Proficiency test for ergot alkaloids in cereals

EURL-PT-MP03 (2019)

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Contents

Summary

A proficiency test (PT) for the determination of ergot alkaloids (EAs) produced by *Claviceps purpurea*: ergocornine/ergocorninine, ergocristine/ergocristinine, α-ergocryptine/α-ergocryptinine, ergometrine/ergometrinine, ergosine/ergosinine, ergotamine/ergotaminine in rye flour and a mix of wheat and oats flours was organised by the European Union Reference Laboratory for mycotoxins & plant toxins (EURL-MP) between September and December 2019. This EURL PT was carried out by Wageningen Food Safety Research (WFSR) in accordance with ISO/IEC 17043 (R013). Harmonised EU regulation for ergot alkaloids in these matrices is being prepared and their inclusion in national monitoring is recommended by EFSA. The primary goal was to assess the proficiency of National Reference Laboratories on mycotoxins (NRLs).

Thirty-three laboratories, among them 28 National Reference Laboratories for mycotoxins in food and feed (from 21 EU Member States, Iceland, Norway and Switzerland) and five Official Laboratories participated in the PT.

Two materials, rye (material A) and a mix of wheat/oat (1:1) (material B), were prepared. Material A was prepared by adding blank rye flour to rye material contaminated with ergot sclerotia containing ergot alkaloids. Material B was a blend of wheat and oat flours, artificially spiked with ergot alkaloid standard solutions. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of each material.

The majority of participants (88%) used LC-MS/MS based methods for detection and quantification of EAs. The others used HPLC-FLD based methods. Out of 33 participants, one did not report results for ergometrine, one did not report results for ergometrine, ergometrinine, ergotamine and ergotaminine, and one reported results only for ergocornine, ergocristinine, ergosine, the sum of α+β-ergocryptine and the sum of α+β-ergocryptinine. In addition, one participant reported the sum of ergosine and ergosinine, therefore for this participant no z-scores could be calculated for the individual epimers.

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 individual EAs in material A ranged from 34 to 126 µg/kg and in material B from 8.5 to 23 µg/kg. Obtained interlaboratory reproducibility (RSD_R) ranged from 14% to 35%. For material A, RSD_R were below the target standard deviation (25%) for nine out of 12 individual EAs and for material B this was the case for five out of 12 individual EAs. For the sum of ergot alkaloids the RSD_R was 16% and 19% 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%. Eighty-nine percent of the results obtained for both materials (A and B) were rated with satisfactory z-scores ($|z| \leq 2$), 5% of the results fell into the questionable range with $2<|z|<3$ and 6% of the results fell into the unsatisfactory range with $|z| \geq 3$. Eleven participants achieved optimal performance for both materials by detecting all ten ergot alkaloids and the sum of α+β-ergocryptine and of α+β-ergocryptinine with the correct quantification, the absence of false positive and false negative results. In this PT, four 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.*

Ergot alkaloid	Matrix	Assigned value (µg/kg)	Uncertainty (µg/kg)	Robust RSD_R[1] (%)	No of labs reporting		
					Quant. value	<LOQ	FN
Ergocornine	A	53.2	1.56	14	33		
	B	12.8	0.993	35	31	2	
Ergocorninine	A	40.9	1.73	19	31	1	1
	B	9.86	0.504	22	30	2	
Ergocristine	A	82.7	3.29	18	32		
	B	13.3	0.634	21	31	1	
Ergocristinine	A	50.4	2.78	25	33		
	B	10.5	0.660	28	30	3	
α+β-Ergocryptine	A	43.9	3.02	32	33		
	B	13.8	0.644	21	32	1	
α+β-Ergocryptinine	A	28.9	1.96	31	32	1	1
	B	8.57	0.634	32	29	4	
Ergometrine	A	37.8	2.67	31	30		
	B	21.1	1.24	26	30		
Ergometrinine	A	34.0	1.35	17	30	1	1
	B	20.3	0.774	17	30	1	1
Ergosine	A	81.9	2.75	15	32		
	B	14.9	0.846	26	32		
Ergosinine	A	46.0	2.58	25	31		
	B	8.54	0.470	24	29	2	
Ergotamine	A	126	5.66	20	31		
	B	22.9	1.55	30	31		
Ergotaminine	A	65.3	3.20	22	31		
	B	13.5	1.05	33	29	2	
Sum of ergot alkaloids	A	701	24.0	16	33		
	B	165	6.86	19	33		

Ergot alkaloid	Matrix	Assigned value (µg/kg)	z-scores[2] satisfactory (% of z-scores)	z-scores[2] questionable (% of z-scores)	z-scores[2] unsatisfactory (% of z-scores)	Labs out of 33 with Acceptable z-score No[3]	Labs out of 33 with Acceptable z-score %[3]
Ergocornine	A	53.2	97	0	3	32	97
	B	12.8	87	10	3	27	82
Ergocorninine	A	40.9	88	9	3	28	85
	B	9.86	93	0	7	28	85
Ergocristine	A	82.7	88	6	6	28	85
	B	13.3	90	3	7	28	85
Ergocristinine	A	50.4	85	9	6	28	85
	B	10.5	87	3	10	26	79
α+β-Ergocryptine	A	43.9	100	0	0	33	100
	B	13.8	91	6	3	29	88
α+β-Ergocryptinine	A	28.9	88	6	6	29	88
	B	8.57	93	0	7	27	82
Ergometrine	A	37.8	73	10	17	22	67
	B	21.1	73	10	17	22	67
Ergometrinine	A	34.0	90	3	7	28	85
	B	20.3	90	7	3	28	85
Ergosine	A	81.9	94	6	0	30	91
	B	14.9	94	3	3	30	91
Ergosinine	A	46.0	94	0	6	29	88
	B	8.54	93	0	7	27	82
Ergotamine	A	126	84	6	10	26	79
	B	22.9	90	7	3	28	85
Ergotaminine	A	65.3	81	6	13	25	76
	B	13.5	83	7	10	24	73
Total sum	A	701	94	6	0	31	94
	B	165	94	3	3	31	94

Matrix: A= Rye, B= Mix wheat/oat (1:1)

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

Ergot alkaloids (EAs) are produced by fungi of the genus *Claviceps*, most notably by *C. purpurea*, which parasitise the seed heads of living plants at the time of flowering. Fungal infections are most commonly found in rye, triticale, wheat, barley, oat and millet. The fungus replaces the developing grain or seed with a characteristic dark coloured crescent shaped alkaloid-containing wintering body, known as ergot or sclerotium. The total ergot alkaloid content of sclerotia may vary considerably, as well as the pattern of alkaloids produced and that are determined by the individual fungal strain in a geographical region and the host plant [1,2]. Sclerotia are harvested together with the cereals or grass and may thus lead to contamination of cereal-based food and feed products with ergot alkaloids. Ergotism remains an important veterinary problem, particularly in cattle, horses, sheep, pigs and chicken. Ergometrine and ergotamine are drug precursors and therefore classified as Category 1 substances requiring a license for their handling [15].

Ergot alkaloids can be sub-classified in two major types: ergopeptines and simple lysergic acid derivatives [1]. There are over 40 ergot alkaloids known, the most important ones are the 8(R)-ergopeptines: ergocornine, ergocristine, ergocryptine (which occurs as a mixture of α- and β-isomers), ergosine and ergotamine; and the 8(R)-lysergic acid derivative ergometrine. The corresponding 8(S)-epimers are also considered relevant because they can epimerise to the 8(R) analogues under various conditions. At the moment there are no maximum limits set for ergot alkaloids in food in the European Union. Currently, the European Commission is considering harmonised legislation on the presence of ergot alkaloids in various food products with priority on barley, wheat, spelt, oats and rye milling products and on processed cereal-based food for infants and young children.

European Directive 2002/32/EC [3] stipulates that the maximum allowed amount of sclerotia in unground cereals intended for animal feed is 1000 mg/kg. Although the concentration may vary considerably, the average concentration of ergot alkaloids may be around 800 µg/g sclerotia [1, 2]. Based on the six ergot alkaloids predominantly present in the sclerotia of *C. purpurea* the EFSA Panel on Contaminants in the Food Chain concluded that chemical analysis should focus on ergocornine, ergocristine, ergocryptine (α- and β-form), ergometrine, ergosine and ergotamine and their respective "-inine" forms. As mentioned above, harmonised EU regulation is currently under preparation for the 12 ergot alkaloids in food and the possible maximum levels for ergot alkaloids will be related to the sum of these ergot alkaloids. The possible maximum levels for ergot alkaloids in milling products of barley, wheat, spelt, oats grains under discussion at the time of drafting this report were 100 µg/kg (with an ash content lower than 900 mg/100 g), 150 µg/kg (with an ash content equal or higher than 900 mg/100 g) and 500 µg/kg for rye milling products. The ergot alkaloids in feed materials are for the moment not considered for legislation. The Commission Recommendation 2012/154/EU [4] asks for monitoring of the 12 ergot alkaloids mentioned above, in cereals and cereal products intended for human consumption or animal feeding.

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 of the EU Additional Measures Directive 93/99/EEC [5] and is demanded by 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 12 ergot alkaloids (ergocornine/ergocorninine, ergocristine/ ergocristinine, α+β-ergocryptine/α+β-ergocryptinine, ergometrine/ergometrinine, ergosine/ergosinine, ergotamine/ergotaminine) considered for legislation in food, using one material consisting of a rye flour and one material consisting of a mix of wheat and oat flours. The target concentrations (see Table 2) were chosen by taking upcoming regulatory limits and commonly found amounts of EAs into account.

Table 2 *Target concentrations µg/kg of mycotoxins in the PT materials.*

	Target concentrations (µg/kg)	
Ergot alkaloid	**Material A**	**Material B**
Ergocornine	65	15
Ergocorninine	35	10
Ergocristine	80	15
Ergocristinine	40	10
α-Ergocryptine	40	15
β-Ergocryptine	35	-
α+β-Ergocryptinine	30	10
Ergometrine	30	20
Ergometrinine	30	15
Ergosine	100	15
Ergosinine	45	10
Ergotamine	175	25
Ergotaminine	55	15

2.2 Material preparation

For preparation of the two PT materials A and B, blank rye flour, an rye flour artificially contaminated with ground sclerotia and a mix of blank wheat and oat flours were used. Rye, wheat and oat grain samples were visually checked for the presence of sclerotia and the cleaned materials were milled using a centrifugal mill (ZM 200, Retsch, Haan) to obtain a particle size of 500 µm. Material A was a blend of 3600 g of blank rye flour and 1200 g of rye flour artificially contaminated by adding ergot sclerotia. The artificially contaminated rye flour was available from a CEN Collaborative study conducted in 2016. Material B was a blend of blank wheat and oat flours (1:1) spiked with ergot alkaloids standards. For this material 5400 g of a blend of blank wheat and oat flours was fortified with 600 g of spiked premix. This premix was prepared in the following way: 600 g of mixed blank wheat and oat flour was fortified by adding 30 ml solution of ergot alkaloids standards prepared in acetone, aiming at the levels as presented in Table 2. After 30 min the premix was mixed with 600 ml of acetone and homogenised using an industrial mixer according to in-house standard operating procedures [9]. The fortified slurry was air dried and homogenized in a Stephan cutter UMC 5.

Materials A and B were homogenised by mixing in a rotating drum and stored at <-18 °C until use. The homogenisation of the materials was carried out by Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). WEPAL is accredited to ISO/IEC 17043 for the organisation of proficiency tests 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 "2019/EURL PT MP/EAs/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 EAs (EURL-MP-method_003 v1) [10]. In brief, EAs 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 kD ultrafilter. Analysis was performed by high performance liquid chromatography coupled with tandem mass spectrometry (LC-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 EAs in material A and B obtained during the homogeneity testing.*

	Material A		Material B	
Compound	**Conc. (µg/kg)**	**RSDr (%)**	**Conc. (µg/kg)**	**RSDr (%)**
Ergocornine	60.9	6.83	16.0	4.10
Ergocorninine	44.4	5.10	11.6	2.91
Ergocristine	71.2	6.38	17.1	6.45
Ergocristinine	57.1	5.46	10.3	5.78
α-Ergocryptine	36.7	5.04	18.4	4.38
β-Ergocryptine	25.2	6.61		
α-Ergocryptinine	33.9	4.02	8.77	5.43
Ergometrine	35.9	4.78	22.5	3.61
Ergometrinine	36.2	4.38	19.5	4.39
Ergosine	81.6	5.35	18.6	2.45
Ergosinine	42.2	3.47	7.35	7.31
Ergotamine	145	6.68	32.2	2.96
Ergotaminine	60.6	5.49	10.9	3.90

2.5 Stability of the materials

The stability of the EAs in the PT materials was assessed according to [11,12]. On October 14th, 2019, the day of distribution of the PT samples, six randomly selected containers of each material A and B were stored at <-70 °C. Under these conditions it is assumed that EAs are stable in the materials. In addition, six samples of each material were stored at <-18 °C.

On December 9th, 2019, 56 days after distribution of the samples, six samples of materials A and B, stored at <-70 °C and <-18 °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 <-18 °C. A consequential instability is observed when the average value of an analyte in the samples stored at <-18 °C is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-70 °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. In none of the tested storage conditions, a consequential difference was observed. The ergot alkaloids 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 following EAs: ergocornine, ergocristine, ergocryptine (α- and β-form), ergometrine, ergosine and ergotamine and their respective "-inine" forms in food and feed, using rye and a mix of wheat and oat. Invitations to the NRL network were sent out on September 16th, 2019 (Annex 5). Thirty-five participants registered for the PT (Annex 1) and 33 participants reported their results. One participant was unable to report results due to supplier problems and one participant did not report results, without providing a reason. Out of 33 participating laboratories, 28 were NRLs from 21 EU countries plus Iceland, Norway and Switzerland and five were OLs (from EU countries). Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report results through an existing 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 EAs (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 October 14th, 2019. 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 <-18 °C and to analyse the samples according to their routine practice. As reported by participants, all parcels, except one, were received within 24 hours after dispatch. One participant received the parcel after 3 days. All samples were received in good order.

The samples were accompanied by a letter describing 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 ergot alkaloids in each sample was requested. The deadline for submitting the quantitative results was November 25th, 2019, allowing the participants six weeks for analysis of the test samples.

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 [13,14] 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 PT performance assessment' on the EURL-MP website.

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 (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 all analytes in both materials. For ergocryptine and ergocryptinine the concentrations of the sum of α- and β-isomers (i.e. for ergocryptine the sum of α+β-ergocryptine and for ergocryptinine the sum of α+β-ergocryptinine) were taken for the calculations of the assigned value. In case the analytical method of a participant could separate the α- and β-isomers of ergocryptine and/or ergocryptinine, it was asked to also report individual concentrations for α- and β-ergocryptine and/or α- and β-ergocryptinine. Individual results obtained for α- or β-isomers were not benchmarked but evaluated for information purposes only.

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 background document 'EURL-MP PT performance assessment' on the EURL-MP website.

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.

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, the uncertainty of the assigned value for ergocornine and ergotaminine in material B was not negligible and, therefore, this was taken into account in the assignment of the z-score (z'). In all other cases, the uncertainty of the assigned value was negligible. No instability of the analytes in the PT materials was observed.

4.4 Evaluation of non-quantified results

In cases, where participant(s) reported 'detected', '<[value]' or 'not detected', 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 PT performance assessment' (for details see the EURL-MP website), using the LOQ value as a result. Proxy-z-scores are for information only and indicated as a value between brackets. Values below -2 were considered as false negatives (see 4.5) and values above -2 were excluded from the evaluation. Values above 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 not to have a quantitative method available for the applicable analyte/matrix.

4.5 False negatives

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as 'detected', '<[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 ergot alkaloids in rye flour and a mixture of wheat and oat flours. Annex 7 summarises the quantitative scopes of each participant, with an indication of the LOQs for each EA.

Twenty-one participants determined and quantified all ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, sum of α+β-ergocryptine, sum of α+β-ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine) as was requested.

Concerning the individual ergot alkaloids included in the scope of the participants: one participant did not provide results for ergometrine, one participant did not report results for ergometrine, ergometrinine, ergotamine and ergotaminine, and one participant reported results only for ergocornine, ergocristinine, ergosine, α-ergocryptine and α-ergocryptinine. In addition, one participant reported the sum of ergosine and ergosinine and, therefore, for this participant no z-scores could be calculated for the individual epimers. A few results were reported as 'detected'. In case the participant had specified an LOQ (Annex 7), for these results proxy z-scores were calculated. One result was reported as 'nd' without specification of LOQ and therefore, this result was excluded from evaluation (see section 4.4).

It was noted that a number of participants had problems with the determination of α+β-ergocryptine/-inine. This is related to the fact that only the α-isomers are available as analytical standards while the separation of α- and β-ergocryptine and particularly α- and β-ergocryptinine, is challenging under conventional reversed-phase chromatographic conditions. All four compounds were present in material A, while only α-ergocryptine and α-ergocryptinine were spiked to material B. Due to the lack of a suitable standard some participants reported difficulties with the identification (and consequently with the quantification) of β-ergocryptine and β-ergocryptinine. Out of 33 participants, 11 participants indicated that they used an analytical method that could separate the α- and β-isomers of ergocryptine. Of these 11 participants six could also separate the α- and β-isomers of ergocryptinine

The LOQs provided by the participants ranged from 0.15 to 26 µg/kg. A large majority of the reported LOQs (25 participants) for individual EAs fell below 5 µg/kg, some of them being even below 1 µg/kg (Annex 7). Three participants reported LOQs of 10 µg/kg, one reported LOQs of 12.5 µg/kg, two participants reported LOQs in the range of 10 to 26 µg/kg and one reported LOQs in the range of 40 to 617 µg/kg. One participant did not indicate the LOQs of the method used. The median LOQs for individual EAs were between 2.5 and 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). One participant provided no information on accreditation, nor any method details.

Out of 33 laboratories, 18 had their EA analysis method covered by ISO 17025 accreditation, while 14 had not accredited their method and one participant did not provide this information.

Median sample intake reported by the participants was 7.5 g; the most often reported intake was 5 g (11 participants). Three participants used 2.5 g or less, while 11 participants used 20 g or more. The samples where extracted with 50 ml (median volume) of extraction solvent for approximately 30 min

(median time). The volumes most often used were 25 ml (10) and 100 ml (10). Most participants (17) reported an extraction time of 30 min; 13 participants used an extraction time between 45 and 60 min. For the extraction solvent participants used acetonitrile (MeCN) (22) or ethyl acetate (EtOAc) (9) as the main organic phase. One participant used methanol (MeOH) and one participant did not specify the composition. The composition of the extraction solvents was either basic aqueous/organic (24), acidic aqueous/organic (5), neutral aqueous/organic (1) or organic (2); one participant did not indicate the conditions used. The most often used extraction solvent combinations were: acetonitrile in combination with ammonium carbonate (15), acetonitrile in combination with formic or acetic acid (4) and ethyl acetate in combination with methanol/isopropanol and ammonia (9).

