculated using a fit-for-purpose target RSD for proficiency of 25%. False negatives were counted here as unsatisfactory z-score.

3) the number and percentage here means: analyte determined, method with a sufficiently low LOQ to allow quantification, and obtaining a satisfactory z-score.

1 Introduction

Tropane alkaloids (TAs) are secondary metabolites produced by a wide variety of plants from the families of Brassicaceae, Convolvulaceae, Moraceae and Solanaceae. Most important weed species in this respect are *Datura stramonium* (thorn apple) and *Atropa belladonna* (deadly nightshade). TAs are regarded as undesirable substances in food and feed and for that reason have been the subject of an EFSA opinion, published in 2013, in which an acute reference dose of 0.016 µg/kg body weight was derived [1]. More than 200 different TA have been identified in the various plant species. However, sufficient data on toxicity and occurrence in food are available only for the TAs atropine and scopolamine (Figure 1), which are regarded the most important representatives of this class of metabolites. Atropine is the racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine (synonyms D- and L- hyoscyamine) [1]. Atropine and scopolamine are strong antimuscarinic agents.



(a) (b)

Figure 1 *Chemical structures of atropine (a) and scopolamine (b).*

Food crops, such as cereals, can be contaminated when TA containing weeds are co-harvested. Common practices for cleaning cereals are not always sufficient to remove the weed plant parts and seeds. Legal limits for TAs in foods were issued in 2016 with Regulation (EU) 2016/239 on maximum levels of TAs in certain cereal-based foods for infants and young children, amending Regulation (EC) No 1881/2006 [2,3]. The maximum limit for these products at the time of the PT was 1 µg/kg for atropine and 1 µg/kg for scopolamine. An amendment to the legislation is foreseen and therefore, a maize sample containing a higher level of TAs was included in this PT.

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and is demanded by the ISO/IEC 17025:2017 [6]. Organisation of proficiency tests (PT) is one of the tasks of the European Union Reference Laboratories (EURLs) [7]. Here the primary goal is to assess the proficiency of the National Reference Laboratories (NRLs). To facilitate NRLs in their task, official laboratories (OLs) can also participate, in consultation with their NRL.

2 PT Material

2.1 Scope of the PT

This proficiency test focused on the TAs atropine and scopolamine in food and feed, using buckwheat flour and maize flour as representative matrices. The target concentrations aimed for (see Table 2) took the regulatory limits and commonly found concentrations into account.

Table 2 *Target concentrations µg/kg of atropine and scopolamine in the PT materials.*

	Target concentrations (µg/kg)	
Tropane alkaloid	**Material A**	**Material B**
Atropine	1	15
Scopolamine	1	50

2.2 Material preparation

For preparation of the two PT materials A and B, respectively, buckwheat flour and maize flour were used. The materials were milled using a centrifugal mill (ZM 200, Retsch, Haan) to obtain a particle size of 500 µm. Contamination levels were artificially increased by spiking with atropine and scopolamine standard solutions.
To prepare the materials, a premix was prepared by spiking the blank material which was mixed with blank material. For each premix, 2000 g of blank material was fortified by adding a solution of atropine and scopolamine standards prepared in acetone, aiming at the levels as presented in Table 2. After 30 min the premix A was mixed with 1500 ml acetone to prepare a slurry and premix B was mixed with 1200 ml acetone. The slurries were homogenised using an industrial mixer (brand Topcraft) according to an in-house standard operating procedure [9]. The fortified slurry was air dried overnight in a fume hood and homogenised in a Stephan cutter UMC 5.

For the final materials, 4000 g blank material was mixed with 2000 g of the spiked premix. Materials A and B were homogenised by mixing in a rotating drum and were stored at <-18 °C until use. The homogenisation of the materials was carried out at Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). WEPAL is accredited to ISO/IEC 17043 for the preparation of PT materials by the Dutch Accreditation Council (RvA, R002).

2.3 Sample identification

After homogenisation, materials A and B were divided into sub-portions of approximately 50 grams and stored in polypropylene, airtight closed containers at <-18 °C until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was "2020/EURLPT MP/TAs/xxx", in which the three-digit number of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each participant. Each sample set consisted of one randomly selected sample of material A and one of material B. The codes of the samples for each sample set are shown in Annex 2. The samples for homogeneity and stability testing were also randomly selected out of materials A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, ten containers of materials A and B were analysed in duplicate for atropine and scopolamine.

Method in brief, atropine and scopolamine were extracted from the homogenised sample by addition of methanol/water (60/40, v/v) containing 0.4% of formic acid and agitation in an overhead shaker. After centrifugation of the sample extract, a portion of the supernatant was purified by passing it through a 30 kDa ultrafilter. Analysis was performed by liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) using reversed phase chromatography with alkaline conditions.

The homogeneity of both materials was evaluated according to the International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [11] and ISO 13528:2015 [12]. Both materials proved to be sufficiently homogeneous for this PT. The results of the homogeneity study, grand means with the corresponding RSD_r, are presented in Table 3. The statistical evaluation of materials A and B is presented in Annex 3.

Table 3 *Concentrations of atropine and scopolamine in material A and B obtained during the homogeneity testing.*

	Material A		Material B	
Compound	Conc. (µg/kg)	RSDr (%)	Conc. (µg/kg)	RSDr (%)
Atropine	0.882	4.06	13.7	1.70
Scopolamine	0.928	5.58	45.5	1.30

2.5 Stability of the materials

The stability of atropine and scopolamine in the PT materials was assessed according to [11,12]. On August 31st, 2020, the day of distribution of the PT samples, six randomly selected containers of material A and B were stored at <-20 °C. Under these conditions it is assumed that atropine and scopolamine are stable in the materials. In addition, six samples of each material were stored at <4 °C.

On November 9th, 2020, 70 days after distribution of the samples, six samples of materials A and B, stored at <-20 °C and <4°C, were analysed in one batch. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes had occurred [11,12] in the materials stored at <4 °C. A consequential instability is observed when the average value of an analyte in the samples stored at <4 °C is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-20 °C. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 4. None of the tested storage conditions caused a consequential difference for the analytes in both materials. Atropine and scopolamine in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

This proficiency test focused on the TAs atropine and scopolamine in food and feed, using buckwheat flour and maize flour. Invitations to the NRL network were sent out on June 30th, 2020 (Annex 5). Thirty-eight participants registered for the PT (Annex 1) and reported their results. Out of 38 participating laboratories, 29 were NRLs from 22 EU Member States plus Iceland and Norway and 9 were OLs (9 from 4 EU Member states and Switzerland). Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report results through an web application designed for proficiency tests as well as to fill in a questionnaire, where it was asked to provide detailed information on the analytical method used for detection and quantification of atropine and scopolamine (extraction solvent/procedure clean-up, detection technique, limit of detection, limit of quantification).

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sets of samples with the corresponding number, consisting of two coded samples (Annex 2) were sent to the PT participants on August 31st, 2020. The sets of samples were dispatched by courier to the participants in insulation boxes containing dry ice. The participants were asked to store the samples at <4 °C and to analyse the samples according to their routine practice. As reported by participants, most of the parcels (30) were received within 24 hours after dispatch. Eight participants received the parcel after 2 days. All samples were received in good order.

The samples were accompanied by a letter with instructions for the requested analysis (Annex 6) and an acknowledgement of receipt form. In addition, by e-mail, each participant received instructions on how to use the web application to report the results. The questionnaire was intended to gather additional information on limits of quantification (LOQs), method recovery estimates (%) and other method-related aspects (e.g. extraction and clean-up, chromatographic and detection conditions, calibration strategy) to investigate individual and/or general patterns on the submitted results.

A single analysis result for the tropane alkaloids atropine and scopolamine in each sample was requested. The deadline for submitting the quantitative results was October 12th, 2020, allowing the participants six weeks for analysis of the test samples. All results, except one, were submitted within the deadline. Participant PT9159 was unable to report results in time due to COVID-19 issues.

4 Evaluation of results

The statistical evaluation of the submitted results was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [11], elaborated by ISO, IUPAC and AOAC, and ISO 13528:2015 [12] in combination with the insights published by the Analytical Methods Committee [4,5] regarding robust statistics.

The evaluation of results was based on assigned values and the standard deviation for proficiency assessment (σ_P). From this, z-scores were calculated to classify the participants' performance. Detailed information on the methods used for the statistical evaluation can be found in the background document 'EURL-MP-background doc_001 (v1) Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed' available from the EURL mycotoxins & plant toxins website[1].

4.1 Calculation of the assigned value

The robust mean was used as consensus value in this PT. The consensus value based on the participants' results (all participants, both NRLs and OLs) was used as the assigned value. The values and their uncertainties are summarised in Table 1 in the Summary section. Assigned values were established for atropine, scopolamine and the sum of both TAs in both materials.

4.2 Standard deviation for proficiency assessment (σ_P)

A fixed relative target standard deviation for proficiency assessment (σ_P) of 25% was used, irrespective the analyte, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and the best practises for mycotoxin and plant toxin determination in food and feed. The rationale behind this is provided in the before mentioned EURL-MP-background doc_001.

4.3 Quantitative performance (z-scores)

For evaluation of numerical results submitted by each participant, z-scores were calculated based on the assigned value, its uncertainty, and the standard deviation for proficiency assessment (σ_P). In cases when the uncertainty of the assigned value was negligible and no instability of the analytes in the PT material was observed, z-scores were calculated using the following equation:

$$z = \frac{x - C}{\sigma_p} \qquad \text{Equation 1}$$

where:

z = z-score;
x = the result of the laboratory;
C = assigned value, here the consensus value;
σ_p = standard deviation for proficiency assessment.

