Proficiency test for ergot alkaloids in cereals

EURLPT-MP08 (2022)

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Wageningen, 6 September 2023

WFSR Report 2023.010

This report can be downloaded for free at https://doi.org/10.18174/636591 or at www.wur.eu/food-safety-research (under WFSR publications).

WFSR report 2023.010

Distribution list:
• Participating laboratories
• Mr. F. Verstraete, European Commission, DG SANTE

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Summary

A proficiency test (PT) for the quantitative determination of ergot alkaloids (EAs) in the cereals wheat and rye was organised by the European Union Reference Laboratory for mycotoxins & plant toxins in food and feed (EURL-MP) between December 2022 and February 2023. This PT was carried out by Wageningen Food Safety Research (WFSR) under accreditation (R013, Dutch Accreditation Council RvA, ISO/IEC 17043:2010). In August 2021 Commission Regulation (EU) 2021/1399 on maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs was published. This regulation has now been integrated in Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food. The primary goal of this proficiency test was to assess the proficiency of the National Reference Laboratories for mycotoxins & plant toxins in food and feed (NRLs) and Official Laboratories (OLs) that participated.

The participants were asked to quantify EAs in the 2 materials and to report for each material 7 results, comprised of 6 groups of epimer pairs and the sum of total ergot alkaloids mentioned in the legislation. The participants' performance was assessed as z-score in both materials for the ergot epimer pairs groups (maximum score 12 out of 12) and for the sum of the EAs in the samples (maximum score 2 out of 2).

Thirty-three laboratories, of which 27 National Reference Laboratories for mycotoxins and/or plant toxins in food and feed (from 18 EU Member States plus Serbia and the EFTA MS Iceland, Norway and Switzerland) and 6 Official Laboratories (all EU Member States) participated in the PT.

Two materials were prepared. Material A consisted of wheat that was artificially spiked with a solution containing a mix of ergot alkaloids standards. Material B consisted of rye that was artificially spiked with a solution consisting of an extract from sclerotia containing ergot alkaloids. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of 50 gram of each material. The participants were requested to report their results within 6 weeks after the dispatch of the samples.

From the provided information on the identification and quantification of the EAs almost all participants used LC-MS/MS (28), except for two participants, of which one applied fluorescence detection and one reported to have used another detection technique. Out of 33 participants, one did not report results for ergometrine and ergometrinine. Twenty-five participants reported Limit of Quantification (LOQ) values of 4 µg/kg or less for individual EAs. Two participants reported LOQs of 5 µg/kg, one reported LOQs in the range of 10 to 15 µg/kg, one reported LOQs in the range of 25 to 50 µg/kg and 4 laboratories did not report LOQs.

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 proficiency of the participants was assessed as z-scores in both materials, calculated using the assigned values and a relative target standard deviation of 25%. Characteristics of the PT materials and the outcome of this PT are summarised in Table 1. Results were calculated for the 6 groups of epimer pairs and the sum of 12 EAs. For material A, the assigned values ranged from 6.85 to 9.98 µg/kg for the groups of epimer pairs. For material B, the assigned values ranged from 21.6 to 89.8 µg/kg. For both materials, the interlaboratory reproducibility (RSD_R) values of the reported results (ranging between 10-23%) were below the target standard deviation of 25%. For the sum of 12 EAs mentioned in legislation the RSD_R values were 19% and 14% for material A and B, respectively.

For both materials (A and B) combined, 90% of the results for the 6 groups of epimer pairs of EAs were rated with satisfactory z-scores ($|z| \leq 2$), 3% of the results fell into the questionable range with $2 < |z| < 3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$. Twenty-one participants achieved optimal performance for both materials by reporting for all the 6 groups of epimer pairs of EAs quantitative results that were satisfactory, the absence of false negative results and reporting within the indicated deadline. With respect to the sum of the 12 EAs, for both materials combined, 89% of submitted results were satisfactory

and 27 participants showed satisfactory performance for both materials. In this PT, 1 false negative result was reported.

From the results obtained in this PT on EAs it can be concluded that most of the participants have an adequate analytical method available that includes the 12 EAs mentioned in legislation and with sufficiently low LOQs. Two participants reported relatively high LOQs, but nevertheless reported values below the stated LOQs. Four participants could not quantify all 6 epimer pairs in material A, due to their relatively high LOQs.

Compared to the previous proficiency test on EAs (EURLPT-MP03), which was conducted in 2019, it can be concluded that progress has been made. All robust RSD_R values were below the target RSD_R of 25%. In part this may be attributed to the fact that in this PT the sum of the epimer pairs was reported, instead of the individual EAs.

Nevertheless, some room for improvement remains, as the results also reveal that a number of laboratories could not quantify some of the epimer pairs due to the relatively high LOQs of their method. Lowering the LOQs would be required for the analysis of cereal-based products for infants and young children, and in line with EFSA's recommendation for monitoring and to enable a better compound exposure evaluation.

Table 1a   *Summary of proficiency materials parameters and participants' performance – number of laboratories reporting quantitative values, <LOQ and false negative (FN).*

EA epimer groups	Matrix	Assigned value (µg/kg)	Uncertainty (µg/kg)	Robust RSD_R[1] (%)	No of labs out of 33 reporting Quant. value	<LOQ	FN
Sum epimers	A	6.85	0.222	14	29	4	
ergocornine/ergocorninine	B	39.0	1.26	15	33		
Sum epimers	A	7.54	0.360	21	31	2	
ergocristine/ergocristinine	B	71.5	1.55	10	33		
Sum epimers	A	7.86	0.410	23	30	3	
ergocryptine/ergocryptinine (sum of α- and β-form)	B	36.5	1.14	14	33		
Sum epimers	A	7.03	0.256	16	29	3	
ergometrine/ergometrinine	B	21.6	1.06	22	31	1	1
Sum epimers	A	8.16	0.335	19	32	1	
ergosine/ergosinine	B	48.3	1.26	12	33		
Sum epimers	A	9.98	0.472	21	32	1	
ergotamine/ergotaminine	B	89.8	3.68	19	33		
Sum 12 ergot alkaloids	A	46.8	1.96	19	33		
	B	307	9.53	14	33		

Matrix: A= wheat, B= rye.

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

Table 1b *Summary of proficiency materials parameters and participants' performance – evaluation of results, satisfactory, questionable and unsatisfactory z and z'-scores.*

EA epimer groups	Matrix	Assigned Value (µg/kg)	z-scores[1] Satisfact. (% of z or z-scores)	Quest. (% of z or z-scores)	Unsatisf. (% of z or z-scores)	Labs out of 33 with Accept. z -score No[2]	%[2]
Sum epimers	A	6.85	89.7	0.0	10.3	26	78.8
ergocornine/ergocorninine	B	39.0	97.0	0.0	3.0	32	97.0
Sum epimers	A	7.54	90.3	0.0	9.7	28	84.8
ergocristine/ergocristinine	B	71.5	87.9	9.1	3.0	29	87.9
Sum epimers	A	7.86	90.0	3.3	6.7	27	81.8
ergocryptine/ergocryptinine (sum of α- and β-form)	B	36.5	93.9	3.0	3.0	31	93.9
Sum epimers	A	7.03	79.3	6.9	13.8	23	69.7
ergometrine/ergometrinine	B	21.6	90.6	0.0	9.4	29	87.9
Sum epimers	A	8.16	84.4	6.3	9.4	27	81.8
ergosine/ergosinine	B	48.3	93.9	0.0	6.1	31	93.9
Sum epimers	A	9.98	90.6	3.1	6.3	29	87.9
ergotamine/ergotaminine	B	89.8	93.9	3.0	3.0	31	93.9
Sum 12 ergot alkaloids	A	46.8	84.8	6.1	9.1	28	84.8
	B	307	93.9	3.0	3.0	31	93.9

Matrix: A= wheat, B= rye.

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

2) 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 mycotoxins produced by fungi of the genus *Claviceps*, most notably by *C. purpurea*, which parasitize the seed heads of various cereals at the time of flowering. Fungal infections are most often found in rye, triticale, wheat, barley, oat and millet. The fungus replaces the developing grain or seed with a characteristic dark colored 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 and, when not properly removed, may 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 chickens. Because of this concern the European Commission (EC) has recently lowered the maximum level for ergot sclerotia and has established maximum levels for the total concentration of ergot alkaloids in certain food products as described in Commission Regulation (EU) 2021/1399 [14], which has recently been replaced by Commission Regulation (EU) 2023/915 [17].

For feed, harmonised EU regulation for rye ergot (*Claviceps purpurea*) in feed is laid down in Regulation (EU) No. 574/2011 amending Annex 1 to Directive 2002/32/EC [3]. It 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]. It should be noted that ergometrine and ergotamine are considered drug precursors and therefore classified as Category 1 substances requiring a license for their handling [15].

The following 12 EAs are included in Commission Regulation (EU) 2021/1399 (applicable during the PT) and the Commission Regulation (EU) 2023/915: ergocornine/ergocorninine, ergocristine/ergocristinine, ergocryptine/ergocryptinine (α- and β-isomers), ergometrine/ergometrinine, ergosine/ergosinine and ergotamine/ergotaminine and the maximum levels for the ergot alkaloids are related to the sum of these ergot alkaloids. The limit of quantification (LOQ) requirement for individual EAs included in the sum definition of ML is specified at ≤ 4 µg/kg for cereals and cereal-based foods and ≤ 2 for cereal-based food for infants and young children. This requirement is laid down in the EC working document SANTE 10673R3/2021, regulation on methods of sampling and analysis for the control of mycotoxins in food and repealing Regulation (EC) No 401/2006 [16]. However, this regulation is not yet formally endorsed or published.

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and is demanded by ISO/IEC 17025:2017 [5]. Organisation of proficiency tests (PT) is one of the tasks of European Union Reference Laboratories (EURLs) [6]. 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 (PT) focused on the EAs in food and feed matrices, using wheat and rye as representative matrices. The scope includes the 12 EAs, (ergocornine/ergocorninine, ergocristine/ergocristinine, α+β-ergocryptine/α+β-ergocryptinine, ergometrine/ergometrinine, ergosine/ergosinine, ergotamine/ergotaminine) as mentioned in Commission Regulation (EU) 2021/1399. The wheat and rye materials were spiked to reach target concentrations (see Table 2) taking the regulatory limits into account.

Table 2   *Target concentrations (µg/kg) of ergot alkaloids in the PT materials.*

	Target concentrations (µg/kg)	
Ergot alkaloid	**Material A (wheat)**	**Material B (rye)**
Ergocornine	5	30
Ergocorninine	2	7
Ergocristine	4	60
Ergocristinine	3	10
α-Ergocryptine	2	20
β-Ergocryptine	4	20
α+β-Ergocryptinine	4	6
Ergometrine	4	15
Ergometrinine	3	4
Ergosine	5	50
Ergosinine	3	7
Ergotamine	7	110
Ergotaminine	4	15
Sum of 12 EAs	50	354

2.2 Material preparation

Wheat and rye, respectively, were used for preparation of the two materials A and B. The grain samples were visually checked for the presence of sclerotia and these and other contaminations were removed. The cleaned materials were milled using a centrifugal mill (ZM 200, Retsch, Haan, the Netherlands) to obtain a particle size of 500 µm. For material A, contamination levels were artificially increased by spiking the material with EA standards and for material B, by spiking with an extract of ground sclerotia.

To prepare the materials, a premix was prepared by spiking part of the ground blank material, and then mixing it with a larger portion of blank material. For material A 50 mL of a solution of EA standards in acetone was prepared, aiming at the levels as presented in Table 2. For material B, an extract was prepared in the following way: 11 g of ground sclerotia was extracted with 55 mL of 0.4% formic acid in methanol on a rotary tumbler for 30 minutes followed by centrifugation (15 min, 3500 g). The extraction was repeated, and the extracts were combined. From this extract a final solution for spiking was prepared, by mixing 25 mL of the extract with 25 mL acetone. The premix for material A was prepared in the following way: 1000 grams of blank wheat was fortified by adding the solution of the EA standards. Premix B was fortified by adding the final solution of the sclerotia extract to 1000 grams of blank rye. After 30 min. premix A was mixed with 1 L of acetone and premix B was mixed with 800 mL of acetone and both were homogenised using an industrial mixer (supplier Topcraft) according to in-house standard operating procedures [9]. The fortified slurries were air dried in a fume hood and subsequently homogenised in a Stephan cutter UMC12 and stored in a freezer until use.

For the final materials, 3500 g blank material was mixed with 1000 g of the spiked premix. Materials A and B were homogenised by mixing in a rotating drum and were stored in a freezer until use. The homogenisation of the final materials was carried out at Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). WEPAL is accredited to ISO/IEC 17043 for the preparation of PT materials by the Dutch Accreditation Council (RvA, R002).

2.3 Sample identification

After homogenisation, materials A and B were divided into sub-portions of approximately 50 grams and stored in polypropylene, airtight closed containers in the freezer until use.

The samples for the participants were randomly selected and coded using a web application designed for proficiency tests. The code used was "2022/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, 10 containers of both materials were analysed in duplicate for the EAs (EURL-MP-method_003 v1) [9].

Method in brief: EAs were extracted from the homogenised sample (4 g) by addition of 40 ml 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 [10] and ISO 13528:2015 [11]. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) were compared with the standard deviation for proficiency assessment (σ_P). The method applied for homogeneity testing is considered suitable if $s_w < 0.5 \times \sigma_P$ and a material is considered adequately homogeneous if $s_s < 0.3 \times \sigma_P$. 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 materials A and B obtained during homogeneity testing.*

	Material A: wheat		Material B: rye	
	Conc.	RSD	Conc.	RSD
Compound	(µg/kg)	(%)	(µg/kg)	(%)
Ergocornine	4.32	5.97	25.8	3.28
Ergocorninine	2.19	7.27	15.0	4.00
Ergocristine	3.72	6.16	43.0	3.24
Ergocristinine	3.23	8.87	29.9	3.14
α-Ergocryptine	2.27	7.93	16.3	3.79
β-Ergocryptine	3.24	6.86	14.0	4.34
α+β-Ergocryptinine	3.79	5.66	16.1	3.44
Ergometrine	3.57	6.79	17.2	2.07
Ergometrinine	2.72	4.95	3.78	1.99
Ergosine	4.82	4.67	33.1	2.84
Ergosinine	2.66	5.00	15.8	2.67
Ergotamine	7.12	5.25	77.1	3.65
Ergotaminine	3.88	4.99	31.9	2.95
Sum of 12 EAs	47.5	4.44	339	2.70

2.5   Stability of the materials

The stability of the EAs in the materials was assessed according to [10, 11]. On December 12th, 2022, the day of distribution of the PT samples, 6 randomly selected containers of material A and B were stored in an ultra-freezer. Under these conditions it is assumed that the EAs are stable in the materials. In addition, 6 samples of each material were stored in a freezer.