Solid phase extraction (SPE) was used by 14 participants for sample extract purification, seven participants applied dispersive SPE (d-SPE) with primary secondary amine (PSA) and one participant used liquid-liquid extraction (LLE). Two participants reported that they diluted the sample extracts and nine participants reported that no clean-up was used. The following clean-up cartridges were reported: Sep-Pak Alumina B plus (8), Mycosep 150 Ergot (5) and Roma (1).

All participants used liquid chromatography (in combination with MS or FLD detection) for separation of EAs. Acetonitrile as an organic mobile phase modifier was used by 25 participants while 6 participants used methanol. Two participants did not specify the mobile phase composition. The majority of participants (27; 82%) indicated that alkaline chromatography had been used. For the preparation of the alkaline mobile phase the following buffers were used: ammonium carbonate (11), ammonium carbamate (8), ammonium bicarbonate (3) and ammonium hydroxide (1). One participant combined ammonium carbonate in water mobile phase A with formic acid added to the organic mobile phase B. Six participants used acidic chromatography: three used ammonium formate with or without addition of formic acid, two used ammonium acetate with or without addition of acetic or formic acid and one used formic acid to acidify the mobile phase.

For alkaline chromatography a wide variety of columns, mostly with C18 based stationary phase, from different suppliers were used: Waters: Acquity BEH (6), XBridge (3); Phenomenex: Gemini (3), Luna (1), Kinetex (1), Kinetex EVO (1); Agilent: Zorbax Eclipse Plus (1), Zorbax Eclipse XDB (1) and Poroshell HPH (1). In addition, the following non-C18 stationary phase columns were used by a number of participants: Phenomenex: Gemini C6 Phenyl (4), hexyl-phenyl (3), Synergi C12 (1); Supelco: Ascentis Express Phenyl-hexyl (1); and Macherey Nagel: Nucleodur pentafluorophenyl (1).

For chromatography with acidic conditions participants also used mostly C18 type stationary phases and from different suppliers: Waters: Acquity UPLC BEH C18 (1); Agilent: Zorbax Eclipse Plus (2), Phenomenex: hexyl-phenyl (1) and Kinetex® F5 pentafluorophenyl (1). One participant used a C18 column without further information.

For the identification and quantification of the EAs most participants used LC-MS/MS (29) and a few participants used HPLC-FLD (4).

The quantification approach followed by the participants is summarised in Table 5. Out of 29 participants that employed an LC-MS-based methodology, 13 used multi-level standard addition: two of them used standard addition after extraction, four before extraction and seven did not indicate when the standards were added. Four participants used a single-point standard addition approach: one added standards after extraction, two before extraction and one did not indicate. Twelve participants performed multi-level calibration with standards in a pure solvent. Most of participants (20) that used LC-MS/MS, have corrected their results for recovery. Regarding the participants that used HPLC-FLD, one used multi-level standard addition before extraction and three used multi-level calibration in pure solvent. None of them corrected results for recovery.

Table 5 *Analytical strategies followed by the participants.*

Detection	Quantification approach	Calibration/quantification	No. of participants	Corrected for recovery
LC-MS/MS	matrix-matched standards*	single point	1	1
	standard addition before extraction	single point	2	1
	standard addition after extraction	single point	1	-
LC-MS/MS	matrix-matched standards*	multi-level	7	5
	standard addition before extraction	multi-level	4	4
	standard addition after extraction	multi-level	2	2
LC-MS/MS	standards in pure solvent	multi-level	12	7
HPLC-FLD	standard addition before extraction	multi-level	1	-
HPLC-FLD	standards in pure solvent	multi-level	3	-

*Calibration standards prepared in blank matrix.

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for EAs in materials A (rye flour) and B (mixture of wheat and oat flours) 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 Tables 6 and 7. These tables include 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. In most cases the uncertainty of the assigned value did comply with the criterion $u \leq 0.3\sigma_p$ and was therefore considered as negligible. Uncertainty of the assigned value (u) in the material B exceeded $0.3\sigma_p$ for ergocornine and ergotaminine, therefore, the uncertainty of the assigned value was taken into account in the evaluation of the z-scores.

Table 6 *Parameters of the individual ergot alkaloids and summary for material A.*

	Ergocornine	Ergo-corninine	Ergocristine	Ergo-cristinine	α+β-Ergo-cryptine	α+β-Ergo-cryptinine
A (µg/kg)	53.2	40.9	82.7	50.4	43.9	28.9
u (µg/kg)	1.56	1.73	3.29	2.78	3.02	1.96
σ_p (µg/kg) (25%)	13.3	10.2	20.7	12.6	11.0	7.23
$u>0.3\sigma_p$	No	No	No	No	No	No
robust σ (µg/kg)	7.18	7.69	14.9	12.8	13.9	8.87
robust σ (%)	13.5	18.8	18.0	25.3	31.6	30.7
# reported	33	32	32	33	33	33
"<", nd		1				1
detected						
# quantitative results	33	31	32	33	33	32
$\|z\|\leq 2$	32	28	28	28	33	29
$2<\|z\|<3$		3	2	3		2
$\|z\|\geq 3$	1		2	2		1
FN		1				1
satisfactory z-scores (%)	97	88	88	85	100	88

	Ergo-metrine	Ergo-metrinine	Ergosine	Ergosinine	Ergo-tamine	Ergo-taminine	Total sum
A (µg/kg)	37.8	34.0	81.9	46.0	126	65.3	701
u (µg/kg)	2.67	1.35	2.75	2.58	5.66	3.20	24.0
σ_p (µg/kg) (25%)	9.43	8.51	20.5	11.5	31.5	16.3	175
$u>0.3\sigma_p$	No	No	No	No	No	No	No
robust σ (µg/kg)	11.7	5.92	12.5	11.5	25.2	14.3	110
robust σ (%)	31.0	17.4	15.2	25.0	20.0	21.9	15.8
# reported	30	31	32	31	31	31	33
"<", nd							
detected		1					
# quantitative results	30	30	32	31	31	31	33
$\|z\|\leq 2$	22	28	30	29	26	25	31
$2<\|z\|<3$	3	1	2		2	2	2
$\|z\|\geq 3$	5	1		2	3	4	
FN		1					
satisfactory z-scores (%)	73	90	94	94	84	81	94

Table 7 *Parameters of the individual ergot alkaloids and summary for material B.*

	Ergocornine	Ergo-corninine	Ergocristine	Ergo-cristinine	α+β-Ergo-cryptine	α+β-Ergo-cryptinine
A (µg/kg)	12.8	9.86	13.3	10.5	13.8	8.57
u (µg/kg)	0.993	0.504	0.634	0.660	0.644	0.634
σ_p (µg/kg) (25%)	3.20	2.47	3.32	2.63	3.44	2.14
$u>0.3\sigma_p$	Yes	No	No	No	No	No
robust σ (µg/kg)	4.42	2.21	2.82	2.89	2.92	2.73
robust σ (%)	34.5	22.4	21.2	27.5	21.2	31.9
# reported	33	32	32	33	33	33
"<", nd	2	2	1	3	1	2
detected						2
# quantitative results	31	30	31	30	32	29
$\|z\|\leq 2$	27	28	28	26	29	27
$2<\|z\|<3$	3		1	1	2	
$\|z\|\geq 3$	1	2	2	3	1	2
FN						
satisfactory z-scores (%)	87	93	90	87	91	93

	Ergo-metrine	Ergo-metrinine	Ergosine	Ergosinine	Ergo-tamine	Ergo-taminine	Total sum
A (µg/kg)	21.1	20.3	14.9	8.54	22.9	13.5	165
u (µg/kg)	1.24	0.77	0.846	0.470	1.55	1.05	6.86
σ_p (µg/kg) (25%)	5.29	5.07	3.72	2.14	5.72	3.37	41.2
$u>0.3\sigma_p$	No	No	No	No	No	Yes	No
robust σ (µg/kg)	5.45	3.39	3.83	2.03	6.90	4.50	31.5
robust σ (%)	25.8	16.7	25.7	23.7	30.2	33.4	19.1
# reported	30	31	32	31	31	31	33
"<", nd				2		2	
detected		1					
# quantitative results	30	30	32	29	31	29	33
$\|z\|\leq 2$	22	28	30	27	28	24	31
$2<\|z\|<3$	3	2	1		2	2	1
$\|z\|\geq 3$	5		1	2	1	3	1
FN		1					
satisfactory z-scores (%)	73	90	94	93	90	83	94

For both materials, 89% of the results were rated with satisfactory z-scores ($|z|\leq 2$), 5% of the results fell into the questionable range with $2<|z|<3$ and 6% of the results fell into the unsatisfactory range with $|z|\geq 3$.

In material A, ergocorninine was present at 41 µg/kg, the sum of α+β-ergocryptinine at 29 µg/kg and ergometrinine at 34 µg/kg and in material B ergometrinine was present at 30 µg/kg. Nevertheless, two participants reported these analytes as below their LOQs. As the proxy z-scores (see 4.5) were <-2, these results were classified as false negatives.

As indicated in section 5.1, some participants had problems with the separation of α- and β-isomers of ergocryptine/-inine. Twenty-five participants reported the sum of α+β-ergocryptine/-inine correctly as it was requested in the provided instructions. Eight participants did not report results for the sum of α+β-ergocryptine and α+β-ergocryptinine. Of these eight participants, five participants were able to separate the α- and β-isomers of ergocryptine and ergocryptinine. These participants indicated that they had no analytical standards to quantify the β-isomers. Therefore, four participants reported only the results for the individual α-isomers. However, this could have been solved by using the α-isomer as a standard for quantification of β-isomer. One participant did it this way but nevertheless reported the results as α-isomers instead of the sum of α+β-isomers.

The results reported by these eight participants for α-ergocryptine, β-ergocryptine and α-ergocryptinine were used as the sum of α+β-isomers, otherwise no z-scores could be calculated.

In total 11 out of 33 participants reported individual results for α-ergocryptine, seven reported results for β-ergocryptine, six reported results for α-ergocryptinine and two reported results for β-ergocryptinine. For material A only α- and β-ergocryptine were statistically evaluated. Satisfactory z-scores were calculated, except one questionable result for β-ergocryptine. The results for α- and β-ergocryptinine could not be evaluated since an insufficient number of results had been submitted. For material B the β-isomers were not spiked to the material. Therefore, the results of the individual α-isomers should be the same as the sum of α+β-isomers. For this reason, the results of the individual α- and β-isomers of ergocryptine/-inine were not statistically evaluated.

The lack of chromatographic resolution of the abovementioned isomers was mostly due to the analytical conditions used by the participants. It was noted that the chromatographic column with a phenyl-hexyl stationary phase might be the most suitable to achieve an acceptable resolution between α- and β-isomers of ergocryptine/-inine.

No clear explanation could be found for the lower satisfactory performance for ergometrine in both materials. No relationship with a specific analytical method could be found. It was noted however that five participants obtained z-scores in the range of 2.7-9.5 for both materials. Possibly it could be related to differences in the analytical reference standards obtained from different suppliers.

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 24 satisfactory z-scores could be obtained, and '24 out of 24' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. The number of participants that analysed the materials for all ten ergot alkaloids and the sum of α+β-ergocryptine/-inine was 21. Out of these 21 participants, eleven participants achieved optimal performance for both materials by detecting all ten EAs and the sum of α+β-ergocryptine/-inine with correct quantification, the absence of false negative results. For the other 12 participants either the scope was incomplete, 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 total sum of the EAs, 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 each EA in both materials are shown in Annex 9 and 10, in Tables 6 and 7 (Section 5.3) and also in Table 1 (Summary section).

For material A, the robust standard deviations (RSD_R) of the reported results (14-25%) for the individual EAs were well below the target standard deviation (25%), except for α+β-ergocryptine (32%), α+β-ergocryptinine (31%) and ergometrine (31%). For material B, only five out of 12 RSD_R values (17-24%) were below the target standard deviation (25%). The RSD_R values exceeded the target standard deviation for ergocornine (35%), ergocristinine (28%), α+β-ergocryptinine (32%), ergometrine (26%), ergosine (26%), ergotamine (30%) and ergotaminine (33%). The higher RSD_R values obtained for material B are likely related to the lower concentrations present in material B. The assigned values for individual EAs in material A ranged from 34 to 126 µg/kg and in material B from 8.5 to 23 µg/kg.

The RSD_R values for the total sum of EAs was well below the target standard deviation (25%) for material A (16%) as well as material B (19%).

6 Conclusions

Thirty-three participants, 28 NRLs (from 21 member states, plus one from Iceland, one from Norway, one from Switzerland) and five OLs, participated in the EURL-PT-MP03 on the quantitative determination of ergot alkaloids in cereals (rye flour and a mix of wheat and oat flours). Out of these thirty-three participants, one did not report results for ergometrine, one did not report results for ergometrine, ergometrinine, ergotamine and ergotaminine, and one reported results just for ergocornine, ergocristinine and ergosine. One participant reported the sum of ergosine and ergosinine, therefore for this participant no z-scores could be calculated for the individual epimers.

Out of 33 participants, 21 provided results for all ten ergot alkaloids and the sum of α+β-ergocryptine/-inine, but only ten of them showed a fully satisfactory performance. Additionally, one participant achieved a satisfactory performance, but did report the results after the deadline.

For individual EAs in material A, satisfactory results varied from 81 to 100% except for ergometrine (73%). The robust standard deviations (RSD_R) of the reported results (14-25%) for individual EAs were below the target standard deviation (25%), except for α+β-ergocryptine (32%), α+β-ergocryptinine (31%) and ergometrine (31%).

For individual EAs in material B, satisfactory results varied from 83 to 94%, except for ergometrine (73%). Five out of 12 RSD_R values (17-24%) were below the target standard deviation (25%). The larger variation might be related to the lower concentrations (8.5 to 23 µg/kg) of the individual EAs in this material.

Overall, for individual EAs in both materials combined, 89% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 5% of the results fell into the questionable range with $2<|z|<3$ and 6% of the results fell into the unsatisfactory range with $|z| \geq 3$. In case of the total sum of the ergot alkaloids, for both materials, 94% of submitted results were satisfactory and 26 participants had a satisfactory performance.

The majority of the participants (25) reported correctly the sum of α+β-ergocryptine/-inine, as requested. Eight participants did not report the sum of α+β-ergocryptine/-inine. Some of them stated that they had no standards for β-ergocryptine and β-ergocryptinine, however in principle the β-isomers could have been quantified based on the α-isomer reference standards.

Twenty-nine participants used LC-MS/MS in their determinations and another four participants used HPLC-FLD. The reported LOQs by the participants varied between 0.15 and 26 µg/kg, except for one participant, which reported LOQs between 40 and 617 µg/kg. The median LOQs for individual EAs were in the range of 2.5 to 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 close to 4 µg/kg, at least for cereal milling products.

The main issue noted during this PT was resolution of the α+β isomers of ergocryptine/-inine. For the α+β isomers of ergocryptine this can be solved by employing specific alkaline conditions on C18 based columns, but the α+β isomers of ergocryptinine at the moment can be separated only on a phenyl-hexyl type column. On one hand this is not a major issue, as according to upcoming legislation, separation of α+β isomers is not requested. On the other hand, lower LOQs and resolution between all analytes would align with EFSA's recommendation for monitoring and to enable a better individual compound exposure evaluation.

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Annex 1 List of participants

Country	Organisation
AUSTRIA*	AGES GmbH
BELGIUM*	Sciensano
BULGARIA*	Bulgarian Food Safety Agency
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*	National Food Institute
FINLAND*	Finnish Food Authority
FRANCE	Labocea
FRANCE*	SCL
GERMANY	State Institute for Chemical and Veterinary Analysis of Food (CVUA) Sigmaringen
GERMANY**	Eurofins WEJ Contaminants
GERMANY	Agrolab LUFA GmbH
GERMANY	Chemisches und Veterinaruntersuchungsamt Rheinland
GERMANY*	Federal Institute fur Risk Assessment (BfR)
GERMANY	CVUA-Westfalen
GERMANY	Thuringian State Institute for Agriculture
GERMANY	Lower Saxony State Office for Consumer Protection and Food Safety (LAVES)
GREECE*	General Chemical State Laboratory
HUNGARY*	National Food Chain Safety Office
IRELAND*	The State Laboratory
IRELAND*	The Public Analyst's Laboratory
ITALY*	Instituto superiore di sanita
LUXEMBOURG*	Laboratoire national de Sante
NORWAY**	Norwegian Veterinary Institute
POLAND*	National Veterinary Research Institute
POLAND*	National Institute of Public Health - National Institute of Hygiene
ROMANIA*	Directia Sanitara Veterinara si pentru Siguranta Alimentelor (DSVSA) Bucuresti
SLOVENIA*	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
SPAIN*	Spanish Agency for Consumer Affairs, Food Safety and Nutrition
SWEDEN*	National Food Agency
SWEDEN*	National Veterinary Institute, SVA
SWITZERLAND**	Kantonales Laboratorium Bern
UNITED KINGDOM*	FERA Science Ltd

* National Reference Laboratory of EU Member State.

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

Annex 2 Codification of the samples

Participants code	Material A*	Material B*
PT9446	753	813
PT9447	142	810
PT9448	966	146
PT9449	537	650
PT9450	597	792
PT9451	238	295
PT9452	447	329
PT9453	272	265
PT9454	821	680
PT9455	660	758
PT9456	632	923
PT9457	788	958
PT9458	258	950
PT9459	882	965
PT9460	666	429
PT9461	774	140
PT9462	153	462
PT9463	706	474
PT9464	849	543
PT9465	894	878
PT9466	149	484
PT9467	436	657
PT9468	422	928
PT9469	949	723
PT9470	502	985
PT9471	863	611
PT9472	345	510
PT9473	662	201
PT9474	374	648
PT9475	674	971
PT9476	482	868
PT9477	507	339
PT9478	291	197
PT9479	125	684
PT9480	664	980

* All sample codes start with 2019/EURLPT MP/EAs/.

Annex 3 Statistical evaluation of the homogeneity data

Sample No.	Ergocornine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	25.9	28.8
Hom/A002	25.4	26.0
Hom/A003	24.5	23.5
Hom/A004	24.4	24.6
Hom/A005	23.8	24.4
Hom/A006	23.2	24.7
Hom/A007	23.2	26.0
Hom/A008	24.4	27.6
Hom/A009	27.6	25.4
Hom/A010	23.1	27.7
Grand mean	25.2	
Cochran's test		
C	0.379	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.30	
s_x	1.16	
s_w	1.69	
s_s	0.000	
Critical= 0.3 σ_P	1.89	
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.