The z-score compares the participants' deviation from the assigned value, taking the target standard deviation accepted for the proficiency test into account, and is interpreted as indicated in Table 4.

[1] Website EURLMP

Table 4 *Classification of z-scores.*

$\|z_a\| \leq 2$	Satisfactory
$2 < \|z_a\| < 3$	Questionable
$\|z_a\| \geq 3$	Unsatisfactory

If the uncertainty of the assigned value and, if applicable, instability of the analyte in the PT material, is not negligible, then this is taken into account in the determination of the z-score. If applicable, this is indicated by assigning a z'-, z_i-or z_i'-score. For details see the background document 'EURL-MP PT performance assessment' on the EURL-MP website.

In this PT, for both materials, the uncertainty of the assigned value for atropine, scopolamine and the sum was negligible. No instability of the analytes in the PT materials was observed during the PT period.

4.4 Evaluation of non-quantified results

In cases, where participant(s) reported '<[value]' or 'not detected' (nd) (i.e. below their limit of quantification (LOQ)), 'proxy-z-scores' were calculated to assess possible false negatives and to benchmark the LOQ relative to the assigned value and the LOQ of the other participants.

A proxy-z-score was calculated by using equation IV and equation V of the background document 'EURL-MP-background doc_001' (for details see the EURL-MP website), using the reported LOQ value as a result. Proxy-z-scores are for information only and indicated as a value between brackets.

Values ($z < -2$) were considered as false negatives (see 4.5). Values ($z > 2$) indicate that the LOQ is high in relation to the assigned value and high in comparison to other participants.

Other types of reported results, e.g. 'detected' or 'not detected', without specification of LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the applicable analyte/matrix.

4.5 False positive and false negative results

A false positive is a quantitative result reported by the participant while the analyte is not detected in the PT material by the organiser, and/or not detected by the majority of the other participants. A threshold is then applied, above which results are considered false positives, indicated as FP. False positives are to be interpreted as unsatisfactory performance.

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', or 'not detected', an assessment is made to judge whether such results should be classified as a false negative. This is the case when the proxy-z-score (see 4.4) is <-2. False negatives are indicated as 'FN'. False negatives are to be interpreted as unsatisfactory performance.

5 Performance assessment

5.1 Scope and LOQ

This PT was dedicated to atropine and scopolamine in buckwheat flour and maize flour. Annex 7 summarises the quantitative scopes of each participant, with an indication of the LOQs for atropine and scopolamine. Five participants provided no details of their LOQs of the method used. The median LOQs for atropine and scopolamine were 0.5 µg/kg.

All the participants determined and quantified atropine and scopolamine as was requested. One participant analysed only material A.

Several results were reported as <LOQ or 'nd'. In case the participant had specified an LOQ (Annex 7), for these results proxy z-scores were calculated.

The LOQs provided by the participants ranged from 0.025 to 5 µg/kg. The recommended LOQ for this proficiency test was 0.5 µg/kg or lower. Twenty-four participants reported an LOQ of 0.5 µg/kg or less: twelve participants reported LOQs of 0.5 µg/kg, five participants reported LOQs in the range of 0.26 to 0.4 µg/kg, six participants reported LOQs in the range of to 0.1 to 0.25 µg/kg and one as low as 0.025 µg/kg. Five participants reported LOQs in the range of 0.53 to 1 µg/kg and 4 participants reported LOQs in the range of 2-5 µg/kg.

5.2 Analytical methods

All participating laboratories were asked to fill in a questionnaire addressing their accreditation, the conditions used for sample preparation, chromatographic separation, detection, quantification and calibration (Annex 8). Three participants did not complete the questionnaire. Two of these participants provided very limited information about the analysis and analytical method via the web application. One participant provided no information at all. The questionnaire of the following participants were submitted after the deadline of October 12th 2020: PT9154-PT9155-PT9156-PT9157-PT9158-PT9161-PT9163-PT9165-PT9169-PT9170-PT9171-PT9174-PT9178-PT9180-PT9183-PT9184 and PT9188. Because this could be due to reasons related to COVID-19 it was not evaluated.

Out of 38 laboratories, 12 had their analytical method covered by ISO/IEC 17025:2017 accreditation, while 17 had not accredited their method and nine participants did not provide this information.

Median sample intake reported by the participants was 4 g; the most often reported intake was 2 g (9 participants). Fourteen participants used 2.5 g or less, 14 participants used between 4 and 10 g, 3 participants used between 20 and 25 g and seven participants provided no details. The samples were extracted with 25 ml (median volume) of extraction solvent for approximately 30 min (median extraction time). The volumes most often used were 20 ml (9), 40 ml (8) and 100 ml (5). Most participants (16) reported an extraction time of 30 min; seven participants used an extraction time between 2 and 20 min; 6 participants used an extraction time of 60 min; one participant used 90 min and 8 participants provided no details. (Aqueous) methanol (21) was used as the extraction solvent by the majority of participants, followed by acidified (aqueous) acetonitrile (ACN) (7). One participant used a mixture of methanol/dichloromethane/ammonia ($MeOH/CH_2Cl_2/NH_3$), one used aqueous methanol without the addition of acid, one used pure acetonitrile, one used acetonitrile in combination with an ammoniumhydrogencarbonate (NH_4HCO_3) buffer, one used acetonitrile with ammonium carbonate ($(NH_4)_2)CO_3$) buffer, one used acidified water and one participant used the QuEChERS extraction, while three participants did not provide information. Formic acid was most often used (23) as acidifier, followed by sulfuric acid (4) and acetic acid (2).

Solid phase extraction (SPE) was used by 8 participants for sample extract purification, two participants applied dispersive SPE (d-SPE) of which one used primary secondary amine (PSA). Four participants reported that they diluted the sample extracts, three participants reported that they filtered the extract, one participant reported that the extracts had been frozen for two hours, one participant reported that they used magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl) with the extraction, four participants reported that another clean-up was used, 11 participants reported that no clean-up was used and four provided no details on the clean-up used. The following clean-up cartridges were reported: Mycosep (1), Oasis MCX (2), Bond Elut Plexa (2), HF Bond Elut LRC-SCX (1) and Strata-X (1) and one participant did not specify the cartridge they had used.

All participants used liquid chromatography in combination with MS for separation of atropine and scopolamine. Participants used either acetonitrile (20) or methanol (14) as an organic mobile phase modifier. One participant reported only the mobile phase A and three participants did not provide information. The majority of participants (25) indicated that acidic chromatography had been used: 18 participants used formic acid to acidify the mobile phase and six used ammonium formate with addition of formic acid and one used ammonium acetate with addition of formic acid. Nine participants used alkaline chromatography. For the preparation of the alkaline mobile phase the following buffers were used: ammonium carbonate (3), ammonium bicarbonate (1), ammonium hydroxide (2) and ammonia (2). One participant used ammonium hydroxide in combination with ammonium acetate.

A wide variety of columns from different suppliers was used for chromatography with acidic conditions, mostly with C18 based stationary phase: Waters: Acquity UPLC BEH (5), Atlantis T3 (1), HSS T3 (1); Agilent: Zorbax Eclipse Plus (1), Poroshell 120 (1); Phenomenex: Synergi Polar RP (2), Kinetex (3), Luna (1); Thermo Scientific: hypersil Gold (3). In addition, the following non-C18 stationary phase columns were used by number of participants: Supelco: Ascentis Express pentafluorophenyl (4); Phenomenex: Kinetex F5 pentafluorophenyl (2), Kinetex biphenyl (1), Waters: Xbridge BEH amide (1). For alkaline chromatography participants used only C18 based stationary phase mostly from one supplier Waters: Acquity BEH (4), XBridge (4); Phenomenex: Gemini (1).

LC-MS/MS was used by most participants (34) for the identification and quantification of atropine and scopolamine. One participant used LC-single quadrupole MS and two participants used LC-high resolution mass spectrometry (HRMS). One provided no information.

The quantification approach followed by the participants is summarised in Table 5. Four participants did not indicate what they used as quantification approach. Out of 34 participants, 17 participants used an external standards calibration curve: five prepared the standards in solvent, five prepared the standards in blank matrix extract and seven prepared the calibration standards from a range of spiked blank samples. Seventeen participants used an internal standard addition approach: 7 used single level standard addition to the sample, five used multi-level standard addition to the sample and five used multi-level standard addition to the sample extract. Twenty-eight participants (82%) have corrected their results for recovery while 18% reported that they didn't.

Table 5 *Analytical strategies followed by the participants.*

Quantification approach	No. of participants	Recovery	
		Corrected	Not corrected
external blank solvent	5	5	
external blank extract	5	3	2
external blank samples	7	5	2
internal single level to sample	7	7	
internal multi-level to sample	5	5	
internal multi-level to extract	5	3	2

Out of 38 participants, twenty-two participants used isotope-labelled internal standards for atropine and scopolamine for quantification. The majority of them added the internal standards before the extraction (19), two added the internal standards to the final extract and one did not indicate when

the internal standards were added. The first approach provides more benefits as the internal standards can correct the results simultaneously for the losses during the extraction step and compensate the matrix effects during MS analysis.

