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Determination of ergot alkaloids in rye

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Determination of ergot alkaloids in rye

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268-PT Accredited by the
Belgian Accreditation Body (BELAC)

Contents

Executive summary

Throughout history, there have been several deadly episodes of food poisoning by ergot alkaloids (EAs) (known as St. Anthony's fire or ergotism). EAs are secondary metabolites produced by fungi of the Claviceps genus (chiefly *Claviceps purpurea*), which are common pathogens of cereals and pasture grasses. During harvest, the fungal body is collected together with the crop leading to the contamination of cereal-based food and feed products. Although this event is highly attenuated nowadays by the physical cleaning techniques in the mills, the detection of EAs in food and feed commodities is not infrequent.

Since 2002, the EU legislation (Directive 2002/32/EC) sets up the maximum content for rye ergot (sclerotia) in all feed containing unground cereals. However, the visual determination of sclerotia in cereals is often inaccurate. Moreover, this visual determination is impossible in processed food and feed. Additionally, the pattern of EAs levels in relation to fungal strains, geographical distribution and host plant is not fully known and they cannot be directly related to the sclerotia amount visually determined. The Commission Recommendation 2012/154/EU additionally recommends the monitoring of the presence of individual EAs in feed and food by chemical analytical methods.

A proficiency test (PT) was organised by the European Union Reference Laboratory (EURL) for Mycotoxins targeting the determination of the most prominent EAs in *Claviceps purpurea*: ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their related –inine epimers, as listed in the above Recommendation. The levels in the rye test material varied from 116 (ergometrine/inine) to 752 µg/kg (ergocristine/inine).

Thirty-seven laboratories, among them 26 National Reference Laboratories for mycotoxins in food and feed from 21 EU Member States plus Iceland and Norway, and 11 Official Control Laboratories participated in the PT. The rating of the laboratories' performance was done by means of z-scores considering the sum of the -ine/-inine pairs of epimers with respect to the values assigned at the JRC-Geel and a σ_{pt} of 22 %. Ninety-one percent of the results were classified as satisfactory ($|z| \leq 2$), while 3.7 % fell into the unsatisfactory range ($|z| \geq 3$). All the results received for ergocornine/inine were satisfactory, whereas 76 and 86 % of the results for α-ergocryptine/inine and ergometrine/inine, respectively, were classified as satisfactory. Despite the overall good performance of the laboratories analysing EAs in the test item, this PT highlighted the need to seek a harmonised approach for quantifying α- and β-ergocryptine/inine, as currently only the α-isomers of this EA type are available as pure reference materials.

Acknowledgements

The organisers of the study would like to thank the JRC colleagues who contributed to the project, in particular the Reference Materials Unit. The laboratories that participated in this exercise, listed in **Table 1**, are also immensely acknowledged.

Table 1. Participating laboratories

Department	Country
AGES GmbH	Austria
CODA-CERVA	Belgium
Central laboratory for chemical testing and control	Bulgaria
Andrija Stampar Teaching Institute of Public Health	Croatia
Inspecto d.o.o. Laboratorij	Croatia
State General Laboratory	Cyprus
Czech Agriculture and Food Inspection Authority	Czech Republic
Central Institute for Supervising and Testing in Agriculture (UKZUZ)	Czech Republic
Finnish Food Safety Authority Evira	Finland
Laboratoire SCL de RENNES	France
LAVES	Germany
Federal Institute for Risk Assessment (BfR)	Germany
LLG - Landesanstalt für Landwirtschaft und Gartenbau	Germany
Landesuntersuchungsamt	Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	Germany
LUFA-ITL GmbH	Germany
General Chemical State Laboratory	Greece
National Food Chain Safety Office	Hungary
Matís Ltd. - Icelandic Food and Biotech R&D	Iceland
State Laboratory	Ireland
Public Analyst's Laboratory	Ireland
Istituto Zooprofilattico Sperimentale Dell'Umbria e Delle Marche	Italy
Agenzia provinciale per l'ambiente di Bolzano	Italy
Italian National Institute of Health	Italy
IZSLER	Italy
Laboratoire National de Santé	Luxembourg
RIKILT - Wageningen University & Research	The Netherlands
Norwegian Veterinary Institute	Norway
National Institute of Public Health - National Institute of Hygiene	Poland
National Veterinary Research Institute	Poland
Sanitary Veterinary and Food Safety Directorate Bucharest	Romania
University of Ljubljana, Veterinary Faculty, National Veterinary Institute	Slovenia
National Food Centre (Spanish Agency for Consumer Affairs, Food Safety and Nutrition)	Spain
Laboratorio de Salud Pública de Valencia	Spain
National Veterinary Institute (SVA)	Sweden
National Food Agency	Sweden
Fera Science Ltd.	UK

List of abbreviations

CEN	European Committee for Standardization
EAs	Ergot alkaloids
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
HPLC-FLD	High performance liquid chromatography-fluorescence detection
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC-HRMS	Liquid chromatography-high resolution mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MS	Member States
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PT	Proficiency test
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe (sample preparation approach)
U_{ref}	Expanded uncertainty of the reference value
X_{ref}	Reference value
σ_{pt}	Standard deviation for proficiency assessment

1 Introduction

Ergot alkaloids (EAs) are mycotoxins produced by fungi belonging to the Claviceps genus. It includes about 36 fungal species which are responsible for the production of over 40 known EAs [1-3]. In Europe, *Claviceps purpurea* is the most widespread Claviceps species that contaminate food supplies, although *C. africana*, *C. fusiformis* and *C. sorghi* might also be relevant in ethnic foods, special diets or imported feed [4].

Oat, wheat, barley, triticale, millet and sorghum can be infected by these fungi, although rye is the most susceptible crop. The dark-coloured crescent-shaped bodies (sclerotia or ergot) that result from the infected kernels contain a mixture of EAs, the composition of which depends on the maturity of the sclerotia, fungal strain, host plant, geographical region and prevailing weather conditions [2,3,5].

Poisoning by EA-contaminated cereal based food is known in Europe since the Middle Ages commonly referred to as St. Anthony's fire (nowadays called ergotism) [4,6]. The most well-known acute effects of ergot poisoning involve strong and very painful vasoconstrictive effects in the extremities, often leading to gangrene, worsening to loss of limbs and possibly death. Chronic exposure may induce symptoms such as: abdominal pain, vomiting, dizziness, burning sensation of the skin, insomnia, paralysis, dementia, convulsions and hallucinations [1,5-8]. EAs were used in medicine in the past centuries for induction of child-birth, control of post-partum bleeding and treatment of migraines. Ergometrine and ergotamine are drug precursors; therefore they are classified as Category 1 substances requiring a license for their handling [9,10].

Based on the six EAs predominantly present in the sclerotia of *C. purpurea,* the EFSA Panel on Contaminants in the Food Chain concluded that chemical analysis should focus mainly on ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (mixture of α- and β-isomers) and ergocornine (along with the corresponding -inine epimers) [4]. The European Commission recommends the monitoring of the presence of the above EAs in feed and food [11].

Although nowadays advanced cleaning procedures prior milling are rather effective, EAs are still found in food and feed commodities, sometimes at relatively high levels [3,12].

A survey on EAs in cereals and cereal products intended for human consumption and animal feeding conducted across the EU indicated that EAs were present in 84 % of rye, 67 % of wheat and 48 % of multigrain (food); as well as in 52 % of rye, 27 % of wheat and 44 % of triticale intended as animal feed. The total EA levels ranged from ≤1 to 1121 µg/kg in food, and from ≤1 to 12340 µg/kg in feed. In food, the majority of samples fell into the range of 1-100 µg/kg total EAs while in feed, they mostly had below 1 µg/kg. The highest level found of a single EA was 3270 µg/kg (ergotamine). In 2009, Crews *et al.* [5] found 25 out of 28 (89 %) rye-based food samples (bread, crispbread, flakes and crackers) contaminated with EAs. The total EAs levels ranged from 1 to 340 µg/kg. Mulder *et al.* [2] reported average levels of EAs in cereal-based products for infants and young children in the Netherlands in 2011, 2012 and 2014 of 10.6, 6.2 and 8.6 µg/kg, respectively. Fifty-four percent of the samples were contaminated with EAs up to a maximum of 115 µg/kg. The occurrence data obtained by EFSA indicated that ergotamine, ergocristine, ergosine and ergocornine are generally more abundant than α- and β-ergocryptine, and ergometrine [4].

2 Scope

As stated in Article 32 of the Regulation (EC) No 882/2004 of the European Parliament and of the Council [13], one of the core duties of the European Union Reference Laboratory (EURL) is to organise proficiency tests for the benefit of the National Reference Laboratories (NRLs) and to ensure an appropriate follow-up.

A proficiency test (PT) was organized to assess the measurement performance of the EU Member States' laboratories regarding the determination of the six ergot alkaloids (and respective –inine epimers) identified in the Commission Recommendation 2012/154/EU [11]. A naturally contaminated rye material containing all proposed analytes in measurable amounts was prepared for that purpose.

The participants were given the possibility to report the individual ergot alkaloids' -ine and respective -inine mass fractions; however, the performance assessment was based on the sum of the ergot alkaloid epimer pairs.

The proficiency test was addressed to all NRLs for mycotoxins and to designated Official Control Laboratories (OCLs). Thirty-eight laboratories from 22 EU Member States, Iceland and Norway registered for the PT.

The EURL Mycotoxins carried-out the planning, execution and assessment of the measurement results based on the requirements laid down in ISO/IEC 17043:2010 [14]. Participants' results were evaluated using the ProLab software package (Quodata, Dresden, DE). The JRC Unit managing the EURL for Mycotoxins is an ISO/IEC 17043:2010 accredited PT provider [15].

3 Confidentiality

All the procedures used for the organisation of the PT are accredited according to ISO/IEC 17043:2010 [14] and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the NRLs designated in line with the Regulation (EC) No 882/2004 will be disclosed to DG SANTE upon request for performance assessment.

4 Time frame

The PT was announced to the National Reference Laboratories by email and through the EURL Mycotoxins web page [15] on 7th March 2017. Registration for this PT was open until 05th May 2017 (**Annex 1**) to provide the laboratories sufficient time to obtain the license to handle Category 1 drug precursors. The participants were given six weeks after the dispatch of the samples (16th and 17th May 2016) for analysing them and reporting back the results together with the duly filled questionnaire. The deadline for reporting the results was 30th June 2017.

5 Materials

5.1 Preparation

The rye test material was produced by combining a contaminated rye batch (approx. 8 mg/kg total EAs) with a blank one in the proportion of 1:3. The material obtained was blended and submitted to cryo-milling, resulting in a powder with a particle size <500 µm. The material was packed in 75 plastic bottles each containing approximately 50 g and stored in the freezer (-18 ºC) until being dispatched.

The test material was produced with the aim to contain relevant levels of EAs that could be quantified with state-of-the-art analytical techniques.

5.2 Homogeneity

For checking the homogeneity of the test materials, 10 units were randomly selected from the production lot (after bottling). Two independent determinations were performed

per bottle using a high performance liquid chromatography-fluorescence detection (HPLC-FLD) method (Working Instruction WI-D-00632/3) accredited under ISO/IEC 17025. The order of the measurements was randomised. Homogeneity was evaluated according to ISO 13528:2015 [16]. The material proved to be adequately homogeneous (**Annex 2**).

5.3 Stability study

The stability of the test material was assessed following an isochronous experimental design [17]: -18 °C was chosen as the reference temperature for sample storage. The stability was checked at the following test temperatures: room temperature (≈20 °C) and 4 °C. The time periods considered in this study were: 14, 28 and 48 days. The stability was evaluated considering the mass fractions of the sum of the EAs' –ine and -inine epimers and followed the requirements of ISO 13528:2015 [16]. A linear regression was drawn for each tested temperature over the duration of the study, and the significance of the slope departure from zero at 95 % confidence level was verified. The material proved to be adequately stable at 4 °C and at room temperature for the period between dispatch (t=0) and the submission date of the last results (t=48 days). (**Annex 3**).

5.4 Distribution

The test material was dispatched to the participants in polystyrene boxes with cooling elements on 16th and 17th May 2017. The parcels were received within 24 hours after dispatch. Storage was required to be at -18 °C until analysis.

Each participant received:

a) one test material for analysis packed in a plastic bottle (approx. 50 g)
b) one ampoule containing a calibration standard solution (≈0.5/0.25 µg/mL EAs in acetonitrile + ammonium carbamate 0.2 g/L (1+1))
c) an accompanying letter with instructions on sample handling and reporting (**Annex 4**)
d) a sample receipt form (**Annex 5**) and
e) laboratory specific files for reporting with a lab code (by email).

6 Instructions to the participants

The scope of the PT and the instructions for sample handling and reporting of the results were communicated to the participants via an accompanying letter (**Annex 4**). The laboratories were required to report the mass fractions of the 6 ergot alkaloids and the corresponding –inine epimers in µg/kg accompanied by the measurement uncertainties (k=2) in µg/kg for the material as received. The participants were advised to use the provided calibration solution for the quantification of the sample.

In addition, the participants were asked to submit validation data, such as: the method's limits of detection (LODs), limits of quantification (LOQs) and method recovery estimates (%). Additionally, they were offered the possibility to compare the response of their in-house standard to the one dispatched and to express it in % (assuming that the dispatched solution reflected 100 %). Specific instructions and the concentration of the dispatched calibration solution were given in the accompanying letter.

The results were reported by the participants using the RingDat software, which is part of the ProLab software [18]. Laboratory specific files generated by ProLab were sent to each laboratory by email for that purpose. A detailed questionnaire was also included (**Annex 6**). The questionnaire was intended to gather additional information on the laboratories' capabilities (e.g. experience, the range of matrices, work-load) and method-related aspects (e.g. extraction and clean-up protocols, chromatographic and detection conditions, and calibration strategy) to investigate individual and/or general patterns on the submitted results. Such data can aid in identifying reasons for underperformances.

Participants were informed about the shipment of the materials under cooling conditions and that upon arrival, they should be transferred immediately to -18 °C. Participants were also encouraged to perform the analysis as soon as possible to allow enough time for acquiring the requested data and for resolving any unexpected instrumental issue before the deadline for reporting.