Sample No.	Ergocorninine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	44.4	44.3
Hom/A002	42.3	47.9
Hom/A003	45.8	45.7
Hom/A004	43.1	41.4
Hom/A005	44.3	39.9
Hom/A006	41.7	49.5
Hom/A007	45.0	44.0
Hom/A008	44.4	47.4
Hom/A009	44.0	45.2
Hom/A010	44.5	43.1
Grand mean	44.4	
Cochran's test		
C	0.477	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	11.1	
s_x	1.35	
s_w	2.55	
s_s	0.000	
Critical= 0.3 σ_P	3.33	
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.

Sample No.	Ergocristine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	66.7	73.6
Hom/A002	79.8	70.9
Hom/A003	76.2	71.4
Hom/A004	69.4	63.9
Hom/A005	72.6	68.1
Hom/A006	71.5	66.6
Hom/A007	72.4	76.6
Hom/A008	67.4	65.2
Hom/A009	68.2	79.3
Hom/A010	69.5	75.5
Grand mean	71.2	
Cochran's test		
C	0.303	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	17.8	
s_x	3.25	
s_w	4.50	
s_s	0.650	
Critical= 0.3 σ_P	5.34	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergocristinine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	54.7	58.4
Hom/A002	61.0	54.5
Hom/A003	60.5	56.7
Hom/A004	54.9	52.3
Hom/A005	53.0	56.8
Hom/A006	58.9	54.5
Hom/A007	59.1	62.4
Hom/A008	55.3	53.2
Hom/A009	60.8	57.2
Hom/A010	61.7	55.5
Grand mean	57.1	
Cochran's test		
C	0.238	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	14.3	
s_x	2.30	
s_w	2.99	
s_s	0.898	
Critical= 0.3 σ_P	4.28	
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.

Sample No.	α-Ergocryptine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	34.5	36.3
Hom/A002	36.1	39.2
Hom/A003	37.1	36.4
Hom/A004	36.9	35.9
Hom/A005	37.9	32.6
Hom/A006	36.6	39.3
Hom/A007	35.2	36.8
Hom/A008	36.0	40.7
Hom/A009	34.8	35.3
Hom/A010	37.7	38.2
Grand mean	36.7	
Cochran's test		
C	0.372	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	9.17	
s_x	1.24	
s_w	1.92	
s_s	0.000	
Critical= 0.3 σ_P	2.75	
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.

Sample No.	α-Ergocryptinine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	32.1	34.7
Hom/A002	34.4	35.0
Hom/A003	33.5	35.5
Hom/A004	32.4	33.5
Hom/A005	35.0	31.2
Hom/A006	33.5	35.7
Hom/A007	33.2	33.3
Hom/A008	32.0	35.4
Hom/A009	34.3	33.1
Hom/A010	36.1	34.5
Grand mean	33.9	
Cochran's test		
C	0.298	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.48	
s_x	0.806	
s_w	1.54	
s_s	0.000	
Critical= 0.3 σ_P	2.54	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	β-Ergocryptine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	25.9	28.8
Hom/A002	25.4	26.0
Hom/A003	24.5	23.5
Hom/A004	24.4	24.6
Hom/A005	23.8	24.4
Hom/A006	23.1	24.7
Hom/A007	23.2	26.0
Hom/A008	24.4	27.6
Hom/A009	27.6	25.4
Hom/A010	23.1	27.7
Grand mean	25.2	
Cochran's test		
C	0.379	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.30	
s_x	1.16	
s_w	1.69	
s_s	0.000	
Critical= 0.3 σ_P	1.89	
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.

Sample No.	Ergometrine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	33.9	39.2
Hom/A002	38.3	38.0
Hom/A003	39.1	35.4
Hom/A004	35.7	34.0
Hom/A005	35.1	34.4
Hom/A006	34.8	34.3
Hom/A007	34.2	37.3
Hom/A008	35.8	33.8
Hom/A009	35.7	36.0
Hom/A010	36.4	35.4
Grand mean	35.9	
Cochran's test		
C	0.464	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.96	
s_x	1.20	
s_w	1.72	
s_s	0.000	
Critical= 0.3 σ_P	2.69	
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.

Sample No.	Ergometrinine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	35.2	38.1
Hom/A002	39.3	37.8
Hom/A003	36.9	37.3
Hom/A004	35.2	34.2
Hom/A005	37.9	34.3
Hom/A006	36.0	34.3
Hom/A007	34.9	36.8
Hom/A008	35.0	34.6
Hom/A009	34.6	38.2
Hom/A010	36.4	37.1
Grand mean	36.2	
Cochran's test		
C	0.286	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	9.05	
s_x	1.18	
s_w	1.50	
s_s	0.515	
Critical= 0.3 σ_P	2.72	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergosine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	74.6	83.8
Hom/A002	83.9	91.3
Hom/A003	86.7	88.2
Hom/A004	79.7	84.3
Hom/A005	81.7	80.0
Hom/A006	78.1	80.4
Hom/A007	77.2	79.8
Hom/A008	76.8	75.4
Hom/A009	79.9	86.4
Hom/A010	82.6	80.7
Grand mean	81.6	
Cochran's test		
C	0.375	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	20.4	
s_x	3.71	
s_w	3.38	
s_s	2.84	
Critical= 0.3 σ_P	6.12	
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.

Sample No.	Ergosinine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	41.6	41.6
Hom/A002	42.1	43.3
Hom/A003	44.0	43.1
Hom/A004	42.5	41.6
Hom/A005	42.3	42.8
Hom/A006	41.4	40.8
Hom/A007	40.4	43.7
Hom/A008	39.7	40.1
Hom/A009	40.5	44.0
Hom/A010	45.0	43.1
Grand mean	42.2	
Cochran's test		
C	0.399	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.6	
s_x	1.19	
s_w	1.23	
s_s	0.815	
Critical= 0.3 σ_P	3.16	
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.

Sample No.	Ergotamine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	138	149
Hom/A002	144	155
Hom/A003	152	167
Hom/A004	135	144
Hom/A005	142	154
Hom/A006	135	147
Hom/A007	135	159
Hom/A008	137	154
Hom/A009	131	153
Hom/A010	141	134
Grand mean	145	
Cochran's test		
C	0.259	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	36.3	
s_x	6.17	
s_w	10.5	
s_s	0.000	
Critical= 0.3 σ_P	10.9	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergotaminine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	57.5	62.9
Hom/A002	61.3	64.8
Hom/A003	64.7	65.9
Hom/A004	59.4	56.5
Hom/A005	62.7	61.7
Hom/A006	57.6	59.0
Hom/A007	59.3	66.9
Hom/A008	61.2	61.2
Hom/A009	55.0	60.6
Hom/A010	57.4	56.9
Grand mean	60.6	
Cochran's test		
C	0.399	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	15.2	
s_x	2.78	
s_w	2.68	
s_s	2.03	
Critical= 0.3 σ_P	4.55	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergocornine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	15.6	16.0
Hom/B002	15.2	16.2
Hom/B003	15.4	16.4
Hom/B004	16.5	16.2
Hom/B005	16.9	16.4
Hom/B006	15.6	16.6
Hom/B007	16.3	15.0
Hom/B008	16.0	16.5
Hom/B009	15.6	16.6
Hom/B010	14.7	17.1
Grand mean	16.0	
Cochran's test		
C	0.471	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.01	
s_x	0.319	
s_w	0.797	
s_s	0.000	
Critical= 0.3 σ_P	1.20	
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.

Sample No.	Ergocorninine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	10.9	11.6
Hom/B002	11.3	11.4
Hom/B003	11.7	11.8
Hom/B004	12.3	11.7
Hom/B005	12.1	11.6
Hom/B006	11.8	11.4
Hom/B007	12.0	11.5
Hom/B008	11.2	11.7
Hom/B009	11.7	11.5
Hom/B010		
Grand mean	11.6	
Cochran's test		
C	0.302	
Ccrit	0.638	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.91	
s_x	0.245	
s_w	0.332	
s_s	0.069	
Critical= 0.3 σ_P	0.872	
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.

Sample No.	Ergocristine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	16.3	16.9
Hom/B002	16.4	18.1
Hom/B003	16.5	18.0
Hom/B004	18.1	17.7
Hom/B005	17.8	17.8
Hom/B006	16.8	18.5
Hom/B007	17.1	13.7
Hom/B008	16.1	17.8
Hom/B009	16.9	17.3
Hom/B010	16.4	18.3
Grand mean	17.1	
Cochran's test		
C	0.419	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.28	
s_x	0.730	
s_w	1.17	
s_s	0.000	
Critical= 0.3 σ_P	1.28	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergocristinine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	10.0	10.5
Hom/B002	10.4	10.6
Hom/B003	10.1	9.42
Hom/B004	9.17	10.9
Hom/B005	10.9	10.6
Hom/B006	10.7	10.1
Hom/B007	10.3	9.53
Hom/B008	10.8	10.6
Hom/B009	10.6	11.0
Hom/B010	8.96	10.4
Grand mean	10.3	
Cochran's test		
C	0.441	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.57	
s_x	0.420	
s_w	0.594	
s_s	0.000	
Critical= 0.3 σ_P	0.771	
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.

Sample No.	α-Ergocryptine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	18.0	18.2
Hom/B002	17.1	18.7
Hom/B003	18.1	18.0
Hom/B004	18.9	18.8
Hom/B005	19.3	18.5
Hom/B006	18.5	19.4
Hom/B007	18.2	17.1
Hom/B008	17.8	19.3
Hom/B009	18.7	18.4
Hom/B010	17.3	20.3
Grand mean	18.4	
Cochran's test		
C	0.540	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.60	
s_x	0.474	
s_w	0.912	
s_s	0.000	
Critical= 0.3 σ_P	1.38	
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.

Sample No.	α-Ergocryptinine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	8.35	8.79
Hom/B002	8.53	9.41
Hom/B003	7.65	8.91
Hom/B004	9.14	9.22
Hom/B005	9.48	8.74
Hom/B006	8.67	8.54
Hom/B007	9.50	8.40
Hom/B008	8.36	8.58
Hom/B009	8.92	8.94
Hom/B010	8.10	9.12
Grand mean	8.77	
Cochran's test		
C	0.292	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.19	
s_x	0.298	
s_w	0.521	
s_s	0.000	
Critical= 0.3 σ_P	0.658	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergometrine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	21.9	22.4
Hom/B002	21.4	22.9
Hom/B003	21.6	21.7
Hom/B004	24.2	22.8
Hom/B005	22.9	23.4
Hom/B006	22.7	21.9
Hom/B007	22.9	23.1
Hom/B008	21.2	23.5
Hom/B009	22.8	22.0
Hom/B010	21.2	22.7
Grand mean	22.5	
Cochran's test		
C	0.393	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	5.62	
s_x	0.573	
s_w	0.810	
s_s	0.024	
Critical= 0.3 σ_P	1.69	
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.

Sample No.	Ergometrinine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	18.3	21.1
Hom/B002	18.4	20.9
Hom/B003	18.6	18.9
Hom/B004	20.1	20.6
Hom/B005	20.1	19.1
Hom/B006	19.0	19.3
Hom/B007	19.9	19.2
Hom/B008	18.7	20.7
Hom/B009	18.9	19.1
Hom/B010	18.8	20.0
Grand mean	19.5	
Cochran's test		
C	0.359	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.87	
s_x	0.434	
s_w	1.03	
s_s	0.000	
Critical= 0.3 σ_P	1.46	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergosine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	18.0	19.3
Hom/B002	17.9	19.4
Hom/B003	18.3	18.9
Hom/B004	18.7	19.0
Hom/B005	18.8	18.5
Hom/B006	18.4	19.1
Hom/B007	18.7	18.1
Hom/B008	17.9	19.0
Hom/B009	18.8	18.5
Hom/B010	18.2	19.2
Grand mean	18.6	
Cochran's test		
C	0.313	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.66	
s_x	0.132	
s_w	0.604	
s_s	0.000	
Critical= 0.3 σ_P	1.40	
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.

Sample No.	Ergosinine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	6.69	7.22
Hom/B002	6.80	7.29
Hom/B003	6.77	7.10
Hom/B004	7.39	7.06
Hom/B005	8.10	7.62
Hom/B006	7.23	7.50
Hom/B007	7.32	8.65
Hom/B008	7.14	8.21
Hom/B009	7.06	7.70
Hom/B010	6.48	7.74
Grand mean	7.35	
Cochran's test		
C	0.296	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.84	
s_x	0.374	
s_w	0.545	
s_s	0.000	
Critical= 0.3 σ_P	0.551	
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.

Sample No.	Ergotamine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	31.0	32.7
Hom/B002	34.3	32.1
Hom/B003	31.1	31.7
Hom/B004	33.2	32.5
Hom/B005	33.1	31.9
Hom/B006	31.6	32.4
Hom/B007	33.8	30.8
Hom/B008	32.4	32.2
Hom/B009	32.4	31.5
Hom/B010	31.0	31.3
Grand mean	32.2	
Cochran's test		
C	0.445	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.04	
s_x	0.622	
s_w	1.01	
s_s	0.000	
Critical= 0.3 σ_P	2.41	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Ergotaminine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	11.1	10.7
Hom/B002	10.5	11.1
Hom/B003	10.4	11.0
Hom/B004	11.1	10.5
Hom/B005	11.2	11.0
Hom/B006	10.3	11.0
Hom/B007	11.1	11.7
Hom/B008	10.7	11.6
Hom/B009	10.5	11.2
Hom/B010	10.4	11.7
Grand mean	10.9	
Cochran's test		
C	0.313	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	2.73	
s_x	0.235	
s_w	0.495	
s_s	0.000	
Critical= 0.3 σ_P	0.820	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_H?	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 ergocornine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	57.2	54.5
	74.5	55.2
	56.7	63.9
	54.2	53.4
	55.8	51.0
	57.1	61.7
Average amount (µg/kg)	59.2	56.6
n	6	6
st. dev (µg/kg)	7.54	5.03
Difference		2.62
$0.3*\sigma_P$		4.44
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocorninine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	41.7	40.9
	51.0	41.7
	43.3	46.8
	41.2	39.5
	43.8	38.2
	41.7	45.0
Average amount (µg/kg)	43.8	42.0
n	6	6
st. dev (µg/kg)	3.67	3.31
Difference		1.75
$0.3*\sigma_P$		3.28
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	69.5	62.0
	71.7	71.9
	66.5	63.9
	67.2	62.3
	76.5	73.0
	66.0	75.7
Average amount (µg/kg)	69.5	68.1
n	6	6
st. dev (µg/kg)	4.02	6.05
Difference		1.41
$0.3*\sigma_P$		5.22
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristinine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	51.2	47.9
	52.5	54.2
	49.9	50.2
	51.2	44.3
	55.2	55.5
	48.2	51.2
Average amount (µg/kg)	51.4	50.5
n	6	6
st. dev (µg/kg)	2.36	4.11
Difference		0.82
$0.3*\sigma_P$		3.85
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	37.8	32.4
	42.0	35.7
	37.0	39.6
	33.5	32.1
	34.6	32.4
	34.6	38.4
Average amount (µg/kg)	36.6	35.1
n	6	6
st. dev (µg/kg)	3.11	3.32
Difference		1.49
$0.3*\sigma_P$		2.74
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptinine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	31.8	30.2
	35.0	30.4
	32.6	34.0
	29.9	29.2
	31.3	28.0
	28.6	31.6
Average amount (µg/kg)	31.5	30.6
n	6	6
st. dev (µg/kg)	2.23	2.06
Difference		0.95
$0.3*\sigma_P$		2.36
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for β-Ergocryptine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	28.0	23.5
	29.0	22.7
	26.6	26.9
	21.8	23.0
	24.1	21.4
	22.8	27.4
Average amount (µg/kg)	25.4	24.1
n	6	6
st. dev (µg/kg)	2.90	2.44
Difference		1.24
$0.3*\sigma_P$		1.90
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	34.9	31.7
	38.1	36.5
	32.4	34.6
	35.4	34.6
	34.1	31.2
	32.5	37.1
Average amount (µg/kg)	34.6	34.3
n	6	6
st. dev (µg/kg)	2.13	2.42
Difference		0.28
$0.3*\sigma_P$		2.59
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrinine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	34.1	32.0
	37.0	35.0
	32.9	33.7
	34.7	33.2
	33.0	31.6
	32.1	36.6
Average amount (µg/kg)	34.0	33.7
n	6	6
st. dev (µg/kg)	1.75	1.90
Difference		0.28
$0.3*\sigma_P$		2.55
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	80.7	78.1
	90.9	81.6
	80.5	83.0
	85.2	86.2
	80.4	78.5
	75.8	79.7
Average amount (µg/kg)	82.3	81.2
n	6	6
st. dev (µg/kg)	5.17	3.08
Difference		1.11
$0.3*\sigma_P$		6.17
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosinine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	37.8	36.5
	40.7	41.3
	38.8	40.4
	39.2	39.4
	39.7	37.0
	36.1	38.4
Average amount (µg/kg)	38.7	38.8
n	6	6
st. dev (µg/kg)	1.61	1.87
Difference		-0.13
$0.3*\sigma_P$		2.90
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotamine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	148	135
	137	147
	149	146
	152	138
	149	143
	142	162
Average amount (µg/kg)	146	145
n	6	6
st. dev (µg/kg)	5.48	9.70
Difference		1.14
$0.3*\sigma_P$		11.0
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotaminine in the material A