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for atropine and scopolamine in materials A (buckwheat flour) and B (maize flour) are summarised in Annex 9 and 10, respectively. A summary of the performance of the participants in this PT is provided in Annex 11.

A summary of the statistical evaluation of the PT results is presented in Table 6. The table includes all relevant parameters: the assigned value (A), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results.

Table 6 *Parameters of the individual tropane alkaloids and summary for materials A and B.*

	Material A (buckwheat flour)			Material B (maize flour)		
	Atropine	Scopolamine	Sum	Atropine	Scopolamine	Sum
A (µg/kg)	1.15	1.16	2.36	15.3	52.7	68.4
u (µg/kg)	0.049	0.063	0.099	0.439	2.21	2.35
σ_p (µg/kg) (25%)	0.287	0.290	0.591	3.81	13.2	17.1
$u>0.3\sigma_p$	No	No	No	No	No	No
robust σ (µg/kg)	0.229	0.297	0.475	2.11	10.6	11.4
robust σ (%)	19.9	25.6	20.1	13.8	20.1	16.7
# reported	38	38	38	37	37	37
"<", nd	4	3	2	1	1	
# quantitative results	34	35	36	36	36	37
$\|z\|\leq 2$	30	27	30	34	34	34
$2<\|z\|<3$	1	6	2		1	1
$\|z\|\geq 3$	3	2	4	2	1	2
FN				1	1	
satisfactory z-scores (%)	88.2	77.1	83.3	91.9	91.9	91.9

FN = False negative

nd = not detected

For atropine and scopolamine in both materials, the uncertainty of the assigned value did comply with the criterion $u\leq 0.3\sigma_p$ and was, therefore, considered as negligible in the evaluation of the z-scores.

For the individual TAs in material A, 83% of the results were rated with satisfactory z-scores ($|z|\leq 2$), 10% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z|\geq 3$.
For the individual TAs in material B, 92% of the results were rated with satisfactory z-scores ($|z|\leq 2$), 1% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z|\geq 3$.
In case of the sum of both TAs, for material A, 83% of the submitted results were satisfactory and for material B 92%.

Overall, 87% percent of the atropine and scopolamine results obtained for both materials (A and B) were rated with satisfactory z-scores ($|z|\leq 2$), 6% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z|\geq 3$.

For material A, four participants reported atropine as not detected or <LOQ and for scopolamine three participants. Out of these participants the LOQ was in the range 1 to 5 µg/kg while the recommended LOQ for this PT was 0.5 µg/kg or lower. In material B atropine was present at 15.3 µg/kg and scopolamine at 52.7 µg/kg. Nevertheless, one participant reported these analytes as below their LOQ. As the proxy z-scores (see 4.4) were <-2, these results were classified as false negatives.
Besides the false negatives, for atropine in total one questionable and five unsatisfactory results and for scopolamine seven questionable and three unsatisfactory results were observed, mostly for buckwheat flour that contained the lower concentration. The results shows that the laboratories' performance for atropine were slightly better than for scopolamine.

Participant PT9186 reported very high results for material A and did not detect the analytes in material B. Based on the results of participant PT9186, it can be speculated that the samples have been interchanged by the participant.

Participants PT9160, PT9165 and PT9174 reported for material A, one of the two TAs as '<'. For the sum of the TAs, participant PT9160 reported the value for scopolamine (1.87 µg/kg), participant PT9165 reported the sum of the LOQ for atropine and the quantitative result for scopolamine (7 µg/kg) and participant PT9174 reported a sum value of 4.5 µg/kg. For material B participant PT9186 reported a sum value of 0.62 µg/kg while reporting both TAs below the LOQ.

In Annex 11 an overview of the overall performance of each participant in this PT is summarised. For the two materials combined, a maximum of 4 satisfactory z-scores could be obtained for the individual TAs, and '4 out of 4' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. All the participants analysed the materials for atropine and scopolamine. Out of 38 participants, 26 participants achieved optimal performance for both materials by detecting atropine and scopolamine with correct quantification and the absence of false negative results. One participant analysed only material A and achieved optimal performance for that material. For the other 11 participants either the indicated LOQs were too high, false negative results were reported, or one or more non-satisfactory z-scores were obtained.
With respect to the sum of the TAs, 30 participants showed satisfactory performance.

5.4 Robust relative standard deviation

The robust relative standard deviation (RSD_R) was calculated according to ISO13528:2015 [12] for informative purposes only. In this study it was used as a good estimation of the interlaboratory variability. The RSD_R values for atropine and scopolamine in both materials are shown in Annex 9 and 10, in Table 6 (Section 5.3) and also in Table 1 (Summary section).

The robust standard deviation (RSD_R) of the reported results for both TAs are in good agreement with the target standard deviation (25%). For material A, the robust standard deviation for atropine was 20% and for scopolamine 26%, the latter just above the target standard deviation of 25%. For material B, the RSD_R for both atropine (14%) and scopolamine (20%) were well below the target standard deviation of 25% and atropine almost even two times lower. The higher RSD_R values obtained for material A are most likely related to the lower concentrations of the TAs present in material A. The lower RSD_R for atropine in both materials shows that the laboratories' performance for atropine was slightly better than for scopolamine. The assigned values for both TAs in material A were respectively 1.15 and 1.16 µg/kg and in material B 15.3 and 52.7 µg/kg.

The RSD_R values for the sum of TAs are also below the target standard deviation (25%) for material A (20%) as well as material B (17%).

6 Conclusions

Thirty-eight participants, 29 NRLs (from 22 EU Member States plus Iceland and Norway) and 9 OLs (9 from four EU countries and Switzerland) participated in the EURLPT-04 on the quantitative determination of the TAs atropine and scopolamine, in buckwheat flour and maize flour. Both the sample matrices and measurand levels were targeted to provide insight in the capabilities of EU Member States' NRLs concerning the implementation of published legislation in this field (maximum limits of atropine and scopolamine of 1 µg/kg, Commission Regulation (EU) 2016/239). In addition to the food product, a maize sample containing a higher level of TAs was included in this PT, since an amendment to the legislation is foreseen.

All laboratories determined both atropine and scopolamine and reported individual levels and the sum.

For individual TAs in material A, the percentage of satisfactory results for atropine was 88% and for scopolamine 77%. The robust standard deviation (RSD_R) of the reported results for atropine (20%) was below the target standard deviation (25%) and for scopolamine (26%) it was just above the target standard deviation (25%). The larger variation might be related to the lower concentrations (1.15 µg/kg for atropine and 1.16 µg/kg for scopolamine) of the individual TAs in this material.

For material B, for both TAs the satisfactory results were 92% and the RSD_R were also below the target standard deviation (25%). One participant did not analyse the TAs in material B.

Overall, for individual TAs in both materials combined, 87% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 6% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$ and 26 participants had a satisfactory performance. In case of the sum of atropine and scopolamine in both materials combined, 88% of submitted results were satisfactory and 30 participants had a satisfactory performance.

Thirty-four participants used methods based on LC-MS/MS, one used LC-MS and two LC-HRMS, either with or without clean-up. One participant provided no information. Four participants followed the analytical protocol supplied by the EURLMP, while one of them used a different analytical column than the recommended one. The reported LOQs by the participants varied between 0.025 and 5 µg/kg. The median LOQs for atropine and scopolamine were both 0.5 µg/kg. Since NRLs are expected to have analytical methods in place not only for compliance testing of regulatory limits, but also in the framework of data generation for risk assessment, it is advised to set target LOQs of individual analytes to 0.5 µg/kg, at least for cereal-based foods for infants and young children.

Sixty-eight percent of the participants performed satisfactorily for both TAs in buckwheat flour and maize flour and 79% for the sum of both TAs. Based on the results of this test it is concluded that there is still a need for improvement of the quantification of atropine and scopolamine in buckwheat flour at the levels regulated by the Commission Regulation (EU) 2016/239.

References

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[9] WFSR SOP-A0989 – Preparation of PT materials and PT samples.

[10] EURLMP-method_004 (version 1), Determination of tropane alkaloids in processed cereal-based foods for infants and young children by LC-MS/MS, WFSR Wageningen University & Research. https://www.wur.nl/en/show/EURL-MP-Method_004-Tropane-alkaloids-by-LCMSMS_v1.htm

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[12] ISO 13528:2015. Statistical methods for use in proficiency testing by inter-laboratory comparison, 1st edition.