7 Assigned values and their uncertainties

The assigned values for the ergot alkaloids in the test material (Table 2) were generated at the JRC-Geel. Eight samples randomly selected from the batch were quantified by standard addition comprising four calibration levels plus the sample as such. The analytical determination was done by HPLC-FLD following the WI-D-00632/3. These results were confirmed with those obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following a procedure studied in a collaborative trial in CEN/TC 327/WG5 for the determination of EAs in unprocessed cereals and cereal-based compound feeds. Therefore, the results obtained by HPLC-FLD served as reference values. The assigned values were then computed summing the respective –ine and –inine mass fractions and combining their uncertainties. The HPLC-FLD procedure offered enough resolution to quantify α-ergocryptine and α-ergocryptinine separately from the respective β-isomers.

Table 2. Assigned values of the EAs' mass fractions in the test item and their associated expanded uncertainties

Parameter	**Mass fraction (µg/kg)**	**U (k=2) (µg/kg)**
Ergometrine/-inine SUM	116	11
Ergosine/-inine SUM	242	21
Ergotamine/-inine SUM	695	51
Ergocornine/-inine SUM	295	15
α-Ergocryptine/-inine SUM	231	14
Ergocristine/-inine SUM	752	41
Analyte	**Mass fraction (µg/kg)**	**U (k=2) (µg/kg)**
Ergometrine	85	10
Ergometrinine	31.3	3.2
Ergosine	178	20
Ergosinine	63.6	5.6
Ergotamine	539	49
Ergotaminine	156	16
Ergocornine	189	12
Ergocorninine	106.1	8.4
α-Ergocryptine	175	13
α-Ergocryptinine	56.0	4.7
Ergocristine	531	36
Ergocristinine	221	20

8 Evaluation of the results

8.1 General observations

Thirty-eight participants from 22 EU Member States plus Iceland and Norway registered for the exercise and 37 datasets were reported back. Twenty-six laboratories were NRLs for mycotoxins and 11 were OCLs. Both NRLs for food and feed from the Czech Republic, Ireland, Poland and Sweden had participated in this PT. Two laboratories were unable to provide a license for handling Category 1 drug precursors and didn't report results for ergometrine, ergometrinine, ergotamine and ergotaminine while one laboratory just reported results for the –ine epimers. Denmark, Lithuania, Latvia, Estonia, Portugal, Malta and Slovakia did not participate in the present PT.

The laboratories were free to use their method of choice reflecting their routine procedures. Most of the laboratories (29) used LC-MS/MS-based methods while 6 laboratories used HPLC-FLD and 2 used LC-HRMS (Orbitrap). One laboratory used both HPLC-FLD and LC-MS/MS, depending on the analyte. All but two laboratories submitted the measurement uncertainty associated with each determination. On the other hand, just 8-12 valid results were received for each analyte regarding the comparison of the in-house and the supplied standard.

8.2 Scores and evaluation criteria

The individual participant performance relevant for fulfilling the mandate of the EURL was assessed based on z-scores following ISO 13528:2015 [16] for the summed mass fractions of the respective –ine and –inine epimers of an EA. This takes note of the Commission Recommendation 2012/154/EU on monitoring of EAs and the fact that epimerisation can occur during the analysis. For information purposes the individual results of each epimer are also included in this report.

The z- and zeta (ζ)-scores (Equations 1 and 2) were also calculated for the results of the individual epimers. They should be regarded as indicative only and are aimed to help in the identification of appropriate follow-up actions.

$$z = \frac{X_{lab} - X_{ref}}{\sigma_{pt}} \qquad \textbf{Equation 1}$$

$$\zeta = \frac{X_{lab} - X_{ref}}{\sqrt{u_{lab}^2 + u_{ref}^2}} \qquad \textbf{Equation 2}$$

where:

X_{lab} is the measurement result reported by a participant

X_{ref} is the reference value (the assigned value)

u_{lab} is the standard uncertainty reported by a participant

u_{ref} is the standard uncertainty of the reference value

σ_{pt} is the standard deviation for proficiency assessment (target standard deviation)

σ_{pt} was calculated as 22 % of the assigned value. The coefficient derived from the Horwitz equation for a mass fraction of 120 µg/kg ($\sigma_p = 0.22\,C$) was applied regardless of the actual mass fraction of each given analyte. The data collected from previous PTs on regulated mycotoxins and plant toxins indicated that this coefficient often closely resembles the reproducibility standard deviation of the participants' data.

The z-score compares the participants' deviation from the reference value with the target

standard deviation accepted for the proficiency test, σ_{pt}. The z-score is interpreted as:

$\|z\| \leq 2$	indicates satisfactory performance
$2 < \|z\| < 3$	indicates questionable performance
$\|z\| \geq 3$	indicates unsatisfactory performance

The interpretation of the ζ-score is similar to the interpretation of the z-score. The ζ-score indicates whether the participants' estimate of the measurement uncertainty is consistent with the observed deviation from the assigned value. An unsatisfactory performance based on a |ζ-score| ≥ 3 might be due to an underestimation of the uncertainty, a large deviation from the reference value (bias) or to a combination of the two factors.

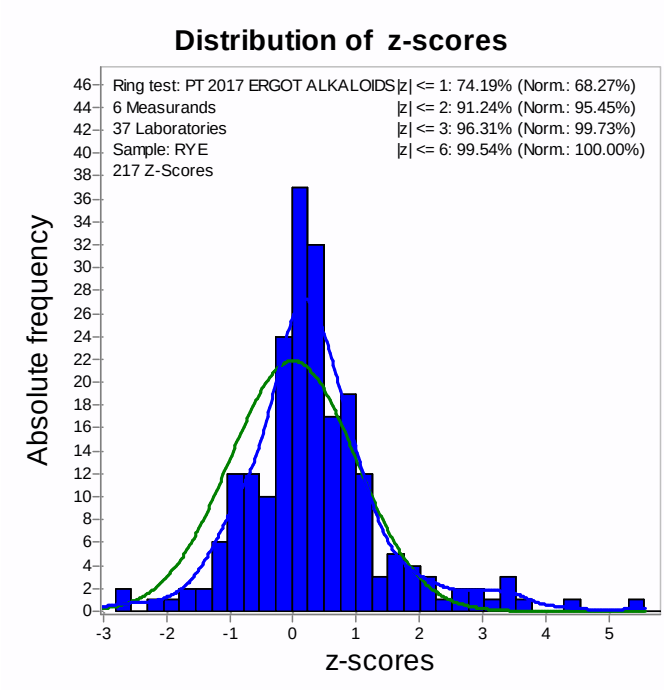
8.3 Laboratory results and scoring

The statistical evaluation of the results was performed using the ProLab software [18]. Z- and ζ-scoring was based on the reference values (and respective uncertainties) assigned by JRC-Geel rather than on the consensus values (robust mean of participants' results). The robust mean and the reproducibility standard deviation were computed according to the Algorithm A of ISO 13528:2015 and are intended for information purposes only [16]. A summary of the statistical evaluation of the PT results is presented in Table 3. The distribution of the z-scores across the six PT parameter pairs is displayed in Figure 1.

91.2 % of the results were rated with satisfactory z-scores (|z| ≤ 2).
3.7 % of the results fell into the unsatisfactory range with |z| ≥ 3.

The reproducibility standard deviations of the reported results (13-17 %) were well below the target standard deviation (22 %) for all parameter pairs except ergometrine/inine (23 %) and α-ergocryptine/inine (34 %). This overall scenario indicates that the methodologies employed by the laboratories, based on liquid chromatography coupled to three different detection systems, generated results in a very narrow range. The cases of ergometrine/ine and α-ergocryptine/inine will be discussed in the course of this report.

Figure 1. Overall distribution of the z-scores of the ergot alkaloids' summed parameters.



The kernel density plots of the parameters covered in the PT are depicted in **Annex 7**. The confidence intervals of the robust means calculated from the participants' results overlap with the confidence intervals of the assigned values for all parameters, except α-ergocryptine/inine, which shows a bimodal distribution and is discussed on page 13. Besides, the major modes in the kernel density plots of ergometrine, ergosine, ergocornine and α-ergocryptine epimeric sum match closer to the respective assigned values than the consensus values.

The distribution of the z-scores for the results of the EAs' –ine and –inine epimers is given in Figure 2. About 20 % more laboratories had a satisfactory performance in the determination of the –ine than in the –inine epimers. This can be explained by the fact that the amount of –inine epimers in the test material was lower than the amount of -ine epimers by a factor of 2.7, on average, rendering the analysis more challenging.

Overall, the analytical performance on the determination of the –ine epimer, which is the biologically active form, was similar to the one obtained considering the sum parameter. In both cases the rate of satisfactory z-scores (|z| ≤ 2) was 91.2%. This finding supports the strategy adopted in the present PT for the assessment of the laboratories' performance based on the epimeric sum parameter.

Figure 2. Distribution of the z-scores for the results on the EAs' –ine (left) and –inine epimers (right)

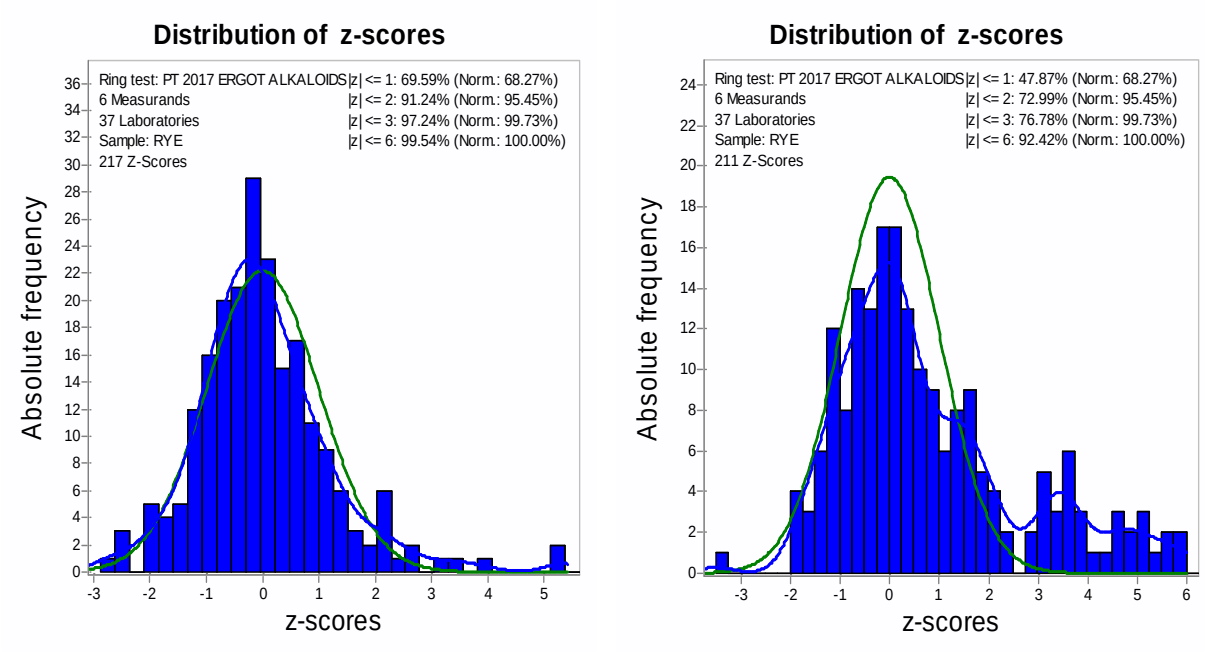


Figure 3 presents an overview of the individual z-scores assigned to the results provided by each laboratory. The longer the triangles, the larger were the differences to the assigned values. Blue triangles represent z-scores in the satisfactory range, yellow triangles in the questionable range and red triangles in the unsatisfactory performance range. The unsatisfactory z-scores are shown next to the red triangles.

The numerical values of the calculated z-scores and ζ-scores are compiled in Table 4 and **Annex 8**. All z- and ζ-scores in the satisfactory performance range are shown with a green background; those in the questionable range are displayed with a yellow background and scores indicating unsatisfactory performance are presented with a light-red background. The sigmoidal distribution of the results for each parameter is given in Figure 4. Parameter values are shown as bars. The green line corresponds to X_{ref}; the green shadow covers the boundary of the reference interval ($X_{ref} \pm u_{ref}$), and the red lines mark the boundary of the target interval ($X_{ref} \pm 2\sigma_{pt}$). Green bars represent results with |z-score| ≤2, yellow bars represent results with 2 < |z-score| < 3, while the red bars represent results with |z-score| ≥ 3.