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	56.4	50.1
	52.3	56.7
	54.6	57.1
	57.6	53.2
	57.5	55.0
	53.9	57.5
Average amount (µg/kg)	55.4	54.9
n	6	6
st. dev (µg/kg)	2.14	2.84
Difference		0.41
$0.3*\sigma_P$		4.15
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocornine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	16.4	16.4
	16.4	17.1
	16.3	15.9
	16.6	15.5
	17.0	17.0
	15.8	15.8
Average amount (µg/kg)	16.4	16.3
n	6	6
st. dev (µg/kg)	0.377	0.683
Difference		0.145
$0.3*\sigma_P$		1.23
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocorninine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	10.9	10.3
	10.7	9.83
	10.4	9.75
	10.8	9.72
	10.2	10.7
	10.0	10.7
Average amount (µg/kg)	10.5	10.2
n	6	6
st. dev (µg/kg)	0.367	0.484
Difference		0.328
$0.3*\sigma_P$		0.789
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	15.0	14.7
	16.1	15.3
	15.0	16.0
	14.7	15.1
	15.2	15.5
	13.7	14.1
Average amount (µg/kg)	14.9	15.1
n	6	6
st. dev (µg/kg)	0.763	0.668
Difference		-0.163
$0.3*\sigma_P$		1.12
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristinine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	9.77	9.44
	9.72	9.21
	9.19	9.63
	9.19	8.74
	9.11	9.86
	8.39	8.88
Average amount (µg/kg)	9.23	9.29
n	6	6
st. dev (µg/kg)	0.500	0.436
Difference		-0.064
$0.3*\sigma_P$		0.692
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	18.4	17.5
	18.4	18.6
	17.5	17.2
	17.6	16.5
	18.0	18.4
	16.2	17.1
Average amount (µg/kg)	17.7	17.5
n	6	6
st. dev (µg/kg)	0.810	0.819
Difference		0.153
$0.3*\sigma_P$		1.33
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptinine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	7.63	7.00
	7.66	7.03
	7.58	7.64
	7.30	6.91
	7.92	7.59
	7.08	7.50
Average amount (µg/kg)	7.53	7.28
n	6	6
st. dev (µg/kg)	0.295	0.331
Difference		0.251
$0.3*\sigma_P$		0.565
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	22.8	22.2
	22.3	23.1
	21.6	21.5
	23.4	20.6
	22.6	23.0
	21.1	22.0
Average amount (µg/kg)	22.3	22.1
n	6	6
st. dev (µg/kg)	0.843	0.949
Difference		0.226
$0.3*\sigma_P$		1.67
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrinine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	19.4	19.4
	19.1	20.1
	19.0	19.1
	20.2	18.6
	19.9	20.1
	19.0	19.0
Average amount (µg/kg)	19.4	19.4
n	6	6
st. dev (µg/kg)	0.509	0.607
Difference		0.031
$0.3*\sigma_P$		1.46
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	18.9	18.2
	19.0	18.4
	18.3	18.3
	19.2	17.6
	19.2	19.6
	16.9	18.6
Average amount (µg/kg)	18.6	18.5
n	6	6
st. dev (µg/kg)	0.880	0.664
Difference		0.114
$0.3*\sigma_P$		1.39
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosinine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	6.01	6.31
	6.52	6.31
	6.30	6.26
	6.30	6.25
	6.40	6.83
	6.03	6.31
Average amount (µg/kg)	6.26	6.38
n	6	6
st. dev (µg/kg)	0.204	0.222
Difference		-0.120
$0.3*\sigma_P$		0.470
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotamine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	31.5	31.2
	32.3	32.7
	31.6	31.3
	33.4	30.9
	32.5	32.8
	29.0	32.9
Average amount (µg/kg)	31.7	32.0
n	6	6
st. dev (µg/kg)	1.51	0.921
Difference		-0.262
$0.3*\sigma_P$		2.38
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotaminine in the material B

Storage temperature	<-70 °C	<-18 °C
Time (days)	0	56
Calculated amounts (µg/kg)	9.88	9.34
	9.53	9.58
	9.83	9.43
	9.77	9.46
	10.4	9.73
	8.86	9.21
Average amount (µg/kg)	9.71	9.46
n	6	6
st. dev (µg/kg)	0.508	0.182
Difference		0.257
$0.3*\sigma_P$		0.729
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

Wageningen Food Safety Research

Date
September 16, 2019

Subject
Invitation EURL mycotoxins & plant toxins proficiency test ergot alkaloids in food and feed matrices EURLPT-MP03

Postal address
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

Visitors address
Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB WAGENINGEN

Internet
www.wur.nl

Our number
09098104

Handled by
Diana Pereboom

Telephone
+31 (0) 614323017

Email
pt.wfsr@wur.nl

Dear colleague,

The EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), will organize a proficiency test (PT) regarding ergot alkaloids in food and feed matrices (EURLPT-MP03). This test will focus on the quantification of ergot alkaloids and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013). Harmonised EU regulation for ergot alkaloids in these matrices is being prepared and their inclusion in national monitoring is recommended by EFSA.

This PT will focus on quantification of 12 ergot alkaloids (ergocornine/ ergocorninine, ergocristine/ ergocristinine, α-ergocryptine/ α-ergocryptinine, ergometrine/ergometrinine, ergosine/ ergosinine, ergotamine/ ergotaminine) 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 strongly encouraged to participate. I would like to invite you to participate in this PT.

1. Test materials:
   - As a feed matrix one test item will be provided consisting of a naturally contaminated rye flour.
   - As a food matrix one test item will be provided consisting of a mix of wheat and oats flours.

   The test amount sent will be approximately 50 g.
2. Test materials will be sent in October 2019. The distribution of the test materials will be announced by e-mail. The deadline for reporting will be six weeks after the shipment of the samples.
3. As some ergot alkaloids are considered drug precursors (ergonovine, ergonovinine, ergotamine and ergotaminine) the participants may need a lincence in order to obtain ergot alkaloid reference standards. The participant should arrange the necessary import permits for the sample materials (if necessary).

Wageningen Research Foundation/Wageningen Food Safety Research (WFSR) is part of Wageningen University & Research. WFSR carries out research and analysis contributing to the safety and reliability of food and feed. WFSR is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014, L335 and R012).

4. Scope of analysis
- The materials may contain one or more of the following analytes:
  - Ergocornine/ergocorninine
  - Ergocristine/ergocristinine
  - α-Ergocryptine/α-ergocryptinine
  - Ergometrine/ergometrinine
  - Ergosine/ergosinine
  - Ergotamine/ergotaminine
  - β-Ergocryptine

The ergot alkaloid β-ergocryptine may be present in the test materails, and laboratories may report it, but it will not be benchmarked. It will be only evaluated for information purposes.

5. Questionnaire

A questionnaire will be send 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.

6. Report
- A report of the proficiency test will be dispatched in May 2020.
- Results of the proficiency test will be presented anonymously.
- The follow-up protocol on proficiency test from DG Santé will be applied.

7. 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.

8. 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 7th of October 2019 to: pt.wfsr@wur.nl.

Hoping to welcome you for this test,

D. Pereboom

Diana Pereboom-de Fauw
Proficiency tests

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

Annex 6 Instruction letter

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

Dear Madam/Sir,

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

The parcel shipped to you should contain:

- One feed material consisting of rye flour and one food material consisting of a mix of wheat and oats flours. Each test material unit contains approximately 50 grams of the homogenised test material.

Instructions:

- After arrival the samples should be stored at -20°C.
- Please fill in the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples by e-mail (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 ergot alkaloids ergocornine/ ergocorninine, ergocristine/ ergocristinine, ergocryptine/ ergocryptinine, ergometrine/ergometrinine, ergosine/ ergosinine, ergotamine/ ergotaminine are considered for legislation in food and feed products. Ergocryptine and ergocryptinine occur naturally as α- and β-isomers. For ergocryptine and ergocryptinine the concentrations should be reported as a sum of α- and β-isomers (i.e. for ergocryptine the sum of α- + β-ergocryptine and for ergocryptinine the sum of α- + β-ergocryptinine). In case your laboratory uses an analytical method that can separate the α- and β-isomers of ergocryptine, we ask you to report, in addition to the reported sum of α- + β- ergocryptine, also the concentrations determined for α- + β- ergocryptine individually. These results will not be benchmarked, but only evaluated for information purposes. In case your laboratory uses an analytical method that can separate the α- and β-isomers of ergocryptinine, we ask you to report, in addition to the reported sum of α- or β- isomers of ergocryptinine, also the

Wageningen Food Safety Research

DATE
October 14, 2019

SUBJECT
Instructions proficiency test ergot alkaloids in food and feed matrices

YOUR REFERENCE
NR

OUR REFERENCE
WFSR/EURLPT-MPGS/2019

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

VISITORS' ADDRESS
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DOCUMENT NUMBER
09098104

HANDLED BY
Diana Poreboom

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+31 (0) 614333017

EMAIL
pt.wfsr@wur.nl

concentrations determined for α- and β-ergocryptinine individually. These results will not be benchmarked, but only evaluated for information purposes.

- 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. Do not use the option 'detected' from the web application. When an analyte is 'not detected' or the result is below your LOQ, please report the result as '<LOQ-value' and specify the value (e.g. <10 µg/kg).
- 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 ergot alkaloids and the analytical method used and send it back to us by e-mail (pt.wfsr@wur.nl).
- You can download the EURL method "EURL-MP-method-003 Ergot alkaloids-food-feed, for the analysis of ergot 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 25th of November 2019.**

- 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), part of Wageningen University & Research
The Netherlands

Annex 7 Scope and LOQ

Lab code	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	β-Ergo-cryptine	α+β-Ergo-cryptine	α-Ergo-cryptinine	β-Ergo-cryptinine	α+β-Ergo-cryptinine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine
	LOQ (µg/kg)															
PT9446	11	22	18	24	22			26			17	19	16	19	18	13
PT9447	4	4	4	4	4			4			4	4	4	4	4	4
PT9448	5	5	5	5	5	5		5		5	5	5	5	5	5	5
PT9449	10	10	10	10			10			10	10	10	10	10	10	10
PT9450	1	1	1	1	1			1			1	1	1	1	1	1
PT9451	10			10	10			10					10			
PT9452	2	2	2	2			2			2	2	2	2	2	2	2
PT9453	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9454	3	3	3	3	3		3	3		3	3	3	3	3	3	3
PT9455	5	5	5	5	5		5			5	5	5	5	5	5	5
PT9456	40		80		617						183		44		131	
PT9457	0.35	0.20	0.39	0.38	0.15			0.29			0.36	0.29	0.34	0.29	0.36	0.31
PT9458	1	1	1	1			1			1			1	1		
PT9459	0.25	0.25	0.25	0.25	0.25	0.25	0.5			0.25	0.25	0.25	0.25	0.25	0.25	0.25
PT9460	0.5	0.5	0.5	0.5			0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5
PT9461	2.5	2.5	2.5	2.5			2.5			2.5		2.5	2.5	2.5	2.5	2.5
PT9463	1	1	1	1			1			1	1	1	1	1	1	1
PT9464	4	4	4	4			4			4	4	4	4	4	4	4
PT9465	2	2	2	2			2			2	2	2	2	2	2	2
PT9466	2	2	2	2	2	2		2	2		2	2	2	2	2	2
PT9467	0.7	0.4	0.6	0.6			1.1			0.5	0.5	0.4	0.3	0.5	0.8	0.4
PT9468	5	5	5	5			5			5	5	5	5	5	5	5
PT9469	10	10	10	10	10			10			2	2	2	10	2	10
PT9470	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
PT9472	5	5	5	5	5			5			5	5	5	5	5	5
PT9473	12.5	12.5	12.5	12.5	12.5			12.5			12.5	12.5	12.5	12.5	12.5	12.5
PT9474	2	2	2	2			2			2	2	2	2	2	2	2
PT9475	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT9476	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PT9477	13.3	13.3	10.2	10.2	17.6			17.6			17.1	17.1	11.9	11.9	12.0	12.0
PT9478																
PT9479	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
PT9480	0.5	0.5	0.5	0.5	0.5			0.5			10	0.5	0.5	5	0.5	0.5

Annex 8 Method details

Lab code	Method	Column	Column length (mm)	Total run time (min)	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	α-Ergocryptine	β-Ergocryptine	α+β-Ergocryptine	α-Ergocryptinine	β-Ergocryptinine	α+β-Ergocryptininine	Ergometrine	Eregometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine
					Retention time (min)															
PT9446	Acid	Phenomenex, hexyl-phenyl, 100 x 2.0 mm	100	16	6.22	7.19	6.17	7.67	6.39			7.46			2.28	4.28	5.84	6.73	5.99	6.97
PT9451	Acid	Eclipse Plus C18, 150 x 2.1 mm, 1.8 µm	150	30	6			8.5	6.3			8.07					5.65			
PT9453	Acid	Acquity UPLC BEH C18, 100 × 2.1 mm, 1.7 µm	100	11	2.01	2.38	2.41	2.68	2.37	2.37	2.37	2.64	2.64	2.64	1.09	1.18	1.87	1.80	1.94	1.89
PT9456	Acid	Kinetex®F5, 150 x 2.1 mm, 2.6 µm, 100Å,	150	43	26.19	27.62	26.96	28.77	26.79			28.15			20.04	22	25.72	25.72	26	26.23
PT9465	Acid	Zorbax Eclipse Plus C18 RRHD, 2.1 x 50 mm, 1,8 µm	50	27	9.0	10.4	9.7	11.1			9.6			10.9	3.3	5.0	8.7	8.7	9.1	9.1
PT9475	Acid	C18		15	7.2	7.71	8.39	9.05	7.9	8.1		8.4	8.6		2.26	2.36	6.83	6.93	7.09	7.72
PT9447	Alkaline	Zorbax Eclipse XDB-C18, 250 x 4.6 mm, 5 µm	250	50	8.96	20.895	12.52	31.381	11.341			27.111			2.675	3.64	5.754	15.234	6.491	17.992
PT9448	Alkaline	Waters X-Bridge C18, 150 x 3.0 mm, 5 µm	150	17	8.1	9.98	8.69	10.66	8.56	8.71		10.51	10.51	10.51	4.46	5.49	7.13	9.17	7.37	9.5
PT9449	Alkaline	Supelco Ascentis Express Phenyl-hexyl, 10 x 2.1 cm, 2.7 µm	100	12	7.22	7.69	7.95	8.62			7.6			8.16	4.53	4.87	6.72	6.91	7.08	7.35
PT9450	Alkaline	Luna Phenomenex C18, 150 x 4.6mm, 3 µm	150	20	8.9	13.7	10.3	16.9	9.9			15.8			3.2	5.2	7.3	11.4	7.7	12.5
PT9452	Alkaline	Agilent Poroshell HPH, 100 x 3.0 mm; 2.7 µm	100	25	12.3	14.9	13.6	16.5			13.3			15.9	5.5	7.1	11.4	13.7	12.1	14.7
PT9454	Alkaline	Macherey Nagel Nucleodur PFP, 125 x 3.0 mm, 5 µm	125	10	5.2	7	6	7.8			5.8			7.5	2.3	3.1	4.1	6	4.3	6.4
PT9455	Alkaline	Phenomenex Gemini C18 150 x 3.0 mm, 3 µm, 100 Å	150	15	9.265	10.416	9.683	10.828	9.582	9.664	9.582			10.721	4.063	6.924	8.541	9.923	8.737	10.151
PT9457	Alkaline	Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	12	3.23	4.86	3.77	5.48	3.62	3.74		5.30			1.65	2.00	2.64	4.08	2.79	4.46
PT9458	Alkaline	Zorbax Eclipse Plus RRHD C18, 150 x 3.0 mm, 1.8 µm	150	26	13.0	14.5	13.4	15.2			13.3			15.0			12.2	13.9		
PT9459	Alkaline	Waters Acquity UHPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	15	3.3	5	3.85	5.65	3.7	3.85				5.45	1.65	2.05	2.7	4.2	2.85	4.6
PT9460	Alkaline	Phenomenex Gemini NX-C18, 100 x 2.0 mm, 3 µm	100	15	8.8	9.5	9.2	10			9.1			9.8	5	6.4	8.5	9.1	8.7	9.4
PT9461	Alkaline	BEH		15	8.68	10.51	9.34	11.27			9.18			11.05		5.43	7.69	9.67	7.99	10.06
PT9463	Alkaline	Phenomenex Kinetex C18, 150 x 4.6 mm, 2.6 µm, 100A	150	17	7.61	9.22	8.58	10.33			8.36			9.97	3.76	5.3	6.79	8.33	7.16	8.9
PT9464	Alkaline	Phenomenex Gemini-NX C18, 150 x 2.0 mm, 5 µm	150	22	7.09	9.89	8.11	12.00			7.78			11.24	4.56	4.99	6.10	8.27	6.42	9.09
PT9466	Alkaline	Phenomenex; Gemini C6 Phenyl, 150 x 2.0 mm, 3 µm	150	30	11.5	13.8	12.2	15.5	11.9	12.1		14.75	14.95		7	8.6	10.6	12.6	10.9	13.2
PT9467	Alkaline	Phenomenex® Gemini® C6-Phenyl, 150 x 2.0 mm, 3 µm	150	33	8.8	14.1	9.8	17.8	9.5		9.5	16.5		16.5	4.7	5.9	7.5	10.5	7.9	12.1
PT9468	Alkaline	Kinetex phenyl/hexyl, 100 x 2.1 mm, 2,6 µm	100	15	9.54	10.62	10.02	11.07			9.82			10.89	6.41	7.49	8.92	10.16	9.20	10.45
PT9469	Alkaline	Phenomenex:Luna Phenyl_Hexyl, 150 x 2.0 mm, 3 µm	150	30	7.68	9.24	8.12	10.5	7.94			9.71			5.51	6.45	6.94	8.58	7.16	8.92
PT9470	Alkaline	Phenomenex Kinetex EVO		10	4.15	4.88	4.46	5.22	4.37			5.12			2.03	2.7	3.72	4.53	3.87	4.72
PT9472	Alkaline	Waters BEH, C18, 150 x 2.1 mm	150	15	5.2	6.73	5.76	7.26	5.64			7.13			2.66	3.1	4.22	6.08	4.44	6.35
PT9473	Alkaline	Phenomenex Synergi Max RP, 150 x 3.0 mm, 4 µm	150	23	6.7	12.4	8.2	17.4	7.9			16			2.6	3.4	4.9	9.1	5.2	10.2
PT9474	Alkaline	Waters XBridge C18, 150 x 3.0 mm, 5 µm	150	20	9.3	11.5	10.4	13			9.8			12.1	4.9	6.2	8	10.5	8.4	10.9
PT9476	Alkaline	Acquity UPLC®BEH, 100 x 2.1 mm, 1.7 µm	100	6	3.33	3.78	3.48	3.95	3.46	3.49		3.92			1.09	1.92	3.04	3.56	3.09	3.63
PT9477	Alkaline	Phenomenex Gemini C6-Phenyl, 150 x 2.0 mm, 3 µm, 110Å	150	24	10.46	15.44	12.27	19.09	11.49			17.44			3.30	5.39	8.47	13.07	9.35	14.48
PT9478	Alkaline	Phenomenex Gemini C6-Phenyl, 150 x 2.0 mm, 3 µm, Precolumn Phenomenex C6-Phenyl, 4.0 x 2.0 mm,	150	16	6.29	7.83	6.9	8.4			6.66			8.06	3.24	3.94	5.39	7.2	5.75	7.56
PT9479	Alkaline	Waters, Xbridge C18, 150 x 3.0 mm, 3.5 µm	150	27	12.64	14.42	13.2	15.06			13.08			14.92	8.28	9.74	11.7	13.64	11.94	13.96
PT9480	Alkaline	Waters Acquity BEH C18, 100 x 2.1 mm, 1.7 µm	100	7	1.6	3	1.9	3.9	1.8			3.6			0.72	0.86	1.2	2.3	1.3	2.6