Annex 1 List of participants

Country	Organisation
AUSTRIA*	Institute for Food Safety Innsbruck
BELGIUM*	Sciensano
CROATIA*	A. Stampar Teaching Institute of Public Health
CYPRUS*	State General Laboratory
CZECH REPUBLIC*	UKZUZ (Central Institute for Supervising and Testing in Agriculture
CZECH REPUBLIC*	Czech Agriculture and Food Inspection Authority (CAFIA)
DENMARK*	Danish Veterinary and Food Administration
FINLAND*	Finnish Customs Laboratory
FINLAND*	Finnish Food Authority
FRANCE***	LABOCEA
FRANCE*	Laboratoire SCL de Strasbourg
FRANCE*	SCL
GERMANY***	CVUA-Mel
GERMANY***	Landesbetrieb Hessisches Landeslabor (LHL)
GERMANY***	Bavarian Health and Food Safety Authority
GERMANY***	Chemisches und Veterinaruntersungungsamt (CVUA-RRW)
GERMANY**	Eurofins WEJ Contaminants
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GREECE*	General Chemical State Laboratory
HUNGARY*	National Food Chain Safety Office
IRELAND*	The State Laboratory
IRELAND*	The Public Analyst's Laboratory
ITALY*	Istituto Superiore di Sanita
LUXEMBOURG*	Laboratoire national de Sante
NORWAY**	Norwegian Institute of Bioeconomy Research (NIBIO)
POLAND*	National Institute of Public Health - National Institute of Hygiene
POLAND***	Wojewodzka Stacja Sanitarno - Epidemiologiczna w Bydgoszczy
POLAND***	Voivodship Sanitary Epidemiological Station
POLAND*	National Veterinary Research Institute
ROMANIA*	Institute for Hygiene and Veterinary Public Health
SERBIA*	SP Laboratorija A.D.
SLOVAKIA*	State veterinary and food institute Dolny Kubin Veterinary and food institute in Kosice
SLOVENIA*	National Laboratory of Health, Environment and Food (NLZOH, Slovenia)
SPAIN***	Laboratori Agencia Salut Publica Barcelona
SPAIN*	Spanish Agency for Consumer Affairs, Food Safety and Nutrition
SWEDEN*	National Food Agency
SWITZERLAND***	Kantonales Laboratorium
UNITED KINGDOM*	FERA Science Ltd

* National Reference Laboratory (NRL) of EU Member State.

** National Reference Laboratory (NRL) of the European Free Trade Association (Eurofins WEJ Contaminants = Iceland).

*** Official Laboratory (OL)

Annex 2 Codification of the samples

Participants code	Material A*	Material B*
PT9064	308	315
PT9151	151	234
PT9152	988	676
PT9153	473	734
PT9154	522	196
PT9155	493	438
PT9156	931	829
PT9157	116	266
PT9158	122	879
PT9159	508	157
PT9160	408	679
PT9161	270	410
PT9162	449	221
PT9163	749	673
PT9164	697	471
PT9165	461	633
PT9166	173	722
PT9167	388	756
PT9168	397	571
PT9169	446	779
PT9170	623	883
PT9171	718	945
PT9172	675	818
PT9173	825	215
PT9174	199	403
PT9175	325	764
PT9176	762	442
PT9177	926	469
PT9178	606	103
PT9179	253	178
PT9180	114	262
PT9181	184	613
PT9182	599	798
PT9183	399	766
PT9184	171	381
PT9185	932	288
PT9186	735	572
PT9187	791	206
PT9188	464	817

* All sample codes start with 2020/EURLPT MP/TAs/.

Annex 3 Statistical evaluation of the homogeneity data

	Atropine in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	0.952	0.887
Hom/A002	0.856	0.880
Hom/A003	0.904	0.878
Hom/A004	0.904	0.927
Hom/A005	0.851	0.854
Hom/A006	0.857	0.882
Hom/A007	0.785	0.868
Hom/A008	0.845	0.897
Hom/A009	0.891	0.911
Hom/A010	0.914	0.896
Grand mean	0.882	
Cochran's test		
C	0.400	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.220	
s_x	0.030	
s_w	0.029	
s_s	0.021	
Critical= 0.3 σ_P	0.058	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	Scopolamine in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	0.977	0.944
Hom/A002	0.892	0.964
Hom/A003	0.894	0.954
Hom/A004	0.921	0.934
Hom/A005	0.818	0.941
Hom/A006	0.921	0.897
Hom/A007	0.819	0.918
Hom/A008	0.947	0.910
Hom/A009	0.966	0.912
Hom/A010	1.028	1.004
Grand mean	0.928	
Cochran's test		
C	0.374	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.23	
s_x	0.041	
s_w	0.045	
s_s	0.026	
Critical= 0.3 σ_P	0.061	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	Atropine in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	13.5	13.7
Hom/B002	13.4	14.2
Hom/B003	13.8	13.5
Hom/B004	13.7	13.9
Hom/B005	13.7	13.6
Hom/B006	14.1	14.1
Hom/B007	13.7	13.9
Hom/B008	13.7	13.6
Hom/B009	13.6	13.6
Hom/B010	13.4	14.0
Grand mean	13.7	
Cochran's test		
C	0.542	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.43	
s_x	0.144	
s_w	0.258	
s_s	0.000	
Critical= 0.3 σ_P	0.907	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	Scopolamine in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	45.4	45.2
Hom/B002	45.1	45.8
Hom/B003	45.6	45.2
Hom/B004	45.8	44.5
Hom/B005	45.7	44.3
Hom/B006	46.2	46.0
Hom/B007	46.3	45.2
Hom/B008	45.8	45.6
Hom/B009	44.8	45.3
Hom/B010	44.9	46.5
Grand mean	45.5	
Cochran's test		
C	0.307	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.37	
s_x	0.359	
s_w	0.658	
s_s	0.000	
Critical= 0.3 σ_P	3.00	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Annex 4 Statistical evaluation of the stability data

*Stability evaluation for **atropine in material A***

Storage temperature	<-20 °C	<4 °C
Time (days)	0	70
Calculated amounts (µg/kg)	1.14	1.08
	1.15	1.08
	1.02	0.913
	0.990	1.00
	0.930	0.962
	1.05	1.00
Average amount (µg/kg)	1.047	1.007
n	6	6
st. dev (µg/kg)	0.086	0.066
Difference		0.040
$0.3*\sigma_P$		0.079
Consequential difference? Diff < $0.3*\sigma_P$		No

*Stability evaluation for **scopolamine in material A***

Storage temperature	<-20 °C	<4 °C
Time (days)	0	70
Calculated amounts (µg/kg)	1.11	1.06
	1.08	1.01
	1.08	1.06
	1.06	0.99
	1.14	1.12
	0.979	0.982
Average amount (µg/kg)	1.08	1.03
n	6	6
st. dev (µg/kg)	0.053	0.052
Difference		0.040
$0.3*\sigma_P$		0.081
Consequential difference? Diff < $0.3*\sigma_P$		No

*Stability evaluation for **atropine in material B***

Storage temperature	<-20 °C	<4 °C
Time (days)	0	70
Calculated amounts (µg/kg)	13.3	13.4
	13.5	13.7
	13.2	14.4
	13.3	13.8
	13.1	13.5
	13.7	13.4
Average amount (µg/kg)	13.3	13.7
n	6	6
st. dev (µg/kg)	0.229	0.369
Difference		-0.357
$0.3*\sigma_P$		1.000
Consequential difference? Diff < $0.3*\sigma_P$		No

*Stability evaluation for **scopolamine in material B***

Storage temperature	<-20 °C	<4 °C
Time (days)	0	70
Calculated amounts (µg/kg)	47.4	49.2
	48.4	45.3
	44.9	48.0
	47.4	49.1
	48.6	48.1
	46.1	48.9
Average amount (µg/kg)	47.1	48.1
n	6	6
st. dev (µg/kg)	1.43	1.44
Difference		-0.965
$0.3*\sigma_P$		3.54
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Invitation letter

WAGENINGEN
UNIVERSITY & RESEARCH

European Union Reference Laboratory

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

NRLs mycotoxins & plant toxins

Dear colleague,

The EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), will organize a proficiency test (PT) regarding tropane alkaloids in food and feed matrices (EURLPT-MP04). This test will focus on the quantification of the tropane alkaloids atropine and scopolamine and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013). Harmonised EU regulation for atropine and scopolamine in these matrices is being prepared and their inclusion in national monitoring is recommended by EFSA.

This PT will focus on quantification of atropine and scopolamine considered for legislation in food and feed products. The primary goal of this proficiency test is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of these compounds in food and feed matrices.

According to Regulation (EU) 2017/625 all EU National Reference Laboratories (NRLs) mycotoxins & plant toxins in food and/or feed are mandatory to participate. I would like to invite you to participate in this PT.

1. Test materials
   One test sample of maize four and one test sample of buckwheat flour will be provided. The test materials maize flour and buckwheat flour are representatives for food and feed. The test amount sent will be approximately 50 g.

2. Shipment of test materials
   Test materials will be sent in the last week of August 2020. The distribution of the test materials will be announced by e-mail. The deadline for reporting is strict and will be six weeks after the date of shipment of the samples.

3. Scope of analysis
   The materials contain the following analytes:
   - Atropine
   - Scopolamine

   Minimal LOQ 0.5 µg/kg.

Wageningen Food Safety Research

DATE
June 30, 2020

SUBJECT
Invitation EURL mycotoxins & plant toxins proficiency test tropane alkaloids in food and feed matrices (EURLPT-MP04)

POSTAL ADDRESS
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

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Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB WAGENINGEN

INTERNET
www.wur.nl/wfsr

CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31 (0) 614323017

EMAIL
pt.wfsr@wur.nl

4. Questionnaire
   A questionnaire will be sent electronically. In this questionnaire the particants will be asked to provide information about the laboratory method used. This information is necessary to conduct a more in depth analysis of the results obtained in this proficiency test.

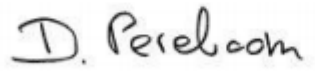
5. Report
   - A report of the proficiency test will be dispatched early in 2021
   - Results of the proficiency test will be presented anonymously
   - The follow-up protocol on proficiency test from DG Santé will be applied

6. Additional information
   - WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications
   - WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first

7. Costs
   - Participation is free of charge for the NRLs.
   - Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee is € 270,- (ex. VAT) as a compensation for the preparation and transportation of the samples.
   - If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the accompanying participation form (preferably digitally) and send it back before the 24th of July 2020 to: pt.wfsr@wur.nl.