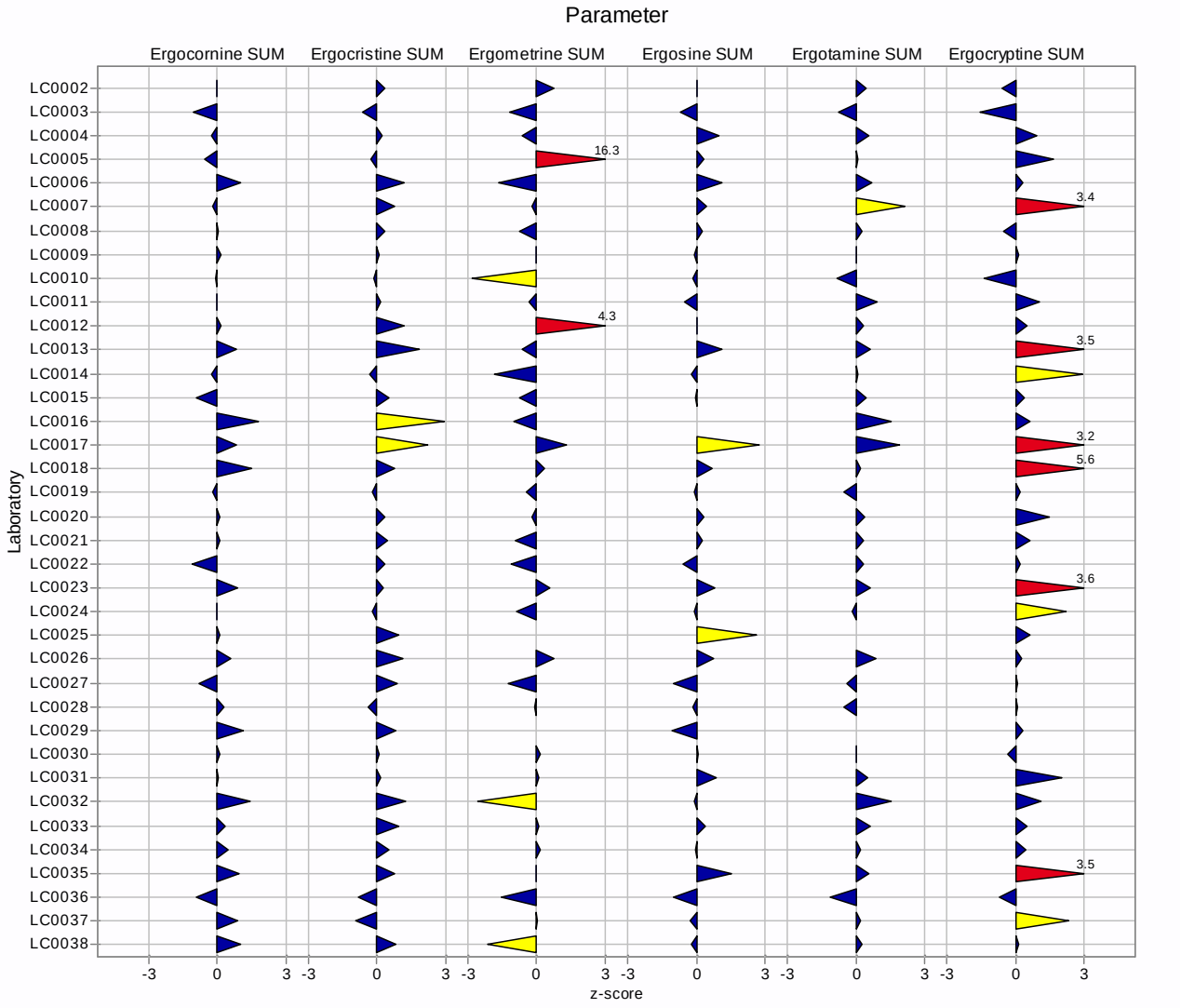
Table 3. Summary statistics of the proficiency test on ergot alkaloids in rye

	Units	Ergometrine/inine SUM	Ergosine/inine SUM	Ergotamine/inine SUM	Ergocornine/inine SUM	Ergocryptine/inine SUM	Ergocristine/inine SUM
No. of participants		37	37	37	37	37	37
No. of laboratories that submitted results		35	36	35	37	37	37
Assigned value (X_{ref})	**µg/kg**	**116**	**242**	**695**	**295**	**231**	**752**
Expanded uncertainty of the assigned value (U_{ref}, k=2)	**µg/kg**	**11**	**21**	**51**	**15**	**14**	**41**
Robust mean	µg/kg	105	252	744	310	280	827
σ_{pt}	µg/kg	26	53	153	65	51	165

Table 4. Ergot alkaloids' results (sum of epimers) and respective z-scores

	Ergometrine/inine		Ergosine/inine		Ergotamine/inine		Ergocornine/inine		Ergocryptine/inine		Ergocristine/inine	
Lab code	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score	Result (µg/kg)	z-score
LC0002	136.1	0.8	244.1	0	761.4	0.4	294.4	0	200.4	-0.6	812.7	0.4
LC0003	86.3	-1.2	204.1	-0.7	581.7	-0.7	228.8	-1	150.6	-1.6	645	-0.6
LC0004	99.7	-0.7	295.1	1	779.8	0.6	278.7	-0.3	276.8	0.9	791.8	0.2
LC0005	533.7	16.3	258.6	0.3	711.9	0.1	258.8	-0.6	315.8	1.7	709.9	-0.3
LC0006	73.7	-1.7	300.6	1.1	803.3	0.7	362.4	1	247.5	0.3	947.3	1.2
LC0007	111.6	-0.2	267.5	0.5	1022.9	2.1	283	-0.2	401.8	3.4	874	0.7
LC0008	97.3	-0.7	256.4	0.3	732	0.2	300	0.1	204.9	-0.5	811	0.4
LC0009	115	-0.1	238	-0.1	697	0	306	0.2	238	0.1	770	0.1
LC0010	44.4	-2.8	232.4	-0.2	569.5	-0.8	291.5	-0.1	161.8	-1.4	731.4	-0.1
LC0011	108.1	-0.3	215.9	-0.5	836.1	0.9	293.5	0	284	1.1	779.3	0.2
LC0012	227	4.3	245	0.1	748	0.3	307	0.2	255	0.5	948	1.2
LC0013	100	-0.6	301	1.1	793	0.6	351	0.9	408	3.5	1060	1.9
LC0014	69.3	-1.8	232	-0.2	708.4	0.1	277	-0.3	379	2.9	701.7	-0.3
LC0015	96.8	-0.8	241	0	761	0.4	234.2	-0.9	251.4	0.4	841.6	0.5
LC0016	90	-1			928	1.5	411	1.8	261	0.6	1237	2.9
LC0017	149.5	1.3	389	2.8	987	1.9	349	0.8	391	3.2	1119	2.2
LC0018	124.3	0.3	278	0.7	729	0.2	392	1.5	513	5.6	874	0.7
LC0019	105.4	-0.4	236.2	-0.1	611	-0.6	283	-0.2	242	0.2	713	-0.2
LC0020	111	-0.2	259.9	0.3	753.8	0.4	301.8	0.1	307	1.5	812.2	0.4
LC0021	93	-0.9	257	0.3	749	0.4	302	0.1	262	0.6	830	0.5
LC0022	87	-1.1	210	-0.6	750	0.4	223	-1.1	240	0.2	810	0.4
LC0023	130.8	0.6	287	0.8	795	0.7	353	0.9	411	3.6	802	0.3
LC0024	93.9	-0.9	235.6	-0.1	674.4	-0.1	294.3	0	343.4	2.2	722.7	-0.2
LC0025			382.3	2.6			302.4	0.1	263.9	0.7	909.2	0.9
LC0026	135.7	0.8	283.9	0.8	828.71	0.9	333.03	0.6	244.6	0.3	937.61	1.1
LC0027	85	-1.2	189	-1	638	-0.4	243	-0.8	236	0.1	901	0.9
LC0028	114.7	-0.1	233.9	-0.2	615.6	-0.5	312.1	0.3	235.2	0.1	690.2	-0.4
LC0029			183.7	-1.1			370.7	1.2	247.6	0.3	890	0.8
LC0030	120.6	0.2	245.2	0.1	697.7	0	301.7	0.1	211.8	-0.4	763.4	0.1
LC0031	119	0.1	287.9	0.9	770.8	0.5	297.5	0	332	2	778.3	0.2
LC0032	50.6	-2.6	238.6	-0.1	933.2	1.6	388.5	1.4	287.6	1.1	957.1	1.2
LC0033	119	0.1	263.8	0.4	788.8	0.6	316	0.3	254.9	0.5	908.7	0.9
LC0034	120	0.1	241.5	0	723	0.2	324	0.4	254	0.5	843	0.5
LC0035	115	-0.1	325	1.6	784	0.6	358.2	1	407.8	3.5	879.4	0.8
LC0036	77.01	-1.5	187.97	-1	518.6	-1.2	236.13	-0.9	194.8	-0.7	616.07	-0.8
LC0037	116.9	0	228.5	-0.3	724.1	0.2	355.2	0.9	348.1	2.3	598.5	-0.9
LC0038	60.6	-2.2	230.7	-0.2	736.5	0.3	360.6	1	236.8	0.1	888.6	0.8

Figure 3. Individual laboratory z-scores for the results on the EAs' sum of epimers in rye.



An interesting case for discussion is that of α-ergocryptine/inine where a number of participants underperformed. The inspection of the individual epimer z-scores indicated that this finding was mostly influenced by the scoring of α-ergocryptinine for which the content was often overestimated. The kernel density plots displayed in Figure 5 elucidate this finding. From our experience, confirmed by some PT participants, the separation of α- and β-ergocryptinine, both present in the sample, is particularly challenging under conventional reverse-phase chromatographic conditions. Therefore, it is plausible that most laboratories reported the sum of α- and β-ergocryptinine as α-ergocryptinine. This, in turn, has led to a mean value fairly disparate from the target value.

For α-ergocryptine, on the other hand, the target and mean values were in good agreement, but the kernel density plot revealed a second mode at a higher mass fraction. Seven out of the nine implicated laboratories confirmed that the result submitted corresponded to the sum α- and β-ergocryptine. The main reason put forward was the poor chromatographic resolution of the isomers, although one laboratory did it intentionally. An investigation of the analytical conditions used by the participants indicated that a chromatographic column with a phenyl-hexyl stationary phase might be the most suitable to achieve an acceptable resolution. While for this PT the separation of the α- and β-isomers was crucial, a joint quantification might still be acceptable, in routine monitoring.

Figure 4. Sigmoidal plots of laboratory results reported for the ergot alkaloids' sum of epimers.

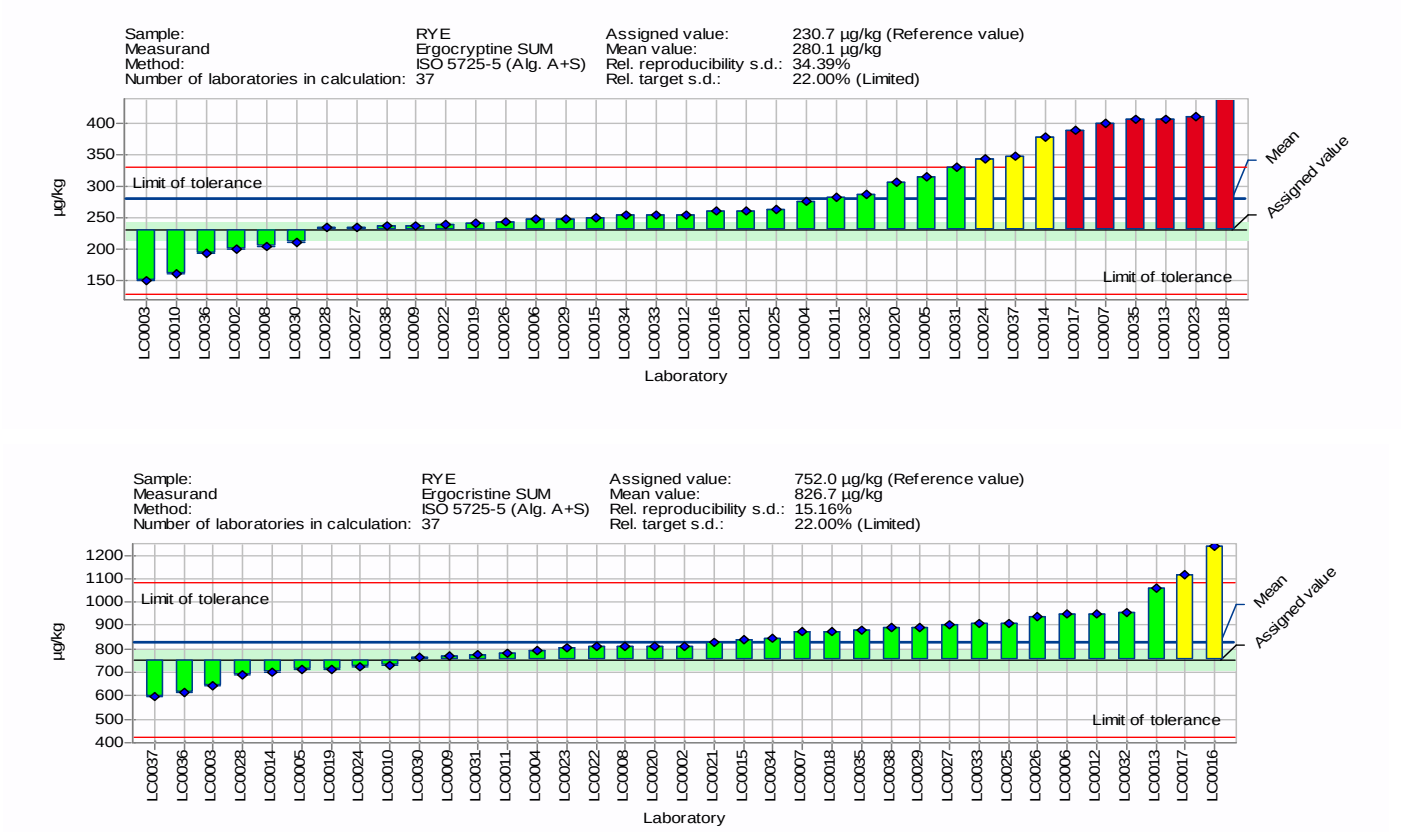
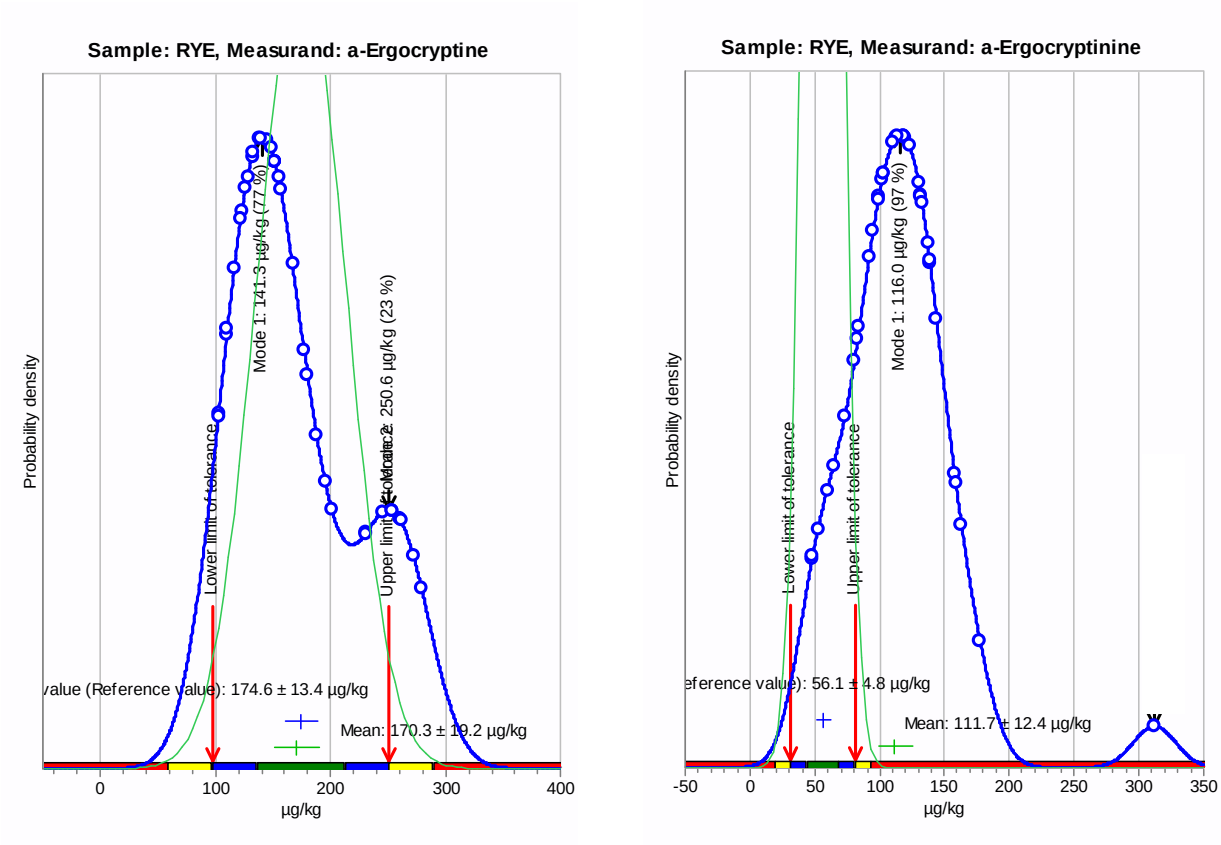


Figure 5. Kernel density plots of the results for α-ergocryptine and α-ergocryptinine.