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)	Mobile phase	Detection technique
PT9446	5	MeCN-0,2M ammonium carbonate (64/18, v/v)	25	mechanical shaking	30	SPE	Roma	4	0.8	A: MeCN/H2O/ammonium formate 0,2M: 10/85/5, v/v/v B: MeCN/H2O/ammonium formate 0,2M: 90/5/5, v/v/v	LC-MS/MS
PT9451	20	MeCN/ammonium carbonate	100	shaking (hand/vortex)	30	SPE	Mycosep	4ml/1ml	1	A: 445 ml distilled water, 50 ml MeOH, 5 ml acetic acid, 0.192 g ammonium acetate B: 495 ml MeOH, 5 ml acetic acid, 0.192 g ammonium acetate	LC-MS/MS
PT9453	2	MeCN + 0.1% FA in H2O (1/1, v/v)	20	mechanical shaking	20	dilution			0.1	A: H2O + 0.1% FA B: MeOH + 0.1% FA + 1Mm ammonium formate	LC-MS/MS
PT9456	2.5	MeCN/H2O + FA	20	mechanical shaking	60	none				A: ammonium acetate + HOAc in H2O B: ammonium acetate + HOAc in MeOH/H2O	LC-MS/MS
PT9465	25	MeCN/H2O/HOAc (79/20/1, v/v/v)	100	mechanical shaking	30	none			0.25	A: 10 Mm ammonium formate Ph 3,0 B: MeOH with 0.2% FA	LC-MS/MS
PT9475	5	MeCN	40	ultraturrax	3	dilution	n.a.	n.a.	0.04	A: H2O + 0,1% FA B: MeCN + 0,1% FA	LC-MS/MS
PT9447	10	EtOAc/MeOH/NH3 25%/2-Propanol (75/5/7/7, v/v/v/v)	50	mechanical shaking	45	SPE	SepPak AluminaB	7	0.2	MeCN/ammonium carbamate 0.2 g/L: (50/50, v/v)	HPLC-FLD
PT9448	4	0.4% FA in MeOH/H2O (60/40, v/v)	40	mechanical shaking	30	none	n.a.	n.a.	0.1	A: 6 Mm ammonium hydroxide in H2O B: 6 Mm ammonium hydroxide in MeCN	LC-MS/MS
PT9449	20	MeCN/ammonium carbonate (aq) (84/16, v/v)	100	mechanical shaking	30	SPE	MycoSep® 150 Ergots	4	0.40	A: ammonium carbonate in H2O B: MeCN with 0.1% FA	LC-MS/MS
PT9450	5	MeCN	25	mechanical shaking	30	other				A: 1 Mm ammonium carbonate B: MeCN	HPLC-FLD
PT9452	10	EtOAc/MeOH/ammonium hydroxide/ isopropanol (75/5/7/7, v/v/v/v)	50	mechanical shaking	45	SPE	Sep_Pak Alumina B Cartridges (Waters)	1	0,07	A: ammonium bicarbonate 1Mm in MeOH/H2O (5/95, v/v) B: MeOH	LC-MS/MS
PT9454	7.5	EtOAc, ammonium hydroxyde	75	ultrasonic	30	LLE			0.1	ammonium carbamate buffer/MeCN	LC-MS/MS
PT9455	20	MeCN: 200 mg/L ammonium carbonate (84/16, v/v)	100	mechanical shaking	60	other			0.2	A: ammonium carbonate 200 mg/L B: MeCN	LC-MS/MS
PT9457	5	MeCN/ammonium carbonate (84/16, v/v)	25	mechanical shaking	30	other	PSA 40um, Bondesil	1	0.2	A: 100% MeCN, B: ammonium carbonate (200 mg/l)	LC-MS/MS
PT9458	5	MeCN/200 mg/l ammonium carbonate in H2O (84/16, v/v)	25	mechanical shaking	30	other	filter paper and PSA	25	0.2	A: 0.003M ammonium carbonate in H2O	LC-MS/MS
PT9459	20	MeCN/2.0 mmol/l ammonium carbonate solution (84/16, v/v)	100	mechanical shaking	30	SPE=other	Varian Bondesil PSA dispersive SPE material	1	0.2		LC-MS/MS
PT9460	20	Ammonium carbonate 200mg/L-MeCN (15/85, v/v)	100	mechanical shaking	30	none				A: ammonium bicarbonate 3 mmol/L B: MeOH	LC-MS/MS
PT9461	10	MeCN/ ammonium carbonate (84/16, v/v)	50	mechanical shaking	30	other		1 (PSA)	0.5	A: H2O/(10mM ammonium carbonate) B: MeCN	LC-MS/MS
PT9463	20	MeCN/ammonium-carbonate in H2O (200mg/l) (84/16, v/v)	100	mechanical shaking	90	SPE	MycoSep 150				LC-MS/MS

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)	Mobile phase	Detection technique
PT9464	5	MeCN/H2O (84/16. v/v)	25	shaking (hand/vortex)	30	other	PSA (quetchers)	1	0.2	A: H2O/ammonium carbonate buffer pH 10 B: MeCN	LC-MS/MS
PT9466	20	EtOAc/MeOH/ammonium hydroxide25%/2-Propanol (75/5/7/7, v/v/v/v)	100	mechanical shaking	60	SPE	Waters Sep-Pak Alumina B Plus	5	0.2	A: 0,2g/L ammonium carbamate B: MeCN	LC-MS/MS
PT9467	10	EtOAc/MeOH/ammoniak. 25%/2-Propanol (75/5/7/7, v/v/v/v)	50	mechanical shaking	45	SPE	"Sep-Pak® Alumina B Plus / Waters	2.5	0.2	A: ammonium carbamate buffer (0,2 g/l) B: MeCN	LC-MS/MS
PT9468	2	1% FA in MeCN	10	mechanical shaking	30	none			0.2	A: 3mM ammonium bicarbonate; B: MeCN	LC-MS/MS
PT9469	20	75VT EtOAc, 5VT MeOH, 7VT ammoniak. 25%	100	mechanical shaking	45	SPE	Sep-Pak Plus Alumina B Cartridges	5	0.2	A: 0,2g/l ammonium carbamate, B: MeCN	LC-MS/MS
PT9470	4		30	mechanical shaking	45	none	NA	NA	0.16	A: aqueous ammonia buffer B: MeCN	LC-MS/MS
PT9472	5	MeCN/0.2g ammonium carbonate solution at pH 8.9 (84/16, v/v)	25	shaking (hand/vortex)	30	other	Addition of 50mg Bondesil PSA 40um	Solution filtered	0.2	A: 0.2g qmmonium carbonate B: MeCN	LC-MS/MS
PT9473	10	750 ml EtOAc, 50 ml MeOH, 70 ml isopropanol, 70ml ammonia solution 25%	50	mechanical shaking	45	SPE	Waters Sep-Pak Alumina B Cartridges	5	0.2	A: 200mg/l ammonium carbamate solution, B: MeCN = 50/50	LC-MS/MS and HPLC-FLD
PT9474	25	MeCN/ammonium carbonate 0,2 g/l (84/16, v/v)	125	mechanical shaking	30	SPE=other	"Bondesil PSA		0.1	Gradient of MeCN/ ammonium carbonate 0,2 g/l	LC-MS/MS
PT9476	5	MeCN/3 mM ammonium carbonate pH 9 (84/16, v/v)	25	mechanical shaking	30	SPE	Mycosep 150 Ergot	4	0.2	A: 3 mM ammonium carbonate pH 9 B: MeCN	LC-MS/MS
PT9477	5	EtOAc/MeOH/ammonium hydroxide solution 25%/isopropanol (75/5/7/7, v/v/v/v)	25	mechanical shaking	45	SPE	Waters Sep-Pak Alumina B Plus	5	0.04	A: ammonium carbamate solution 0.02% B: MeCN	HPLC-FLD
PT9478	5	EtOAc/MeOH/ammonia /isopropanol (75/5/7/7, v/v/v/v)	25	mechanical shaking	45	SPE	Sep-Pak®Plus-Aluminia B Cartridges	5	0.2	A: MeCN B: ammonium carbamate buffer 0,2g/L in H2O	LC-MS/MS
PT9479	5	MeCN/ ammonium carbonate (aq)	25	mechanical shaking	60	other			0.2	A: MeCN B: 3 mM ammonium carbonate (aq)	LC-MS/MS
PT9480	20	MeCN/ammonium carbonate	100	shaking (hand/vortex)	60	SPE	Mycosep 150 Ergot	4/1	1.6	A: ammonium carbonate buffer (200 mg/l, pH 8.9) B: MeCN	LC-MS/MS

MeCN = acetonitrile; MeOH = methanol; EtOAc = ethylacetate; H2O = water; FA (HCOOH) = formic acid; HOAc (CH3COOH) = acetic acid; HCOONH4 = ammonium formate; CH3COONH4 = ammonium acetate; (NH4)2CO3 = ammonium carbonate; NH4HCO3 = ammonium bicarbonate; CH3COONH4 = ammonium acetate.

Annex 9 Results: Material A (rye)

	Material A							
	Ergocornine **A: 53.2 µg/kg** **u: 1.56 µg/kg** **σ_p: 13.3 µg/kg (25%)** **robust σ: 7.18 µg/kg (14%)**		**Ergocorninine** **A: 40.9 µg/kg** **u: 1.73 µg/kg** **σ_p: 10.2 µg/kg (25%)** **robust σ: 7.69 µg/kg (19%)**		**Ergocristine** **A: 82.7 µg/kg** **u: 3.29 µg/kg** **σ_p: 20.7 µg/kg (25%)** **robust σ: 14.9 µg/kg (18%)**		**Ergocristinine** **A: 50.4 µg/kg** **u: 2.78 µg/kg** **σ_p: 12.6 µg/kg (25%)** **robust σ: 12.8 µg/kg (25%)**	
Lab code	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**
PT9446	76	1.71	45	0.40	66	-0.81	32	-1.46
PT9447	58.5	0.40	34	-0.67	81.5	-0.06	40	-0.82
PT9448	54	0.06	38	-0.28	91	0.40	44	-0.51
PT9449	35.1	-1.36	44.6	0.36	93	0.50	77.7	**2.17**
PT9450	48.7	-0.34	24.5	-1.60	87.5	0.23	35.3	-1.20
PT9451	50.32	-0.22					43	-0.59
PT9452	58.1	0.37	41	0.01	82.3	-0.02	59.4	0.72
PT9453	33.31	-1.50	37.65	-0.32	96.79	0.68	64.04	1.08
PT9454	37.88	-1.15	28.28	-1.23	59.87	-1.10	33.95	-1.30
PT9455	54.2	0.07	36.9	-0.39	78.6	-0.20	55	0.37
PT9456	94	**3.06**	63.4	**2.20**	149.1	**3.21**	**87.9**	**2.98**
PT9457	52.4	-0.06	36.7	-0.41	91.3	0.42	45.9	-0.36
PT9458	69.4	1.22	<1.0	**(-3.90) FN**	66.8	-0.77	51	0.05
PT9459	49	-0.32	41.3	0.04	81.4	-0.06	40.1	-0.82
PT9460	52.1	-0.08	35.4	-0.54	72.3	-0.50	46.6	-0.30
PT9461	54.0	0.06	66.6	**2.52**	140.0	**2.77**	66.2	1.26
PT9463	28.5	-1.86	40	-0.09	41.6	-1.99	44.4	-0.48
PT9464	50.7	-0.19	57.2	1.60	57.2	-1.23	65.6	1.21
PT9465	50.2	-0.23	51.1	1.00	84.1	0.07	67.7	1.37
PT9466	69	1.19	33	-0.77	85	0.11	54	0.29
PT9467	27.17	-1.96	53.41	1.23	40.25	**-2.05**	87.29	**2.93**
PT9468	55.8	0.19	38.2	-0.26	84.3	0.08	47.7	-0.21
PT9469	73.7	1.54	55.5	1.43	149	**3.21**	141	**7.19**
PT9470	56.1	0.22	40.2	-0.07	74.4	-0.40	47.4	-0.24
PT9472	52.8	-0.03	36.6	-0.42	88.1	0.26	39.4	-0.87
PT9473	48.8	-0.33	32	-0.87	101.5	0.91	46.5	-0.31
PT9474	49	-0.32	43	0.21	74	-0.42	40	-0.82
PT9475	71.2	1.35	32.05	-0.86	91.25	0.41	32.73	-1.40
PT9476	59.7	0.49	42.9	0.20	86.1	0.17	47.2	-0.25
PT9477	50.9	-0.17	40.9	0.00	73.8	-0.43	56.3	0.47
PT9478	55.8	0.19	38.71	-0.21	75.77	-0.33	39.49	-0.87
PT9479	39.6	-1.02	65.3	**2.39**	73.2	-0.46	129.1	**6.25**
PT9480	75	1.64	45	0.40	110	1.32	58	0.60

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

	Material A							
	α+β-Ergocryptine A: 43.9 µg/kg u: 3.02 µg/kg σ_p:11.0 µg/kg (25%) robust σ: 13.9 µg/kg (32%)		α+β-Ergocryptinine A: 28.9 µg/kg u: 1.96 µg/kg σ_p: 7.23 µg/kg (25%) robust σ: 8.87 µg/kg (31%)		Ergometrine A: 37.7 µg/kg u: 2.67 µg/kg σ_p: 9.43 µg/kg (25%) robust σ: 11.7 µg/kg (31%)		Ergometrinine A: 34.0 µg/kg u: 1.35 µg/kg σ_p: 8.51 µg/kg (25%) robust σ: 5.92µg/kg (17%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9446	41	-0.27	19	-1.37	81	**4.59**	33	-0.12
PT9447	37	-0.63	14.5	-1.99	43.5	0.61	27.5	-0.77
PT9448	62	1.65	22	-0.96	34	-0.40	33	-0.12
PT9449	36.6	-0.67	40.9	1.66	128.2	**9.59**	32	-0.24
PT9450	30.7	-1.20	23	-0.82	16	**-2.30**	17.3	-1.97
PT9451	36.36	-0.69	13.47	**-2.14**				
PT9452	58.1	1.29	36	0.98	30.8	-0.74	34.7	0.08
PT9453	55.21	1.03	24.39	-0.63	49.94	1.29	32.98	-0.12
PT9454	31.87	-1.10	20.15	-1.21	45.93	0.87	33.62	-0.05
PT9455	37	-0.63	32	0.43	47.9	1.08	40.1	0.71
PT9456	56.3	1.13	43.2	1.98	40.3	0.27	71.8	**4.44**
PT9457	52.5	0.78	27.4	-0.21	40.2	0.26	34.3	0.03
PT9458	31.5	-1.13	< 1.0	**(-3.86) FN**	nt		nt	
PT9459	62.4	1.68	25.8	-0.43	29.1	-0.92	34	0.00
PT9460	45.4	0.13	25.2	-0.51	75	**3.95**	33.2	-0.10
PT9461	54.5	0.96	56.1	**3.76**	nt		58.5	**2.87**
PT9463	28.3	-1.42	28.6	-0.04	10.7	**-2.87**	23.3	-1.26
PT9464	26	-1.63	39.1	1.41	41.1	0.36	38.8	0.56
PT9465	48.3	0.40	37.8	1.23	41	0.35	44.3	1.21
PT9466	64	1.83	31	0.29	40	0.24	39	0.58
PT9467	26.01	-1.63	47.6	**2.58**	31.87	-0.62	32.58	-0.17
PT9468	44.6	0.06	27.4	-0.21	31.7	-0.64	28.3	-0.67
PT9469	60.9	1.55	43.2	1.98	31.1	-0.70	37.5	0.41
PT9470	28.8	-1.38	26.6	-0.32	27	-1.14	38.4	0.51
PT9472	32	-1.09	27.7	-0.17	67.2	**3.12**	31.1	-0.35
PT9473	32.4	-1.05	30	0.15	81.9	**4.68**	32.9	-0.13
PT9474	30	-1.27	23	-0.82	26	-1.24	29	-0.59
PT9475	43.63	-0.03	18.24	-1.48	16.76	**-2.22**	detected,<5	**(-3.41) FN**
PT9476	55.8	1.08	31.1	0.30	32	-0.61	40.9	0.81
PT9477	54.2	0.94	34.6	0.79	44.3	0.70	36.2	0.25
PT9478	48.02	0.37	19.13	-1.35	34.39	-0.35	25.87	-0.96
PT9479	41.9	-0.18	32.9	0.55	29.9	-0.83	27.5	-0.77
PT9480	56	1.10	29	0.01	31	-0.71	36	0.23

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nt = not tested.