Looking forward to welcome you for this test,



Diana Pereboom-de Fauw
Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research
Wageningen
The Netherlands

Annex 6 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam, Sir,

Thank you very much for your participation in the proficiency test for the analysis of tropane alkaloids in food and feed matrices.

The parcel shipped to you should contain:

- One feed material consisting of maize flour and one food material consisting of buckwheat flour. Each test material unit contains approximately 50 grams of the homogenised test material.

Instructions:

- After arrival the samples should be stored at +4 °C.
- Please fill in the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples by e-mail to pt.wfsr@wur.nl.
- Before analysis, homogenise the samples according to your laboratory's procedure.
- Treat the test material as a sample for routine analysis. Report one result and not an average of multiple measurements.
- The tropane alkaloids atropine and scopolamine are considered for legislation in food products. The concentrations of atropine and scopolamine should be reported individually and as a sum of atropine and scopolamine.
- Please report all analytical results in µg/kg. If an analyte is not included in the scope of the method, please report 'nt (not tested)' in the corresponding place of the web application. When the result for an analyte is below your LOQ, 'please report the result as '<LOQ-value' and specify the value (e.g. <10 µg/kg). Do not report these results as 'not detected'. Please note that the recommended LOQ for this proficiency test is 0.5 µg/kg or lower.
- Please use the following web application for entering your results for the test samples (https://crlwebshop.wur.nl/apex/f?p=107:LOGIN). Instructions for use of this web application were sent to you earlier by e-mail. If you didn't receive these instructions or you have a question, please contact us.
- Provide detailed information in the questionnaire on the analysis of the tropane alkaloids and the analytical method used and send it back to us by e-mail (pt.wfsr@wur.nl).

Wageningen Food Safety Research

DATE
August 31, 2020

SUBJECT
Instructions proficiency test tropane alkaloids in food and feed matrices

OUR REFERENCE
2023291/WFSR

POSTAL ADDRESS
P.O. Box 230
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The Netherlands

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INTERNET
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CoC NUMBER
09098104

HANDLED BY
D.P.K.H. Pereboom - de Fauw

TELEPHONE
+31 (0)614 323 017

EMAIL
pt.rikilt@wur.nl

- You can download the EURL method "EURL-MP-method-004 Determination of tropane alkaloids in processed cereal-based foods for infants and young children by LC-MS/MS, for the analysis of tropane alkaloids using LC-MS/MS", from the EURL mycotoxins & plant toxins website: (https://www.wur.nl/en/Research-Results/Research-Institutes/food-safety-research/Reference-laboratory/European-Union-Reference-Laboratory-1/EURL-mycotoxins-plant-toxins/Library-EURL-MP.htm).
- The deadline for submitting test-results for this test is **the 12th of October, 2020.** Please note that this will be a strict deadline; results reported after the deadline will not be considered. The EURL should be contacted at least 2 weeks in advance, if for exceptional reasons the deadline cannot be met.

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

Please contact me in case you have any questions or need any assistance.

With kind regards,

D. Pereboom

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins
Wageningen Food Safety Research (WFSR)
The Netherlands

Annex 7 Scope and LOQ

Lab code	Atropine	Scopolamine
	LOQ (µg/kg)	
PT9064	0.5	0.5
PT9152	0.5	0.5
PT9153	0.5	0.5
PT9154		
PT9155		
PT9156	0.4	0.4
PT9157		
PT9158		
PT9159	0.1	0.1
PT9160	1	1
PT9161	0.4	0.4
PT9162	5	5
PT9163	0.72	0.72
PT9164	0.8	0.8
PT9165		
PT9166	0.025	0.05
PT9167	0.2	0.2
PT9168	0.53	0.52
PT9169	0.25	0.25
PT9170	0.5	0.5
PT9171	5	5
PT9172	0.26	0.27
PT9173	0.3	0.3
PT9174	2	2
PT9175	0.2	0.2
PT9176	1	1
PT9177	0.5	0.5
PT9178	0.5	0.5
PT9179	0.5	0.5
PT9180	0.40	0.36
PT9181	0.25	0.25
PT9182	0.5	0.5
PT9183	0.5	0.5
PT9184	0.1	0.1
PT9185	0.5	0.5
PT9186	0.5	0.5
PT9187	0.5	0.5
PT9188	5	5

Annex 8 Method details

Lab code	Method	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Atropine RT (min)	Scopolamine RT (min)
PT9064	acid	Supelco Ascentis express F5, 100 x 2.1 mm, 2.7 µm	100	16		HRMS	3.62	2.95
PT9152	acid	Waters Atlantis T3, 150 x 3.0 mm, 3 µm	150	22	A: 95+5 MeOH/H_2O v/v + 1% FA + 5 mM $HCOONH_4$; B: H_2O +1% FA + 5 mM $HCOONH_4$	MS/MS	9.7	8.8
PT9153	acid	Phenomenex Luna C18, 100 x 2 mm	100	13	A and B: 5 mM AmAc/ACN/formic acid	MS/MS	6.5	6.2
PT9154						MS/MS		
PT9155	acid	Phenomenex Kinetex F5, 100 x 2.1 mm, 2.6 µm	100		A: H_2O + 0.1% formic acid; B: ACN + 0.1% formic acid	MS/MS		
PT9156	acid	Supelco Ascentis Express F5, 100 x 2.1 mm, 2.7 µm	100	13	A: H_2O + 0.1% HCOOH; B: ACN + 0.1% HCOOH	MS/MS	6.74	5.59
PT9157	alkaline	Waters UPLC BEH C18, 100 x 2.1 mm	100	10	A: Ammoniumhydrogencarbonate 10 mM; B: ACN + 10mM Ammoniumhydrogencarbonate	MS/MS		
PT9158						UPLC/MS/MS		
PT9159	acid	Agilent Poroshell 120 EC-C18, 100 x 4.6 mm, 2.7 µm	100	12	A: 0.2% formic acid in H_2O; B: 0.2% formic acid in MeOH	MS/MS	9.17	5.58
PT9160	acid	Waters Acquity UPLC BEH, C18, 50 x 2.1 mm, 1.7 µm	50	15	0.1% formic acid in H_2O/ 0.1% formic acid in MeOH	MS/MS	4.8	2.5
PT9161	acid	Waters Xbridge Amide, 150 x 2.1 mm, 3.5 µm	150	20	A: 0.05% formic acid in H_2O; B: ACN	MS/MS	2.56	2.69
PT9162	acid	Waters Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	10	A: H_2O + 0.1% HCOOH; B: MeOH + 0.1% HCOOH + 1 mM $HCOONH_4$	MS/MS	1.336	1.174
PT9163	acid	Phenomenex Biphenyl, 100 x 2.1 mm, 2.6 µm	100	10	A: H_2O + 0.1% formic acid; B: ACN + 0.1% formic acid	MS/MS	7.1	6.9
PT9164	acid	Phenomenex Synergi Polar RP, 100 x 2 mm, 80 Å, 4 µm	100	9.1	A: H_2O + 0.1% formic acid; B: ACN + 0.1% formic acid	MS/MS	4.5	3.5
PT9165								
PT9166	acid	Thermo Scientific, Hypersil Gold C18, 200 x 2.1 mm, 1.9 µm	200	26	A: H_2O, HCOOH 0.1%, $HCOONH_4$+: 315 mg/L; B: MeOH, HCOOH 0.1%, $HCOONH_4$+: 315 mg/L	MS/MS	10.56	8.59
PT9167	acid	Phenomenex, Kinetex C18, 100 x 2.1 mm, 2.6 µm	100	45	A: 5 mmol ammonium formiate and 1 ml formic acid in 1000 ml H_2O; B: 5 mmol ammonium formiate and 1 ml formic acid in 1000 ml MeOH	MS/MS	16.44	10.82
PT9168	acid	Phenomenex Synergi Polar-RP 50 x 2 mm, 2.5 µm	50	16	A: 0.1% HCOOH (aq); B: 0.1% HCOOH (MeOH)	MS/MS	2.4	1.9
PT9169	alkaline	Waters UPLC BEH C18, 150 x 2.1 mm, 1.7 µm	150		A: 10 mM ammonium carbonate; B: ACN	MS/MS	8.63	7.47
PT9170	alkaline	Waters Xbridge C18, 75 x 3 mm	75	12	A: 6 mM NH_4OH; B: MeOH/6 mM NH_4OH	MS/MS	6.8	5.8
PT9171	acid	Thermo Hypersil Gold aQ		6	A: H_2O/formic acid; B: MeOH	MS/MS	3.7	3.6