The rate of satisfactory ζ-scores was lower than for the z-scores. The plausibility of the uncertainty statements of the laboratories was assessed by classifying every reported uncertainty into three groups (see column C, in Tables of **Annex 8**) according to the following scenarios:

1) The standard measurement uncertainty of a result ($u(x_i)$) is most likely to fall within a range between a minimum and a maximum uncertainty (case "a": $u_{min} \leq u(x_i) \leq u_{max}$). The minimum uncertainty (u_{min}) is set for the respective analyte to the standard uncertainty of the assigned value ($u(x_{ref})$). This is based on the assumption that it is unlikely that a laboratory carrying out the analysis on a routine basis would determine the measurand with a smaller measurement uncertainty than that achieved in the experiments for the characterisation of the test material. The maximum uncertainty is set to the standard deviation accepted for the assessment of results (σ_{pt}). Consequently, case "a" becomes: $u(x_{ref}) \leq u(x_i) \leq \sigma_{pt}$.
2) If $u(x_i)$ is smaller than $u(x_{ref})$ (case "b": $u(x_i) < u(x_{ref})$), the laboratory might have underestimated its measurement uncertainty.
3) If $u(x_i)$ is larger than σ_{pt} (case "c": $u(x_i) > \sigma_{pt}$), the laboratory might have overestimated its measurement uncertainty or applied an analytical method that was not fit-for-purpose.

The participants in categories "b" and "c" are encouraged to assess their uncertainty estimation in line with the above observations. The uncertainty is an integral part of the measurement result and has major implications on the assessment of the compliance of food according to the European Union legislation.

9 Evaluation of the questionnaire

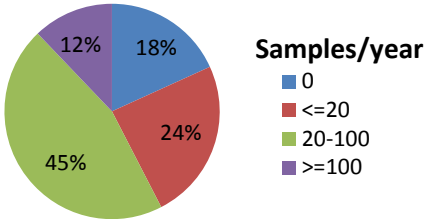
The questionnaire distributed to the participants (**Annex 6**) has provided very useful information concerning the capabilities and analytical approaches followed by the laboratories regarding the determination of ergot alkaloids in cereals.

9.1 Experience and organisational aspects

The vast majority of the participants (81 %) stated that they had prior experience on the determination of ergot alkaloids, spanning from 1 to more than 10 years (typically 3 years). The ergot alkaloids covered in their methods were mainly those included in the present PT. However, three laboratories stated that they could also analyse β-ergocryptine and one laboratory extended its analytical range to include agroclavine, chanoclavine-1, elymoclavine, ergine, erginine, festuclavine and lysergol.

The matrices where the ergot alkaloids are monitored are mainly cereals (wheat, barley, buckwheat, rye, triticale and oat) but also cereal products (cereal flour, bakery products) and compound feed. Two laboratories also perform this determination on silage, hay and grass. Typically, the laboratories analyse between 20 and 50 samples per year; however, there are some laboratories that despite having the method implemented, do not analyse ergot alkaloids by routine. The annual analysis of ergot alkaloids is depicted in Figure 6. Forty-three percent of the laboratories are accredited for this determination.

Figure 6. Number of samples analysed for ergot alkaloids on a yearly basis.



The majority of the participants (86 %) did not experience any difficulties in the execution of this PT. Those who found hindrances related them to:

- matrix effects
- matrix interferences for ergometrine/ine and ergosine/ine
- retention time fluctuation
- ergot alkaloids' levels exceeding the normal calibration range
- unusual recoveries and the sensitivity of the instrument being not as good as it used to be

The platform for reporting the results (ProLab/RingDat) worked smoothly for most of the participants. Only one remark was received indicating that the instructions for the submission of the final report were unclear. Ninety-three percent of the participants found the instructions for conducting the PT sufficiently explanatory. Still, one participant commented that the information on whether the distributed sample was feed or food would have been crucial while another found it unclear as to whether only α-ergocryptine/inine should be reported, or if α+β-isomer should be integrated. The matrix offered to the participants was, indeed, rye flour that could be considered either as food or feed.
Some participants shared their analytical findings in the Comments section of the questionnaire, which was highly appreciated. A compilation can be found in Table 5.
Several participants observed chromatographically unresolved double peaks for ergocryptine and ergocryptinine, corresponding to the α- and β-isomers. In some cases, it was still possible to report the individual result for α-ergocryptine, as requested in this PT, while for ergocryptinine the sum α+β was reported. Two laboratories noted a lower response of their ergometrine standard compared with the one provided by the EURL. Further discussion among the delegates to the annual EURL Mycotoxins workshop indicated that the reason behind such behaviour might lie in the insufficient solubility of the supplied ergometrine salt in the recommended solvent. Indeed, the median response of the ergometrine standards held by the laboratories compared with the EURL standard amounted to just 77 %, indicating that this standard requires careful preparation. For the remaining analytes both standards were comparable (90-96 %, see Table 7).

Table 5. Comments submitted by the participants

In our experience the exposition to light is a critical factor
Problems with the supplied standard for ergometrine, different amount than our standard (see results). Results for beta-ergocryptine (incl. recovery, estimated with the alpha-ergocryptine standard) = 79.1 µg/kg. Results for beta-ergocryptinine (incl. recovery, estimated with the alpha-ergocryptinine standard) = 46.8 µg/kg.
We analyzed samples using our standard but in a different batch.
The method was developed in 2016 but never applied in routine For alfa-ergokryptine we had two peaks that were badly separated
The data provided for Ergosine is a sum of ergosine and ergosinine. Recoveries were calculated based on the supplied calibration standards prepared with matrix components. Calibration curves were constructed employing internal standard procedures. LOD and LOQ values were calculated based on the matrix assisted calibrations using the data collected during the last year. LOQ and LOD values based on internal standard procedures are not available.
We got a double peak in the ergocryptine and ergocryptinine chromatograms for the sample, not in our spiked sample. Possibly some matrix contaminant that almost coeluted with these alkaloids? This might have affected our results.
We were surprised to see that our ergometrine standard concentration was very different from yours. And only for ergometrine! We diluted our stock solution and we obtained the same. Our molecules are bought from BIOPURE. At the moment we have not found the reason. We are waiting for other new standards (all the -ine)
Ergocryptine is recalculated using REC obtained from CRM and IRM measurement
The laboratory does not have a precursor license for analyzing ergometrine, ergometrinine, ergotamine and ergotaminine and for that reason we didn't perform it.
As the method is not in routine use in our laboratory we have not established values for LOD, LOQ, MU.
a-ergocryptine is reported as this only, quantified against the a-ergocryptine standard. a-ergotcryptinine is actually the sum of a+b ergocryptinine as we cannot separate the two compounds, quantified against the a-ergocryptinine standard.
The method is not yet validated for cereals and based cereals products. Std % for ergometrine, ergometrinine, ergotamine and ergotaminine can't be calculated because of the lack of our own standards.
The scarce experience with these mycotoxins didn't allow us to prepare a proper experimental plan good for assessing recoveries and signal suppression/enhancement

9.2 Overview of the analytical methodologies

The majority of the laboratories (29) used LC-MS/MS-based methodologies for analysing the PT sample, while a few resorted to HPLC-FLD (6) or LC-HRMS (2). Statistically, the 3 analytical principles produced comparable results. The most used methods followed (or were derived from) the §64 LFGB L 15.01/02 method, the draft CEN standards for ergot alkaloids in food or feed, papers published in scientific journals or application notes accompanying clean-up materials (e.g. MycoSep 150). The need for a prior clean-up step was unanimous. The most used clean-up materials were: MycoSep 150 > SPE Alumina > Bondesil PSA. More details on the type of method, extraction, chromatographic and detection conditions, amongst others, can be found in **Annex 10**.

The quantification approaches followed by the laboratories are summarised in Table 6. Of those that have employed an LC-MS-based methodology, more than half performed a calibration with standards in a pure solvent and 2/3 reported recovery-corrected results. The remaining laboratories preferred a matrix-compensated calibration approach and the majority also corrected their results for recoveries. Conversely, the laboratories that chose HPLC-FLD as the analytical system calibrated the method exclusively with standards in a pure solvent and the majority did not find the need to correct the results for recoveries. The recoveries were estimated mostly by spiking a blank or contaminated cereal sample, whereas one laboratory used a CRM and another used a FAPAS test material for that purpose.

Table 6. Analytical strategies followed by the participants

			%
LC-MS	Standards in pure solvent	Corrected for recoveries	32
		Not corrected	16
LC-MS	Standard addition or Matrix-matched calibration	Corrected for recoveries	22
		Not corrected	14
HPLC-FLD	Standards in pure solvent	Corrected for recoveries	3
		Not corrected	13

The preferred approach for estimating the measurement uncertainty was using method validation data (71 %), whereas three laboratories relied on the Horwitz model and one laboratory used the GUM approach. A summary of the analytical figures of merit of the employed methodologies along with the outcome of the comparison of the calibration standard (Std) provided by the EURL and those existing in the participants' laboratories is given in Table 7.

Table 7. Summary of the figures of merit of the methods employed in the PT

	LOD (Median, µg/kg)	LOQ (Median, µg/kg)	Recoveries (Mean, %)	U (k=2) (Median, %)	Analyt. signal ratio Labs vs EURL Std (%)
Ergometrine	1.5	5	90	25	77
Ergometrinine	1	5	94	27	96
Ergosine	1	5	90	25	94
Ergosinine	1	5	98	27	91
Ergotamine	1.5	5	91	24	93
Ergotaminine	1	5	97	26	91
Ergocornine	1.6	5	94	25	96
Ergocorninine	1	5	95	25	94
α-Ergocryptine	1.2	5	98	25	95
α-Ergocryptinine	1	5	100	25	90
Ergocristine	1	5	91	25	96
Ergocristinine	1	5	99	26	95

Annex 11 compiles all the validation data supplied by the participants. The LOQs declared by the participants were sufficient to analyse the levels of ergot alkaloids contained in the sample, the lowest being ergometrinine at 31 μg/kg.

10 Conclusions

A total of 37 laboratories representing 21 EU Member States and Norway and Iceland submitted their results for the PT on ergot alkaloids. Two laboratories didn't provide results for ergometrine, ergotamine and respective -inine epimers, since they didn't hold a license for handling Category 1 drug precursors whereas, one laboratory didn't submit results for ergosine/inine.

Overall, 91.2 % of the results were classified as satisfactory. The rate of satisfactory z-scores for each EA pair was ranked as follows: ergocornine/inine - 100 %, ergotamine/inine – 97.1 %, ergocristine/inine - 94.6 %, ergosine/inine - 94.4 %, ergometrine/inine - 85.7 % and α-ergocryptine/inine - 75.7 %. The rate of satisfactory z-scores was better for the –ine (biologically active) than for the –inine epimers.

The majority of the participants reported correctly α-ergocryptine, as requested. Still, six participants mentioned a poor resolution of the α- and β-isomers, leading to a joint quantification. On the other hand, the majority of participants had most likely reported the sum α+β-ergocryptinine leading to a considerable overestimation of the parameter. This is known to be a challenging chromatographic separation. According to the information provided by the participants, a phenyl-hexyl stationary phase enables the best chromatographic resolution of the isomers.

Twenty-nine laboratories used an LC-MS/MS-based methodology while six used HPLC-FLD and 2 used LC- LC-HRMS (Orbitrap). The three quantification techniques, preceded by a variety of extraction and clean-up protocols, provided comparable results. Likewise, the results produced using the provided calibration solution didn't differ statically (t-student test, 95 %) from those produced using the laboratories' calibration standards. Still, two participants noted a lower response of their ergometrine standard compared with the EURL one which may be related to insufficient redissolution of the dry film. This was substantiated by the fact that recently a supplier of EA reference materials changed the protocol for re-dissolution taking note that the salt form (maleate) of the EA might have re-dissolution issues in acetonitrile. The responses for the remaining analytes in both standards were in a comparable range (90-96 %).

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Annexes

Annex 1. Opening of the registration

Proficiency test on the determination of ergot alkaloids in cereals

Fields marked with * are mandatory.

On behalf of the operating manager of the European Union Reference Laboratory for Mycotoxins (EURL Mycotoxins), I have the pleasure to announce the opening for registration to the proficiency test (PT) on the determination of ergot alkaloids in cereals.

The aim of this study is to evaluate the proficiency of the European National Reference Laboratories (NRLs) and Official Food Control Laboratories (OCLs) on the determination of ergot alkaloids which were identified as priority by the European Food Safety Authority and for which maximum levels can be expected in the near future.