	Material A									
	Ergosine A: 81.9 µg/kg u: 2.75 µg/kg σ_p: 20.5 µg/kg (25%) robust σ: 12.5 µg/kg (15%)		Ergosinine A: 46.0 µg/kg u: 2.58 µg/kg σ_p: 11.5 µg/kg (25%) robust σ: 11.5 µg/kg (25%)		Ergotamine A: 126 µg/kg u: 5.66 µg/kg σ_p: 31.5 µg/kg (25%) robust σ: 25.2 µg/kg (20%)		Ergotaminine A: 65.3 µg/kg u: 3.20 µg/kg σ_p: 16.3 µg/kg (25%) robust σ: 14.3 µg/kg (22%)		Total sum A: 701 µg/kg u: 24.0 µg/kg σ_p: 175 µg/kg (25%) robust σ: 110 µg/kg (16%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9446	95	0.64	34	-1.04	137	0.35	64	-0.08	723	0.12
PT9447	77.5	-0.22	45	-0.09	160.5	1.10	62	-0.20	682	-0.11
PT9448	84	0.10	34	-1.04	157	0.99	52	-0.82	705	0.02
PT9449	75.9	-0.29	103.8	**5.03**	232.8	**3.40**	70	0.29	971	1.54
PT9450	69.3	-0.62	30.4	-1.36	103.6	-0.71	36.5	-1.76	523	-1.02
PT9451	87	0.25							230	**-2.69**
PT9452	83.7	0.09	56.7	0.93	125	-0.03	80.6	0.94	746	0.26
PT9453	88.42	0.32	38.33	-0.67	97.89	-0.89	62.26	-0.19	681	-0.12
PT9454	78.41	-0.17	34.03	-1.04	113.3	-0.40	47.6	-1.08	565	-0.78
PT9455	140.3	**2.85**	64.8	1.64	213.8	**2.79**	55	-0.63	856	0.88
PT9456	177.7	**sum**			248	**3.88**	180.1	**7.03**	1212	**2.91**
PT9457	82.6	0.03	40.9	-0.44	143.7	0.57	59.5	-0.36	707	0.03
PT9458	108.7	1.31	32.7	-1.16	nt		nt		360	-1.95
PT9459	92	0.49	41.1	-0.42	141	0.48	57.1	-0.50	694	-0.04
PT9460	59.8	-1.08	38.1	-0.69	110.2	-0.50	58.6	-0.41	652	-0.28
PT9461	101.1	0.94	47.6	0.14	272.5	**4.66**	119.7	**3.33**	1037	1.91
PT9463	44	-1.85	46.6	0.05	53.6	**-2.30**	64.3	-0.06	454	-1.41
PT9464	71.9	-0.49	51.3	0.46	105	-0.66	65.6	0.02	670	-0.18
PT9465	80.8	-0.05	85.3	**3.42**	120.3	-0.18	120.4	**3.37**	831	0.74
PT9466	89	0.35	54	0.70	125	-0.03	65	-0.02	748	0.27
PT9467	52.95	-1.41	45.4	-0.05	84.21	-1.32	72.43	0.44	601	-0.57
PT9468	95.8	0.68	58.8	1.11	110.2	-0.50	63.2	-0.13	686	-0.09
PT9469	123	**2.01**	62.6	1.45	140	0.45	91.8	1.62	1009	1.76
PT9470	76.9	-0.24	43.4	-0.22	118.4	-0.24	145.8	**4.93**	723	0.13
PT9472	85.3	0.17	45.2	-0.07	180.7	1.74	38.1	-1.67	724	0.13
PT9473	73.9	-0.39	40	-0.52	117.1	-0.28	57.9	-0.45	695	-0.04
PT9474	74	-0.39	40	-0.52	110	-0.50	54	-0.69	592	-0.62
PT9475	68.91	-0.63	22.97	-2.00	95.28	-0.97	25.32	**-2.45**	518	-1.04
PT9476	85	0.15	40.1	-0.51	130	0.13	64.7	-0.04	716	0.08
PT9477	79.5	-0.12	50.2	0.37	112	-0.44	72.6	0.45	706	0.02
PT9478	79.44	-0.12	45.36	-0.05	116.62	-0.29	60.96	-0.27	640	-0.35
PT9479	57.5	-1.19	67.2	1.85	116.3	-0.30	97.3	1.96	778	0.44
PT9480	93	0.54	59	1.13	139	0.42	101	**2.19**	832	0.74

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nt = not tested.

sum = participant reported sum of ergosine and ergosinine. The values are not included in the calculation of the A

	Material A					
	α-Ergocryptine A: 35.7 µg/kg u: 1.01 µg/kg σ_p: 8.93 µg/kg (25%) robust σ: 2.69 µg/kg (7.5%)		β-Ergocryptine A: 21.9 µg/kg u: 1.89 µg/kg σ_p: 5.47 µg/kg (25%) robust σ: 4.01 µg/kg (18%)		α-Ergocryptinine	β-Ergocryptinine
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z'-score	Result (µg/kg)	Result (µg/kg)
PT9446	41	0.59			19	
PT9447	37	0.15			14.5	
PT9448	36	0.03	25	0.54		
PT9449						
PT9450						
PT9451						
PT9452						
PT9453						
PT9454						
PT9455	37	0.15				
PT9456						
PT9457	34.5	-0.13	18.8	-0.53		
PT9458						
PT9459	36.2	0.06	26.2	0.75		
PT9460						
PT9461						
PT9463						
PT9464						
PT9465						
PT9466	42	0.71	22	0.02	17	14
PT9467						
PT9468						
PT9469						
PT9470						
PT9472	32	-0.41			27.7	
PT9473						
PT9474						
PT9475	35.1	-0.07	8.53	**-2.31**	18.24	
PT9476	32.9	-0.31	22.9	0.18		
PT9477	32.2	-0.39	22	0.02	19.9	14.7
PT9478						
PT9479						
PT9480						

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

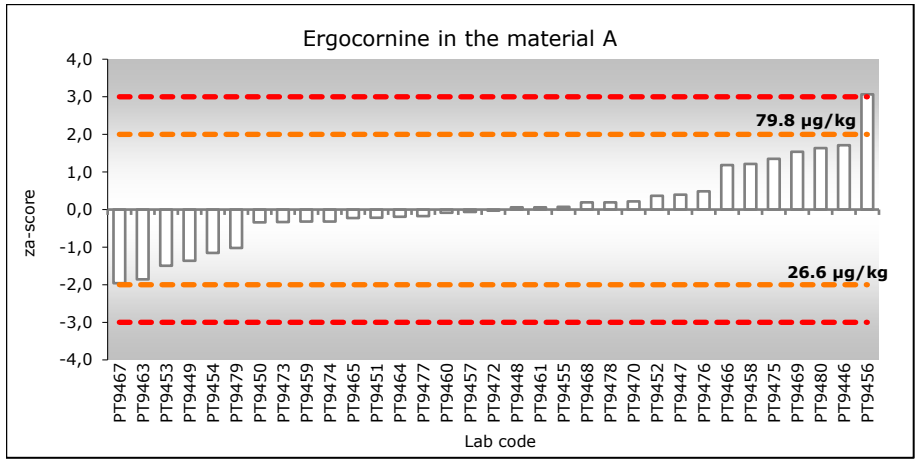

Figure 1 *Graphical representation of the z-scores for ergocornine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

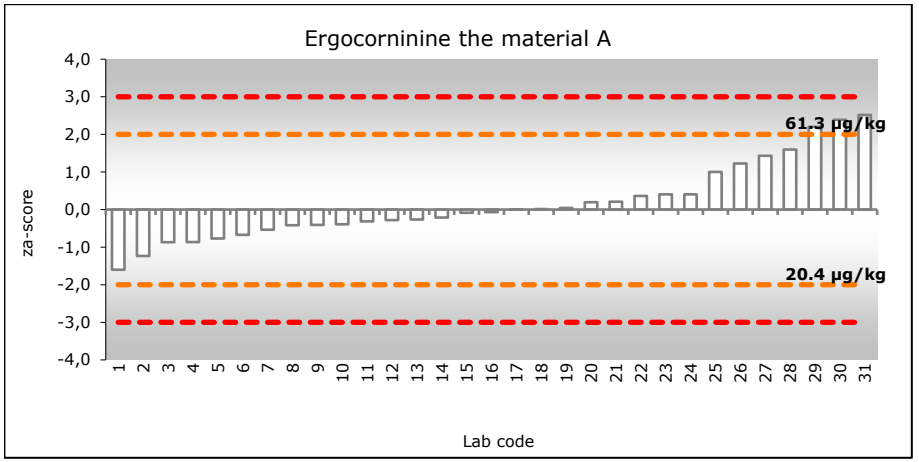

Figure 2 *Graphical representation of the z-scores for ergocorninine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

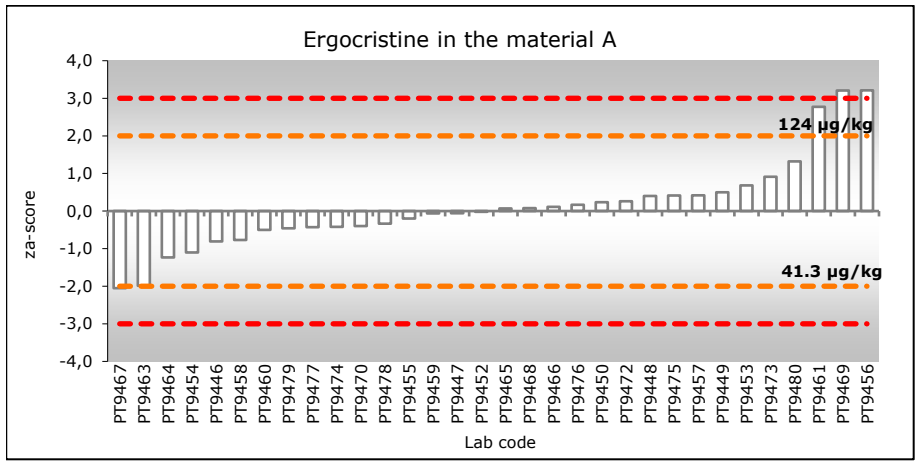

Figure 3 *Graphical representation of the z-scores for ergocristine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

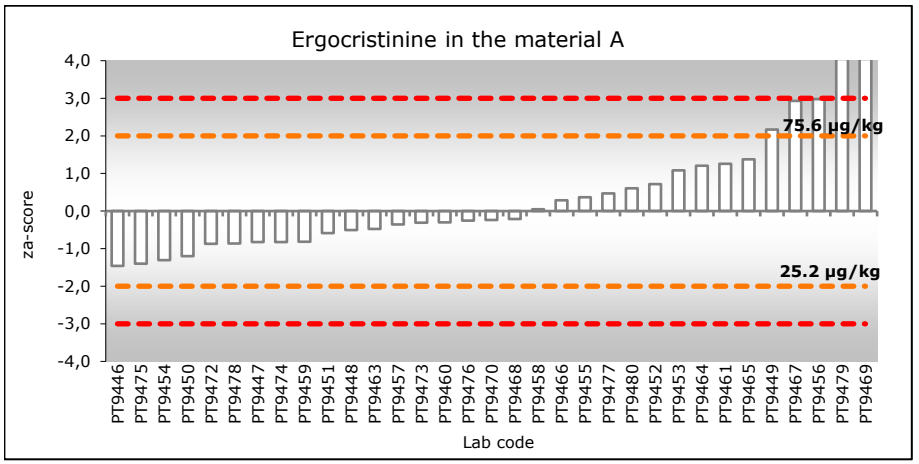

Figure 4 *Graphical representation of the z-scores for ergocristinine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

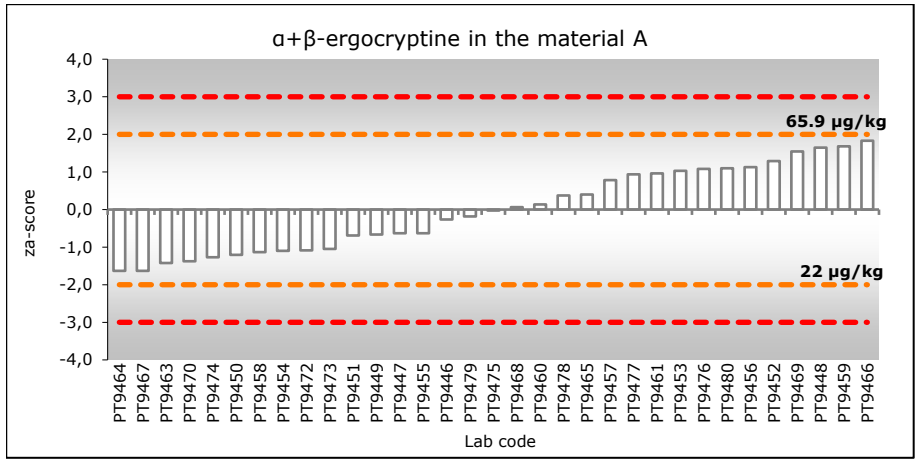

Figure 5 *Graphical representation of the z-scores for α+β-ergocryptine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

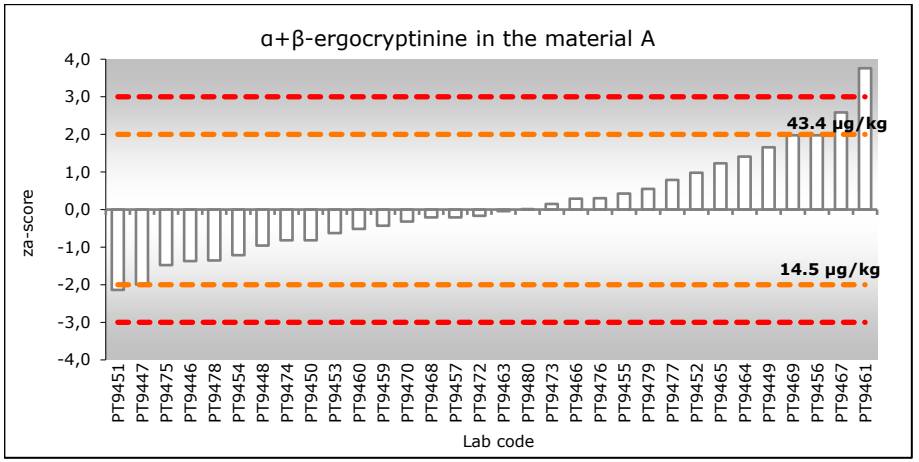

Figure 6 *Graphical representation of the z-scores for α+β-ergocryptinine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

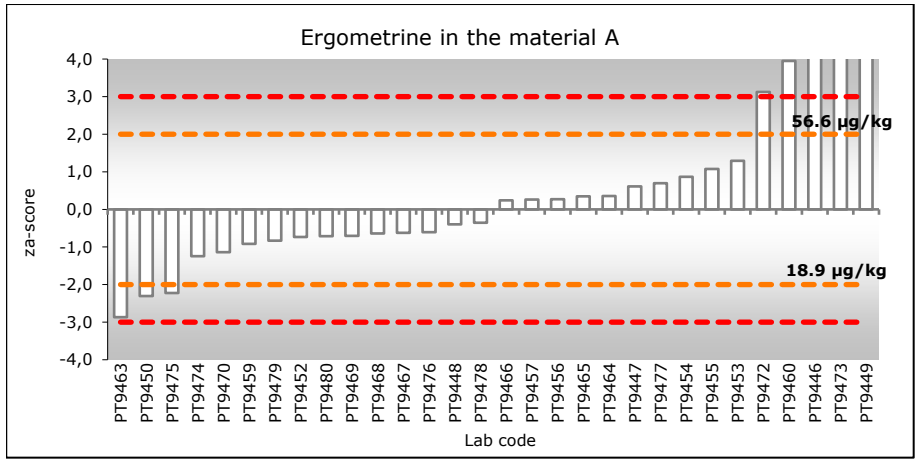

Figure 7 *Graphical representation of the z'-scores for ergometrine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

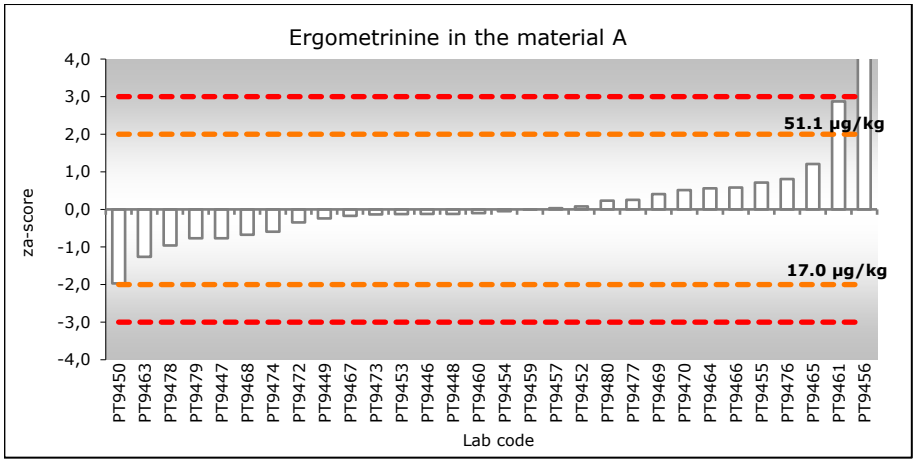

Figure 8 *Graphical representation of the z-scores for ergometrinine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

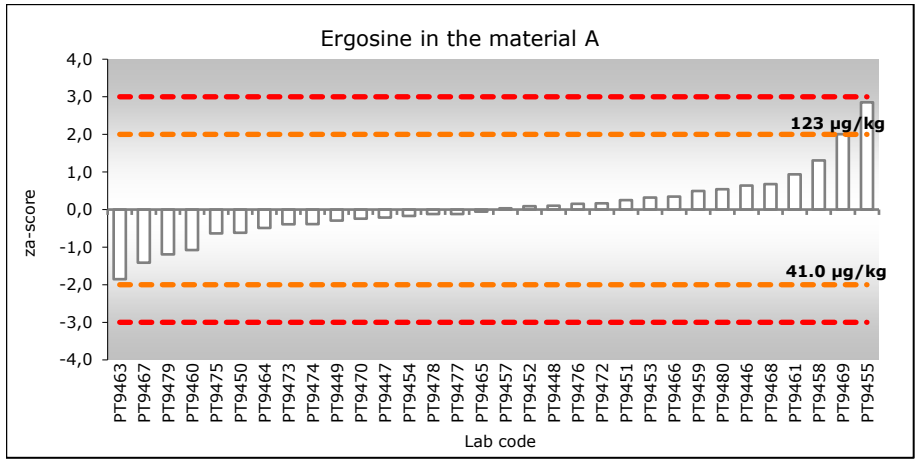

Figure 9 *Graphical representation of the z'-scores for ergosine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

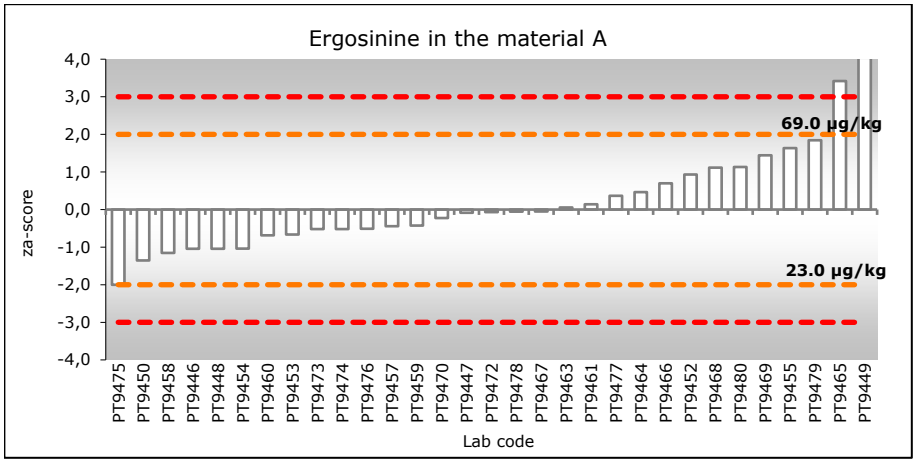

Figure 10 *Graphical representation of the z'-scores for ergosinine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