Lab code	Method	Column	Column length (mm)	Total run time (min)	Mobile phase	Detection technique	Atropine RT (min)	Scopolamine RT (min)
PT9172	acid	Supelco Ascentis Express F5, 100 x 2.1 mm, 2.7 µm	100	16	A: H_2O + 0.1% formic acid; B: ACN + 0.1% formic acid	MS/MS	2.88	2.28
PT9173	alkaline	Phenomenex Gemini C18, 100 x 2.0 mm, 3 µm	100	11	A: MQ-H_2O with NH_3 (pH 10.7); B: ACN with NH_3	HRMS	4.07	3.15
PT9174	acid	Agilent Zorbax Eclipse Plus C18 RRHD, 50 x 2.1 mm, 1.8 µm	50	27	A: 10 mM ammoniumformiate; B: 0.2% formic acid in MeOH	MS/MS	4.1	2.7
PT9175	acid	Waters HSS T3, 100 x 2.1 mm, 1.7 µm	100	22	A: H_2O, 5 mM ammonium acetate, 0.1% acetic acid	MS/MS	3	2.5
PT9176	alkaline	Waters Xbridge C18, 150 x 3 mm, 5 µm	150	20	A: Ammonium carbonate 0.2 g/l; B: ACN	MS/MS	5.7	4.7
PT9177	acid	Phenomenex Kinetex C18, 150 x 4.6 mm; 2.6 µm, 100 Å	150	12	A: 0.2% HCOOH/H_2O; B: MeOH	MS/MS	6.94	6.1
PT9178	alkaline	Waters Acquity UPLC BEH C18, 150 x 2.1 mm, 1.7 µm	150	21	A: 10 mM ammonium carbonate in H_2O pH 9.0; B: ACN	MS/MS	11.08	11.29
PT9179	acid	Phenomenex Kinetex, C18, 100 x 4.6 mm; 2.6 µm	100	10.5	A: 0.3% formic acid in H_2O; B: MeOH	MS (single)	7.2	6
PT9180	alkaline	Waters Xbridge C18, 150 x 3 mm, 5 µm	150	25	A: ammonia 6 mM in H_2O; B: ammonia 6 mM in ACN	MS/MS	8.7	6.8
PT9181	acid	Thermo Hypersil GOLD C18, 150 mm x 2.1 mm, 1.9 µm	150	15	A: 5 mmol Ammoniumformate + 0.1% Formic Acid in H_2O; B: 5 mmol Ammoniumformate + 0.1% Formic Acid in 95% MeOH	MS/MS	6.2	5.1
PT9182	acid	Supelco Ascentis Express F5		10	A: 98% H_2O + 0.1% FA, 2% ACN; B: ACN + 0.1% FA	MS/MS	2.71	2.46
PT9183	alkaline	Waters, X-Bridge C18, 150 x 3 mm	150	17	A: 6 mM Ammonium Hydroxide in H_2O; B: 6 mM Ammonium Hydroxide in ACN	MS/MS	6.34	4.98
PT9184	acid	Waters Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	6.5	A: 0.1% Formic Acid in UPW; B: 0.1% Formic Acid in ACN	MS/MS	2.15	1.95
PT9185	alkaline	Waters Acquity BEH C18, 100 x 2.1 mm, 1.7 µm	100	6	A: H_2O + 0.05% ammonium hydroxide (v/v) + 5 mmol/L ammonium acetate; B: MeOH	MS/MS	3.48	3.23
PT9186	acid	Phenomenex Kinetex PFP, 100 Å, 75 x 2.1 mm, 2.6 µm	75	16	A: 0.1% Formic acid (H_2O); B: 0.1% Formic acid (ACN)	MS/MS	3.34	2.78
PT9187	acid	Waters Acquity BEH-C18, 150 x 2.1 mm, 1.7 µm	150	15	A: H_2O + 0.1% FA; B: ACN + 0.1% FA	MS/MS	10.6	6.4
PT9188	acid	Waters Acquity BEH C18, 50 x 2.1 mm, 1.7 µm	50	10	A: H_2O + formic acid 0.1%; B: ACN + formic acid 0.1%	MS/MS	2.5	2.8

ACN = acetonitrile; MeOH = methanol; H_2O = water; FA (HCOOH) = formic acid; HOAc (CH_3COOH) = acetic acid; NH_3 = ammonia; NH_4OH = ammonium hydroxide; $HCOONH_4$ = ammonium formate; CH_3COONH_4 = ammonium acetate; $(NH_4)_2CO_3$ = ammonium carbonate; NH_4HCO_3 = ammoniumhydrogencarbonate; NH_4OH = ammonium hydroxide.

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)
PT9064	4	H_2O:ACN 0.5% HCOOH (50:50, v/v)		mechanical shaking	30	other			1
PT9152	2	ACN/H_2O/FA 50+50+1 (vol.)	20	mechanical shaking	30	other, shaking with 4 g MgSO4 and 1 g NaCl, centrifugation, concentration 2.5 ml (ACN layer) and reconstitution in 0,5 ml of methanol/water/FA 60+39+1 (vol.)			0.5
PT9153	10	MeOH/CH_2Cl_2/NH_3	100		2	x			10g/100mL-20mL/2mL
PT9154	2	0.4% formic acid in MeOH / H_2O (60/40, v/v)	20			filtration			
PT9155	1	0.5% formic acid in ACN/H_2O (1:1)	4	mechanical shaking	30	none			0.25
PT9156	4	MeOH/H_2O/FA: 60:40:0.4	40	mechanical shaking	30	SPE	SPE OASIS MCX 150 mg, 6 ml	10	2
PT9157		ACN/ ammoniumhydrogencarbonate				SPE	Mycosep Romer Labs		
PT9158		0.2% formic acid in H_2O		mechanical shaker		SPE			
PT9159	5	ACN HPLC	20	mechanical shaking	60	none			0.25
PT9160	2	MeOH/ H_2O/ formic acid (60/40/4)	20	mechanical shaking	30	dilution			0.02
PT9161	1	ACN, 0.05% formic acid in H_2O 1:1	5	shaking (hand/vortex)	10	other			0,2
PT9162	2	ACN + 0.1% HCOOH in H_2O (1:1)	20	mechanical shaking	20	dilution			0.1
PT9163		Quechers extraction	x			dSPE			
PT9164	2.5	MeOH/H_2O/formic acid 39/60/1	25	mechanical shaking	30	none			
PT9165									
PT9166	8	MeOH (with H_2SO_4 0.05M)	40	ultrasonic	15	SPE	HF Bond Elut LRC-SCX, 500 mg Agilent	10	2
PT9167	20	0.05 M sulfuric acid in MeOH	100	ultrasonic	15	SPE	Bond Elut Plexa PCX, 500 mg 6ml/Agilent	10 ml/1 ml	2
PT9168	1.5	MeOH/H_2O/Formic acid (60/40/0.4)	25	mechanical shaking	30	dilution			0.012
PT9169	4	MeOH/H_2O/formic acid solution (75/25/0.4%)	40	mechanical shaking	30	SPE	Strata-X-C 200 mg/6 ml, 33 µm	10	2

Lab code	Sample weight (g)	Extraction solvent	Extraction solvent volume (ml)	Extraction conditions	Extraction time (min)	Sample clean-up	SPE cartridge	Volume extract loaded on SPE (ml)	Matrix equivalent final extract (g/ml)
PT9170	4	MeOH (with H_2SO_4 0.05M)	40	mechanical shaking	30	none			
PT9171	2	MeOH/H_2O/Formic acid	20	ultrasonic	30	dilution			
PT9172	2	MeOH:H_2O:Formic acid (39:60:1)	20	mechanical shaking	60	none			0.1
PT9173	4	0.4% Formic acid in MeOH:H_2O (60:40)	40	mechanical shaking	60	SPE	Oasis MCX	6	0.3
PT9174	25	ACN:H_2O:Acetic acid (79:20:1)	100	mechanical shaking	30	none			0.25
PT9175	5	H_2O + ACN (0.5% acetic acid)	20	mechanical shaking	30	other			1
PT9176	25	ACN / Ammonium carbonate 0.2 g/l (86/14)	125	mechanical shaking	30	SPE	100 mg dSPE Bondesil PSA	2 ml / 4 ml	0.1
PT9177	1-2	MeOH/H_2O 60:40 + 4 ml HCOOH/l	20-50	mechanical shaking	90	none	"		
PT9178	4	0.4% formic acid in MeOH/H_2O (60/40, v/v)	40	shaking (hand/vortex)	30	Ultrafilter (Amicon Ultra-4 Ultracel 30kD)		2	0.1
PT9179					x				
PT9180	2	MeOH/H_2O/formic acid (60/40/0.4)	8	mechanical shaking	15	none			
PT9181	10	0.05 M H_2SO_4 in MeOH	100	ultrasonic	10	SPE	HF Bond Elute Plexa PCX; 500 mg/6 ml	10	1
PT9182	10	0.4% formic acid in MeOH/H_2O (60/40, v/v)	100	mechanical shaking	30	other, freeze out for at least 2 hours			0,1
PT9183	4	0.4% formic acid in MeOH:H_2O 60:40	40	mechanical shaking	30	other	N/A	N/A	0.1
PT9184	2	MeOH:UPW:Formic Acid (39:60:1)	20	shaking (hand/vortex)	60	None, Sample was centrifuged using a Costar Spin X Tube and the supernatant injected			0.1
PT9185	4	MeOH/H_2O (60:40) + 0.4% formic acid	40	mechanical shaking	60	none	N/A	N/A	0.1
PT9186	2	MeOH/H_2O/formic acid (39:60:1)	20	mechanical shaking	60	none			0.1
PT9187									
PT9188	1	H_2O + MeOH	10	30		filtration			

ACN = acetonitrile; MeOH = methanol; H_2O = water; CH_2Cl_2 = dichloromethane; H_2SO_4 = sulfuric acid; FA (HCOOH) = formic acid; HOAc (CH_3COOH) = acetic acid; NH_3 = ammonia; $(NH_4)_2CO_3$ = ammonium carbonate; NH_4HCO_3 = ammoniumhydrogencarbonate.