On the 13th of February 2023, 63 days after distribution of the samples, 6 samples of materials A and B, stored in the ultra-freezer and freezer, 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 [10,11] in the materials stored in the freezer. A consequential instability is observed when the average value of an analyte in the samples stored in the freezer is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored in the ultra-freezer. 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. For the analytes in both materials none of the tested storage conditions caused a consequential difference. The EAs in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

This PT focused on the determination of EAs in food and feed, using wheat and rye. Invitations to the NRL network were sent out on November 14th, 2022 (Annex 5). Thirty-four laboratories registered for the PT and 33 participants (Annex 1) reported their results of which 1 reported their results after the deadline. One participant was unable to report results due to instrument problems. Out of 33 participating laboratories, 27 were NRLs from 18 EU Member States plus Iceland, Norway, Switzerland, Serbia and 6 were Official Laboratories (all EU Member States). Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report the results through a 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 and limit of quantification).

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding numbers, consisting of 2 coded samples (Annex 2) were sent to the participants on December 12th, 2022. The sample sets were dispatched immediately by courier to the participants in insulation boxes containing dry ice. The participants were asked to store the samples in the freezer and to analyse the samples according to their routine method. As reported by participants, all parcels 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, each participant received instructions by e-mail on how to use the web application to report the results. The questionnaire was intended to gather additional information on Limits of Quantification (LOQ), 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.

For each material a total of 7 results, comprised of 6 groups of epimer pairs of EAs and the sum of total EAs was requested. The deadline for submitting the quantitative results was February 6th, 2023, allowing the participants 7 weeks for analysis of the test samples. All results, except from 1 participant, were submitted within the deadline.

4 Evaluation of results

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [10], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [11] in combination with the insights published by the Analytical Methods Committee [12, 13] regarding robust statistics.

The evaluation of results was based on assigned values and the standard deviation for proficiency assessment (σ_P). From this, z-scores were calculated to classify the participants' performance. Detailed information on the methods used for the statistical evaluation can be found in the background document 'EURL-MP-background doc_001 (v1) Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed' which is available at from the EURL mycotoxins & plant toxins website [4].

4.1 Calculation of the assigned value

The robust mean was used as consensus value in this PT. The consensus value based on the participants' results (all participants, both NRLs and OLs) was used as the assigned value. The values and their uncertainties are summarised in Table 1 in the Summary section. Assigned values were established for all 6 groups of epimer pairs and for the sum of 12 EAs in both materials.

4.2 Standard deviation for proficiency assessment (σ_P)

A fixed relative target standard deviation for proficiency assessment of 25% was used, irrespective of the plant toxin, matrix or concentration. This generic fit-for-purpose value is considered to reflect current analytical capabilities and 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].

4.3 Quantitative performance (z-scores)

For evaluation of numerical results submitted by the participant, z-scores are calculated based on the assigned value, its uncertainty, and the standard deviation for proficiency assessment. When the uncertainty of the assigned value is negligible and no instability of the analytes in the material is observed, z-scores are calculated by:

$$z = \frac{x - C}{\sigma_p}$$

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.*

$\lvert z \rvert \leq 2$	Satisfactory
$2 < \lvert z \rvert < 3$	Questionable
$\lvert z \rvert \geq 3$	Unsatisfactory

If the uncertainty of the assigned value and, if applicable, instability of the analyte in the PT material, is not negligible, 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 [4]. In this PT, the uncertainty of the assigned value for all 6 groups of epimer pairs and the sum of total EAs in both materials A and B were negligible.

4.4 Evaluation of non-quantified results

In cases, where participant(s) reported '<[value]', 'detected' or 'not detected' (nd) (i.e. below their 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 of the background document 'EURL-MP-background doc_001' (for details see the EURL-MP website), using the reported LOQ value as a result [4]. Proxy-z-scores are for information only and are indicated as a value between brackets. Proxy-z-scores are not included in the evaluation of the results and do not count as a satisfactory result.

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

Reported results, e.g. 'detected' or 'not detected', without specification of LOQ, were excluded from the evaluation. In these cases, the participant was considered to have no quantitative method available for the specific analyte or analyte group/matrix. Non reported results for analytes or analyte groups are to be interpreted as unsatisfactory performance.

4.5 False positive and false negative results

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

When an analyte is present in the material, i.e. an assigned value has been established, and the participant reports the analyte as '<[value]', 'detected' 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 value (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 the quantification of EAs in wheat and rye. Annex 7 summarises the quantitative scope of each participant, with an indication of the LOQ for each EA. Four participants provided no details on the LOQs of the individual EAs.

Thirty-two participants reported for both material A and B a total of 7 results, comprised of 6 groups of epimer pairs of EAs and the sum of total EAs, as was requested. One participant reported for both materials a total of 5 results, due to lack of availability of the standards ergometrine, ergometrinine and ergotaminine. Therefore, for this participant the content of the epimer pair ergotamine/ergotaminine consists only of the concentration of ergotamine.

The LOQs provided by the participants ranged from 0.15 to 50 µg/kg. A large majority of the reported LOQs (25 participants) for individual EAs were below 4 µg/kg, some of them even below 1 µg/kg (Annex 7). All these participants reported LOQs that are in line with the upcoming regulation on methods of sampling and analysis of plant toxins which states that the LOQ should at least be 4 µg/kg for individual EAs (EC working document SANTE 10672R3/2021) [16]. Two participants reported LOQs of 5 µg/kg, one reported LOQs in the range of 10 to 15 µg/kg, one reported LOQs in the range of 25 to 50 µg/kg and 4 laboratories provided no LOQs. It should be noted that the two participants reporting the highest LOQs nevertheless reported results below their stated LOQs. It can be concluded that most participants are able to achieve LOQs of 4 µg/kg or lower, which is in line with the requirements of the (future) legislation. Some laboratories need to improve the sensitivity of their method for one or more EAs, in order to comply with the upcoming regulation on the methods of sampling and analysis of EAs in cereals.

5.2   Analytical methods

All participating laboratories were asked to fill in a questionnaire addressing their accreditation, conditions used for sample preparation, chromatographic separation, detection, quantification and calibration (Annex 8). Three participants provided no information about method details.

Out of the 30 laboratories, 19 participants reported their analytical method covered by ISO 17025 accreditation.

Based on the information provided on the laboratory sample preparation procedure, the median sample intake by the participants was 7.5 g; the most often reported intake was 5 g (9 participants). Six participants used 4 g or less, while 15 participants used 10 g or more.

The samples were extracted with 40 mL (median volume) of extraction solvent for approximately 30 min (median extraction time). The volumes most often used were 25 mL (8) and 100 mL (8). Most participants (16) reported an extraction time of 30 min, 4 participants used an extraction time between 3 and 20 min, 4 participants used 45 min, 5 participants used 60 min and 1 participant used 90 min.

For the extraction solvent participants used acetonitrile (23), ethyl acetate (5) or methanol (2) as the main organic phase. The composition of the extraction solvents was either alkaline aqueous/organic (21), acidic aqueous/organic (7) or organic (2). The most often used extraction solvent combinations were acetonitrile in combination with ammonium carbonate (15), acetonitrile in combination with formic or acetic acid (5), methanol in combination with formic acid (2) or ethyl acetate in combination with methanol/isopropanol and ammonia (5).

Solid phase extraction (SPE) was used by 8 participants for sample extract purification, 5 participants applied dispersive SPE (d-SPE) with primary secondary amine (PSA), 2 participants diluted the sample extract, 2 participants used liquid-liquid extraction (LLE), 5 participants used another clean-up, without providing details, and 7 participants reported that no clean-up was applied. The following cartridges were reported: Mycosep 150 Ergot (4), Sep-Pak Alumina B plus (2) and Chromabond Alox (2).

For the identification and quantification of the EAs almost all participants used LC-MS/MS (28). One participant applied fluorescence (FLD) detection and one reported to have used another detection technique.

For chromatography participants used either acetonitrile (25) or methanol (5) as an organic modifier in combination with an aqueous buffer. The majority of participants (25) used alkaline chromatography. For the preparation of the alkaline mobile phase the following buffers were used: ammonium carbonate (16), ammonium carbamate (5), ammonium bicarbonate (3) and ammonium hydroxide (1). Five participants used acidic chromatography: 2 used ammonium formate with or without addition of formic acid, 2 used ammonium acetate with addition of acetic acid and one used formic acid to acidify the mobile phase.

A wide variety of columns from different suppliers was used for chromatography under acidic or alkaline conditions. For methods applying acidic conditions, mostly columns with a C18 based stationary phase were used: Waters: Acquity UPLC BEH (2), Acquity UPLC HSS T3 (1); Thermo Scientific: Hypersil Gold (1). For methods applying alkaline conditions mostly C18 type stationary phases were used as well: Waters: Acquity BEH (12), XBridge (1); Phenomenex: Gemini (4), Kinetex EVO (1) and Agilent: Zorbax Eclipse XDB (1). In addition, the following non-C18 stationary phase columns were used by a number of participants: Phenomenex: Gemini C6 Phenyl (2), Kinetex phenyl/hexyl (2), Kinetex F5 pentafluorophenyl (1); Macherey Nagel: pentafluorophenyl-propyl (1); Supelco: Ascentis Express Phenyl-hexyl (1). The column length mostly used was either 100 mm (15) or 150 mm (7). The total run time reported varied between 5 and 50 min and the medium run time was 15 min.

The quantification approach followed by the participants is summarised in Table 5. Out of 30 participants, 24 used multi-level standard addition: 12 of them performed multi-level calibration with standards in a pure solvent, 9 used multi-level standard addition to the sample, 2 used multi-level standard addition before extraction and 1 after extraction. Three participants used a single-point standard addition approach; 2 of them added the standards before extraction and 1 added the standards after extraction. One participant provided no details if the standard addition approach was multi-level or single-point and two participants provided no details at all on the quantification approach. On questions about the correction of the results for recovery all participants except one participant replied. Fifteen participants (50%) have corrected their results for recovery while 14 (47%) reported that they didn't.

Table 5   *Analytical strategies followed by the participants.*

Quantification approach	Calibration/ quantification	No. of participants	Recovery		
			Corrected	Not corrected	Not reported
standard addition before extraction	single point	2		2	
standards in pure solvent	single point	2	1	1	
standards in pure solvent	multi-level	12	6	5	1
matrix-matched standards	multi-level	9	6	3	
standard addition before extraction	multi-level	2	1	1	
standard addition after extraction	multi-level	2	1	1	
standard addition before extraction	?	1		1	

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for the EAs in materials A (wheat) and B (rye) 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 all the cases the uncertainty of the assigned value did comply with the criterion $u \leq 0.3\sigma_p$ and was therefore considered as negligible.

Table 6   *Summary of statistical evaluation of the PT results on the groups of epimer pairs of EAs in material A.*

	Sum epimer pair ergocornine/ ergocorninine	Sum epimer pair ergocristine/ ergocristinine	Sum epimer pair ergocryptine/ergocryptinine (α- and β-form)	Sum epimer pair ergometrine/ ergometrinine
A (µg/kg)	6.85	7.54	7.86	7.03
u (µg/kg)	0.222	0.360	0.410	0.256
σ_p (µg/kg) (25%)	1.71	1.88	1.97	1.76
$u > 0.3\sigma_p$	No	No	No	No
robust σ (µg/kg)	0.958	1.60	1.79	1.10
robust σ (%)	14.0	21.2	22.8	15.7
# reported	33	33	33	32
"<", nd, detected	4	2	3	3
# quantitative results	29	31	30	29
$|z| \leq 2$	26	28	27	23
$2 < |z| < 3$	0	0	1	2
$|z| \geq 3$	3	3	2	4
FN	0	0	0	0
S z-scores (%)	89.7	90.3	90.0	79.3

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

	Sum epimer pair ergosine/ergosinine	Sum epimer pair ergotamine/ergotaminine	Sum 12 ergot alkaloids
A (µg/kg)	8.16	9.98	46.8
u (µg/kg)	0.335	0.472	1.96
σ_p (µg/kg) (25%)	2.04	2.50	11.7
$u > 0.3\sigma_p$	No	No	No
robust σ (µg/kg)	1.52	2.14	9.00
robust σ (%)	18.6	21.4	19.2
# reported	33	33	33
"<", nd, detected	1	1	0
# quantitative results	32	32	33
$|z| \leq 2$	27	29	28
$2 < |z| < 3$	2	1	2
$|z| \geq 3$	3	2	3
FN	0	0	0
S z-scores (%)	84.4	90.6	84.8

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

Table 7 *Summary of statistical evaluation of the PT results on the groups of epimer pairs of EAs in material B.*

	Sum epimer pair ergocornine/ ergocorninine	Sum epimer pair ergocristine/ ergocristinine	Sum epimer pair ergocryptine/ergocryptinine (α- and β-form)	Sum epimer pair ergometrine/ ergometrinine
A (µg/kg)	39.0	71.5	36.5	21.6
u (µg/kg)	1.26	1.55	1.14	1.06
σ_p (µg/kg) (25%)	9.76	17.9	9.13	5.40
$u > 0.3\sigma_p$	No	No	No	No
robust σ (µg/kg)	5.80	7.14	5.22	4.73
robust σ (%)	14.9	9.99	14.3	21.9
# reported	33	33	33	32
"<", nd, detected	0	0	0	1
# quantitative results	33	33	33	31
$|z| \leq 2$	32	29	31	29
$2 < |z| < 3$	0	3	1	0
$|z| \geq 3$	1	1	1	2
FN	0	0	0	1
S z-scores (%)	97.0	87.9	93.9	90.6

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

	Sum epimer pair ergosine/ergosinine	Sum epimer pair ergotamine/ergotaminine	Sum 12 ergot alkaloids
A (µg/kg)	48.3	89.8	307
u (µg/kg)	1.26	3.68	9.53
σ_p (µg/kg) (25%)	12.1	22.5	76.8
$u > 0.3\sigma_p$	No	No	No
robust σ (µg/kg)	5.77	16.9	43.8
robust σ (%)	12.0%	18.8	14.3
# reported	33	33	33
"<", nd, detected	0	0	0
# quantitative results	33	33	33
$|z| \leq 2$	31	31	31
$2 < |z| < 3$	0	1	1
$|z| \geq 3$	2	1	1
FN	0	0	0
S z-scores (%)	93.9	93.9	93.9

S z-scores = satisfactory z-scores.

FN= False negative.

nd= not detected.

For the 6 groups of epimer pairs in material A, 87% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 3% of the results fell into the questionable range with $2 < |z| < 3$ and 9% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). For material B was this respectively 93%, 3% and 5% (Table 7). Overall, 90% percent of the results obtained for both materials (A and B) were rated with satisfactory z-scores ($|z| \leq 2$), 3% of the results fell into the questionable range with $2 < |z| < 3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$.