One test item will be provided consisting of a naturally contaminated rye product. Participants will be asked to analyse the 6 ergot alkaloids (and the corresponding –inine epimers) mentioned in the Commission Recommendation 2012/154/EU. Additional ergot alkaloids can be reported voluntarily, but will not be benchmarked.

Participants will be provided with a standard solution for calibration. As some ergot alkaloids are considered drug precursors (ergometrine, ergometrinine, ergotamine and ergotaminine), the standard solution will **ONLY** be **DELIVERED** on the condition that a licence allowing the lab to handle drug precursors is submitted to us.

The PT is open to all NRLs and appointed OCLs. Participation is free of charge for the NRLs. The participation fee for OCLs is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. Enrolled OCLs will be contacted for payment details upon registration.

The dispatch of the samples is expected by mid-May 2017. Participants will have 6 weeks from the dispatch date to report back the results. Confidentiality of results is guaranteed. The deadline for registration is 05th May 2017

In order to register, please fill in your laboratory details in the fields below. Thank you in advance for your consideration.

Best regards,

Carlos Goncalves (on behalf of the Operating Manager of the EURL for Mycotoxin)

* Contact person

* Second contact person

* Organisation

Department

* Address

* Postcode

* City

* Country

* Telephone number

Fax

* Email address

Additional Comments

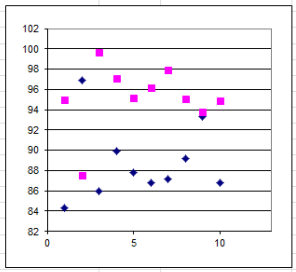
Please upload your import licence for drug precursors

Annex 2. Homogeneity test

Homogeneity according to ISO 13528:2015	Rye: sample EA###					
	Ergometrine	**Ergometrinine**	**Ergosine**	**Ergosinine**	**Ergotamine**	**Ergotaminine**
$\hat{\sigma}$	20.247 (22 %)	8.122 (22 %)	45.408 (22 %)	18.634 (22 %)	137.73 (22 %)	44.484 (22 %)
$0.3\,\hat{\sigma}$ (critical value)	6.074	2.437	13.622	5.590	41.319	13.345
S_X (standard deviation of sample averages)	1.205	0.642	2.503	0.706	9.145	3.607
S_W (within-sample standard deviation)	6.327	0.857	4.450	3.882	14.427	9.192
S_S (between-sample standard deviation)	0.000	0.212	0.000	0.000	0.000	0.000
$S_S < 0.3\,\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**

Ergometrine

			m =	10										
	variances		mean =	92.030										
	1.4529		s_x =	1.205		22.0%	= σ-trg(%)							
MSW =	40.0330		$s_{an}=s_w$ =	6.327	1	20.247	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000		6.074	= 0,3*σ-trg							
		1) Cochran test		0.2344	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528	Ss < 0,3*strg => passed											
		3) IUPAC		0.000	109.80	= Crit = F1*(0,3*σ)²+F2*MSW								
			Ss2 < Crit => passed											
	Bottle	Result_a	Result_b	diff	sum	avg								
	1	84.3	95	-10.7	179.3	89.65								
	2	96.9	87.5	9.4	184.4	92.2								
	3	86	99.7	13.7	185.7	92.85								
	4	89.9	97.1	-7.2	187	93.5								
	5	87.8	95.2	-7.4	183	91.5								
	6	86.8	96.2	-9.4	183	91.5								
minimum 7	7	87.2	97.9	-10.7	185.1	92.55								
	8	89.2	95.1	-5.9	184.3	92.15								
	9	93.3	93.8	-0.5	187.1	93.55								
	10	86.8	94.9	-8.1	181.7	90.85								
	11													
	12													
			SDD=Σ(diff)² =		800.66									
			MSB = var(sum)/2 =		2.9058									



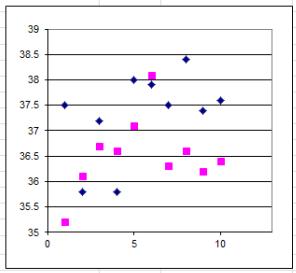
IUPAC

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

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

Ergometrinine

			m =	10										
	variances		mean =	36.920										
	0.4118		s_x =	0.642		22.0%	= σ-trg(%)							
MSW =	0.7340		$s_{an}=s_w$ =	0.857	1	8.122	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0448		s_s =	0.212										
			s_s =	0.212		2.437	= 0,3*σ-trg							
		1) Cochran test		0.3604	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528	Ss < 0,3*strg => passed											
		3) IUPAC		0.045	11.90	= Crit = F1*(0,3*σ)²+F2*MSW								
			Ss2 < Crit => passed											
	Bottle	Result_a	Result_b	diff	sum	avg								
	1	37.5	35.2	2.3	72.7	36.35								
	2	35.8	36.1	-0.3	71.9	35.95								
	3	37.2	36.7	0.5	73.9	36.95								
	4	35.8	36.6	-0.8	72.4	36.2								
	5	38	37.1	0.9	75.1	37.55								
	6	37.9	38.1	-0.2	76	38								
minimum 7	7	37.5	36.3	1.2	73.8	36.9								
	8	38.4	36.6	1.8	75	37.5								
	9	37.4	36.2	1.2	73.6	36.8								
	10	37.6	36.4	1.2	74	37								
	11													
	12													
			SDD=Σ(diff)² =		14.68									
			MSB = var(sum)/2 =		0.8236									



IUPAC

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

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

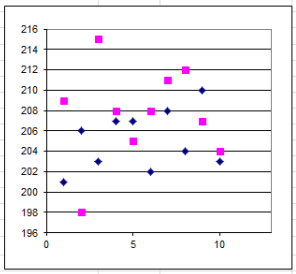
Ergosine

			m =	10										
	variances		mean =	206.400										
	6.2667		s_x =	2.503		22.0%	= σ-trg(%)							
MSW =	19.8000		$s_{an}=s_w$ =	4.450	1	45.408	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000		13.622	= 0,3*σ-trg							
		1) Cochran test		0.3636	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	368.85	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	201	209	-8	410	205
2	206	198	2	404	202
3	203	215	-12	418	209
4	207	208	-1	415	207.5
5	207	205	2	412	206
6	202	208	-6	410	205
minimum 7	208	211	-3	419	209.5
8	204	212	-8	416	208
9	210	207	3	417	208.5
10	203	204	-1	407	203.5
11					
12					

SDD=Σ(diff)² = 396

MSB = var(sum)/2 = 12.5333



IUPAC

Tab1

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

Tab2

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

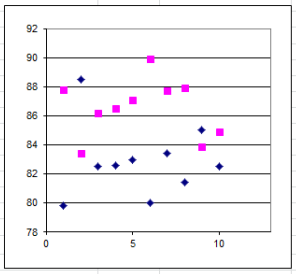
Ergosinine

			m =	10										
	variances		mean =	84.700										
	0.4989		s_x =	0.706		22.0%	= σ-trg(%)							
MSW =	15.0720		$s_{an}=s_w$ =	3.882	1	18.634	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000		5.590	= 0,3*σ-trg							
		1) Cochran test		0.3251	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	73.97	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	79.8	87.8	-8	167.6	83.8
2	88.5	83.4	5.1	171.9	85.95
3	82.5	86.2	-3.7	168.7	84.35
4	82.6	86.5	-3.9	169.1	84.55
5	83	87.1	-4.1	170.1	85.05
6	80	89.9	-9.9	169.9	84.95
minimum 7	83.4	87.7	-4.3	171.1	85.55
8	81.4	87.9	-6.5	169.3	84.65
9	85	83.9	1.1	168.9	84.45
10	82.5	84.9	-2.4	167.4	83.7
11					
12					

SDD=Σ(diff)² = 301.44

MSB = var(sum)/2 = 0.9978



IUPAC

Tab1

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

Tab2

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

Ergotamine

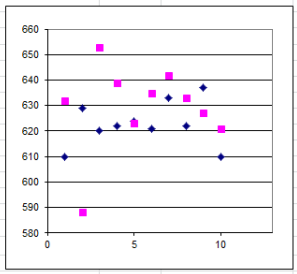
			m =	10										
	variances		mean =	626.050										
	83.6361		s_x =	9.145		1	22.0%	= σ-trg(%)						
MSW =	208.1500		s_{an}=s_w =	14.427			137.731	= σ-trg			Homogeneity Tests			
s^2_{sam}=	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000			41.319	= 0,3*σ-trg						
		1) Cochran test		0.4038	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	3419.77	= Crit = F1*(0,3*σ)^2+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	610	632	-22	1242	621
2	629	588	41	1217	608.5
3	620	653	-33	1273	636.5
4	622	639	-17	1261	630.5
5	624	623	1	1247	623.5
6	621	635	-14	1256	628
minimum 7	633	642	-9	1275	637.5
8	622	633	-11	1255	627.5
9	637	627	10	1264	632
10	610	621	-11	1231	615.5
11					
12					

2

SDD=Σ(diff)^2 = 4163

MSB = var(sum)/2 = 167.2722



IUPAC

Tab1

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

Tab2

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

Ergotaminine

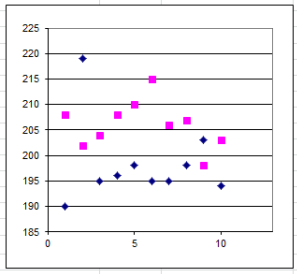
			m =	10										
	variances		mean =	202.200										
	13.0111		s_x =	3.607		1	22.0%	= σ-trg(%)						
MSW =	84.5000		s_{an}=s_w =	9.192			44.484	= σ-trg			Homogeneity Tests			
s^2_{sam}=	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000			13.345	= 0,3*σ-trg						
		1) Cochran test		0.2367	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	420.16	= Crit = F1*(0,3*σ)^2+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	190	208	-18	398	199
2	219	202	17	421	210.5
3	195	204	-9	399	199.5
4	196	208	-12	404	202
5	198	210	-12	408	204
6	195	215	-20	410	205
minimum 7	195	206	-11	401	200.5
8	198	207	-9	405	202.5
9	203	198	5	401	200.5
10	194	203	-9	397	198.5
11					
12					

2

SDD=Σ(diff)^2 = 1690

MSB = var(sum)/2 = 26.0222



IUPAC

Tab1

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

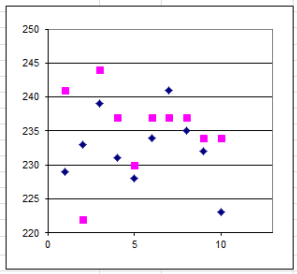
Tab2

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

Homogeneity according to ISO 13528:2015	Rye: sample EA###					
	Ergocornine	Ergocorninine	α-Ergocryptine	α-Ergo cryptinine	Ergocristine	Ergocristinine
$\hat{\sigma}$	51.458 (22 %)	29.458 (22 %)	45.695 (22 %)	17.293 (22 %)	139.14 (22 %)	64.537 (22 %)
0.3 $\hat{\sigma}$ (critical value)	15.437	8.837	13.709	5.188	41.742	19.361
S_X (standard deviation of sample averages)	4.557	1.776	2.470	1.041	9.409	2.427
S_W (within-sample standard deviation)	4.919	3.860	6.238	1.817	13.098	9.584
S_S (between-sample standard deviation)	2.944	0.000	0.000	0.000	1.658	0.000
S_S < 0.3 $\hat{\sigma}$	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**	**Passed**

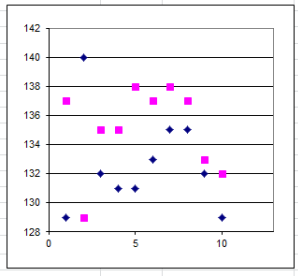
Ergocornine

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1															
2			m =	10											
3		variances	mean =	233.900											
4		20.7667	s_x =	4.557		22.0%	= σ-trg(%)								
5	MSW =	24.2000	s_{an}=s_w =	4.919	1	51.458	= σ-trg			Homogeneity Tests					
6	s^2_{sam}=	8.6667	s_s =	2.944											
7			s_s =	2.944		15.437	= 0,3*σ-trg								
8															
9			1) Cochran test	0.2975	$C=D_{max}^2/SDD$										
10				no outlier	no outlier								IUPAC		
11				0.6020	0.7175	= Crit							Tab1	Cochran	
12				@ 95%	@ 99%							m	Crit-95%	Crit-99%	
13												3	0.9669	0.9933	
14			2) ISO-13528	Ss < 0,3*strg => passed								4	0.9065	0.9676	
15												5	0.8412	0.9279	
16			3) IUPAC	8.667	472.45	= Crit = F1*(0,3*σ)^2+F2*MSW						6	0.7808	0.8828	
17				Ss2 < Crit => passed								7	0.7271	0.8376	
18												8	0.6789	0.7945	
19		Bottle	Result_a	Result_b	diff	sum	avg					9	0.6385	0.7544	
20		1	229	241	-12	470	235					10	0.6020	0.7175	
21		2	233	222	11	455	227.5					11	0.5700	0.684	
22		3	239	244	-5	483	241.5					12	0.5410	0.6528	
23		4	231	237	-6	468	234								
24		5	228	230	-2	458	229								
25		6	234	237	-3	471	235.5						Tab2		
26	minimum 7	7	241	237	4	478	239					m	F1	F2	
27		8	235	237	-2	472	236					3	2.996	4.276	
28		9	232	234	-2	466	233					4	2.605	2.796	
29		10	223	234	-11	457	228.5					5	2.372	2.096	
30		11										6	2.214	1.694	
31		12										7	2.099	1.433	
32				SDD=Σ(diff)^2 =	484							8	2.010	1.250	
33					MSB = var(sum)/2 =	41.5333						9	1.938	1.115	
34												10	1.880	1.010	
35												11	1.831	0.927	
36												12	1.789	0.859	
37															