N/A: not applicable

Annex 9 Results: Material A (buckwheat flour)

	Material A					
	Atropine A: 1.15 µg/kg u: 0.049 µg/kg σ_p: 0.287 µg/kg (25%) robust σ: 0.229 µg/kg (19.9%)		Scopolamine A: 1.16 µg/kg u: 0.063 µg/kg σ_p: 0.290 µg/kg (25%) robust σ: 0.297 µg/kg (25.6%)		Sum A: 2.36 µg/kg u: 0.099 µg/kg σ_p: 0.591 µg/kg (25%) robust σ: 0.475 µg/kg (20.1%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9064	0.9	-0.87	0.4	**-2.62**	1.3	-1.80
PT9152	0.8	-1.22	0.9	-0.90	1.7	-1.12
PT9153	1	-0.52	0.88	-0.97	1.9	-0.79
PT9154	1.71	1.95	0.8	-1.24	2.51	0.25
PT9155	1.4	0.87	1	-0.55	2.4	0.06
PT9156	0.98	-0.59	0.956	-0.71	1.94	-0.72
PT9157	1.029	-0.42	1.347	0.64	2.376	0.02
PT9158	0.98	-0.59	1.44	0.96	2.42	0.09
PT9159	0.99	-0.56	1.18	0.07	2.17	-0.33
PT9160	nd, <1	(-0.52)	1.87	**2.45**	1.87	-0.84
PT9161	1.32	0.59	0.94	-0.76	2.26	-0.18
PT9162	<5	**(13.40)**	<5	**(13.23)**	<5	**(4.46)**
PT9163	1.21	0.21	1.13	-0.11	2.34	-0.04
PT9164	0.93	-0.76	1.16	0.00	2.09	-0.46
PT9165	<5.0	**(13.40)**	2	**2.89**	7	**7.84**
PT9166	1.48	1.15	1.86	**2.41**	3.34	1.65
PT9167	1.12	-0.10	1.14	-0.07	2.26	-0.18
PT9168	1.11	-0.14	1.04	-0.42	2.15	-0.36
PT9169	0.99	-0.56	0.99	-0.59	1.98	-0.65
PT9170	0.92	-0.80	0.97	-0.66	1.89	-0.80
PT9171	1.14	-0.03	0.963	-0.68	2.1	-0.45
PT9172	1.4	0.87	1.1	-0.21	2.5	0.23
PT9173	0.97	-0.62	1.04	-0.42	2.01	-0.60
PT9174	3.2	**7.13**	<2	**(2.89)**	4.5	**3.61**
PT9175	12.8	**40.54**	0.31	**-2.93**	13.1	**18.16**
PT9176	0.94	-0.73	1.6	1.51	2.5	0.23
PT9177	1.07	-0.28	1.01	-0.52	2.08	-0.48
PT9178	0.993	-0.54	1.71	1.89	2.703	0.57
PT9179	1.3	0.52	1.3	0.48	2.6	0.40
PT9180	1.09	-0.21	2.79	**5.62**	3.88	**2.56**
PT9181	1.4	0.87	1.5	1.17	2.9	0.91
PT9182	1.35	0.70	0.98	-0.62	2.33	-0.06
PT9183	0.96	-0.66	0.98	-0.62	1.94	-0.72
PT9184	1.879	**2.54**	1.801	**2.21**	3.68	**2.23**
PT9185	1.04	-0.38	1.01	-0.52	2.05	-0.53
PT9186	8.7	**26.27**	37.2	**124**	45.9	**73.65**
PT9187	1.17	0.07	1.29	0.45	2.46	0.16
PT9188	<5	**(13.40)**	<5	**(13.23)**		

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

nd = not detected.

robust σ = robust (relative) standard deviation based on participants' results.

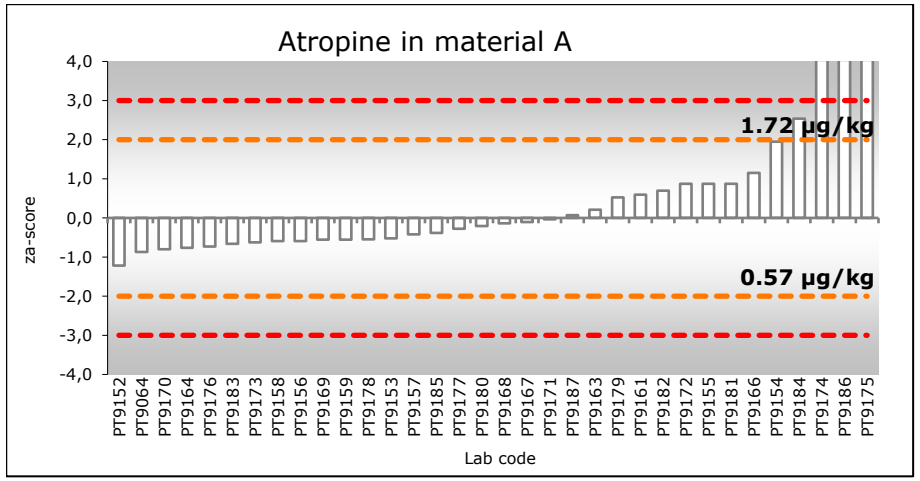

Figure 2 *Graphical representation of the z-scores for atropine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

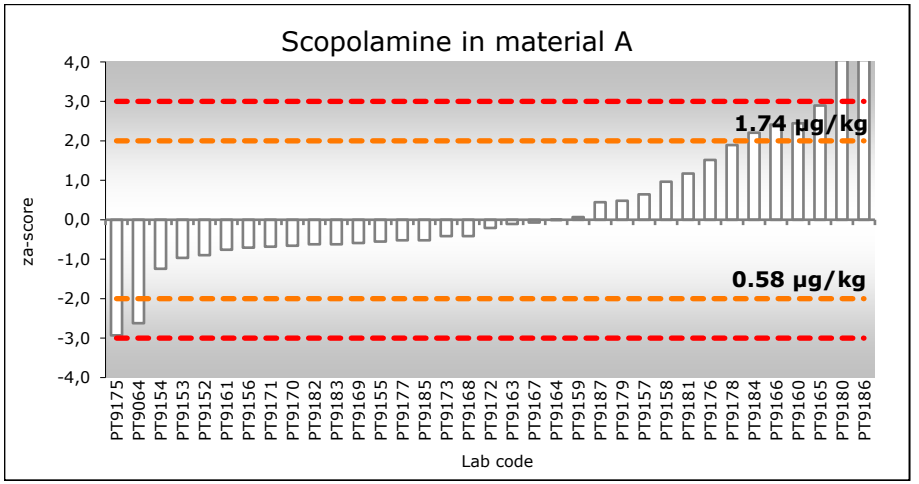

Figure 3 *Graphical representation of the z-scores for scopolamine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

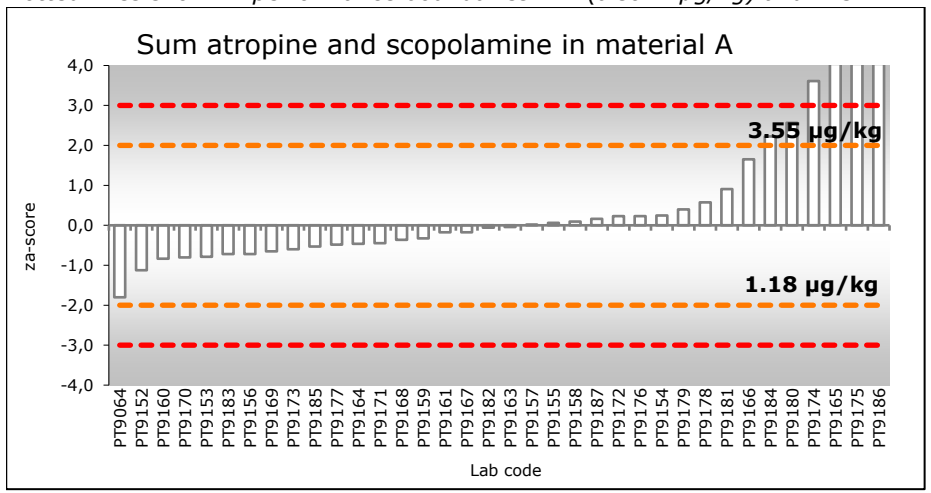

Figure 4 *Graphical representation of the z-scores for the sum atropine and scopolamine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 10 Results: Material B (maize flour)