In case of the sum of the 12 EAs mentioned in legislation, for material A, 85% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 6% of the results fell into the questionable range with $2 < |z| < 3$ and 9% of the results fell into the unsatisfactory range with $|z| \geq 3$ (Table 6). For the sum of 12 EAs in material B was this respectively 94%, 3% and 3% (Table 7). In case of the sum of EAs, for both materials, 89% of submitted results were satisfactory.

In Annex 11 an overview of the overall performance for each participant in this PT is provided. For the 2 materials combined, a maximum of 12 satisfactory z-scores, based on quantitative results for the 6 groups of epimer pairs of EAs could be obtained, and '12 out of 12' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. Out of 33 participants, 21 participants achieved optimal performance for both materials by detecting all groups of epimer pairs with correct quantification, the absence of false positive and false negative results and reporting within the deadline. For the other 12 participants either false negative results were reported, an incomplete scope of compounds was used, or one or more non-satisfactory z-scores were obtained. With respect to the sum of EAs mentioned in legislation, 27 participants showed satisfactory performance.

One false negative (FN) result was reported for material B for the sum of epimer pair ergometrine/ergometrinine. This FN could not be explained based on the LOQ provided by the participant, which was 5 µg/kg and the consensus value for the sum of this epimer pair was 21.6 µg/kg.

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 are included in for Annex 9, 10, in Tables 6 and 7 (Section 5.3) and in Table 1 (Summary section).

For both materials, all the RSD_R of the reported results were below the target standard deviation (25%). For material A, the RSD_R ranged between 14% and 23% and for material B it ranged between 10% and 22%.

The RSD_R values for the sum of the 12 EAs mentioned in legislation was for material A 19% and for material B it was 14%, both below the target standard deviation (25%).

6   Conclusions

Thirty-three laboratories, of which 27 National Reference Laboratories for mycotoxins and/or plant toxins in food or feed (from 18 EU Member States plus Iceland, Norway, Serbia and Switzerland) and 6 Official Laboratories (all EU Member States) participated in the PT on quantitative determination of the 12 EAs, as mentioned in Regulation (EU) 2021/1399, in wheat and rye.

Out of 33 participants, 32 reported a total of 14 results, comprised of 6 groups of epimer pairs of EAs and the sum of all EAs in the two samples, consisting of material A and material B, as was requested. Concerning the individual EAs included in the scope of the participants, one participant could not report results for ergometrine, ergometrinine and ergotaminine due to problems with the availability of these ergot standards. Twenty-five participants used a method with a reported LOQ for individual EAs of 4 µg/kg or lower. Two participants reported LOQs of 5 µg/kg, one reported LOQs in the range of 10 to 15 µg/kg, one reported LOQs in the range of 25 to 50 µg/kg. Nevertheless, the latter two participants reported results below their stated LOQs. Four laboratories did not report LOQs. Three laboratories had problems with reporting quantitative results for several epimeric pairs in material A, due to the relatively high LOQs of their method. Since NRLs are expected to have analytical methods in place not only for compliance testing of regulatory limits, but also in the framework of data generation for risk assessment, it is advised to set target LOQs of individual analytes to ≤4 µg/kg, at least for cereals and cereal-based foods and ≤2 µg/kg for cereal-based food for infants and young children.

The large majority of participants used methods based on LC-MS/MS (93%) either with or without clean-up. The most common clean-up step reported by the participants was use of SPE (45%).

For material A, for the 6 groups of EA epimer pairs, the percentage of satisfactory results varied from 79% to 91%. The RSD_R of the reported results ranged between 14% and 23%, all well below the target standard deviation of 25%. For the 6 groups of epimer pairs in material B, satisfactory results varied from 88% to 97% and the RSD_R (ranging between 10–22%) were also all below the target standard deviation of 25%.

With respect to the sum of 12 EAs considered in legislation, for material A and B, respectively, 85% and 94% of the results were satisfactory. The RSD_R for material A and B was 19% and 14%, respectively.

Overall, for the groups of epimer pairs in both materials combined (12 results), 90% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 3% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$. Twenty-one participants had a satisfactory performance. With respect to the sum of the 12 EAs, in both materials combined (2 results), 89% of submitted results were satisfactory and 27 participants had a satisfactory performance.

With respect to the previous proficiency test (EURLPT-MP03) it can be concluded that progress had been made. This can be seen from the fact that all robust RSD_R values were below the target RSD_R of 25%. In part this may be attributed to the fact that in this PT the sum of the epimer pairs was reported, instead of the individual EAs.

Nevertheless, some room for improvement remains, as the results also indicate that a number of laboratories could not quantify some of the epimer pairs due to the relatively high LOQs of their method. Lowering the LOQs would be required for the analysis of cereal-based products for infants and young children, and they would also be in line with EFSA's recommendation for monitoring and to enable a better exposure evaluation.

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Annex 1   List of participants

Country	Organisation
AUSTRIA	AGES GmbH
BELGIUM	CER Groupe
CROATIA	A. Stampar Teaching Institute of Public Health
CYPRUS	State General Laboratory
CZECH REPUBLIC	Czech Agriculture and Food Inspection Authority (CAFIA)
CZECH REPUBLIC	Central Institute for Supervising and Testing in Agriculture
DENMARK	Danish Veterinary and Food Administration
FINLAND	Finnish Food Authority
FINLAND	Finnish Customs Laboratory
FINLAND***	Natural Resources Institute Finland
FRANCE	SCL
FRANCE***	LABOCEA
GERMANY**	Eurofins WEJ Contaminants
GERMANY	Federal Institute fur Risk Assessment (BfR)
GERMANY***	CVUA Westfalen
GERMANY***	Lower Saxony State Office for Consumer Protection and Food Safety (LAVES)
GERMANY***	Landesuntersuchungsamt Rheinland-Pfalz, ILC Trier
GREECE	General Chemical State Laboratory
HUNGARY	National Food Chain Safety Office
IRELAND	The State Laboratory
IRELAND	The Public Analyst's Laboratory
ITALY***	IZSLER
ITALY	Istituto 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
SERBIA	SP Laboratoria A.D.
SLOVENIA	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
SWEDEN	National Food Agency
SWEDEN	Statens Veterinarmedicinska Anstalt
SWITZERLAND**	Kantonales Laboratorium Bern

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

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

*** Official Laboratory (OL).

Annex 2   Codification of the samples

Participant's code	Material A*	Material B*
PT8317	314	629
PT8318	871	213
PT8319	191	189
PT8320	320	802
PT8321	118	828
PT8322	580	887
PT8323	709	921
PT8324	872	427
PT8325	689	907
PT8326	702	553
PT8327	520	796
PT8328	864	744
PT8329	605	525
PT8330	780	961
PT8331	394	467
PT8332	941	222
PT8333	425	186
PT8334	569	715
PT8335	385	869
PT8336	855	924
PT8337	584	365
PT8338	306	704
PT8339	190	652
PT8340	803	124
PT8341	944	816
PT8342	448	541
PT8343	820	637
PT8344	967	223
PT8345	651	389
PT8346	526	983
PT8347	700	383
PT8348	456	880
PT8349	930	101
PT8350	721	193

* All sample codes start with 2022/EURL PT MP/EAs/.

Annex 3 Statistical evaluation of homogeneity data

Sample No.	Ergocornine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	4.72	4.14
Hom/A002	4.55	4.16
Hom/A003	3.94	4.62
Hom/A004	3.89	4.20
Hom/A005	4.51	4.68
Hom/A006	4.46	4.35
Hom/A007	3.90	4.41
Hom/A008	4.53	4.18
Hom/A009	4.04	4.37
Hom/A010	4.24	4.49
Grand mean	4.32	
Cochran's test		
C	0.284	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.08	
s_x	0.155	
s_w	2.88	
s_s	0.000	
Critical= 0.3 σ_P	0.324	
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	2.48	2.21
Hom/A002	2.14	2.08
Hom/A003	2.15	2.12
Hom/A004	1.98	2.12
Hom/A005	2.55	2.21
Hom/A006	2.19	2.36
Hom/A007	1.88	2.22
Hom/A008	2.02	2.33
Hom/A009	2.22	2.27
Hom/A010	2.09	2.10
Grand mean	2.19	
Cochran's test		
C	0.256	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.547	
s_x	0.119	
s_w	0.150	
s_s	0.054	
Critical= 0.3 σ_P	1.64	
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	4.09	3.65
Hom/A002	3.52	3.69
Hom/A003	3.70	4.12
Hom/A004	3.43	3.58
Hom/A005	3.99	4.02
Hom/A006	3.70	3.55
Hom/A007	3.49	4.04
Hom/A008	3.78	3.90
Hom/A009	3.42	3.54
Hom/A010	3.52	3.66
Grand mean	3.72	
Cochran's test		
C	0.380	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.930	
s_x	0.182	
s_w	0.199	
s_s	0.116	
Critical= 0.3 σ_P	0.279	
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.	Ergocristinine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	3.88	3.48
Hom/A002	3.69	2.91
Hom/A003	2.97	3.45
Hom/A004	2.93	3.02
Hom/A005	3.25	3.18
Hom/A006	3.07	2.97
Hom/A007	3.48	3.20
Hom/A008	3.33	3.37
Hom/A009	2.71	3.46
Hom/A010	3.15	3.20
Grand mean	3.23	
Cochran's test		
C	0.364	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.809	
s_x	0.201	
s_w	0.289	
s_s	0.000	
Critical= 0.3 σ_P	0.243	
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.

	α-Ergocryptine in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	2.55	2.15
Hom/A002	2.39	1.99
Hom/A003	2.15	2.65
Hom/A004	2.07	2.12
Hom/A005	2.56	2.27
Hom/A006	2.43	2.45
Hom/A007	2.05	2.21
Hom/A008	2.19	2.21
Hom/A009	2.26	2.31
Hom/A010	2.20	2.26
Grand mean	2.27	
Cochran's test		
C	0.362	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.569	
s_x	0.122	
s_w	0.187	
s_s	0.000	
Critical= 0.3 σ_P	0.171	
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.

	α-Ergocryptinine in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	4.12	3.70
Hom/A002	3.98	3.90
Hom/A003	3.68	3.80
Hom/A004	3.52	3.94
Hom/A005	4.20	3.90
Hom/A006	3.73	3.98
Hom/A007	3.51	3.64
Hom/A008	3.41	3.76
Hom/A009	3.52	4.02
Hom/A010	3.72	3.70
Grand mean	3.79	
Cochran's test		
C	0.278	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.947	
s_x	0.152	
s_w	0.213	
s_s	0.023	
Critical= 0.3 σ_P	0.284	
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.	β-Ergocryptine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	3.29	3.33
Hom/A002	3.41	3.09
Hom/A003	3.07	3.19
Hom/A004	2.71	3.07
Hom/A005	3.68	3.27
Hom/A006	3.24	3.30
Hom/A007	2.87	3.41
Hom/A008	3.13	3.52
Hom/A009	3.31	3.45
Hom/A010	3.13	3.41
Grand mean	3.24	
Cochran's test		
C	0.310	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.811	
s_x	0.160	
s_w	0.219	
s_s	0.039	
Critical= 0.3 σ_P	0.243	
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	3.84	3.29
Hom/A002	3.48	3.37
Hom/A003	3.24	3.50
Hom/A004	3.33	3.53
Hom/A005	3.87	3.85
Hom/A006	3.50	3.50
Hom/A007	3.39	3.79
Hom/A008	3.36	3.65
Hom/A009	3.44	4.04
Hom/A010	3.39	3.97
Grand mean	3.57	
Cochran's test		
C	0.268	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.892	
s_x	0.155	
s_w	0.261	
s_s	0.000	
Critical= 0.3 σ_P	0.267	
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	2.65	2.63
Hom/A002	2.80	2.53
Hom/A003	2.61	2.77
Hom/A004	2.52	2.93
Hom/A005	2.96	2.79
Hom/A006	2.78	2.76
Hom/A007	2.50	2.80
Hom/A008	2.66	2.80
Hom/A009	2.65	2.93
Hom/A010	2.63	2.77
Grand mean	2.72	
Cochran's test		
C	0.335	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.681	
s_x	0.072	
s_w	0.159	
s_s	0.000	
Critical= 0.3 σ_P	0.204	
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.	Ergosine in A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	5.05	4.98
Hom/A002	4.78	4.39
Hom/A003	4.47	5.21
Hom/A004	4.50	4.77
Hom/A005	5.03	4.72
Hom/A006	4.88	4.75
Hom/A007	4.62	5.11
Hom/A008	4.59	5.03
Hom/A009	4.89	4.99
Hom/A010	4.81	4.82
Grand mean	4.82	
Cochran's test		
C	0.418	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.21	
s_x	0.128	
s_w	0.258	
s_s	0.000	
Critical= 0.3 σ_P	0.362	
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	2.87	2.53
Hom/A002	2.51	2.54
Hom/A003	2.60	2.90
Hom/A004	2.54	2.82
Hom/A005	2.70	2.67
Hom/A006	2.53	2.58
Hom/A007	2.49	2.69
Hom/A008	2.54	2.85
Hom/A009	2.69	2.74
Hom/A010	2.56	2.79
Grand mean	2.66	
Cochran's test		
C	0.248	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.664	
s_x	0.074	
s_w	0.154	
s_s	0.000	
Critical= 0.3 σ_P	0.199	
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	7.47	7.11
Hom/A002	6.65	6.47
Hom/A003	6.56	7.53
Hom/A004	6.86	7.26
Hom/A005	7.48	7.62
Hom/A006	6.85	6.95
Hom/A007	6.70	7.39
Hom/A008	7.13	7.32
Hom/A009	6.91	7.72
Hom/A010	6.91	7.45
Grand mean	7.12	
Cochran's test		
C	0.345	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	1.78	
s_x	0.267	
s_w	0.369	
s_s	0.058	
Critical= 0.3 σ_P	0.534	
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.

	Ergotaminine in A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	4.26	3.91
Hom/A002	3.96	3.82
Hom/A003	3.73	4.22
Hom/A004	3.78	3.81
Hom/A005	4.09	3.82
Hom/A006	3.85	3.43
Hom/A007	3.68	3.77
Hom/A008	3.74	4.09
Hom/A009	3.76	3.94
Hom/A010	3.93	4.03
Grand mean	3.88	
Cochran's test		
C	0.295	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.970	
s_x	0.131	
s_w	0.202	
s_s	0.000	
Critical= 0.3 σ_P	0.291	
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.