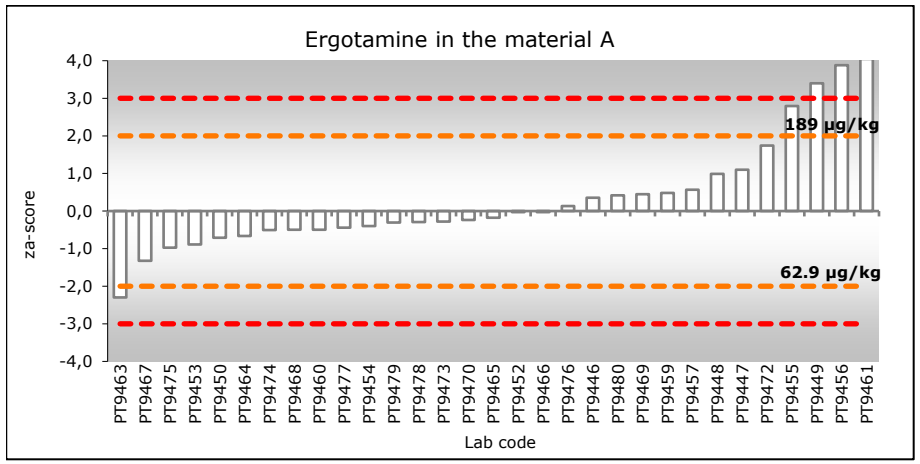

Figure 11 *Graphical representation of the z'-scores for ergotamine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

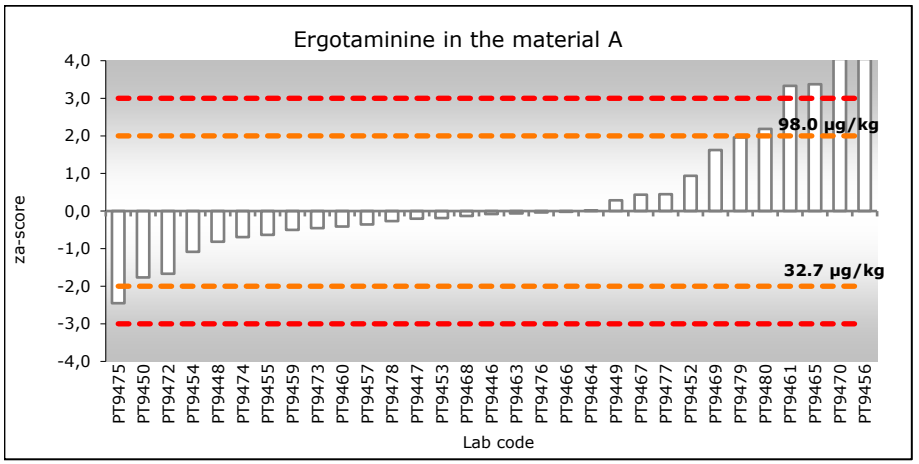

Figure 12 *Graphical representation of the z-scores for ergotaminine in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

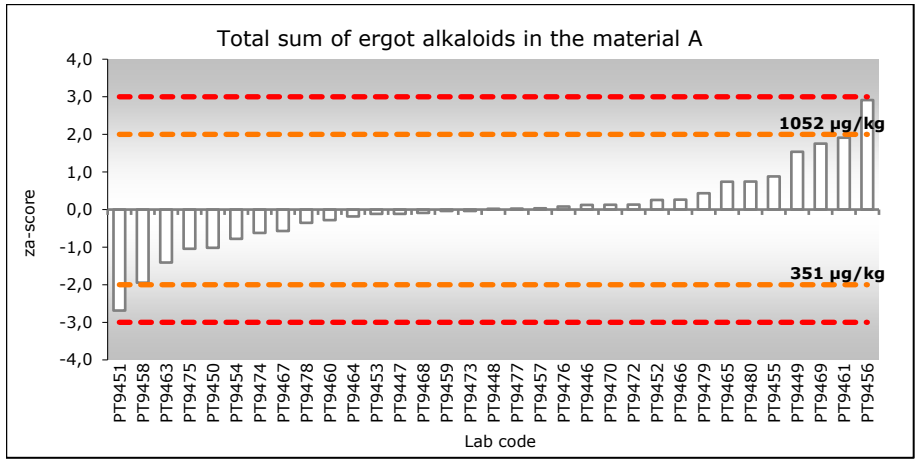

Figure 13 *Graphical representation of the z-scores for total sum in the material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 10 Results: Material B (wheat + oat)

	Material B							
	Ergocornine A: 12.8 µg/kg u: 0.993 µg/kg σ_p: 3.20 µg/kg (25%) robust σ: 4.42 µg/kg (35%)		**Ergocorninine** A: 9.86 µg/kg u: 0.504 µg/kg σ_p: 2.47 µg/kg (25%) robust σ: 2.21 µg/kg (22%)		**Ergocristine** A: 13.3 µg/kg u: 0.634 µg/kg σ_p: 3.32 µg/kg (25%) robust σ: 2.82 µg/kg (21%)		**Ergocristinine** A: 10.5 µg/kg u: 0.660 µg/kg σ_p: 2.63 µg/kg (25%) robust σ: 2.89µg/kg (28%)	
Lab code	**Result (µg/kg)**	**z'-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**	**Result (µg/kg)**	**z-score**
PT9446	<11	(-0.54)	<22	**(4.92)**	<18	(1.42)	<24	**(5.12)**
PT9447	16	0.95	9.2	-0.27	19	1.72	8.5	-0.77
PT9448	16	0.95	9	-0.35	15	0.51	8	-0.96
PT9449	29.6	**5.01**	10.9	0.42	25.5	**3.67**	23.7	**5.01**
PT9450	6.4	-1.91	7.6	-0.92	6	**-2.19**	7.1	-1.30
PT9451	12.9	0.03					8.75	-0.67
PT9452	13.1	0.09	7.6	-0.92	12.3	-0.30	14.7	1.59
PT9453	7.2	-1.67	8.68	-0.48	12.36	-0.28	8.93	-0.61
PT9454	11.3	-0.45	10.7	0.34	13.37	0.02	12.53	0.76
PT9455	11.7	-0.33	8.8	-0.43	12.4	-0.27	8.4	-0.81
PT9456	19.5	2.00	13.3	1.39	11.2	-0.63	nd	
PT9457	11.2	-0.48	10.3	0.18	11.7	-0.48	12.9	0.90
PT9458	16.3	1.04	2	**-3.19**	13.6	0.09	11.9	0.52
PT9459	12.5	-0.09	13.7	1.56	14.6	0.39	10	-0.20
PT9460	13	0.06	10.1	0.10	17.4	1.24	13.5	1.13
PT9461	15.2	0.72	8.5	-0.55	10.5	-0.84	7.9	-1.00
PT9463	7.4	-1.61	9.2	-0.27	8.1	-1.56	9.6	-0.35
PT9464	11.9	-0.27	13	1.27	11.2	-0.63	12.6	0.79
PT9465	10.4	-0.72	10.6	0.30	11.4	-0.57	11.3	0.29
PT9466	17	1.25	7.5	-0.96	14	0.21	8.8	-0.66
PT9467	8.84	-1.18	13.87	1.63	8.38	-1.48	16.9	**2.42**
PT9468	18.8	1.79	8.38	-0.60	13.2	-0.03	8.35	-0.83
PT9469	20.1	**2.18**	13.5	1.48	28.4	**4.55**	27.9	**6.60**
PT9470	9.2	-1.08	10.8	0.38	15.4	0.63	10.4	-0.05
PT9472	11.9	-0.27	9.7	-0.07	14.2	0.27	8.2	-0.88
PT9473	<12.5	(-0.09)	<12.5	(1.07)	14.9	0.48	<12.5	(0.75)
PT9474	12	-0.24	11	0.46	14	0.21	10	-0.20
PT9475	15.82	0.90	6.78	-1.25	17.38	1.23	6.43	-1.56
PT9476	13.1	0.09	11	0.46	14	0.21	9.01	-0.58
PT9477	13.4	0.18	10.6	0.30	14.5	0.36	11.2	0.26
PT9478	13.33	0.16	8.01	-0.75	13.44	0.04	7.14	-1.29
PT9479	5.8	**-2.09**	22.0	**4.92**	6.7	-1.98	27.2	**6.34**
PT9480	4.1	**-2.60**	5.5	-1.77	10	-0.99	13	0.94

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

nd = not detected without specification of an LOQ, is excluded from evaluation.

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

	Material B							
	α+β-Ergocryptine A: 13.8 µg/kg u: 0.644 µg/kg σ_p: 3.44 µg/kg (25%) robust σ: 2.92 µg/kg (21%)		α+β-Ergocryptinine A: 8.57 µg/kg u: 0.634 µg/kg σ_p: 2.14 µg/kg (25%) robust σ: 2.73 µg/kg (32%)		Ergometrine A: 21.1 µg/kg u: 1.24 µg/kg σ_p: 5.29 µg/kg (25%) robust σ: 5.45 µg/kg (26%)		Ergometrinine A: 20.3 µg/kg u: 0.774 µg/kg σ_p: 5.07 µg/kg (25%) robust σ: 3.39 µg/kg (17%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT9446	<22	**(2.39)**	<26	**(8.14)**	41	**3.76**	25	0.93
PT9447	17	0.94	5.05	-1.64	24	0.54	18.5	-0.35
PT9448	19	1.52	6	-1.20	20	-0.22	18	-0.45
PT9449	20.9	**2.07**	19.1	**4.92**	47	**4.89**	30.2	1.95
PT9450	7	-1.97	6.1	-1.15	11.3	-1.86	9.4	**-2.15**
PT9451	38.18	**7.08**	5.95	-1.22				
PT9452	12.8	-0.28	11.8	1.51	17.2	-0.75	19.4	-0.18
PT9453	14.07	0.08	6.61	-0.91	23.13	0.38	18.73	-0.31
PT9454	12.38	-0.41	9.06	0.23	40.25	**3.62**	20.76	0.09
PT9455	15.2	0.41	5.2	-1.57	26.7	1.05	22.6	0.46
PT9456	12.8	-0.28	8.4	-0.08	9.3	**-2.24**	33.8	**2.66**
PT9457	12.7	-0.31	10.0	0.67	21	-0.03	17.2	-0.61
PT9458	14.6	0.24	8.1	-0.22	nt		nt	
PT9459	14.3	0.15	9.1	0.25	17.5	-0.69	20.6	0.06
PT9460	14.5	0.21	10.1	0.71	45.3	**4.57**	18.1	-0.43
PT9461	12.0	-0.52	12.6	1.88	nt		29.1	1.74
PT9463	7.8	-1.74	7.4	-0.55	5.8	**-2.90**	14.3	-1.18
PT9464	6.8	**-2.03**	9	0.20	22	0.16	20	-0.06
PT9465	9.8	-1.16	10.7	0.99	19.6	-0.29	24.9	0.91
PT9466	17	0.94	6.8	-0.83	23	0.35	20	-0.06
PT9467	9.87	-1.13	12.52	1.84	19.97	-0.22	19.85	-0.09
PT9468	17.1	0.96	6	-1.20	18.7	-0.46	26.8	1.28
PT9469	17.1	0.96	detected,<10	(0.67)	19.3	-0.35	22.3	0.40
PT9470	15.6	0.53	9	0.20	18.4	-0.52	21	0.14
PT9472	12.9	-0.26	7.7	-0.41	43.6	**4.25**	17.3	-0.59
PT9473	14	0.06	<12.5	(1.84)	35.3	**2.68**	15.7	-0.90
PT9474	14	0.06	7.8	-0.36	17	-0.78	18	-0.45
PT9475	12.2	-0.46	detected,<5	(-1.67)	15.74	-1.02	detected,<5	**(-3.01) FN**
PT9476	14.2	0.12	7.29	-0.60	16.6	-0.86	22.4	0.42
PT9477	14.1	0.09	8.8	0.11	21.9	0.14	20.6	0.06
PT9478	15.3	0.44	5.29	-1.53	23.11	0.37	17.11	-0.63
PT9479	8.3	-1.59	19.1	**4.92**	24.5	0.64	19.7	-0.12
PT9480	7.1	-1.94	12	1.60	18	-0.59	22	0.34

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nt = not tested.

	Material B									
	Ergosine A: 14.9 µg/kg u: 0.846 µg/kg σ_p: 3.72 µg/kg (25%) robust σ: 3.83 µg/kg (26%)		Ergosinine A: 8.54 µg/kg u: 0.470 µg/kg σ_p: 2.14 µg/kg (25%) robust σ: 2.03 µg/kg (24%)		Ergotamine A: 22.9 µg/kg u: 1.55 µg/kg σ_p: 5.72 µg/kg (25%) robust σ: 6.90 µg/kg (30%)		Ergotaminine A: 13.5 µg/kg u: 1.05 µg/kg σ_p: 3.37 µg/kg (25%) robust σ: 4.50 µg/kg (33%)		Total sum A: 165 µg/kg u: 6.86 µg/kg σ_p: 41.2 µg/kg (25%) robust σ: 31.5 µg/kg (19%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z'-score	Result (µg/kg)	z-score
PT9446	30	**4.06**	<19	**(4.89)**	29	1.07	<13	(-0.14)	125	-0.97
PT9447	16	0.30	7.15	-0.65	37	**2.47**	10.5	-0.85	188	0.56
PT9448	15	0.03	6	-1.19	26	0.54	10	-0.99	168	0.08
PT9449	16.2	0.35	23.8	**7.14**	75.3	**9.16**	24	**2.97**	346	**4.40**
PT9450	9.3	-1.50	6.7	-0.86	16.1	-1.19	8.3	-1.47	101	-1.54
PT9451	11.42	-0.93							77	**-2.13**
PT9452	11.3	-0.97	11.1	1.20	13.8	-1.59	18.6	1.45	164	-0.03
PT9453	18.6	0.99	6.59	-0.91	18.72	-0.73	10.16	-0.94	154	-0.27
PT9454	15.97	0.29	8.29	-0.12	23.74	0.15	19.89	1.81	198	0.81
PT9455	22.7	**2.10**	11.2	1.24	33.6	1.87	8.9	-1.30	187	0.55
PT9456	24.3	**sum**			16.1	-1.19	25.4	**3.37**	174	0.22
PT9457	14.9	0.00	10.0	0.68	18.0	-0.85	14.6	0.31	165	-0.01
PT9458	21.8	1.85	6.2	-1.10	nt		nt		93	-1.76
PT9459	17.8	0.78	10.1	0.73	25.8	0.51	12.3	-0.34	178	0.33
PT9460	12.5	-0.64	8.8	0.12	25	0.37	14.5	0.28	203	0.92
PT9461	13.3	-0.43	9.7	0.54	25.5	0.46	16.0	0.71	160	-0.11
PT9463	8.9	-1.61	8	-0.25	11.2	**-2.04**	11.5	-0.56	109	-1.35
PT9464	12.7	-0.59	9	0.21	22.6	-0.05	14.2	0.20	165	0.00
PT9465	11.5	-0.91	11.6	1.43	16.8	-1.06	15.8	0.65	164	-0.01
PT9466	14	-0.24	7.1	-0.68	23	0.02	11	-0.71	169	0.11
PT9467	11.72	-0.85	9.69	0.54	18.53	-0.76	14.46	0.27	165	-0.01
PT9468	20.2	1.42	7	-0.72	30.1	1.26	10.1	-0.96	185	0.48
PT9469	22.3	1.99	10.1	0.73	31.5	1.51	17.4	1.11	230	1.58
PT9470	16.6	0.46	9.2	0.31	25.9	0.53	26.2	**3.60**	188	0.55
PT9472	13.8	-0.29	7.3	-0.58	30.4	1.31	6.9	-1.87	184	0.46
PT9473	13.4	-0.40	<12.5	(1.85)	21.9	-0.17	<12.5	(-0.28)	115	-1.20
PT9474	15	0.03	8.3	-0.11	23	0.02	12	-0.42	162	-0.07
PT9475	16.77	0.50	5.59	-1.38	18.95	-0.69	5.34	**-2.31**	121	-1.06
PT9476	15.8	0.24	8.1	-0.21	21.6	-0.22	12.8	-0.20	166	0.03
PT9477	16.3	0.38	8.4	-0.07	21.5	-0.24	11.7	-0.51	173	0.20
PT9478	15.86	0.26	7.16	-0.65	25.74	0.50	9.98	-1.00	161	-0.08
PT9479	9.5	-1.45	19.7	**5.22**	15.3	-1.33	31.2	**5.01**	209	1.07
PT9480	8.9	-1.61	7	-0.72	13	-1.73	18	1.28	139	-0.64

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

nt = not tested.

sum = participant reported sum of ergosine and ergosinine. The values are not included in the calculation of the assigned value.

	Material B			
	α-Ergocryptine	β-Ergocryptine	α-Ergocryptinine	β-Ergocryptinine
Lab code	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)	Result (µg/kg)
PT9446	<22		<26	
PT9447	17		5.05	
PT9448	17			
PT9449				
PT9450				
PT9451				
PT9452				
PT9453				
PT9454				
PT9455	15.2			
PT9456				
PT9457	12.7			
PT9458				
PT9459	14.3	<0.5		
PT9460				
PT9461				
PT9463				
PT9464				
PT9465				
PT9466	17	<2	6.8	<2
PT9467				
PT9468				
PT9469				
PT9470				
PT9472	12.9		7.7	
PT9473				
PT9474				
PT9475	12.2			
PT9476	14.2			
PT9477	14.1		8.8	
PT9478				
PT9479				
PT9480				

A = assigned value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency.