	Material B					
	Atropine A: 15.3 µg/kg u: 0.439 µg/kg σ_p: 3.81 µg/kg (25%) robust σ: 2.11 µg/kg (13.8%)		Scopolamine A: 52.7 µg/kg u: 2.21 µg/kg σ_p: 13.2 µg/kg (25%) robust σ: 10.6 µg/kg (20.1%)		Sum A: 68.4 µg/kg u: 2.35 µg/kg σ_p: 17.1 µg/kg (25%) robust σ: 11.4 µg/kg (16.7%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9064	2.8	**-3.27**	32.7	-1.52	35.5	-1.92
PT9152	15.3	0.01	61.3	0.65	76.6	0.48
PT9153	16	0.20	52	-0.05	68	-0.02
PT9154	14.8	-0.12	45.1	-0.58	59.9	-0.50
PT9155	16.3	0.28	37.4	-1.16	53.7	-0.86
PT9156	13.8	-0.38	47.7	-0.38	61.5	-0.40
PT9157	10.587	-1.22	62.375	0.74	72.962	0.27
PT9158	13.7	-0.41	58.3	0.43	72	0.21
PT9159	14.74	-0.13	45.85	-0.52	60.59	-0.46
PT9160	15	-0.07	48.3	-0.33	63.3	-0.30
PT9161	17.4	0.56	43.29	-0.71	60.69	-0.45
PT9162	18.08	0.74	56.71	0.31	74.79	0.37
PT9163	nt		nt		nt	
PT9164	13.63	-0.43	60.52	0.59	74.15	0.34
PT9165	15	-0.07	45	-0.58	60	-0.49
PT9166	13.87	-0.36	48.13	-0.35	62	-0.38
PT9167	16.3	0.28	50.2	-0.19	66.5	-0.11
PT9168	16.1	0.22	48.1	-0.35	64.2	-0.25
PT9169	13.6	-0.43	43.2	-0.72	56.8	-0.68
PT9170	16.4	0.30	52.5	-0.01	68.9	0.03
PT9171	16.95	0.45	65.45	0.97	82.4	0.82
PT9172	18.6	0.88	70.4	1.34	89	1.20
PT9173	11.32	-1.03	49.41	-0.25	60.73	-0.45
PT9174	17.4	0.56	53	0.02	70.4	0.12
PT9175	233	**57.11**	32.7	-1.52	266	**11.55**
PT9176	13	-0.59	66	1.01	79	0.62
PT9177	14.9	-0.09	50.8	-0.14	65.7	-0.16
PT9178	13.48	-0.46	73.1	1.55	86.58	1.06
PT9179	8.5	-1.77	38.7	-1.06	47.2	-1.24
PT9180	18.05	0.73	98.56	**3.48**	116.61	**2.82**
PT9181	18.5	0.85	62.1	0.71	80.6	0.71
PT9182	15.65	0.10	52.16	-0.04	67.81	-0.04
PT9183	15.6	0.09	51.2	-0.11	66.8	-0.09
PT9184	17.212	0.51	61.501	0.67	78.713	0.60
PT9185	14.33	-0.24	44.4	-0.63	58.73	-0.57
PT9186	<0.5	**(-3.87) FN**	<0.5	**(-3.96) FN**	0.62	**-3.96**
PT9187	14.9	-0.09	54.8	0.16	69.8	0.08
PT9188	15.1	-0.04	83.4	**2.33**	98.5	1.76

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

nt = not tested.

robust σ = robust (relative) standard deviation based on participants' results.

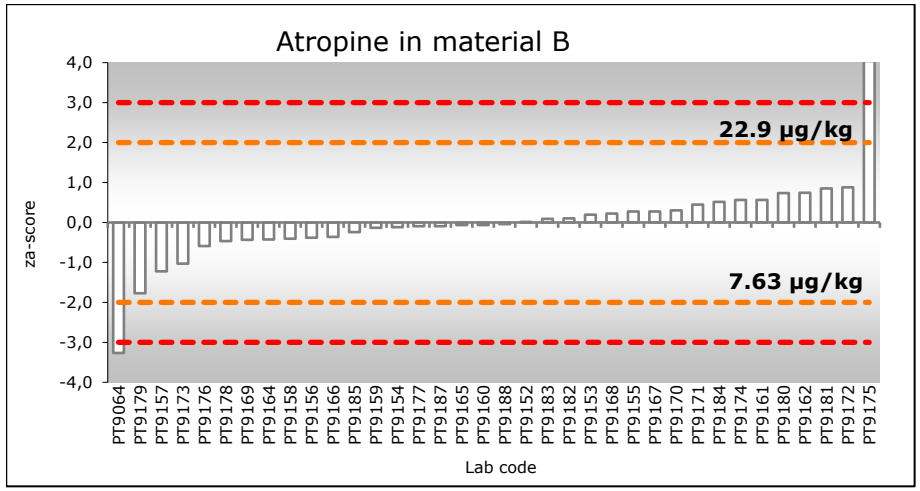

Figure 5 *Graphical representation of the z-scores for atropine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

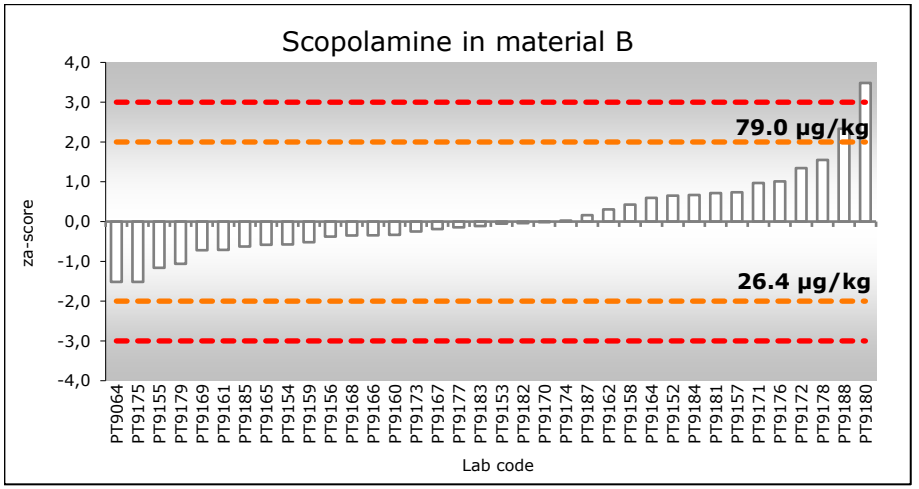

Figure 6 *Graphical representation of the z-scores for scopolamine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

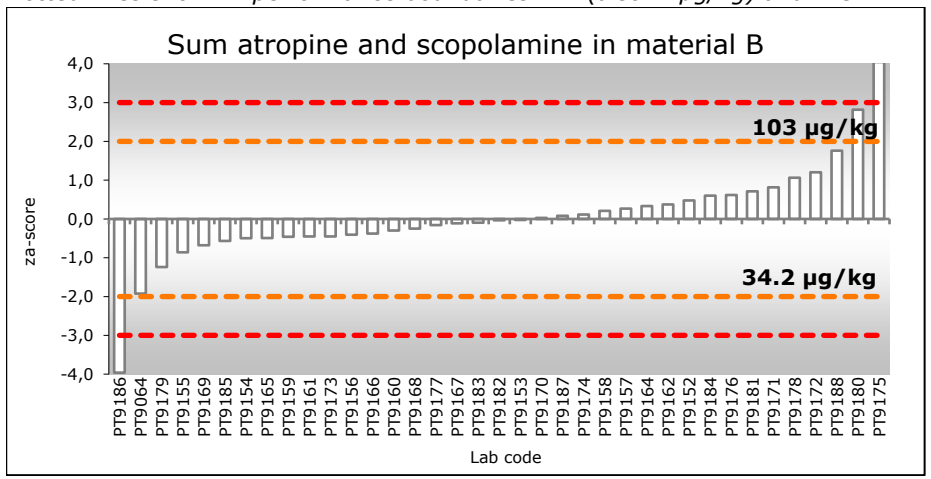

Figure 7 *Graphical representation of the z-scores for the sum of atropine and scopolamine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

WFSR report 2021.005 | 37

Annex 11 Overview performance per participant

Participant code	Individual tropane alkaloids Satisfactory performance *	Sum Satisfactory performance *
PT9064	2 out of 4	**2 out of 2**
PT9152	**4 out of 4**	**2 out of 2**
PT9153	**4 out of 4**	**2 out of 2**
PT9154	**4 out of 4**	**2 out of 2**
PT9155	**4 out of 4**	**2 out of 2**
PT9156	**4 out of 4**	**2 out of 2**
PT9157	**4 out of 4**	**2 out of 2**
PT9158	**4 out of 4**	**2 out of 2**
PT9159	**4 out of 4**	**2 out of 2**
PT9160	2 out of 4	**2 out of 2**
PT9161	**4 out of 4**	**2 out of 2**
PT9162	2 out of 4	1 out of 2
PT9163**	**2 out of 2****	**1 out of 1****
PT9164	**4 out of 4**	**2 out of 2**
PT9165	2 out of 4	1 out of 2
PT9166	3 out of 4	**2 out of 2**
PT9167	**4 out of 4**	**2 out of 2**
PT9168	**4 out of 4**	**2 out of 2**
PT9169	**4 out of 4**	**2 out of 2**
PT9170	**4 out of 4**	**2 out of 2**
PT9171	**4 out of 4**	**2 out of 2**
PT9172	**4 out of 4**	**2 out of 2**
PT9173	**4 out of 4**	**2 out of 2**
PT9174	2 out of 4	1 out of 2
PT9175	1 out of 4	0 out of 2
PT9176	**4 out of 4**	**2 out of 2**
PT9177	**4 out of 4**	**2 out of 2**
PT9178	**4 out of 4**	**2 out of 2**
PT9179	**4 out of 4**	**2 out of 2**
PT9180	2 out of 4	0 out of 2
PT9181	**4 out of 4**	**2 out of 2**
PT9182	**4 out of 4**	**2 out of 2**
PT9183	**4 out of 4**	**2 out of 2**
PT9184	2 out of 4	1 out of 2
PT9185	**4 out of 4**	**2 out of 2**
PT9186	0 out of 4	0 out of 2
PT9187	**4 out of 4**	**2 out of 2**
PT9188	1 out of 4	1 out of 2

* Satisfactory performance means a satisfactory z-score was obtained for the mycotoxins present in material A and B.

** Participant PT9163 did not analyse material B.

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WFSR report 2021.005

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