	Ergocornine in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	26.4	25.2
Hom/B002	26.6	26.0
Hom/B003	25.3	26.3
Hom/B004	25.6	27.3
Hom/B005	25.3	25.4
Hom/B006	24.2	26.0
Hom/B007	26.0	24.2
Hom/B008	27.3	25.4
Hom/B009	24.8	26.4
Hom/B010	26.2	25.9
Grand mean	25.8	
Cochran's test		
C	0.198	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	6.45	
s_x	0.505	
s_w	0.950	
s_s	0.000	
Critical= 0.3 σ_P	1.94	
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	14.5	15.9
Hom/B002	15.2	15.8
Hom/B003	14.6	15.0
Hom/B004	15.4	15.7
Hom/B005	15.0	14.2
Hom/B006	13.6	14.9
Hom/B007	14.9	14.2
Hom/B008	15.8	15.4
Hom/B009	14.8	15.2
Hom/B010	15.5	15.3
Grand mean	15.0	
Cochran's test		
C	0.317	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.76	
s_x	0.473	
s_w	0.534	
s_s	0.285	
Critical= 0.3 σ_P	1.13	
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	43.3	42.6
Hom/B002	44.0	42.5
Hom/B003	43.1	42.8
Hom/B004	42.5	45.4
Hom/B005	44.5	40.7
Hom/B006	41.7	43.2
Hom/B007	42.9	39.3
Hom/B008	44.6	42.4
Hom/B009	42.4	43.3
Hom/B010	44.3	44.1
Grand mean	43.0	
Cochran's test		
C	0.309	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	10.7	
s_x	0.865	
s_w	1.53	
s_s	0.000	
Critral= 0.3 σ_P	3.22	
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.	Ergocristinine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	29.7	29.0
Hom/B002	31.4	30.2
Hom/B003	30.2	29.7
Hom/B004	29.6	30.6
Hom/B005	30.3	28.9
Hom/B006	28.2	30.9
Hom/B007	29.9	27.9
Hom/B008	30.6	29.5
Hom/B009	28.8	30.4
Hom/B010	30.7	30.8
Grand mean	29.9	
Cochran's test		
C	0.362	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.47	
s_x	0.603	
s_w	1.01	
s_s	0.000	
Critical= 0.3 σ_P	2.24	
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	15.7	16.3
Hom/B002	16.8	16.3
Hom/B003	15.5	16.0
Hom/B004	16.3	17.4
Hom/B005	16.6	16.1
Hom/B006	14.9	16.6
Hom/B007	17.1	15.3
Hom/B008	17.0	16.1
Hom/B009	16.3	16.6
Hom/B010	16.7	16.7
Grand mean	16.3	
Cochran's test		
C	0.335	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.08	
s_x	0.374	
s_w	0.689	
s_s	0.000	
Critical= 0.3 σ_P	1.22	
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	16.1	16.3
Hom/B002	16.1	16.1
Hom/B003	15.9	16.2
Hom/B004	16.2	16.6
Hom/B005	16.6	14.9
Hom/B006	15.6	16.2
Hom/B007	16.0	14.6
Hom/B008	16.9	16.4
Hom/B009	15.5	16.2
Hom/B010	16.4	16.4
Grand mean	16.1	
Cochran's test		
C	0.465	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.01	
s_x	0.387	
s_w	0.555	
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.	β-Ergocryptine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	13.6	13.8
Hom/B002	14.4	13.8
Hom/B003	13.8	14.2
Hom/B004	14.1	15.3
Hom/B005	14.3	12.8
Hom/B006	13.5	13.8
Hom/B007	13.7	13.0
Hom/B008	15.1	13.7
Hom/B009	13.5	14.0
Hom/B010	14.6	14.4
Grand mean	14.0	
Cochran's test		
C	0.312	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.49	
s_x	0.450	
s_w	0.578	
s_s	0.189	
Critical= 0.3 σ_P	1.05	
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 B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	17.7	17.4
Hom/B002	17.2	16.7
Hom/B003	17.5	17.7
Hom/B004	17.2	17.1
Hom/B005	17.3	17.2
Hom/B006	16.6	17.5
Hom/B007	16.9	16.5
Hom/B008	17.3	17.4
Hom/B009	16.7	16.9
Hom/B010	17.0	17.3
Grand mean	17.2	
Cochran's test		
C	0.555	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	4.29	
s_x	0.301	
s_w	0.276	
s_s	0.229	
Critical= 0.3 σ_P	1.29	
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	3.73	3.82
Hom/B002	3.66	3.87
Hom/B003	3.81	3.70
Hom/B004	3.83	3.91
Hom/B005	3.72	3.74
Hom/B006	3.70	3.82
Hom/B007	3.75	3.72
Hom/B008	3.78	3.80
Hom/B009	3.72	3.83
Hom/B010	3.80	3.96
Grand mean	3.78	
Cochran's test		
C	0.367	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	0.946	
s_x	0.051	
s_w	0.078	
s_s	0.000	
Critical= 0.3 σ_P	0.284	
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.

	Ergosine in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	34.6	34.0
Hom/B002	33.2	33.5
Hom/B003	32.6	33.2
Hom/B004	34.0	33.0
Hom/B005	33.3	32.7
Hom/B006	31.1	33.9
Hom/B007	32.4	31.4
Hom/B008	34.2	33.1
Hom/B009	32.5	33.1
Hom/B010	32.1	34.3
Grand mean	33.1	
Cochran's test		
C	0.448	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	8.28	
s_x	0.672	
s_w	0.933	
s_s	0.123	
Critical= 0.3 σ_P	2.48	
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.

	Ergosinine in B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	15.8	16.2
Hom/B002	15.9	16.2
Hom/B003	15.3	16.2
Hom/B004	16.0	16.2
Hom/B005	15.9	15.8
Hom/B006	14.8	15.5
Hom/B007	15.5	15.0
Hom/B008	15.5	16.2
Hom/B009	15.6	16.0
Hom/B010	15.8	16.2
Grand mean	15.8	
Cochran's test		
C	0.304	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	3.94	
s_x	0.328	
s_w	0.379	
s_s	0.190	
Critical= 0.3 σ_P	1.18	
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	75.7	77.9
Hom/B002	78.0	79.5
Hom/B003	77.0	83.4
Hom/B004	77.1	78.7
Hom/B005	78.2	74.0
Hom/B006	71.0	78.5
Hom/B007	77.0	71.4
Hom/B008	78.4	79.7
Hom/B009	74.6	78.0
Hom/B010	76.9	77.9
Grand mean	77.1	
Cochran's test		
C	0.332	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	19.3	
s_x	1.91	
s_w	2.92	
s_s	0.000	
Critical= 0.3 σ_P	5.79	
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.	Ergotaminine in B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	32.5	32.1
Hom/B002	32.6	32.7
Hom/B003	31.6	31.8
Hom/B004	31.8	32.8
Hom/B005	32.3	29.8
Hom/B006	30.8	31.6
Hom/B007	32.2	29.8
Hom/B008	33.0	32.1
Hom/B009	30.9	32.4
Hom/B010	32.9	32.1
Grand mean	31.9	
Cochran's test		
C	0.345	
Ccrit	0.602	
C < Ccrit?	NO OUTLIERS	
Target s = σ_P	7.97	
s_x	0.653	
s_w	0.955	
s_s	0.000	
Critical= 0.3 σ_P	2.39	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Annex 4 Statistical evaluation of stability data

Stability evaluation for ergocornine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	4.56	4.48
	4.53	4.44
	4.46	4.48
	4.46	4.17
	4.56	4.44
	4.45	4.47
Average amount (µg/kg)	4.50	4.41
n	6	6
st. dev (µg/kg)	0.053	0.121
Difference		0.092
$0.3 * \sigma_P$		0.338
Consequential difference? Diff < $0.3 * \sigma_P$		No

Stability evaluation for ergocorninine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	2.54	2.50
	2.60	2.47
	2.42	2.43
	2.59	2.50
	2.48	2.39
	2.47	2.67
Average amount (µg/kg)	2.51	2.50
n	6	6
st. dev (µg/kg)	0.072	0.097
Difference		0.019
$0.3 * \sigma_P$		0.189
Consequential difference? Diff < $0.3 * \sigma_P$		No

Stability evaluation for ergocristine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.71	3.99
	3.95	3.65
	3.71	3.84
	3.77	3.68
	3.92	3.94
	3.96	4.03
Average amount (µg/kg)	3.84	3.85
n	6	6
st. dev (µg/kg)	0.120	0.161
Difference		-0.019
$0.3 * \sigma_P$		0.288
Consequential difference? Diff < $0.3 * \sigma_P$		No

Stability evaluation for ergocristinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.40	3.27
	3.46	3.16
	3.11	3.37
	3.19	3.04
	3.24	3.38
	3.27	3.22
Average amount (µg/kg)	3.28	3.24
n	6	6
st. dev (µg/kg)	0.131	0.129
Difference		0.039
$0.3 \cdot \sigma_P$		0.246
Consequential difference? Diff < $0.3 \cdot \sigma_P$		No

Stability evaluation for α-ergocryptine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	2.58	2.46
	2.42	2.39
	2.27	2.36
	2.33	2.22
	2.30	2.35
	2.49	2.35
Average amount (µg/kg)	2.40	2.36
n	6	6
st. dev (µg/kg)	0.120	0.078
Difference		0.043
$0.3 \cdot \sigma_P$		0.180
Consequential difference? Diff < $0.3 \cdot \sigma_P$		No

Stability evaluation for α-ergocryptinine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.61	3.85
	3.85	3.78
	3.50	3.74
	3.73	3.75
	3.73	3.91
	3.65	3.75
Average amount (µg/kg)	3.68	3.80
n	6	6
st. dev (µg/kg)	0.121	0.071
Difference		-0.116
$0.3 \cdot \sigma_P$		0.276
Consequential difference? Diff < $0.3 \cdot \sigma_P$		No

Stability evaluation for β-ergocryptine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.31	3.62
	3.74	3.44
	3.30	3.78
	3.57	3.34
	3.58	3.84
	3.49	3.56
Average amount (µg/kg)	3.50	3.59
n	6	6
st. dev (µg/kg)	0.171	0.191
Difference		-0.096
$0.3*\sigma_P$		0.262
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.68	3.88
	3.77	3.64
	3.84	3.85
	3.84	3.66
	3.78	3.69
	3.78	3.74
Average amount (µg/kg)	3.78	3.74
n	6	6
st. dev (µg/kg)	0.057	0.101
Difference		0.037
$0.3*\sigma_P$		0.284
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrinine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.05	3.10
	3.13	3.09
	3.13	3.09
	3.08	3.07
	3.08	2.96
	3.03	3.09
Average amount (µg/kg)	3.08	3.07
n	6	6
st. dev (µg/kg)	0.040	0.056
Difference		0.017
$0.3*\sigma_P$		0.231
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	4.83	5.17
	5.06	4.84
	5.07	4.85
	4.93	4.86
	5.05	4.91
	4.77	4.95
Average amount (µg/kg)	4.95	4.93
n	6	6
st. dev (µg/kg)	0.129	0.124
Difference		0.019
$0.3*\sigma_P$		0.371
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosinine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	2.75	2.98
	2.81	2.53
	2.81	2.80
	2.78	2.68
	2.73	2.88
	2.85	2.82
Average amount (µg/kg)	2.79	2.78
n	6	6
st. dev (µg/kg)	0.044	0.157
Difference		0.004
$0.3*\sigma_P$		0.209
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotamine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	6.92	6.91
	6.85	6.37
	6.64	6.79
	6.90	6.79
	6.63	6.95
	7.05	6.94
Average amount (µg/kg)	6.83	6.79
n	6	6
st. dev (µg/kg)	0.166	0.217
Difference		0.042
$0.3*\sigma_P$		0.513
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotaminine in material A.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	3.64	3.58
	3.71	3.46
	3.40	3.63
	3.58	4.07
	3.79	3.74
	3.76	3.74
Average amount (µg/kg)	3.65	3.70
n	6	6
st. dev (µg/kg)	0.145	0.210
Difference		-0.059
$0.3*\sigma_P$		0.273
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocornine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	25.8	24.7
	27.2	25.8
	26.0	26.0
	26.3	25.5
	25.9	26.0
	25.7	27.0
Average amount (µg/kg)	26.1	25.8
n	6	6
st. dev (µg/kg)	0.575	0.750
Difference		0.317
$0.3*\sigma_P$		1.96
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocorninine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	14.1	14.4
	14.9	14.6
	14.5	14.9
	14.5	14.4
	15.3	14.9
	14.6	14.9
Average amount (µg/kg)	14.6	14.7
n	6	6
st. dev (µg/kg)	0.391	0.261
Difference		-0.037
$0.3*\sigma_P$		1.10
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	40.5	43.2
	43.7	41.2
	41.3	44.1
	44.0	43.2
	42.4	41.7
	42.6	43.0
Average amount (µg/kg)	42.4	42.7
n	6	6
st. dev (µg/kg)	1.350	1.081
Difference		-0.303
$0.3*\sigma_P$		3.18
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergocristinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	28.1	27.6
	29.2	28.0
	27.5	29.0
	28.5	28.9
	28.5	28.3
	27.6	28.8
Average amount (µg/kg)	28.2	28.4
n	6	6
st. dev (µg/kg)	0.608	0.559
Difference		-0.182
$0.3*\sigma_P$		2.12
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	16.2	16.4
	16.7	15.7
	15.3	16.6
	16.3	17.1
	16.9	16.6
	15.7	16.6
Average amount (µg/kg)	16.2	16.5
n	6	6
st. dev (µg/kg)	0.607	0.437
Difference		-0.307
$0.3*\sigma_P$		1.21
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for α-ergocryptinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	13.5	13.8
	14.4	13.9
	13.9	13.8
	14.2	14.0
	14.3	14.0
	13.8	14.2
Average amount (µg/kg)	14.0	13.9
n	6	6
st. dev (µg/kg)	0.361	0.131
Difference		0.068
$0.3*\sigma_P$		1.05
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for β-ergocryptinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	15.3	16.0
	16.6	15.1
	15.7	16.6
	15.4	16.1
	16.4	15.3
	15.6	16.8
Average amount (µg/kg)	15.8	16.0
n	6	6
st. dev (µg/kg)	0.513	0.716
Difference		-0.140
$0.3*\sigma_P$		1.19
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	18.1	18.8
	19.0	17.9
	18.2	17.2
	18.5	18.4
	18.4	18.4
	17.7	18.8
Average amount (µg/kg)	18.3	18.3
n	6	6
st. dev (µg/kg)	0.426	0.588
Difference		0.034
$0.3*\sigma_P$		1.37
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergometrinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	4.16	4.22
	4.23	4.23
	4.25	4.26
	4.05	4.21
	4.24	4.28
	4.26	4.16
Average amount (µg/kg)	4.20	4.22
n	6	6
st. dev (µg/kg)	0.079	0.041
Difference		-0.024
$0.3*\sigma_P$		0.315
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	32.7	33.2
	32.7	32.0
	32.3	33.6
	33.6	34.0
	32.9	34.3
	32.9	35.7
Average amount (µg/kg)	32.9	33.8
n	6	6
st. dev (µg/kg)	0.419	1.23
Difference		-0.929
$0.3*\sigma_P$		2.46
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergosinine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	14.3	14.8
	15.2	14.9
	14.8	15.0
	15.2	15.3
	15.3	14.8
	14.2	15.3
Average amount (µg/kg)	14.9	15.0
n	6	6
st. dev (µg/kg)	0.493	0.242
Difference		-0.171
$0.3*\sigma_P$		1.11
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotamine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	72.6	74.8
	75.7	73.0
	72.8	75.2
	75.3	78.5
	75.9	74.2
	74.7	74.2
Average amount (µg/kg)	74.5	75.0
n	6	6
st. dev (µg/kg)	1.46	1.88
Difference		-0.486
$0.3*\sigma_P$		5.59
Consequential difference? Diff < $0.3*\sigma_P$		No

Stability evaluation for ergotaminine in material B.