Ergocorninine

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1															
2			m =	10											
3		variances	mean =	133.900											
4		3.1556	s_x =	1.776		22.0%	= σ-trg(%)								
5	MSW =	14.9000	s_{an}=s_w =	3.860	1	29.458	= σ-trg			Homogeneity Tests					
6	s^2_{sam}=	0.0000	s_s =	0.000	MSB<MSW										
7			s_s =	0.000		8.837	= 0,3*σ-trg								
8															
9			1) Cochran test	0.4060	$C=D_{max}^2/SDD$										
10				no outlier	no outlier								IUPAC		
11				0.6020	0.7175	= Crit							Tab1	Cochran	
12				@ 95%	@ 99%							m	Crit-95%	Crit-99%	
13												3	0.9669	0.9933	
14			2) ISO-13528	Ss < 0,3*strg => passed								4	0.9065	0.9676	
15												5	0.8412	0.9279	
16			3) IUPAC	0.000	161.87	= Crit = F1*(0,3*σ)^2+F2*MSW						6	0.7808	0.8828	
17				Ss2 < Crit => passed								7	0.7271	0.8376	
18												8	0.6789	0.7945	
19		Bottle	Result_a	Result_b	diff	sum	avg					9	0.6385	0.7544	
20		1	129	137	-8	266	133					10	0.6020	0.7175	
21		2	140	129	11	269	134.5					11	0.5700	0.684	
22		3	132	135	-3	267	133.5					12	0.5410	0.6528	
23		4	131	135	-4	266	133								
24		5	131	138	-7	269	134.5								
25		6	133	137	-4	270	135						Tab2		
26	minimum 7	7	135	138	-3	273	136.5					m	F1	F2	
27		8	135	137	-2	272	136					3	2.996	4.276	
28		9	132	133	-1	265	132.5					4	2.605	2.796	
29		10	129	132	-3	261	130.5					5	2.372	2.096	
30		11										6	2.214	1.694	
31		12										7	2.099	1.433	
32				SDD=Σ(diff)^2 =	298							8	2.010	1.250	
33					MSB = var(sum)/2 =	6.3111						9	1.938	1.115	
34												10	1.880	1.010	
35												11	1.831	0.927	
36												12	1.789	0.859	
37															



α-Ergocryptine

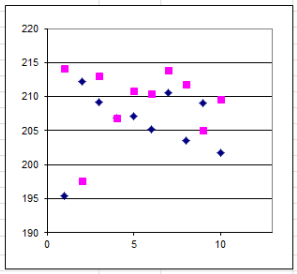
			m =	10										
	variances		mean =	207.705										
	6.0997		s_x =	2.470		22.0%	= σ-trg(%)							
MSW =	38.9095		$s_{an}=s_w$ =	6.238	1	45.695	= σ-trg		Homogeneity Tests					
s^2_{sam} =	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000		13.709	= 0,3*σ-trg							
		1) Cochran test		0.4542	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	392.58	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	195.4	214.2	-18.8	409.6	204.8
2	212.2	197.6	14.6	409.8	204.9
3	209.2	213	-3.8	422.2	211.1
4	206.9	206.9	0	413.8	206.9
5	207.1	210.8	-3.7	417.9	208.95
6	205.2	210.4	-5.2	415.6	207.8
minimum 7	210.5	213.9	-3.4	424.4	212.2
8	203.6	211.8	-8.2	415.4	207.7
9	209	205.1	3.9	414.1	207.05
10	201.7	209.6	-7.9	411.3	205.65
11					
12					

2

SDD=Σ(diff)² = 778.19

MSB = var(sum)/2 = 12.1994



IUPAC

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

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

α-Ergocrytpinine

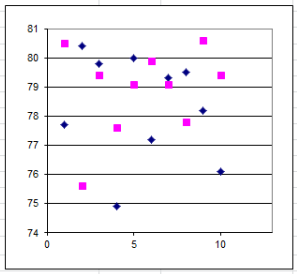
			m =	10										
	variances		mean =	78.605										
	1.0847		s_x =	1.041		22.0%	= σ-trg(%)							
MSW =	3.3005		$s_{an}=s_w$ =	1.817	1	17.293	= σ-trg		Homogeneity Tests					
s^2_{sam} =	0.0000		s_s =	0.000	MSB<MSW									
			s_s =	0.000		5.188	= 0,3*σ-trg							
		1) Cochran test		0.3490	$C=D_{max}^2/SDD$									
				no outlier	no outlier									
				0.6020	0.7175	= Crit								
				@ 95%	@ 99%									
		2) ISO-13528		Ss < 0,3*strg => passed										
		3) IUPAC		0.000	53.93	= Crit = F1*(0,3*σ)²+F2*MSW								
				Ss2 < Crit => passed										

Bottle	Result_a	Result_b	diff	sum	avg
1	77.7	80.5	-2.8	158.2	79.1
2	80.4	75.6	4.8	156	78
3	79.8	79.4	0.4	159.2	79.6
4	74.9	77.6	-2.7	152.5	76.25
5	80	79.1	0.9	159.1	79.55
6	77.2	79.9	-2.7	157.1	78.55
minimum 7	79.3	79.1	0.2	158.4	79.2
8	79.5	77.8	1.7	157.3	78.65
9	78.2	80.6	-2.4	158.8	79.4
10	76.1	79.4	-3.3	155.5	77.75
11					
12					

2

SDD=Σ(diff)² = 66.01

MSB = var(sum)/2 = 2.1694



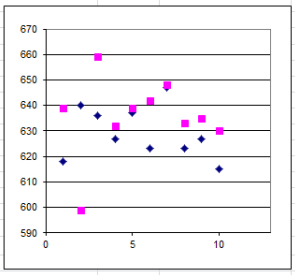
IUPAC

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

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

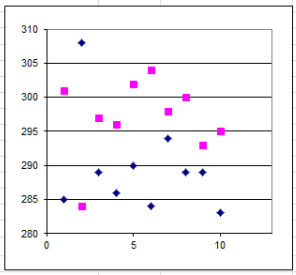
Ergocristine

			m =	10										
	variances	mean =	632.450											
	88.5250	s_x =	9.409			22.0%	= σ-trg(%)							
MSW =	171.5500	$s_{an}=s_w$ =	13.098		1	139.139	= σ-trg			Homogeneity Tests				
s^2_{sam}=	2.7500	s_s =	1.658											
		s_s =	1.658			41.742	= 0,3*σ-trg							
		1) Cochran test	0.4899	$C=D_{max}^2/SDD$										
			no outlier	no outlier										IUPAC
			0.6020	0.7175	= Crit									
			@ 95%	@ 99%										Tab1 Cochran
		2) ISO-13528	Ss < 0,3*strg => passed											m Crit-95% Crit-99%
		3) IUPAC	2.750	3448.76	= Crit = F1*(0,3*σ)²+F2*MSW									3 0.9669 0.9933
			Ss2 < Crit => passed											4 0.9065 0.9676
														5 0.8412 0.9279
	Bottle	Result_a	Result_b	diff	sum	avg								6 0.7808 0.8828
	1	618	639	-21	1257	628.5								7 0.7271 0.8376
	2	640	599	2	1239	619.5								8 0.6789 0.7945
	3	636	659	-23	1295	647.5								9 0.6385 0.7544
	4	627	632	-5	1259	629.5								10 0.6020 0.7175
	5	637	639	-2	1276	638								11 0.5700 0.684
	6	623	642	-19	1265	632.5								12 0.5410 0.6528
minimum 7	7	647	648	-1	1295	647.5								
	8	623	633	-10	1256	628								Tab2
	9	627	635	-8	1262	631								m F1 F2
	10	615	630	-15	1245	622.5								3 2.996 4.276
	11													4 2.605 2.796
	12													5 2.372 2.096
			SDD=Σ(diff)² =	3431										6 2.214 1.694
			MSB = var(sum)/2 =	177.0500										7 2.099 1.433
														8 2.010 1.250
														9 1.938 1.115
														10 1.880 1.010
														11 1.831 0.927
														12 1.789 0.859



Ergocristinine

			m =	10										
	variances	mean =	293.350											
	5.8917	s_x =	2.427			22.0%	= σ-trg(%)							
MSW =	91.8500	$s_{an}=s_w$ =	9.584		1	64.537	= σ-trg			Homogeneity Tests				
s^2_{sam}=	0.0000	s_s =	0.000	MSB<MSW										
		s_s =	0.000			19.361	= 0,3*σ-trg							
		1) Cochran test	0.3136	$C=D_{max}^2/SDD$										
			no outlier	no outlier										IUPAC
			0.6020	0.7175	= Crit									
			@ 95%	@ 99%										Tab1 Cochran
		2) ISO-13528	Ss < 0,3*strg => passed											m Crit-95% Crit-99%
		3) IUPAC	0.000	797.47	= Crit = F1*(0,3*σ)²+F2*MSW									3 0.9669 0.9933
			Ss2 < Crit => passed											4 0.9065 0.9676
														5 0.8412 0.9279
	Bottle	Result_a	Result_b	diff	sum	avg								6 0.7808 0.8828
	1	285	301	-16	586	293								7 0.7271 0.8376
	2	308	284	2	592	296								8 0.6789 0.7945
	3	289	297	-8	586	293								9 0.6385 0.7544
	4	286	296	-10	582	291								10 0.6020 0.7175
	5	290	302	-12	592	296								11 0.5700 0.684
	6	284	304	-20	588	294								12 0.5410 0.6528
minimum 7	7	294	298	-4	592	296								
	8	289	300	-11	589	294.5								Tab2
	9	289	293	-4	582	291								m F1 F2
	10	283	295	-12	578	289								3 2.996 4.276
	11													4 2.605 2.796
	12													5 2.372 2.096
			SDD=Σ(diff)² =	1837										6 2.214 1.694
			MSB = var(sum)/2 =	11.7833										7 2.099 1.433
														8 2.010 1.250
														9 1.938 1.115
														10 1.880 1.010
														11 1.831 0.927
														12 1.789 0.859



Annex 3. Stability study

Rye material: EA0##

	Ergometrine/inine SUM				**Ergosine/inine SUM**			
T (ºC)	Slope	Lower 95 % *	Upper 95 % *	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
4	0.0174	-0.1536	0.1885	**YES**	-0.0225	-0.2516	0.2066	**YES**
20	-0.0258	-0.2237	0.1722	**YES**	-0.2110	-0.4433	0.0212	**YES**

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

	Ergotamine/inine SUM				**Ergocornine/inine SUM**			
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
4	0.0212	-0.5729	0.6153	**YES**	0.0759	-0.0911	0.2428	**YES**
20	-0.4620	-0.9443	0.0203	**YES**	-0.1390	-0.3151	0.0371	**YES**

	α-Ergocryptine/inine SUM				**Ergocristine/inine SUM**			
T (ºC)	Slope	Lower 95 %	Upper 95 %	Null slope	Slope	Lower 95 %	Upper 95 %	Null slope
4	-0.0584	-0.3408	0.2240	**YES**	0.1190	-0.9436	1.1816	**YES**
20	-0.1539	-0.4084	0.1005	**YES**	-0.2615	-1.2462	0.7232	**YES**

Annex 4. Accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Food and Feed Compliance (F.5)
European Union Reference Laboratory for Mycotoxins

Ref. Ares(2017)2502928 - 17/05/2017

Geel, 15th of May 2017

2017 PROFICIENCY TEST FOR THE NATIONAL REFERENCE LABORATORIES (NRLS) AND APPOINTED OFFICIAL CONTROL LABORATORIES (OCLS) REGARDING THE DETERMINATION OF ERGOT ALKALOIDS IN RYE

Dear Participant,

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

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

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

The 2017 EURL PT on Ergot Alkaloids aims to assess the content of 6 ergot alkaloids (and the corresponding –inine epimers) in a naturally contaminated rye.
Although, you will have the chance to **report the individual -ine and respective -inine mass fractions the z–scoring will be based on the sum of the ergot alkaloid pairs**.

Please report their **mass fractions in µg kg^{-1}**, accompanied by the **measurement uncertainty in µg kg^{-1}** considering a coverage factor of 2 (k=2). Please report additionally the respective limits of detection (LODs), limits of quantification (LOQs) and the recoveries (%). In the Questionnaire please mention whether the results **WERE CORRECTED for recoveries OR NOT.** In figure 1 of the Annex ("Measured values" table) you can see a preview of the requested data.

Additional information will be asked in the Questionnaire to give us a chance to interpret methodological trends and therefore allow the deepest insight in laboratory independent method-related aspects.

A calibration standard solution containing the 12 analytes (≈0.5/0.25 µg mL^{-1} in acetonitrile + ammonium carbamate 0.2 gL^{-1} (1+1)) was included in the delivery. The -inines are approx. half of the concentration of the -ines. Their concentrations can be found in Table 1 of the Annex. **Please use this solution to prepare the calibration standards** according to your procedures.
Should you have your own calibration solution (*Your Std*) you have the possibility to report how this solution compares to the one dispatched (in % assuming that the dispatched solution (*EURL Std*) reflects 100%). The following formula can be used:

$$\text{Your Std (\%)} = 100*(\text{Signal}_{\text{Your Std}} * \text{Conc}_{\text{EURL Std}}) / (\text{Signal}_{\text{EURL Std}} * \text{Conc}_{\text{Your Std}})$$

Before starting the analysis please allow the sample and the standard solution to reach room temperature. Homogenise the test material with a spatula before analysis, as segregation might have occurred during transport.

Reporting the results and Questionnaire

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

The deadline for reporting the PT results is the 30th June 2017.

If some incident happens during the analysis that hampers you from submitting the results on time, please let us know as soon as possible.

Please keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to costumers, accreditation bodies and analysts alike.

Should you need any further assistance, please do not hesitate to contact us.

Success with the analysis!

With kind regards,

Carlos Gonçalves
(on behalf of the Operating Manager of the EURL Mycotoxins)

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

Cc: Frans Verstraete, Hendrick Emons, Joerg Stroka

Annex

Table 1 - Concentration of the ergot alkaloids in the calibration solution provided.

Analytes	Conc. (µg mL^{-1})	Analytes	Conc. (µg mL^{-1})
Ergometrine	0.514	Ergometrinine	0.252
Ergosine	0.498	Ergosinine	0.251
Ergotamine	0.508	Ergotaminine	0.252
Ergocornine	0.506	Ergocorninine	0.252
α-Ergocryptine	0.507	α-Ergocryptinine	0.252
Ergocristine	0.502	Ergocristinine	0.254

Instructions for reporting the results using RingDat.