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

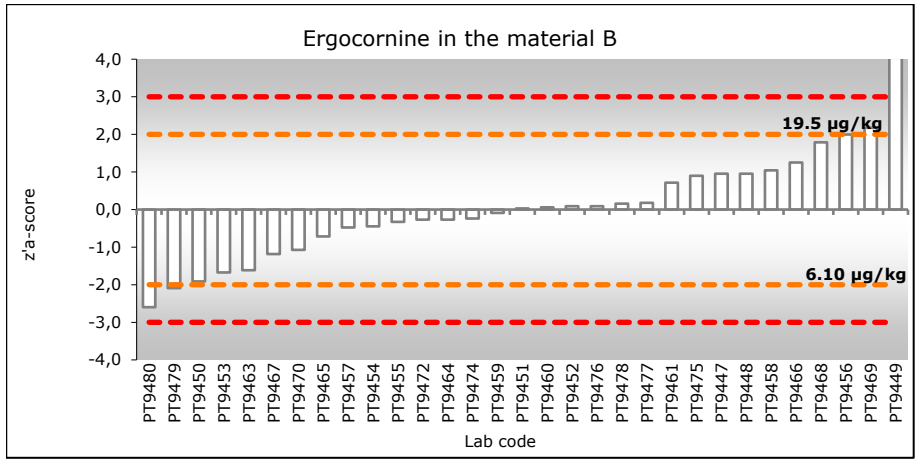

Figure 14 *Graphical representation of the z'-scores for ergocornine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

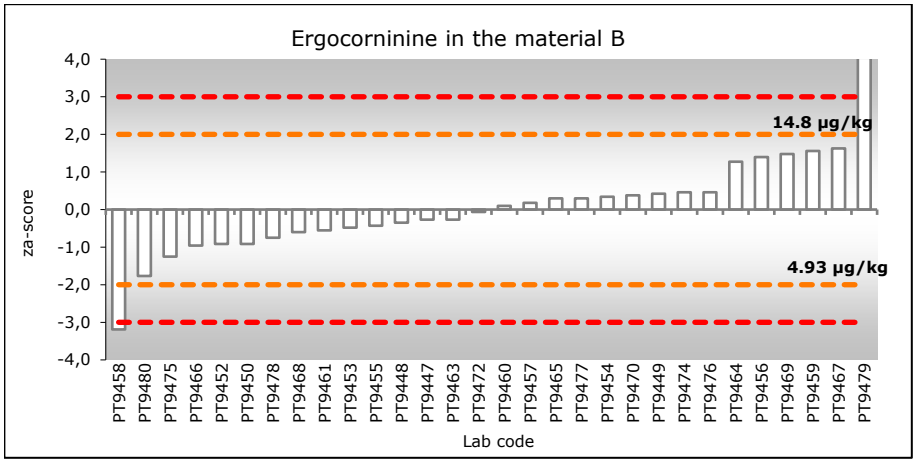

Figure 15 *Graphical representation of the z-scores for ergocorninine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

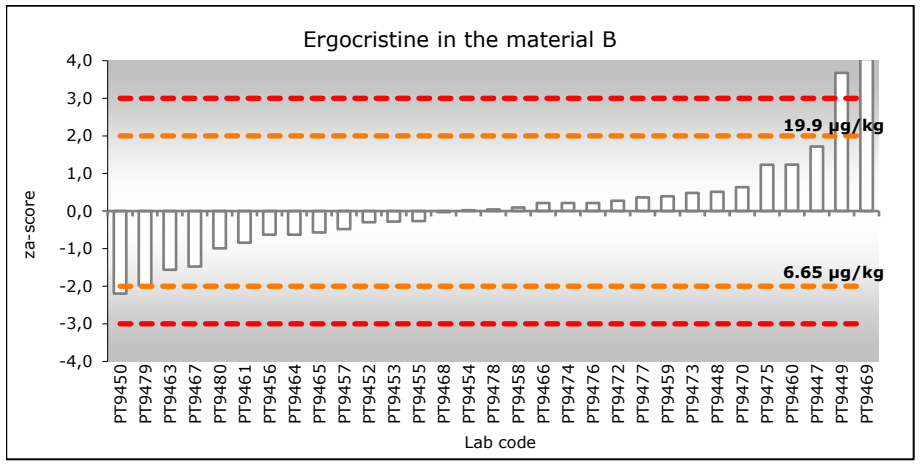

Figure 16 *Graphical representation of the z-scores for ergocristine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

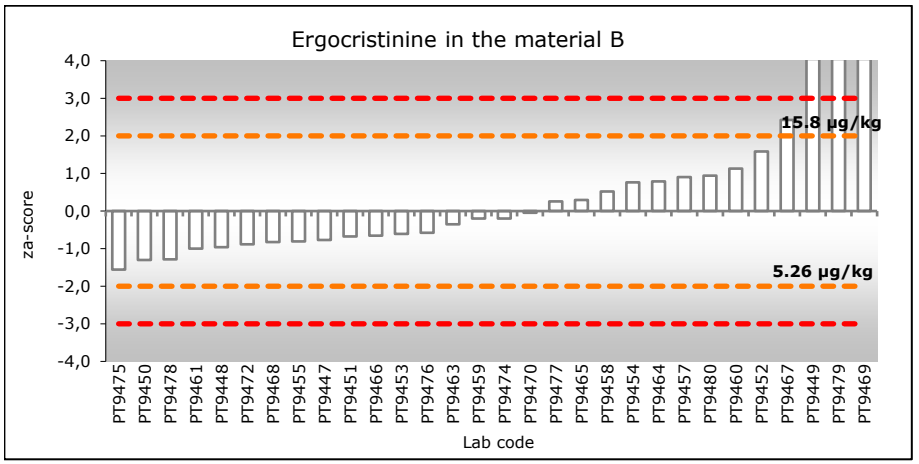

Figure 17 *Graphical representation of the z-scores for ergocristinine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

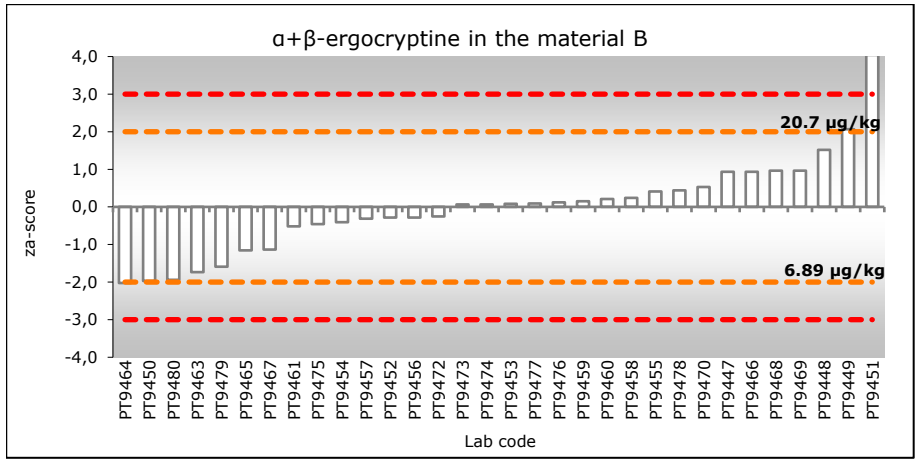

Figure 18 *Graphical representation of the z-scores for α+β-ergocryptine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

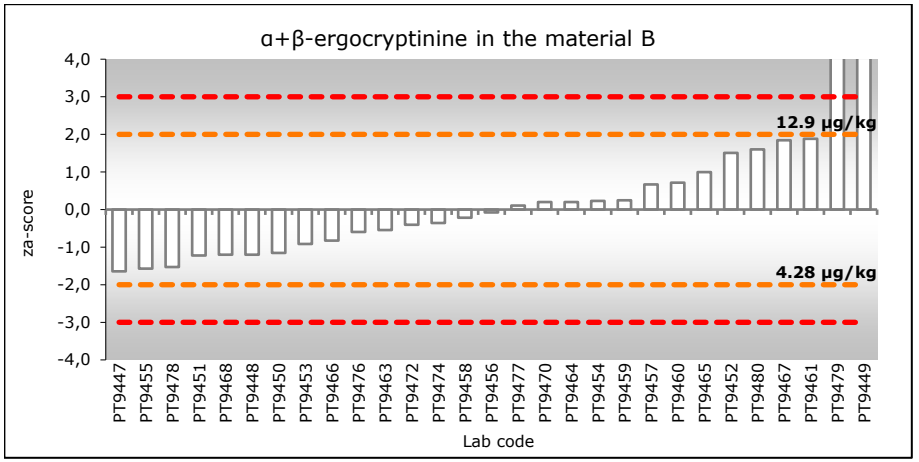

Figure 19 *Graphical representation of the z-scores for α+β-ergocryptinine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

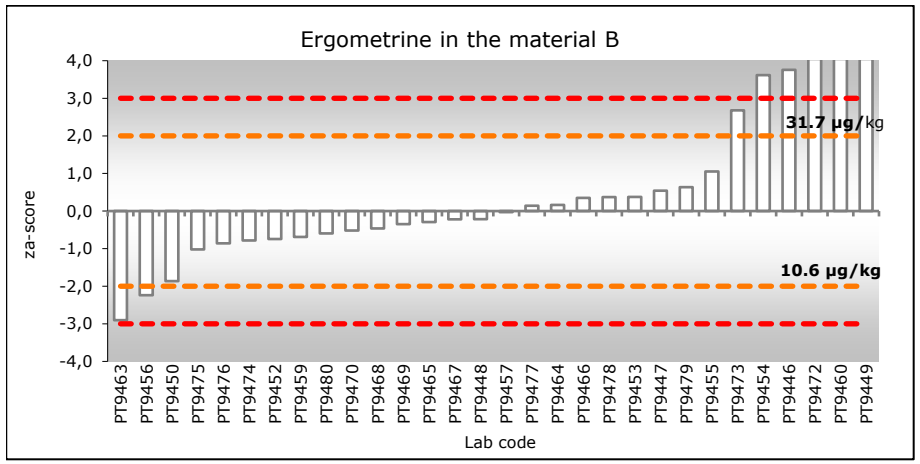

Figure 20 *Graphical representation of the z'-scores for ergometrine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

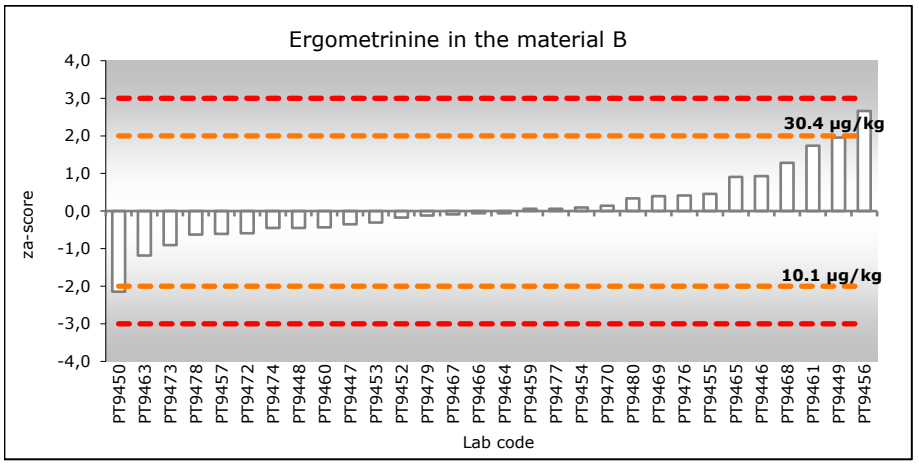

Figure 21 *Graphical representation of the z-scores for ergometrinine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

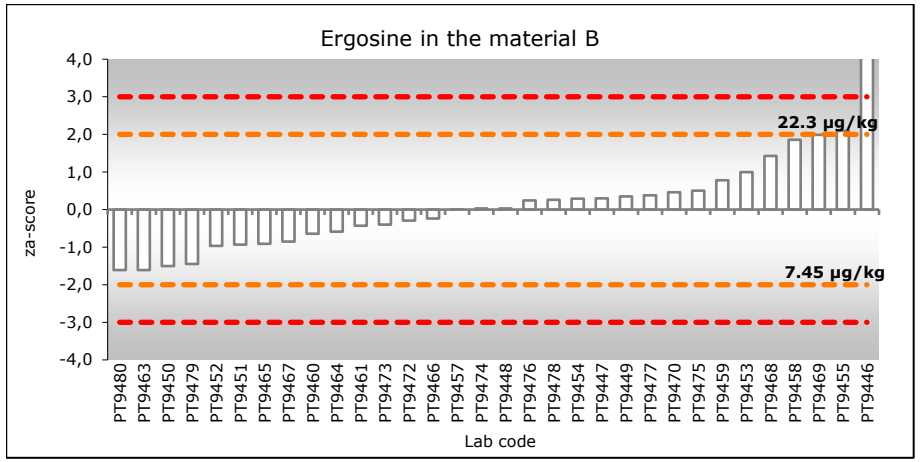

Figure 22 *Graphical representation of the z'-scores for ergosine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

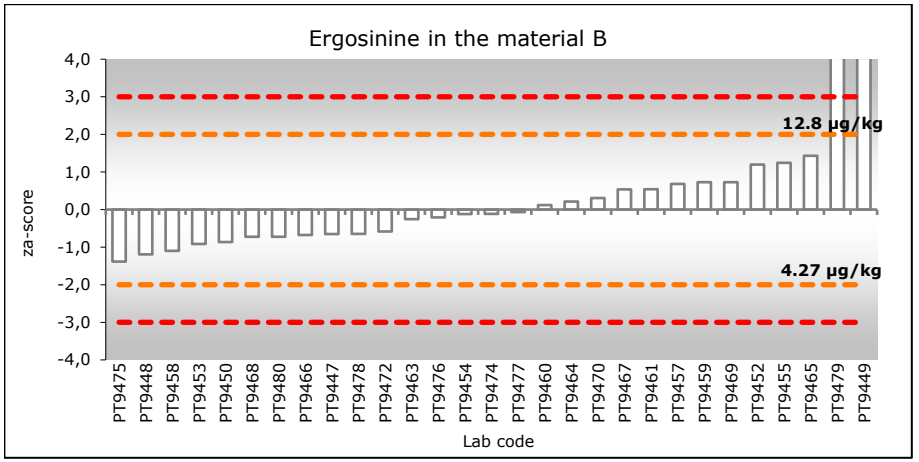

Figure 23 *Graphical representation of the z'-scores for ergosinine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

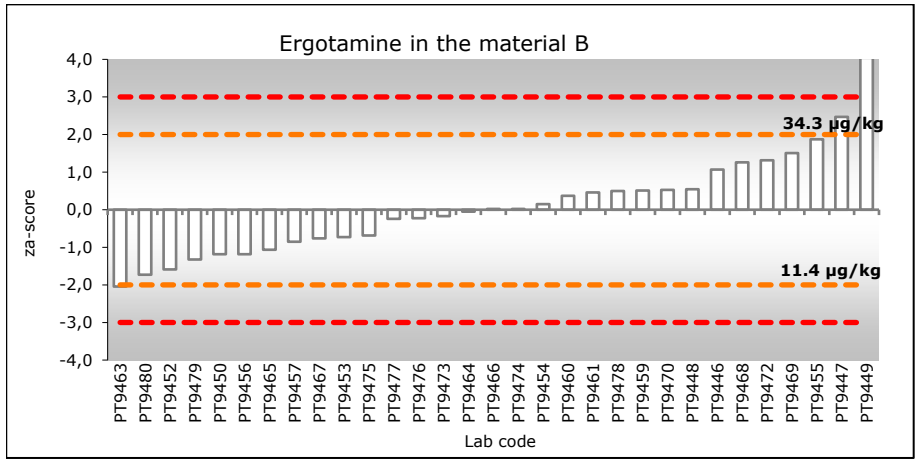

Figure 24 *Graphical representation of the z'-scores for ergotamine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

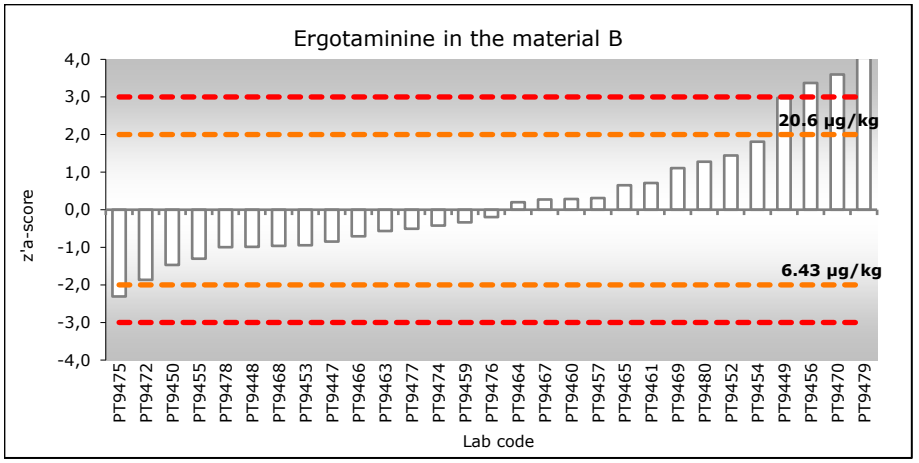

Figure 25 *Graphical representation of the z'-scores for ergotaminine in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

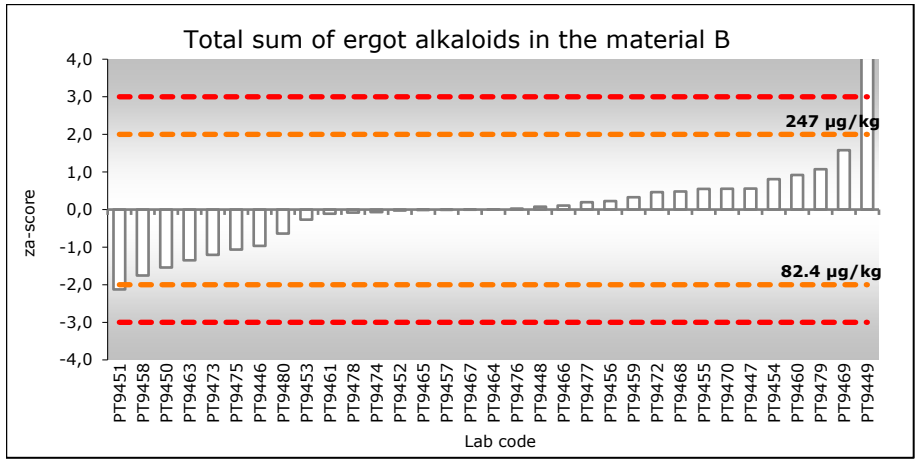

Figure 26 *Graphical representation of the z-scores for the total sum in the material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 11 Overview performance per participant

Participant code	Individual ergot alkaloids Satisfactory performance *	Total sum Satisfactory performance *, **
PT9446	13 of 24	**2 of 2**
PT9447	23 of 24	**2 of 2**
PT9448	**24 of 24**	**2 of 2**
PT9449	11 of 24	1 of 2
PT9450	21 of 24	**2 of 2**
PT9451	8 of 24	(0 of 2)
PT9452	**24 of 24**	**2 of 2**
PT9453	**24 of 24**	**2 of 2**
PT9454	23 of 24	**2 of 2**
PT9455	21 of 24	**2 of 2**
PT9456	9 of 24	(1 of 2)
PT9457	**24 of 24**	**2 of 2**
PT9458	13 of 24	**(2 of 2)**
PT9459	**24 of 24**	**2 of 2**
PT9460	22 of 24	**2 of 2**
PT9461	16 of 24	**(2 of 2)**
PT9463	20 of 24	**2 of 2**
PT9464	23 of 24	**2 of 2**
PT9465	22 of 24	**2 of 2**
PT9466	**24 of 24**	**2 of 2**
PT9467	20 of 24	**2 of 2**
PT9468	**24 of 24**	**2 of 2**
PT9469	17 of 24	**2 of 2**
PT9470	22 of 24	**2 of 2**
PT9472	22 of 24	**2 of 2**
PT9473	16 of 24	**2 of 2**
PT9474	**24 of 24**	**2 of 2**
PT9475	18 of 24	**2 of 2**
PT9476	**24 of 24**	**2 of 2**
PT9477	**24 of 24**	**2 of 2**
PT9478	**24 of 24**	**2 of 2**
PT9479	16 of 24	**2 of 2**
PT9480	22 of 24	**2 of 2**

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

** Scores of participants with an incomplete scope of analytes are between brackets.

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