Storage temperature	Ultra-freezer	freezer
Time (days)	0	63
Calculated amounts (µg/kg)	27.9	28.0
	29.5	28.3
	27.9	28.8
	28.4	30.7
	29.4	29.5
	28.0	29.0
Average amount (µg/kg)	28.5	29.0
n	6	6
st. dev (µg/kg)	0.729	0.978
Difference		-0.515
$0.3*\sigma_P$		2.14
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Invitation letter

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

NRLs mycotoxins & plant toxins

Dear colleague,

The EURL mycotoxins & plant toxins, at Wageningen Food Safety Research (WFSR), will organize a proficiency test regarding ergot alkaloids in cereals in 2022 (EURLPT-MP08). This proficiency test will focus on the quantification of 12 ergot alkaloids as mentioned in Regulation (EU) 2021/1399 and will be organised under accreditation according to ISO 17043 (General requirements for proficiency testing - R013).

I would like to invite you to participate in this proficiency test.

Harmonised EU regulation for rye ergot (*Claviceps purpurea*) in feed is laid down Regulation (EU) No. 574/2011 amending Annex 1 to Directive 2002/32/EC and for sclerotia and ergot alkaloids in food in Regulation (EU) 2021/1399. The primary goal of this proficiency test is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of ergot alkaloids in food and feed matrices.

According to Regulation (EU) 2017/625, it is mandatory for all EU National Reference Laboratories (NRLs) mycotoxins & plant toxins in food and/or feed to participate.

The following matters are important for participation in this proficiency test:

1. Test materials
   Two test materials, rye and wheat will be provided.
   The test amount sent for each material will be approximately 50 gram.

2. Shipment of the test materials
   Test materials will be sent in December 2022. The distribution of the test materials will be announced by e-mail. The deadline for reporting will be eight weeks after the shipment of the samples.

3. Scope of the analysis
   The materials contain one or more of the following analytes, as defined in Regulation (EU) 2021/1399:
   - ergocornine/ergocorninine
   - ergocristine/ergocristinine
   - ergocryptine/ergocryptinine (α- and β-form)
   - ergometrine/ergometrinine
   - ergosine/ergosinine

ergotamine/ergotaminine
The participants should provide their own analytical standards.

4. Questionnaire

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

5. Report

- Preliminary results of this proficiency test will be reported to the participants in May 2023.
- The report is expected to be published in September 2023.
- Results of the proficiency test will be presented anonymously.
- Disclosure of the results of the NRLs to the representative of the European Commission is foreseen after the report is published.
- The follow-up protocol on proficiency test from DG Santé will be applied.

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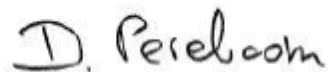
- WFSR is allowed to use the anonymous results of the proficiency test in presentations, seminars and publications.
- WFSR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

7. Costs

- Participation is free of charge for NRLs.
- Official laboratories (OLs) can participate as long as sufficient test material is available, at a first come first serve basis. The participation fee for OLs 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 December 2nd 2022**: pt.wfsr@wur.nl.

Looking forward to welcome you for this proficiency test,

D. Pereboom

Diana Pereboom
Proficiency tests

EURL mycotoxins & plant toxins in food and feed
Wageningen Food Safety Research

Annex 6   Instruction letter

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

Wageningen Food Safety Research

DATE
December 12, 2022

SUBJECT
Instructions proficiency test
ergot alkaloids in cereals

YOUR REFERENCE
2220769/WFSR

OUR REFERENCE
WFSR/EURLPT-MP08/2022

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/rikilt

CoC NUMBER
09098104

HANDLED BY
Diana Pereboom

TELEPHONE
+31 (0) 614323017

EMAIL
pt.wfsr@wur.nl

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of ergot alkaloids in cereals.

The parcel shipped to you should contain:
- One material consisting of rye flour and one material consisting of wheat flour. 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.
- Quantify the 12 ergot alkaloids as defined in Commission Regulation (EU) 2021/1399.
- Report for each material a total of seven results, comprised of six groups of epimer pairs and the sum of total ergot alkaloids:
  1. Sum of the epimer pair ergocornine/ergocorninine
  2. Sum of the epimer pair ergocristine/ergocristinine
  3. Sum of the epimer pair ergocryptine/ergocryptinine (α- and β-form)
  4. Sum of the epimer pair ergometrine/ergometrinine
  5. Sum of the epimer pair ergosine/ergosinine
  6. Sum of the epimer pair ergotamine/ergotaminine
  7. Sum of the 12 ergot alkaloids

DATE
December 12, 2022

OUR REFERENCE
WFSR/EURLPT-MP08/2022

PAGE
2 of 2

- Reporting:
  - Report all analytical results in **µg/kg**
  - Report other results as '**<[value µg/kg]**' or '**nt**' as follows:
    - '**<[value in µg/kg]**': When the result for the analyte is below the LOQ of the method, report the result as below the LOQ value in µg/kg, e.g. as '<10 µg/kg'.
    - '**nt**': If an analyte is not included in the scope of the method, report the result as not tested, 'nt'.
- Results reported in any other format (e.g. nd, detected, <LOQ, etc) will be regarded as not tested, 'nt'.
- Please use the web application to submit the results for the test samples (https://crlwebshop.wur.nl/ordsp/f?p=107:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- 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 6ᵗʰ of February 2022.**

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

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

With kind regards,

D. Pereboom

Diana Pereboom
Proficiency tests

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

Annex 7   Scope and LOQ

Lab code	LOQ (µg/kg)													
	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	β-Ergo-cryptine	α-Ergo-cryptinine	β-Ergo-cryptinine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine
PT8317	5	5	5	5	5	5	5	5	5	5	5	5	5	5
PT8318	50	50	50	50	25	25	25	25	25	25	25	25	25	25
PT8319	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT8320	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT8321	0.5	0.5	0.5	0.5	0.5		0.5		4	0.5	0.5	2	0.5	0.5
PT8322	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
PT8323	0.5	0.5	1.0	1.0	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5	0.5
PT8324	2	2	2	2	2		2		2	2	2	2	2	2
PT8325														
PT8326	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PT8327	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PT8328	3	3	3	3	3		3		3	3	3	3	3	3
PT8329	4	4	4	4	4		4		4	4	4	4	4	4
PT8330	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PT8331														
PT8332	0.35	0.2	0.39	0.38	0.15	0.15	0.29	0.29	0.36	0.29	0.34	0.29	0.36	0.31
PT8333	2	2	2	2	2		2		2	2	2	2	2	2
PT8334	2.8	0.6	2.5	0.6	2.7	2.7	0.6	0.6	2.2	0.6	2.7	0.5	2.7	0.6
PT8335	1	1	1	1	1		1		1	1	1	1	1	1
PT8336	0.5	0.5	0.5	0.5	0.5		0.5		0.5	0.5	0.5	0.5	0.5	0.5
PT8337	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
PT8338	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PT8339	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
PT8340	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PT8341	5	5	5	5	5		5		5	5	5	5	5	5
PT8342	1.0	1.0	1.0	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
PT8343														
PT8344														
PT8346	2	2	2	2	2		2	2	2	2	2	2	2	2
PT8347	10	10	10	10	10		10		15	15	10	10	10	10
PT8348	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PT8349	2	2	2	2	2		2		1.5	1.5	1.5	1.5	1.5	1.5
PT8350	1	1	1	1	1		1		1	1	1	1	1	1

Lab code	Method	Column	Column length (mm)	Total run time (min)	Retention time (min) Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	α-Ergocryptine	β-Ergocryptine	α-Ergocryptinine	β-Ergocryptinine	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine
PT8317	acid	Waters, Acquity BEH C18		15	7.2	7.7	8.2	9	7.8	7.85	7.9	7.95	2.2	2.3	6.8	6.9	7.1	7.2
PT8318	acid	Phenomenex, Kinetex®F5, pore size, 100 x 2.1 mm, 1.7 µm	100	21	9.07	9.48	9.58	10.03	9.5	9.5	9.86	9.86	5.5	6.63	8.82	8.66	8.98	8.75
PT8319	alkaline	Waters, Acquity UPLC BEH C18, 1.7 µm		11	5.3	6.8	5.8	7.4	5.7	5.8	7.3	7.3	1.7	2.8	4.5	6.1	4.7	6.4
PT8320	alkaline	Waters, XBridge C18, 150 x 3.0 mm, 5 µm	150	20	9.3	11.5	10.4	13	10.2	10.5	13.5	13.5	4.9	6.2	8	10.5	8.4	10.9
PT8321	alkaline	Waters, Acquity BEH C18, 100 x 2.1 mm, 1.7 µm	100	7														
PT8322	alkaline	Waters, BEH C18, 100 x 2.1 mm, 1.8 µm	100	10	3.09	4.84	3.61	5.80	3.48	3.61	5.53	5.57	1.57	1.82	2.46	3.92	2.59	4.27
PT8323	alkaline	Waters, ACQUITY Premier BEH C18, 100 x 2.1 mm, 1.7 µm	100	12	3.96	5.64	4.61	6.21	4.44	4.58	6.05		2.05	2.39	3.21	4.94	3.40	5.28
PT8324	acid	Waters, Acquity UPLC HSS T3, 1.8 µm		11	6.86	7.54	7.17	7.84	7.12		7.72		3.86	4.31	6.49	7.06	6.63	7.26
PT8325	alkaline	Waters, Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7 µm	100	15														
PT8326	alkaline	Phenomenex, Gemini NX-C18, 100 x 4.6 mm, 2.6 µm	100	14	6.62	9.87	7.58	11.78	7.4		11.39		3.78	4.58	5.36	7.99	5.61	8.73
PT8327	alkaline	Macherey Nagel PFP, 125 x 3 mm	125	10	5.2	7	6	7.8	5.8	5.8	7.5	7.5	2.05	2.95	3.55	5.3	3.75	5.8
T8328	alkaline	Agilent, Zorbax Eclipse XDB-C18		50	8.00	18.7	11	28	9.9	10.5	24	24	2.7	3.5	2.3	13.4	5.9	16.4
PT8329	alkaline	Supelco, Ascentis Express Phenyl-hexyl, 100 x 2.1 mm, 2.7 µm	100	14	7.15	8.9	7.8	10	7.55		9.5		3.4	4.55	6.25	8.15	6.65	8.55
PT8330	alkaline	Waters, Acquity BEH C18, 100 x 2.1 mm, 1.7 µm	100	11	4.79	5.98	5.2	6.44	5.09	5.19	6.31		2.49	3.08	4.19	5.44	4.36	5.68
PT8331	alkaline	Waters, Acequity UPLC BEH C18, 150 X 2.1 mm, 1.7 µm	150	30	20.92	24.03	22.02	25.41	21.76		25.03		12.1	15.08	19.26	22.63	19.79	23.22
PT8332	alkaline	Waters, Aquity UPLC BEH, 100 x 2.1 mm, 1.7 µm	100	12	3.25	4.96	3.8	5.59	3.64	3.78	5.4	5.4	1.68	2.02	2.67	4.16	2.82	4.57
PT8333	alkaline	Phenomenex, Kinetex phenyl/hexyl, 100 x 2.1 mm, 2.6 µm	100	15	6.83	7.95	7.31	8.38	7.11		8.22		3.71	4.68	6.21	7.47	6.48	7.76
PT8334	alkaline	Waters, Premier BEH C18, (100	100	16	7.2	9.3	7.8	10.0	7.6	7.8	9.8	9.9	4.2	4.9	6.2	8.4	6.5	8.8
PT8335	alkaline																	

Lab code	Method	Column	Column length (mm)	Total run time (min)	Retention time (min)													
					Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	α-Ergocryptine	β-Ergocryptine	α-Ergocryptinine	β-Ergocryptinine	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine
PT8336	alkaline	Phenomenex, Gemini NX-C18, 100 x 2 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
PT8337	alkaline	Phenomenex, Gemini-NX, C18, 150 x 2 mm, 5 µm	150	22	7.43	11.48	8.66	13.85	8.2	8.6	13.2		4.53	5.02	6.22	9.39	6.54	10.45
PT8338	alkaline	Phenomenex Gemini C6 Phenyl, 150 x 2 mm, 3 µm	150	17	7.4	9.9	8.6	10.9	8.1	8.1	10.5	10.5	2.7	4.4	6.1	8.8	6.5	9.4
PT8339	alkaline	Waters, Aquity UPLC BEH, C18, 50 x 2.1 mm, 1.7 µm	50	21	7.8	10	8.6	11.1	8.4		10.8		3.0	3.9	6.5	8.9	6.9	9.4
PT8340	acid	Thermo Scientific, Hypersil Gold, 100 x 2.1 mm	100	13	8.9	9.5	9.6	9.9	9.4	9.4	9.9	9.9	5	6.2	8.6	8.7	8.8	9
PT8341	acid	Waters, Acquity UPLC BEH C18, 100 × 2.1 mm, 1.7 µm	100	11	3.98	4.6	4.63	4.96	4.57		4.91		1.18	1.44	3.72	3.57	3.91	3.83
PT8342	alkaline	Phenomenex, Kinetex EVO C18, 150 x 4.6 mm, 2.6 µm	150	23	10.04	10.75	10.37	11.18	10.31		11.10		7.64	8.41	9.64	10.36	9.78	10.54
PT8343	alkaline																	
PT8344	alkaline																	
PT8346	alkaline	Phenomenex, Gemini C18, 100 Å, 150 x 3 mm, 3 µm	150	15	9.265	10.416	9.683	10.828	9.582	9.664	10.721	10.721			8.541	9.923	8.737	
PT8447	alkaline	Phenomenex, Gemini C6 Phenyl, 110 Å, 150 x 2 mm, 3 µm	150	24	11.1	17.5	13.0	22.9	12.4		20.8		3.0	6.7	9.2	14.1	9.8	15.9
PT8348	alkaline	Phenomenx, Kinetex Phenyl-Hexyl, 150 x 2.1 mm, 2.6 µm	150	28	6.52	10.14	8.16	11.64	7.51	7.86	11.05	11.20	1.28	1.87	4.12	8.58	5.12	9.52
PT8349	alkaline	Waters, ACQUITY Premier BEH C18, 50 x 2.1 mm, 1.7 µm	50	5	0.73	0.91	0.79	0.97	0.79		0.96		0.31	0.43	0.64	0.83	0.66	0.87
PT8350	alkaline	Waters, Acquity UPLC® BEH C18, 100 × 2.1 mm, 1.7 µm	100	6	3.35	3.8	3.51	3.97	3.49		3.95		1.09	1.98	3.07	3.59	3.12	3.65