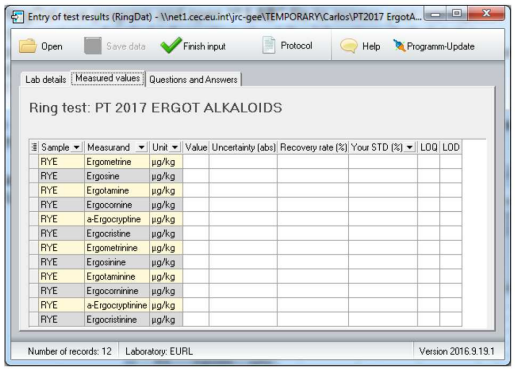
1. Download the updated version of the data entry program (called RingDat) free from the QuoData web page using following link: http://quodata.de/ringdat_en.php
User: *ringdat* Password: *prolabdata*
Alternatively, in case you already have Ringdat you can update it via the "Programm-update" button.

2. Save the two lab specific files with the extension **"*.Lab"** and **"*.LA2",** generated by the ProLab software and provided to each individual laboratory (personal files attached to this email) to the same folder as RingData.exe.
The name of each laboratory and the samples are codified by the software, so that each participant will receive samples with unique codified numbers (i.e., EA016).
- The **"*.LA2"** file contains information about the participant – laboratory name and laboratory code;
- The **"*.LAB"** file is unique to each laboratory and contains information about the samples and measurands that have to be analysed and reported.

3. Start the RingDat.exe program and open **"*.LAB"** file to access your workspace.
- The first tab contains detailed information about the laboratory (Lab details).
- The second tab contains a table for entering the results for every measurand/sample combination (Measured values)
- The third tab contains a general questionnaire (Questions and Answers).

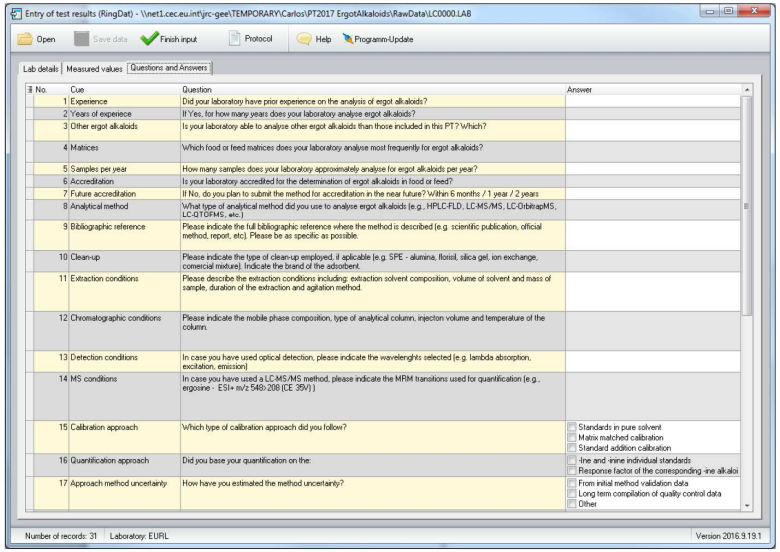
4. Fill in the results table (Measured values) with your data. Please find below some captures of the RingDat pages that have been configured for this PT.

Figure 1 – Capture of the "Measured Values" page



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

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



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

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

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

Annex 5. Materials receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Geel Site

European Union Reference Laboratory for Mycotoxins

Geel, 15th of May 2017

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Address:
Member State:

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

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

Date of receipt	
Sample number (e.g. EA021)	
All items have been received undamaged	YES / NO
If NO, please list damaged items:	

Contents of the parcel:

a) **One test material** for analysis packed in a green lid bottle
b) **One ampoule** containing the calibration solution (4mL)
c)
A bag containing the following document:
- the pro-forma invoice

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

Carlos GONÇALVES

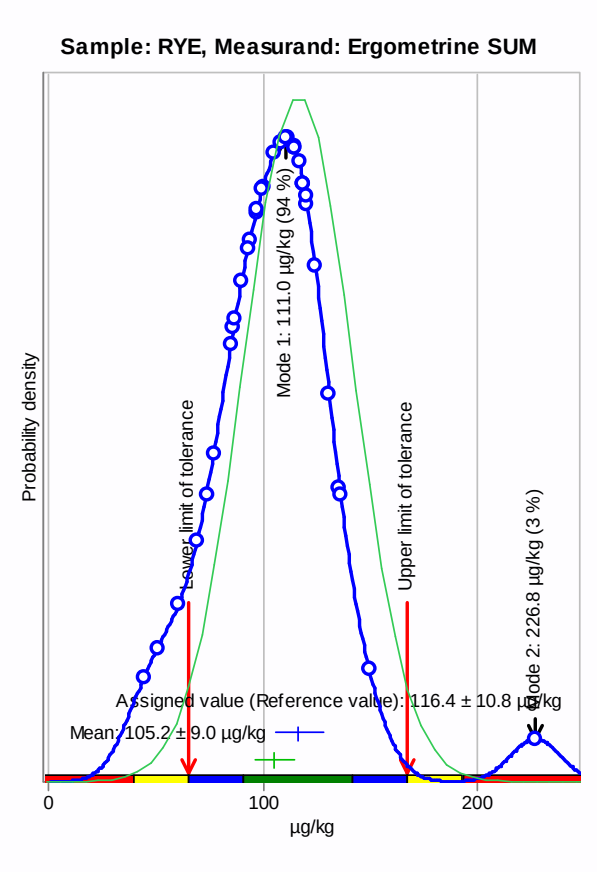
E-mail: JRC-EURL-MYCOTOX@ec.europa.eu

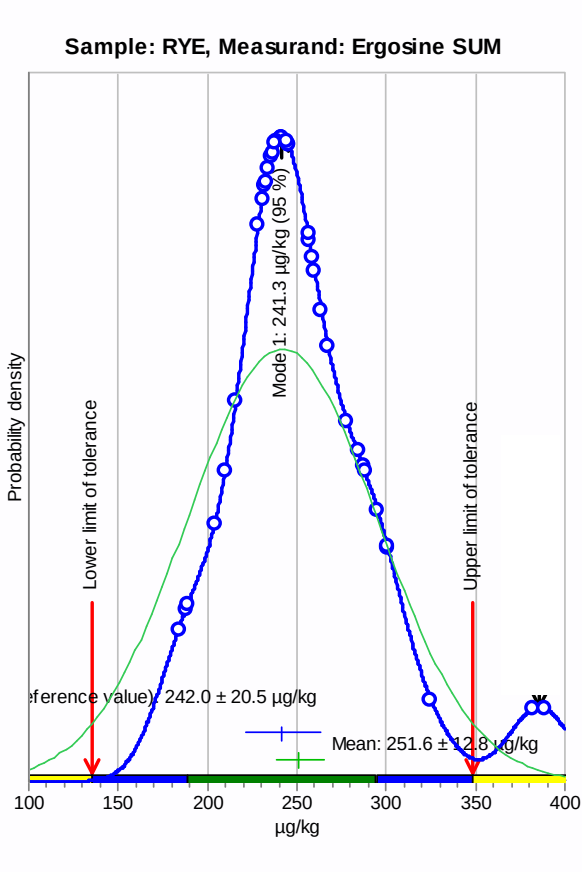
Your Signature / Stamp here:

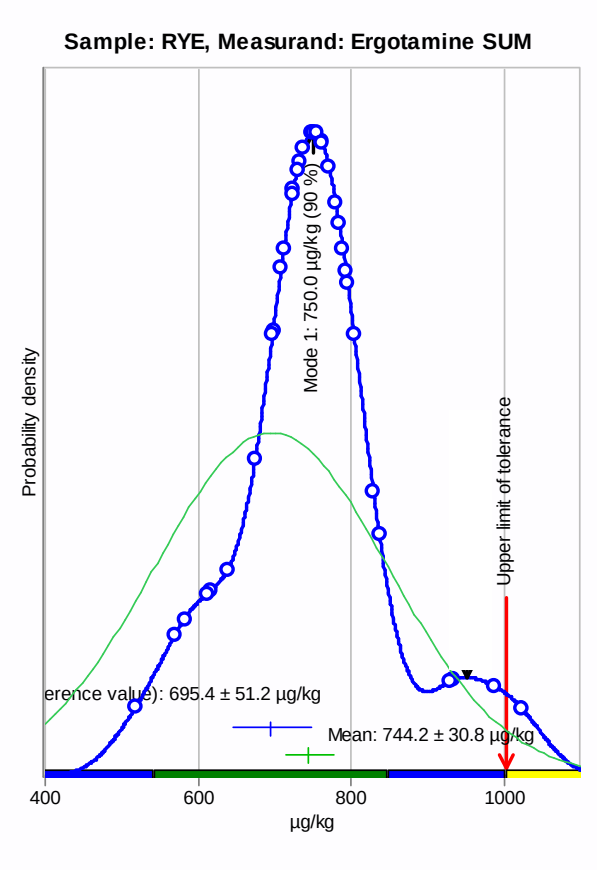
Annex 6. Questionnaire

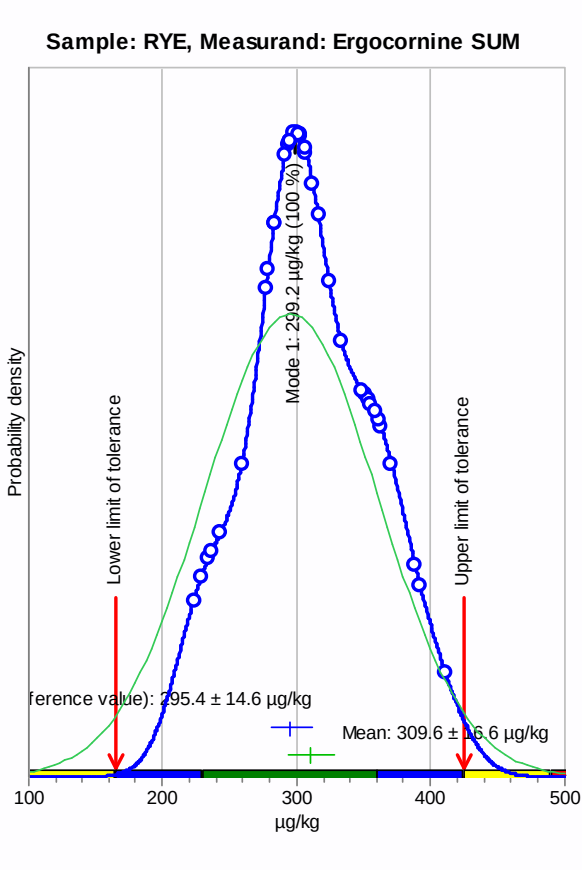
Ring test : PT 2017 ERGOT ALKALOIDS (26 questions, 832 answers)			
No.	**Cue**	**Question**	**Answers**
1	Experience	Does your laboratory have prior experience on the analysis of ergot alkaloids?	37 Answers
2	Years of experience	If Yes, for how many years does your laboratory analyse ergot alkaloids?	31 Answers
3	Other ergot alkaloids	Is your laboratory able to analyse other ergot alkaloids than those included in this PT? Which?	37 Answers
4	Matrices	Which food or feed matrices does your laboratory analyse most frequently for ergot alkaloids?	36 Answers
5	Samples per year	How many samples does your laboratory approximately analyse for ergot alkaloids per year?	36 Answers
6	Accreditation	Is your laboratory accredited for the determination of ergot alkaloids in food or feed?	37 Answers
7	Analytical method	What type of analytical methodology did you use to analyse ergot alkaloids (e.g., HPLC-FLD, LC-MS/MS, LC-OrbitrapMS, LC-QTOFMS, etc.)	37 Answers
8	Bibliographic reference	Please indicate the full bibliographic reference where the method is described (e.g. scientific publication, official method, report, etc). Please be as specific as possible.	34 Answers
9	Clean-up	Please indicate the type of clean-up employed, if applicable (e.g. SPE - alumina, florisil, silica gel, ion exchange, commercial mixture). Indicate the brand of the adsorbent.	36 Answers
10	Extraction conditions	Please describe the extraction conditions including: extraction solvent composition, volume of solvent and mass of sample, duration of the extraction and agitation method.	37 Answers
11	Chromatographic conditions	Please indicate the mobile phase composition, type of analytical column, injection volume and temperature of the column.	37 Answers
12	Detection conditions	In case you have used optical detection, please indicate the wavelengths selected (e.g. lambda absorption, excitation, emission)	18 Answers
13	MS conditions	In case you have used a LC-MS/MS method, please indicate the MRM transitions used for quantification (e.g., ergosine - ESI+ m/z 548>208 (CE 35V))	30 Answers
14	Calibration approach	Which type of calibration approach did you follow? Standards in pure solvent / Matrix-matched calibration / Standard addition calibration	37 Answers
15	Quantification	Did you use for quantification the: Supplied calibration solution / Our own standards	37 Answers
16	Areas in FLD	In case you have used fluorescence detection (FLD), please indicate the peak areas in the sample	14 Answers
17	Approach method uncertainty	How have you estimated the method uncertainty?	33 Answers
18	Recovery estimation	How did you estimate the method's recovery?	36 Answers
19	Recovery correction	The results submitted were: Corrected for recoveries / Not corrected	37 Answers
20	Supplier of standards	You were asked to use the standards provided by the EURL. In case you have your own standards, which is the supplier?	25 Answers
21	Overnight stop	During the analysis did you need to include an overnight stop? If Yes, at which stage of the analysis?	36 Answers
22	Difficulties	Did you have major difficulties analysing the samples?	36 Answers
23	Which difficulties	If Yes, please specify which? e.g sensitivity of the instrument; pumps pressure; chromatographic resolution; tedious sample preparation; complex matrix, insufficient clean-up, etc.	14 Answers
24	Problems with ProLab/RingDat	Did you have any problems using the ProLab/RingDat platform for results reporting? If Yes, describe which?	34 Answers
25	Instructions clear	Did you find the instructions distributed for this PT adequate?. If No, which parts do you think can be improved?	30 Answers
26	Comments	Any other comments you wish to address?	20 Answers