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
PT8317	10	acetonitrile	80	ultraturrax	3	dilution			0.03	A: 0.1 % formic acid; B: acetonitrile + 0.1 % formic acid	MS/MS
PT8318	2.5	acetonitrile/water + formic acid	20	mechanical shaking	60	none				A: ammonium acetate + acetic acid in H_2O; B: MeOH	MS/MS
PT8319	10	acetonitrile: ammonium carbonate 5 mM (85:15)	40	mechanical shaking	30	other			2	A: ammonium carbonate 10 mM; B: acetonitrile	MS/MS
PT8320	25	acetonitrile/ammonium carbonate 0.2 g/L (84/16) = 2 mM	125	mechanical shaking	30	SPE	Bondesil PSA (= dSPE)	50 mg of dSPE / mL of sample extract	0.1	A: ammonium carbonate 2 mM; B: acetonitrile	MS/MS
PT8321	20	acetonitrile:ammonium carbonate (84:16; v/v)	100	mechanical shaking	60	LLE	MycoSep 150 Ergot	4	0.2	A: ammonium carbonate (2 mM); B: acetonitrile	MS/MS
PT8322	5	acetonitrile/0.2 M ammonium hydrogen carbonate (84/16)	25	mechanical shaking	30	other	MycoSep 150	4	0.61	A: 10 mM ammonium bicarbonate; B: ACN, 10 mM ammonium hydrogen carbonate (9/1)	MS/MS
PT8323	10	acetonitrile:ammonium carbonate (84:16)	50	mechanical shaking	30	SPE	dispersed SPE (PSA)	1 mL extract + 50 mg of PSA	1	A: ammonium carbonate; B: acetonitrile	MS/MS
PT8324	5	H_2O/acetonitrile/formic acid (18/80/2)	15	mechanical shaking	60	LLE			0.33	A : 10 mM ammonium formate; B : MeOH + 10 mM ammonium formate	MS/MS
PT8325	25	acetonitrile:water:acetic acid (79:20:1)	100	mechanical shaking	30	none			0.25	A: ammonium carbonate 10 mM; B: acetonitrile	MS/MS
PT8326	5	acetonitrile	25	shaking (hand/vortex)	30	other	d-SPE			A: 0.001 M ammonium carbonate; B: acetonitrile	MS/MS
PT8327	15	solution mix of ethyl acetate & ammonium hydroxide	75	mechanical shaking	15	LLE				A: 0.2 mg ammonium carbamate; B: acetonitrile	MS/MS
PT8328	10	ethyl acetate/methanol/2-propanol/NH_3 25% 75/5/7/7	50	blender	45	SPE	Sep-Pak Alumina B plus	10	0.2	ACN: ammonium carbamate 3 mM 50:50	other
PT8329	20	acetonitrile:ammonium carbonate (aq): 84/16 (v/v%)	100	mechanical shaking	30	none			0.4	A: ammonium carbonate in water; B: acetonitrile	MS/MS
PT8330	5	acetonitrile:$(NH_4)_2CO_3$ pH 8.9 84:16 v/v	25	mechanical shaking	30				0.2	A: ammonium carbonate 10 mM; B: acetonitrile	MS/MS
PT8331	4	MeOH:water=60:40+0.4% HCOOH	40	mechanical shaking	30	other			0.1	A: 10 mM ammonium carbonate; B: acetonitrile	MS/MS
PT8332	5	acetonitrile:ammonium carbonate 84:16	25	mechanical shaking	30	SPE	PSA	1	0.2	A: ammonium carbonate 1 mM; B: acetonitrile	MS/MS
PT8333	2	1% HCOOH in acetonitrile	10	mechanical shaking	30	none			0.2	A: 3 mM ammonium bicarbonate B: acetonitrile	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
PT8334	2	acetonitrile: 3 mM ammonium carbonate (85:15)	20	shaking (hand/vortex)	4	none			0.1	A: 3 mM ammonium carbonate in water; B: acetonitrile	MS/MS
PT8335											
PT8336	20	acetonitrile:ammonium carbonate 200 mg/L (85+15)	100	mechanical shaking	30	none			0.2	A: ammonium bicarbonate 3 mmol/L; B: MeOH	MS/MS
PT8337	5	acetonitrile - ammonium carbonate 20 mmol: 84/16 v/v	25	mechanical shaking	30	none			0.2	A: ammonium carbonate 20 mmol pH 10; B: acetonitrile	MS/MS
PT8338	10	50 mL ethyl acetate-methanol+isopropanol+25% ammonia (150/10/14/14) (v/v/v/v)	50	mechanical shaking	45	SPE	ALOX-SPE (Macherey + Nagel)	5	0.2	A: 2.5 mM ammonium carbamate; B: acetonitrile	MS/MS
PT8339	4	methanol:water (60:40) with 0.4% formic acid	40	mechanical shaking	30	other			0.1	A: 6 mM ammonia in water; B: acetonitrile	MS/MS
PT8340	20	acetonitrile:ammonium carbonate	100	shaking (hand/vortex)	30	SPE	Mycosep	4 mL/1 mL	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	MS/MS
PT8341	2	ACN + 0.1% HCOOH in water (1:1)	20	mechanical shaking	20	dilution			0.1	A: H_2O + 0.1% HCOOH B: MeOH + 0.1% HCOOH + 1 mM $HCOONH_4$	MS/MS
PT8342	20	acetonitrile:ammonium carbonate in water 1 g/L 84:16 = 1 M	100	mechanical shaking	90	other				A: ammonium carbonate in water 2 mM: B: acetonitrile	MS/MS
PT8343											
PT8344											
PT8346	20	acetonitrile: 200 mg/L $(NH_4)_2CO_3$ 84:16	100	mechanical shaking	60	other			0.2	A: ammonium carbonate 2 mM B: acetonitrile	MS/MS
PT8347	5	ethyl acetate/methanol/ammonium hydroxide solution 25%/isopropanol (75/5/7/7)	25	mechanical shaking	45	SPE	ALOX-SPE (Macherey + Nagel)	5	0.04	A: ammonium carbamate solution 0.02%; B: acetonitrile	HPLC-UV/PDA/FLD
PT8348	20	ethyl acetate/MeOH/NH_3-Lsg. 25%/2-propanol; 75/5/7/7 (v/v/v/v)	100	mechanical shaking	45	SPE	waters Sep-Pak Plus Alumina B Carrtridges	5	0.02	A: 2.5 mM ammonium carbamate; B: acetonitrile	MS/MS
PT8349	5	acetonitrile: 3.3 mM $(NH_4)_2CO_3$ (aq) (84:16 v/v)	25	mechanical shaking	60	other	PSA		0.2	A: 3.3 mM ammonium carbonate (aq); B: acetonitrile	MS/MS
PT8350	5	acetonitrile/3 mM ammonium carbonate (84:16, v/v)	25	mechanical shaking	30	SPE	Mycosep® 150 Ergot column	4	1.25	A: 3 mM ammonium carbonate; B: acetonitrile	MS/MS

ACN = acetonitrile; MeOH = methanol; H_2O = water; FA (HCOOH) = formic acid; CH_3COOH = acetic acid; $HCOONH_4$ = ammonium formate; $(NH_4)_2CO_3$ = ammonium carbonate; NH_4HCO_3 = ammonium bicarbonate.

Annex 9   Results material A (wheat)

Lab code	Sum epimer pair ergocornine/ergocorninine A: 6.85 µg/kg u: 0.222 µg/kg σ_p: 1.71 µg/kg (25%) robust σ: 0.958 µg/kg (14%)		Sum epimer pair ergocristine/ergocristinine A: 7.54 µg/kg u: 0.360 µg/kg σ_p: 1.88 µg/kg (25%) robust σ: 1.60 µg/kg (21%)		Sum epimer pair ergocryptine/ergocryptinine (α- and β-form) A: 7.86 µg/kg u: 0.410 µg/kg σ_p: 1.97 µg/kg (25%) robust σ: 1.79 µg/kg (23%)		Sum epimer pair ergometrine/ergometrinine A: 7.03 µg/kg u: 0.256 µg/kg σ_p: 1.76 µg/kg (25%) robust σ: 1.10 µg/kg (16%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8317	<5	[-1.08]	10.8	1.73	<5	[-1.46]	<5	[-1.15]
PT8318	6.3	-0.32	9.8	1.20	9.8	0.99	2.3	**-2.69**
PT8319	7.3	0.26	7.1	-0.23	8.2	0.17	6.4	-0.36
PT8320	7.3	0.26	8.2	0.35	8.4	0.27	6.8	-0.13
PT8321	15	**4.76**	16	**4.49**	16	**4.14**	17	**5.68**
PT8322	6.07	-0.45	6.15	-0.74	6.73	-0.58	8.59	0.89
PT8323	6.52	-0.19	6.26	-0.68	7.09	-0.39	7.65	0.35
PT8324	12.5	**3.30**	14.9	**3.90**	8.8	0.48	4.5	-1.44
PT8325	6.9	0.03	7.0	-0.29	9.0	0.58	8.5	0.84
PT8326	6.5	-0.20	7.4	-0.07	8.2	0.17	7.2	0.10
PT8327	4.9	-1.14	5.0	-1.35	5.6	-1.15	5.6	-0.81
PT8328	7.5	0.38	7.3	-0.13	10.5	1.34	12.7	**3.23**
PT8329	<4	[-1.66]	<4	[-1.88]	<4	[-1.97]	<4	[-1.72]
PT8330	8.8	1.14	8.0	0.24	9.4	0.78	7.1	0.04
PT8331	4.82	-1.18	5.6	-1.03	7.41	-0.23	7.5	0.27
PT8332	6.1	-0.44	6.6	-0.50	7.2	-0.34	8.9	1.07
PT8333	7.23	0.22	6.44	-0.58	7.47	-0.20	7.49	0.26
PT8334	8.60	1.02	11.22	1.95	7.54	-0.16	5.40	-0.93
PT8335	7.7	0.50	7.6	0.03	6.2	-0.85	8.0	0.55
PT8336	6.3	-0.32	7.2	-0.18	8.5	0.32	6.6	-0.24
PT8337	7.5	0.38	8.3	0.40	11.4	1.80	6.9	-0.07
PT8338	6.68	-0.10	7.12	-0.22	7.43	-0.22	6.74	-0.16
PT8339	6.1	-0.44	6.5	-0.55	8.4	0.27	6.4	-0.36
PT8340	4.09	-1.61	10.62	1.63	6.00	-0.95	6.60	-0.24
PT8341	<5	[-1.08]	<5	[-1.35]	<5	[-1.46]	<5	[-1.15]
PT8342	21.8	**8.73**	22.2	**7.78**	22.4	**7.40**	30.8	**13.53**

| | Sum epimer pair ergocornine/ergocorninine | | Sum epimer pair ergocristine/ergocristinine | | Sum epimer pair ergocryptine/ergocryptinine (α- and β-form) | | Sum epimer pair ergometrine/ergometrinine | |
	A: 6.85 µg/kg u: 0.222 µg/kg σ_p: 1.71 µg/kg (25%) robust σ: 0.958 µg/kg (14%)		A: 7.54 µg/kg u: 0.360 µg/kg σ_p: 1.88 µg/kg (25%) robust σ: 1.60 µg/kg (21%)		A: 7.86 µg/kg u: 0.410 µg/kg σ_p: 1.97 µg/kg (25%) robust σ: 1.79 µg/kg (23%)		A: 7.03 µg/kg u: 0.256 µg/kg σ_p: 1.76 µg/kg (25%) robust σ: 1.10 µg/kg (16%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8343	<5.0	[-1.08]	10.8	1.73	2.8	**-2.58**	20.1	**7.44**
PT8344	7.5	0.38	7.0	-0.29	7.9	0.02	7.2	0.10
PT8346	6.6	-0.14	6.2	-0.71	7.8	-0.03	nt	
PT8347	7.32	0.28	10.5	1.57	5.90	-1.00	6.27	-0.43
PT8348	6.05	-0.47	6.18	-0.72	5.58	-1.16	2.96	**-2.32**
PT8349	6.2	-0.38	4.9	-1.40	5.1	-1.41	6.8	-0.13
PT8350	6.44	-0.24	6.74	-0.42	9.26	0.71	6.51	-0.29

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_p = target standard deviation for proficiency test.

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

nt = not tested.

Lab code	Sum epimer pair ergosine/ergosinine A: 8.16 µg/kg u: 0.335 µg/kg σp: 2.04 µg/kg (25%) robust σ: 1.52 µg/kg (19%)		Sum epimer pair ergotamine/ergotaminine A: 9.98 µg/kg u: 0.472 µg/kg σp: 2.50 µg/kg (25%) robust σ: 2.14 µg/kg (21%)		Sum 12 ergot alkaloids A: 46.8 µg/kg u: 1.96 µg/kg σp: 11.7 µg/kg (25%) robust σ: 9.00 µg/kg (19%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8317	<5	[-1.55]	<5	[-2.00]	16.7	**-2.57**
PT8318	3.8	**-2.14**	8.1	-0.75	40.1	-0.57
PT8319	8.2	0.02	9.7	-0.11	46.9	0.01
PT8320	8.1	-0.03	10.0	0.01	49	0.19
PT8321	16	**3.84**	19	**3.61**	98	**4.38**
PT8322	7.67	-0.24	8.77	-0.49	44.0	-0.24
PT8323	7.96	-0.10	10.5	0.21	46.0	-0.07
PT8324	8.9	0.36	8.2	-0.71	57.7	0.93
PT8325	11.9	1.83	12.0	0.81	55.3	0.73
PT8326	7.3	-0.42	9.4	-0.23	46.0	-0.07
PT8327	6.3	-0.91	8.0	-0.79	35.4	-0.97
PT8328	7.2	-0.47	12	0.81	57.1	0.88
PT8329	4.92	-1.59	4.87	**-2.05**	9.72	**-3.17**
PT8330	13.3	**2.52**	13.1	1.25	59.6	1.09
PT8331	6.68	-0.73	8.47	-0.61	40.48	-0.54
PT8332	7.6	-0.28	8.9	-0.43	45.3	-0.13
PT8333	8.8	0.31	11.1	0.45	48.5	0.15
PT8334	10.06	0.93	11.56	0.63	54.38	0.65
PT8335	10.0	0.90	11.2	0.49	50.7	0.33
PT8336	7.6	-0.28	9.0	-0.39	45.2	-0.14
PT8337	8.0	-0.08	12.1	0.85	54.2	0.63
PT8338	7.93	-0.11	9.88	-0.04	45.8	-0.09
PT8339	7.4	-0.37	8.8	-0.47	43.6	-0.27
PT8340	11.10	1.44	11.38	0.56	49.79	0.26
PT8341	5.37	-1.37	6.83	-1.26	12.2	**-2.96**
PT8342	19.8	**5.70**	40.4	**12.19**	157.4	**9.45**
PT8343	17.1	**4.38**	13.5	1.41	64.3	1.50
PT8344	8.7	0.26	8.7	-0.51	47.0	0.02
PT8346	7.7	-0.23	7.5	-1.00	35.8	-0.94
PT8347	8.35	0.09	10.7	0.29	49.0	0.19
PT8348	4.56	-1.77	8.14	-0.74	33.5	-1.14
PT8349	10.2	1.00	10.1	0.05	43.3	-0.30
PT8350	7.56	-0.30	9.32	-0.27	45.83	-0.08

A = consensus value (robust mean).

u = uncertainty of consensus value.

σp = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.
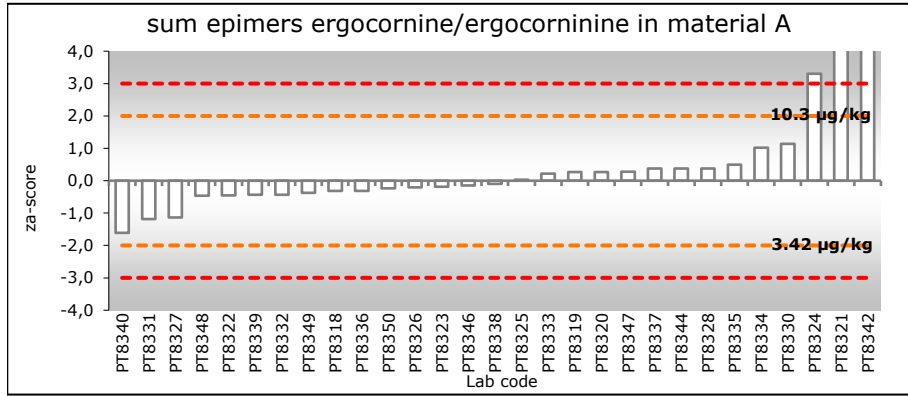
Figure 1 *Graphical representation of the z-scores for the sum of ergocornine/ergocorninine in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
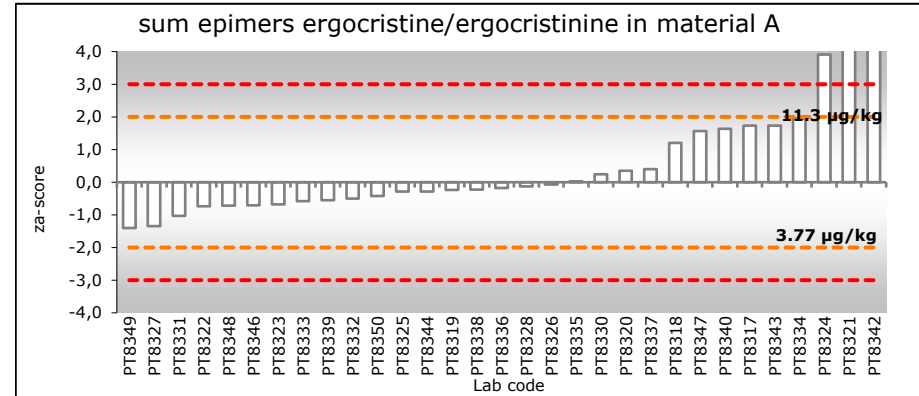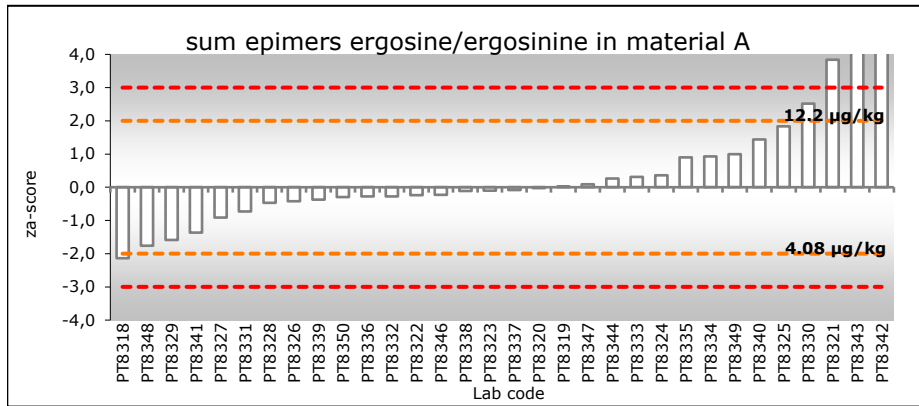
Figure 2 *Graphical representation of the z-scores for the sum of ergocristine/ergocristinine in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
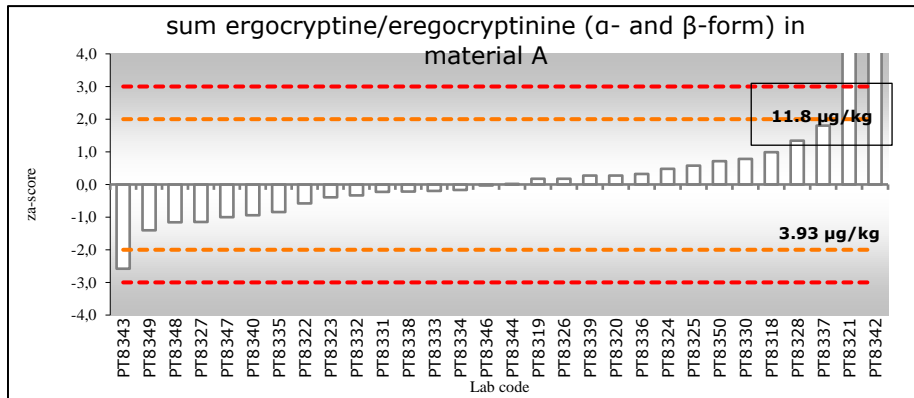
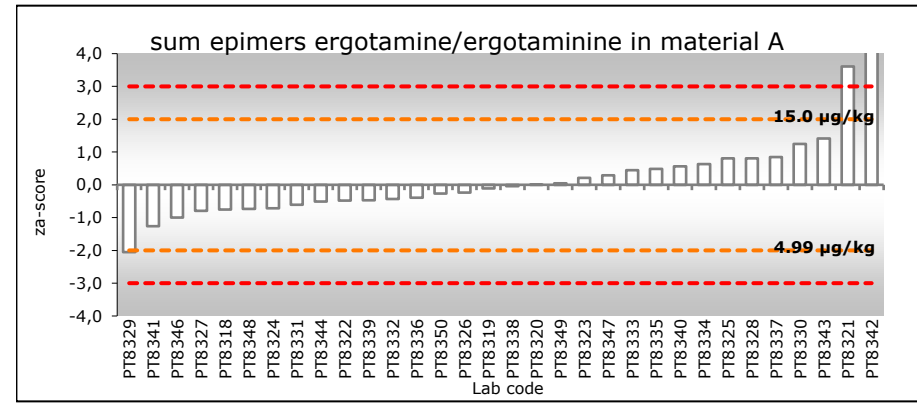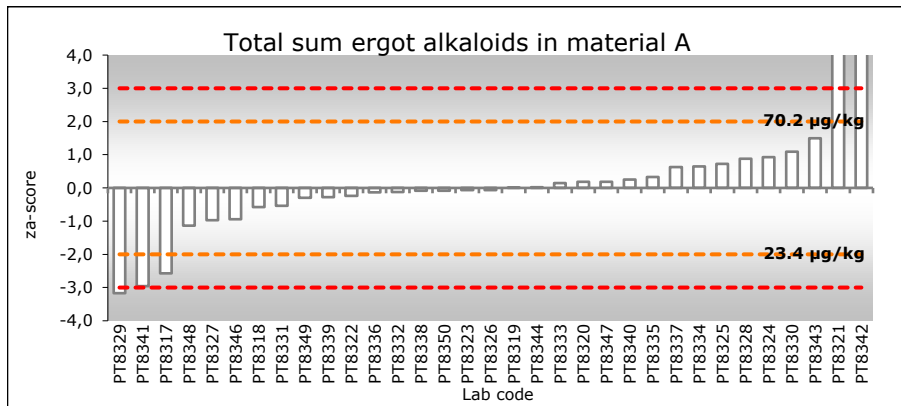**Figure 3** *Graphical representation of the z-scores for the sum of ergocryptine/ergocryptinine (α- and β-form) in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
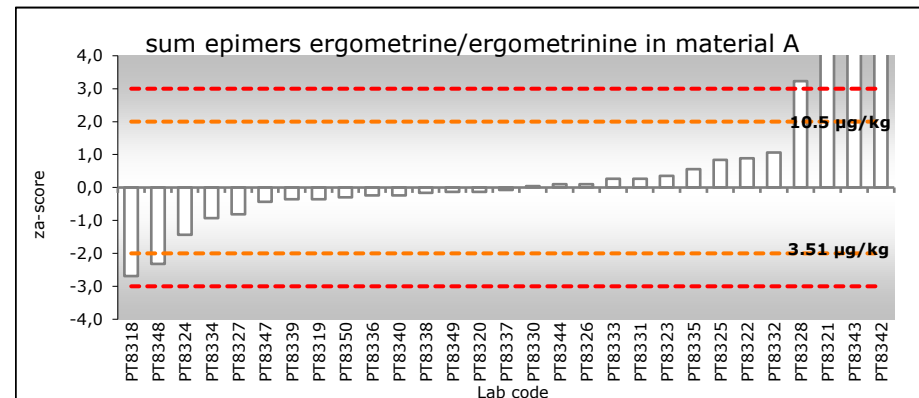
Figure 4 *Graphical representation of the z-scores for the sum of ergometrine/ergometrinine in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

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

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

Figure 7 *Graphical representation of the z-scores for the total sum of 12 EAs in material A. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 10   Results material B (rye)

Lab code	Sum epimer pair ergocornine/ergocorninine A: 39.0 µg/kg u: 1.26 µg/kg σ_P: 9.76 µg/kg (25%) robust σ: 5.80 µg/kg (15%)		Sum epimer pair ergocristine/ergocristinine A: 71.5 µg/kg u: 1.55 µg/kg σ_P: 17.9 µg/kg (25%) robust σ: 7.14 µg/kg (10%)		Sum epimer pair ergocryptine/ergocryptinine (α- and β-form) A: 36.5 µg/kg u: 1.14 µg/kg σ_P: 9.13 µg/kg (25%) robust σ: 5.22 µg/kg (14%)		Sum epimer pair ergometrine/ergometrinine A: 21.6 µg/kg u: 1.06 µg/kg σ_P: 5.40 µg/kg (25%) robust σ: 4.73 µg/kg (22%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8317	43.6	0.47	118.7	**2.64**	22.4	-1.55	<5	**[-3.07]FN**
PT8318	40.3	0.13	81.8	0.58	34.6	-0.21	15.1	-1.20
PT8319	44.0	0.51	66.7	-0.27	35.4	-0.12	24.0	0.45
PT8320	42	0.30	77	0.31	35	-0.17	21	-0.11
PT8321	39	0.00	56	-0.87	31	-0.61	31	1.74
PT8322	36.3	-0.28	65.55	-0.33	37.04	0.06	24.63	0.56
PT8323	36.1	-0.30	66.4	-0.28	34.1	-0.27	21.0	-0.11
PT8324	52.7	1.40	139	**3.78**	37.2	0.07	14.3	-1.35
PT8325	43.2	0.43	65.6	-0.33	42.0	0.60	28.5	1.28
PT8326	34.0	-0.52	66.2	-0.29	38.4	0.20	19.8	-0.33
PT8327	25.54	-1.38	47.67	-1.33	28.6	-0.87	19.03	-0.48
PT8328	38.7	-0.03	70	-0.08	45.1	0.94	22.4	0.15
PT8329	30.3	-0.89	58.0	-0.75	17.5	**-2.08**	14.0	-1.41
PT8330	53.0	1.43	76.1	0.26	41.8	0.58	29.1	1.39
PT8331	32.55	-0.66	68.45	-0.17	29.33	-0.79	23.3	0.32
PT8332	36.3	-0.28	68.8	-0.15	33.5	-0.33	28.2	1.22
PT8333	44.2	0.53	85.7	0.80	36.0	-0.06	23.0	0.26
PT8334	36.77	-0.23	73.36	0.11	35.41	-0.12	17.02	-0.85
PT8335	38	-0.11	68	-0.19	21.2	-1.68	20.9	-0.13
PT8336	38.8	-0.02	70.5	-0.05	37.8	0.14	19.5	-0.39
PT8337	43.1	0.42	93.2	1.22	54.8	2.00	20.3	-0.24
PT8338	37.6	-0.15	72.8	0.07	36.9	0.04	23.7	0.39
PT8339	36.5	-0.26	63.5	-0.45	38.4	0.20	19.3	-0.43
PT8340	31.80	-0.74	68.88	-0.14	23.21	-1.46	18.32	-0.61
PT8341	42.37	0.34	85.52	0.79	39.84	0.36	19.12	-0.46
PT8342	95.8	**5.82**	116.4	**2.52**	68.8	**3.53**	62.4	**7.56**

Lab code	Sum epimer pair ergocornine/ergocorninine		Sum epimer pair ergocristine/ergocristinine		Sum epimer pair ergocryptine/ergocryptinine (α- and β-form)		Sum epimer pair ergometrine/ergometrinine	
	A: 39.0 µg/kg u: 1.26 µg/kg σ_P: 9.76 µg/kg (25%) robust σ: 5.80 µg/kg (15%)		A: 71.5 µg/kg u: 1.55 µg/kg σ_P: 17.9 µg/kg (25%) robust σ: 7.14 µg/kg (10%)		A: 36.5 µg/kg u: 1.14 µg/kg σ_P: 9.13 µg/kg (25%) robust σ: 5.22 µg/kg (14%)		A: 21.6 µg/kg u: 1.06 µg/kg σ_P: 5.40 µg/kg (25%) robust σ: 4.73 µg/kg (22%)	
	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8343	33.1	-0.61	110.3	**2.17**	38.9	0.26	64.0	**7.85**
PT8344	45.0	0.61	75.0	0.20	40.0	0.38	25.0	0.63
PT8346	38.6	-0.04	69.2	-0.13	39.4	0.31	nt	
PT8347	39.4	0.04	74.2	0.15	23.5	-1.43	24.0	0.45
PT8348	35.78	-0.33	68.24	-0.18	36.70	0.02	14.8	-1.26
PT8349	45.2	0.63	75.4	0.22	40.8	0.47	21.8	0.04
PT8350	31.84	-0.74	67.65	-0.21	45.96	1.03	17.84	-0.70

A = consensus value (robust mean).

u = uncertainty of consensus value.

σ_P = target standard deviation for proficiency test.

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

nt = not tested.

FN = False negative.

|	Sum epimer pair ergosine/ergosinine A: 48.3 µg/kg u: 1.26 µg/kg σₚ: 12.1 µg/kg (25%) robust σ: 5.77 µg/kg (12%)		Sum epimer pair ergotamine/ergotaminine A: 89.8 µg/kg u: 3.68 µg/kg σₚ: 22.5 µg/kg (25%) robust σ: 16.9 µg/kg (19%)		Sum 12 ergot alkaloids A: 307 µg/kg u: 9.53 µg/kg σₚ: 76.8 µg/kg (25%) robust σ: 43.8 µg/kg (14%)	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
PT8317	41	-0.60	44.7	**-2.01**	255.4	-0.67
PT8318	44.1	-0.35	86.4	-0.15	302.3	-0.06
PT8319	48.7	0.04	98.7	0.39	317.5	0.14
PT8320	47	-0.10	94	0.19	320	0.17
PT8321	44	-0.35	81	-0.39	280	-0.35
PT8322	49.63	0.11	94.07	0.19	307.2	0.00
PT8323	47.4	-0.07	102	0.54	307	0.00
PT8324	45.6	-0.22	95.1	0.23	384	1.00
PT8325	71.1	1.89	113	1.03	363	0.73
PT8326	47.9	-0.03	85.6	-0.19	291.9	-0.20
PT8327	39.83	-0.70	72.67	-0.76	233.34	-0.96
PT8328	46.6	-0.14	102.2	0.55	324.8	0.23
PT8329	41.3	-0.58	66.3	-1.05	227.4	-1.04
PT8330	67.8	1.62	130.3	1.80	398.1	1.19
PT8331	47.2	-0.09	68.3	-0.96	269.13	-0.49
PT8332	48.0	-0.02	88.9	-0.04	303.6	-0.05
PT8333	52.6	0.36	99.4	0.43	341	0.44
PT8334	51.70	0.28	110.60	0.92	324.85	0.23
PT8335	52	0.31	90	0.01	290	-0.22
PT8336	46.4	-0.15	82.7	-0.32	295.6	-0.15
PT8337	53.3	0.42	125.0	1.57	389.7	1.08
PT8338	50.8	0.21	90.4	0.03	312.2	0.07
PT8339	45.5	-0.23	80.6	-0.41	271	-0.47
PT8340	43.49	-0.40	79.89	-0.44	265.59	-0.54
PT8341	50.70	0.20	98.24	0.37	335.78	0.37
PT8342	113.2	**5.38**	180.2	**4.02**	636.8	**4.30**
PT8343	118.7	**5.84**	120.5	1.37	485.5	**2.32**
PT8344	55.0	0.56	45.0	-2.00	285.0	-0.29
PT8346	48.9	0.05	62.6	-1.21	258.7	-0.63
PT8347	54.0	0.48	96.7	0.31	312	0.06
PT8348	40.2	-0.67	79.67	-0.45	275.38	-0.41
PT8349	45.2	-0.25	81.8	-0.36	310.2	0.04
PT8350	39.52	-0.72	73.40	-0.73	276.21	-0.40

A   = consensus value (robust mean).

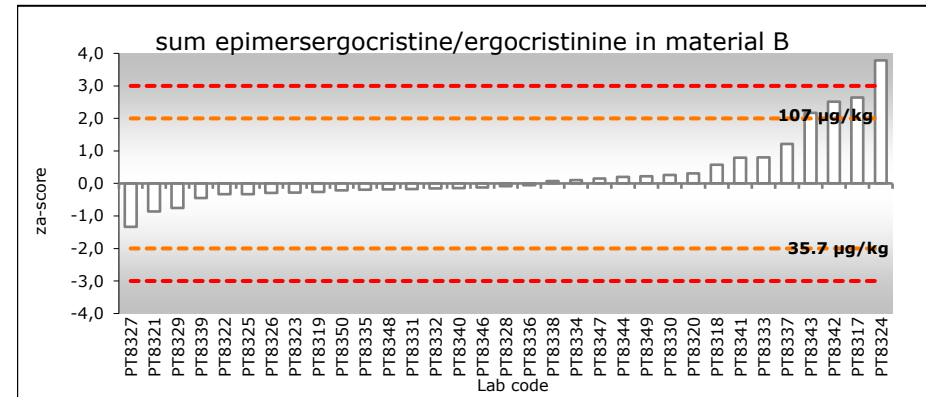u   = uncertainty of consensus value.

σₚ = target standard deviation for proficiency test.

robust σ = robust (relative) standard deviation based on participants' results.
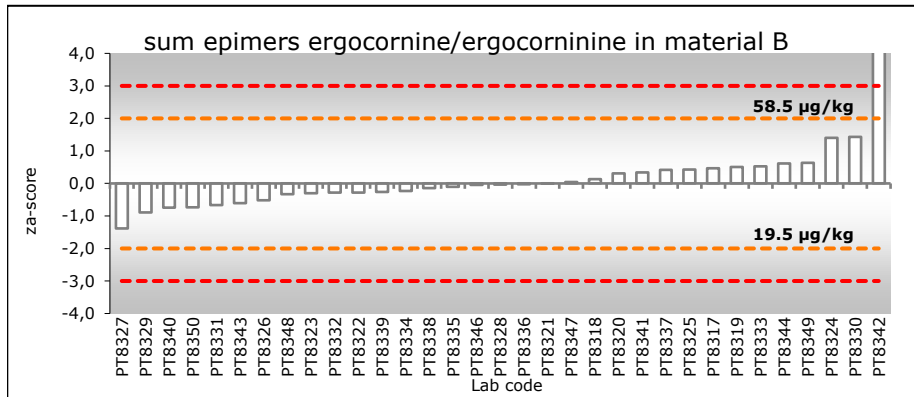
Figure 8 *Graphical representation of the z-scores for the sum of ergocornine/ergocorninine in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
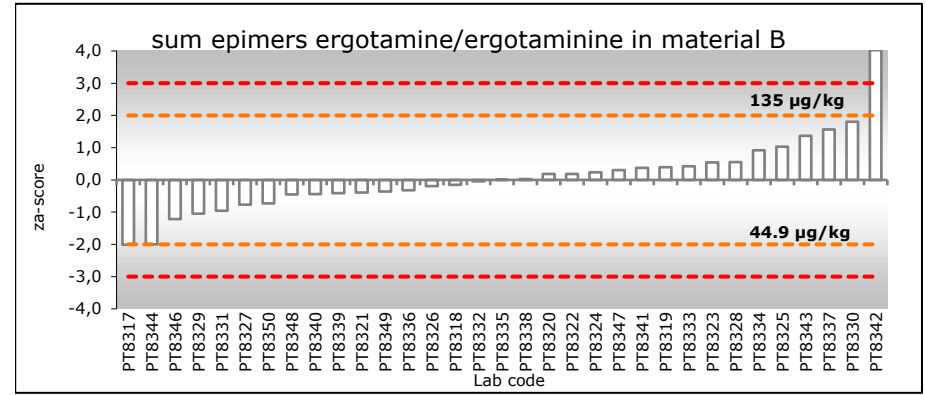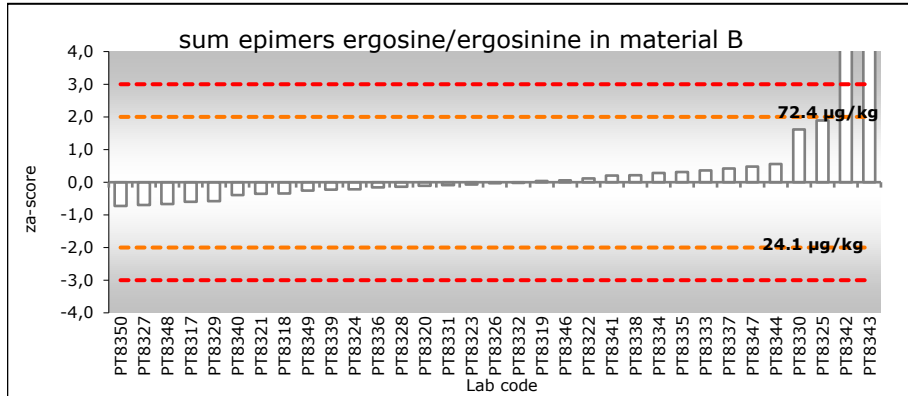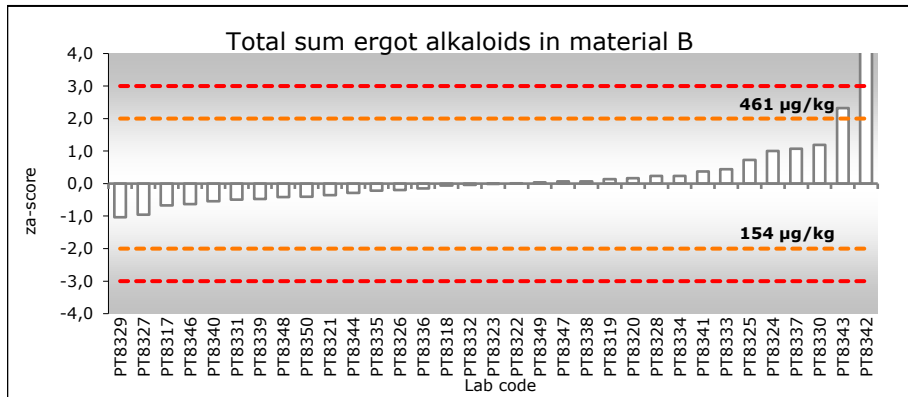

Figure 9 *Graphical representation of the z-scores for the sum of ergocristine/ergocristinine in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
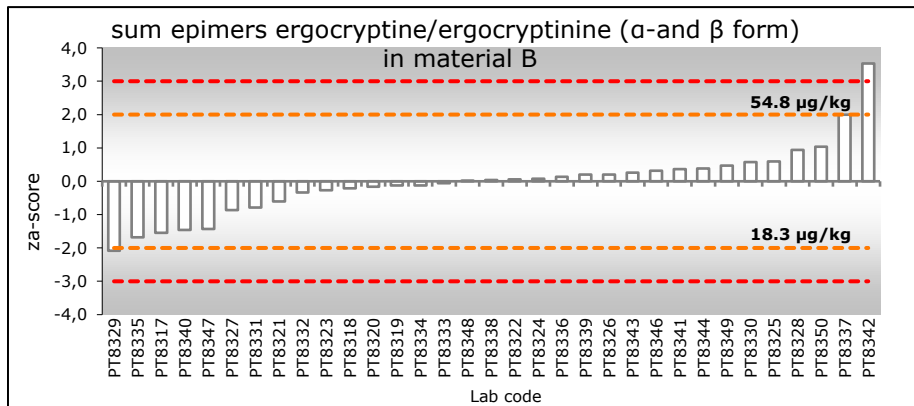
Figure 10 *Graphical representation of the z-scores for the sum of ergocryptine/ergocryptinine (α- and β-form) in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*
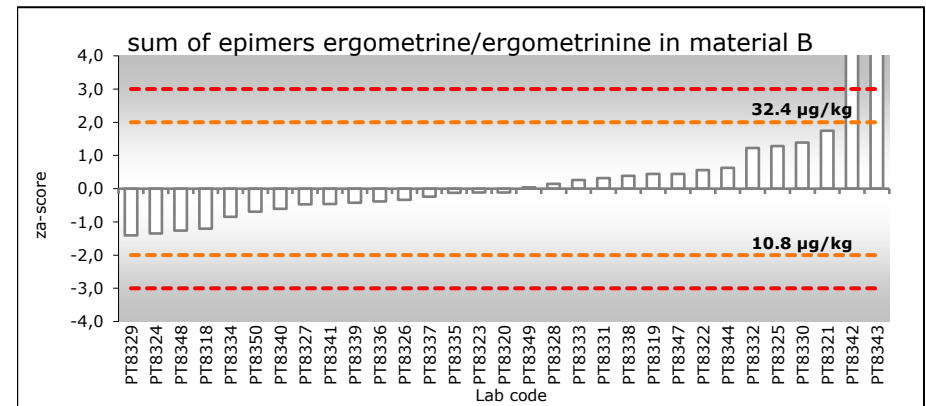
Figure 11 *Graphical representation of the z-scores for the sum of ergometrine/ergometrinine in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

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

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

Figure 14 *Graphical representation of the z-scores for the total sum of 12 EAs in material B. Dotted lines show PT performance boundaries ± 2 (also in µg/kg) and ± 3.*

Annex 11 Overview performance per laboratory

Lab code	Groups of epimer pairs Satisfactory performance *	Total sum Satisfactory performance *	FN
PT8317	4 of 12	1 of 2	1
PT8318	10 of 12	**2 of 2**	
PT8319	**12 of 12**	**2 of 2**	
PT8320	**12 of 12**	**2 of 2**	
PT8321	6 of 12	1 of 2	
PT8322	**12 of 12**	**2 of 2**	
PT8323	**12 of 12**	**2 of 2**	
PT8324	9 of 12	**2 of 2**	
PT8325	**12 of 12**	**2 of 2**	
PT8326	**12 of 12**	**2 of 2**	
PT8327	**12 of 12**	**2 of 2**	
PT8328	11 of 12	**2 of 2**	
PT8329	6 of 12	1 of 2	
PT8330	11 of 12	**2 of 2**	
PT8331	**12 of 12**	**2 of 2**	
PT8332	**12 of 12**	**2 of 2**	
PT8333	**12 of 12**	**2 of 2**	
PT8334	**12 of 12**	**2 of 2**	
PT8335	**12 of 12**	**2 of 2**	
PT8336	**12 of 12**	**2 of 2**	
PT8337	**12 of 12**	**2 of 2**	
PT8338	**12 of 12**	**2 of 2**	
PT8339	**12 of 12**	**2 of 2**	
PT8340	**12 of 12**	**2 of 2**	
PT8341	8 of 12	1 of 2	
PT8342	0 of 12	0 of 2	
PT8343	5 of 12**	1 of 2**	
PT8344	**12 of 12**	**2 of 2**	
PT8346	10 of 12	**2 of 2**	
PT8347	**12 of 12**	**2 of 2**	
PT8348	11 of 12	**2 of 2**	
PT8349	**12 of 12**	**2 of 2**	
PT8350	**12 of 12**	**2 of 2**	

* Satisfactory performance here means a quantitative result with a satisfactory z-score was obtained for each of the 6 groups of ergot alkaloid epimer pairs or the total sum of EAs present in material A and B. Results reported as <LOQ are not considered a satisfactory z-score.

** reported results after the deadline.

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the potential
of nature to
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quality of life

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,600 employees (6,700 fte) and 13,100 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.