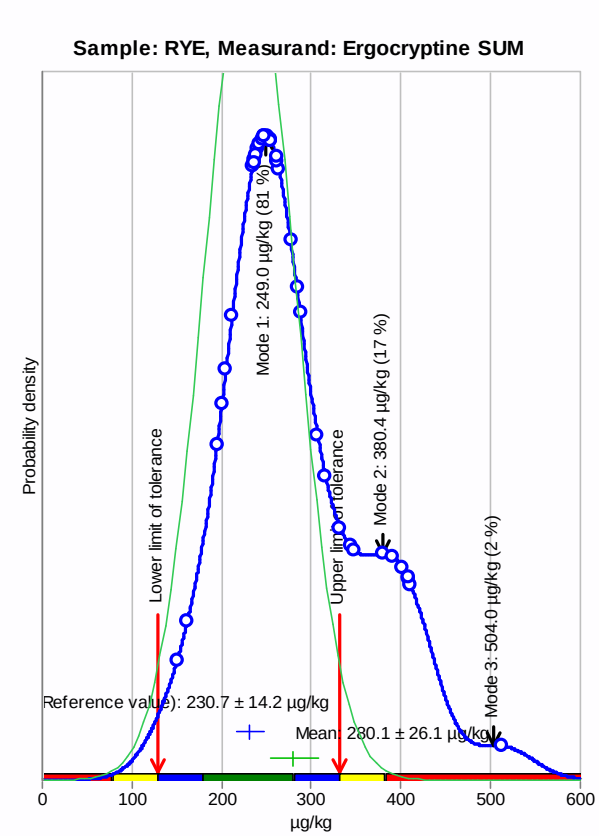
Annex 7. Kernel density plots

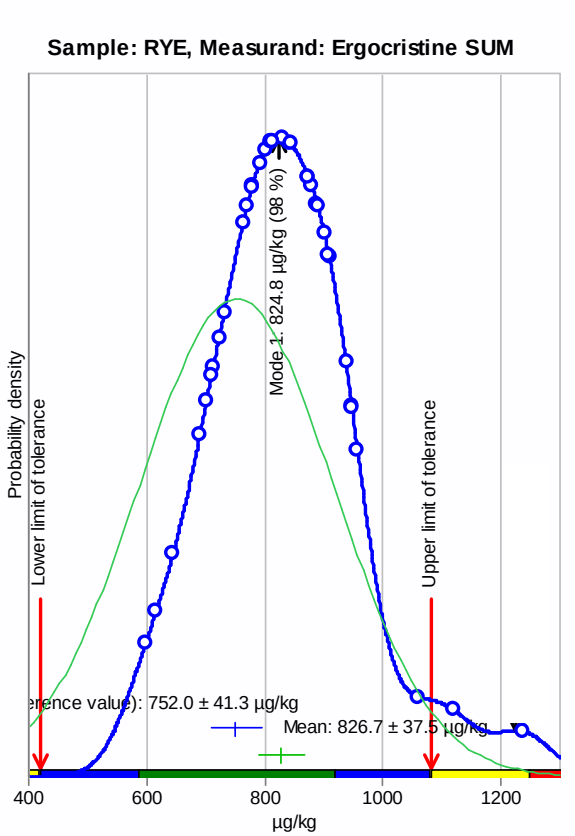









Sample: RYE, Measurand: Ergocryptine SUM
Probability density
Lower limit of tolerance
Upper limit of tolerance
Mode 1: 249.0 µg/kg (81 %)
Mode 2: 380.4 µg/kg (17 %)
Mode 3: 504.0 µg/kg (2 %)
Reference value): 230.7 ± 14.2 µg/kg
Mean: 280.1 ± 26.1 µg/kg
0
100
200
300
400
500
600
µg/kg


Sample: RYE, Measurand: Ergocristine SUM
Probability density
Lower limit of tolerance
Upper limit of tolerance
Mode 1: 824.8 µg/kg (98 %)
erence value): 752.0 ± 41.3 µg/kg
Mean: 826.7 ± 37.5 µg/kg
400
600
800
1000
1200
µg/kg

Annex 8. Z- and ζ-scores assigned to the individual ergot alkaloids

	Ergometrine					Ergosine					Ergotamine					Ergocornine				
Lab code	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C*	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C
LC0002	107.9	25.2	1.2	1.7	a	193.4	48.9	0.4	0.6	a	637	120.5	0.8	1.5	a	210.4	48.2	0.5	0.9	a
LC0003	64.9	28.6	-1.1	-1.3	a	147.1	64.7	-0.8	-0.9	a	429.6	189	-0.9	-1.1	a	147.2	64.8	-1	-1.3	a
LC0004	76.4	22.9	-0.5	-0.7	a	209.6	62.9	0.8	0.9	a	561.9	168.6	0.2	0.3	a	176.6	53	-0.3	-0.5	a
LC0005	53.5	8.9	-1.7	-4.7	b	181.9	30.3	0.1	0.2	a	569.2	94.8	0.3	0.6	a	156	26	-0.8	-2.3	a
LC0006	48.5	9	-2	-5.4	b	217.6	17	1	3	b	594.8	39.2	0.5	1.8	b	228.1	16.8	0.9	3.8	a
LC0007	86	25.8	0	0.1	a	212.2	63.6	0.9	1	a	870.2	261	2.8	2.5	c	180.5	54.1	-0.2	-0.3	a
LC0008	70.5	7	-0.8	-2.4	b	160	16	-0.5	-1.4	b	481	48	-0.5	-1.7	b	172	17	-0.4	-1.6	a
LC0009	91	12.9	0.3	0.7	a	182	19.1	0.1	0.3	b	535	61.5	0	-0.1	a	197	20.5	0.2	0.7	a
LC0010	37.3	11.2	-2.6	-6.3	a	167.4	50.2	-0.3	-0.4	a	423.6	127	-1	-1.7	a	193.9	58.2	0.1	0.2	a
LC0011	84.2	25.3	-0.1	-0.1	a	150	45	-0.7	-1.2	a	650.2	195.1	0.9	1.1	a	173.8	52.1	-0.4	-0.6	a
LC0012	158	0	3.9		b	171	0	-0.2		b	524	0	-0.1		b	194	0	0.1		b
LC0013	68	33	-0.9	-1	a	154	65	-0.6	-0.7	a	401	147	-1.2	-1.8	a	164	69	-0.6	-0.7	a
LC0014	51.8	18	-1.8	-3.2	a	171.6	50	-0.2	-0.3	a	552.5	135	0.1	0.2	a	179.4	52	-0.2	-0.4	a
LC0015	77	23	-0.4	-0.6	a	173.8	52	-0.1	-0.2	a	607.2	182	0.6	0.7	a	137.2	41	-1.2	-2.4	a
LC0016	90	26	0.3	0.3	a						928	30	3.3	13.6	b	411	25	5.3	16	a
LC0017	108	0	1.2		b	389	0	5.4		b	666	0	1.1		b	191	0	0		b
LC0018	87.6	61.3	0.1	0.1	c	160	40	-0.5	-0.8	a	408	106	-1.1	-2.3	a	274	137	2	1.2	c
LC0019	79.3	14	-0.3	-0.7	a	168	34	-0.3	-0.5	a	437	78	-0.9	-2.2	a	177	26	-0.3	-0.8	a
LC0020	83.7	16.7	-0.1	-0.1	a	194.2	38.8	0.4	0.7	a	603.4	120.7	0.5	1	a	178.4	35.7	-0.3	-0.6	a
LC0021	70	35	-0.8	-0.8	a	190	60	0.3	0.4	a	590	180	0.4	0.5	a	180	60	-0.2	-0.3	a
LC0022	68	36	-0.9	-0.9	a	100	38	-2	-3.7	a	310	42	-1.9	-7.1	b	83	64	-2.5	-3.3	a
LC0023	97.8	10	0.7	1.8	b	217	23	1	2.5	a	599	60	0.5	1.5	a	212	21.2	0.6	1.9	a
LC0024	70.96	35.5	-0.8	-0.8	a	171.7	85.9	-0.2	-0.2	c	512.9	256.4	-0.2	-0.2	c	176.4	88.2	-0.3	-0.3	c
LC0025						314.4	47.2	3.5	5.3	a						191.3	28.7	0.1	0.1	a
LC0026	107.23	0	1.2		b	176.3	0	-0.1		b	603.43	0	0.5		b	239.76	0	1.2		b
LC0027	64	0	-1.1		b	127	0	-1.3		b	487	0	-0.4		b	153	0	-0.9		b
LC0028	88.4	17.7	0.2	0.3	a	177.5	35.5	0	0	a	474.3	94.9	-0.5	-1.2	a	167.8	33.6	-0.5	-1.2	a
LC0029						129	27	-1.3	-3	a						130.7	50	-1.4	-2.3	a
LC0030	94.1	14.1	0.5	1	a	168.7	42.2	-0.2	-0.4	a	499.5	99.9	-0.3	-0.7	a	186.6	37.3	-0.1	-0.1	a
LC0031	87.4	48.1	0.1	0.1	c	182	100.1	0.1	0.1	c	493.7	271.5	-0.4	-0.3	c	165.2	90.9	-0.6	-0.5	c
LC0032	31.4	6.3	-2.9	-8.9	b	151.8	33.4	-0.7	-1.4	a	671.2	161.1	1.1	1.6	a	241.7	53.2	1.3	1.9	a
LC0033	90.1	22.5	0.3	0.4	a	203.6	50.9	0.6	0.9	a	662.6	165.7	1	1.4	a	203.5	50.9	0.3	0.6	a
LC0034	88.9	22.2	0.2	0.3	a	175	43.8	-0.1	-0.1	a	569	142	0.3	0.4	a	212	53	0.6	0.8	a
LC0035	86.9	34.8	0.1	0.1	a	203	81.2	0.6	0.6	c	473	189.2	-0.6	-0.7	a	201	80.4	0.3	0.3	a
LC0036	58.62	0	-1.4		b	142.18	0	-0.9		b	416.32	0	-1		b	155.37	0	-0.8		b
LC0037	95.7	42.1	0.6	0.5	c	154.2	68	-0.6	-0.7	a	550.7	242	0.1	0.1	c	221.9	98	0.8	0.7	c
LC0038	39.5	23.7	-2.4	-3.5	a	146.3	87.8	-0.8	-0.7	c	518.2	310.9	-0.2	-0.1	c	222	133.2	0.8	0.5	c

* Classification of the uncertainty reported by the participant

	Ergocryptine					Ergocristine					Ergometrinine					Ergosinine				
Lab code	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C
LC0002	152	42.9	-0.6	-1	a	644.8	164.1	1	1.3	a	28.2	6.8	-0.4	-0.8	a	50.7	11.7	-0.9	-2	a
LC0003	102.7	45.2	-1.9	-3.1	a	457.5	201.3	-0.6	-0.7	a	21.4	9.4	-1.4	-2	a	57	25.1	-0.5	-0.5	a
LC0004	156.9	47.1	-0.5	-0.7	a	497.7	149.3	-0.3	-0.4	a	23.3	7	-1.2	-2.1	a	85.5	25.6	1.6	1.7	a
LC0005	177.1	29.5	0.1	0.2	a	470.9	78.5	-0.5	-1.4	a	480.2	80	65.2	11.2	c	76.7	12.8	0.9	1.9	a
LC0006	167	10.9	-0.2	-0.9	b	643.7	26.2	1	5	b	25.2	4.8	-0.9	-2.1	a	83	7.1	1.4	4.3	a
LC0007	278.9	83.6	2.7	2.5	c	713.1	213.9	1.6	1.7	a	25.6	7.6	-0.8	-1.4	a	55.3	16.5	-0.6	-1	a
LC0008	122	12	-1.4	-5.8	b	499	50	-0.3	-1.1	a	26.8	3	-0.7	-2.1	b	96.4	10	2.3	5.7	a
LC0009	139	15.3	-0.9	-3.5	a	548	55.9	0.1	0.5	a	24	3.5	-1.1	-3.1	a	56	11.9	-0.5	-1.2	a
LC0010	102.5	30.7	-1.9	-4.3	a	507.9	152.3	-0.2	-0.3	a	7.1	2.2	-3.5	-12.6	b	65	19.5	0.1	0.1	a
LC0011	126.3	37.9	-1.3	-2.4	a	521.1	156.3	-0.1	-0.1	a	23.9	7.2	-1.1	-1.9	a	65.9	19.8	0.2	0.2	a
LC0012	123	0	-1.3		b	693	0	1.4		b	69	0	5.5		b	74	0	0.7		b
LC0013	245	97	1.8	1.4	c	477	171	-0.5	-0.6	a	32	17	0.1	0.1	c	147	63	6	2.6	c
LC0014	260.1	71	2.2	2.4	a	531.6	131	0	0	a	17.5	7.3	-2	-3.5	a	60.4	21	-0.2	-0.3	a
LC0015	152	46	-0.6	-0.9	a	601.7	181	0.6	0.8	a	19.8	6	-1.7	-3.4	a	67.2	20	0.3	0.3	a
LC0016	261	20	2.2	7.2	a	1237	30	6	30	b										
LC0017	254	0	2.1		b	723	0	1.6		b	41.5	0	1.5		b					
LC0018	201	111	0.7	0.5	c	586	410	0.5	0.3	c	36.7	11	0.8	0.9	a	118	35	3.9	3.1	c
LC0019	128	22	-1.2	-3.6	a	498	128	-0.3	-0.5	a	26.1	4.6	-0.8	-1.9	a	68.2	16.4	0.3	0.5	a
LC0020	148.4	29.7	-0.7	-1.6	a	633.4	126.7	0.9	1.5	a	27.3	5.5	-0.6	-1.3	a	65.7	13.1	0.2	0.3	a
LC0021	152	46	-0.6	-0.9	a	600	190	0.6	0.7	a	23	12	-1.2	-1.3	a	67	34	0.2	0.2	c
LC0022	110	37	-1.7	-3.3	a	420	46	-1	-3.8	a	19	42	-1.8	-0.6	c	110	30	3.3	3	c
LC0023	272	28	2.5	6.3	a	521	53	-0.1	-0.3	a	33	3.6	0.3	0.7	a	70	8	0.5	1.3	a
LC0024	231.1	115.5	1.5	1	c	524.1	262.1	-0.1	-0.1	c	22.89	11.4	-1.2	-1.4	a	63.92	32	0	0	c
LC0025	132.1	19.8	-1.1	-3.6	a	661.7	99.3	1.1	2.5	a						67.9	10.2	0.3	0.7	a
LC0026	180.17	0	0.1		b	779.94	0	2.1		b	28.46	0	-0.4		b	107.63	0	3.1		b
LC0027	133	0	-1.1		b	718	0	1.6		b	21	0	-1.5		b	62	0	-0.1		b
LC0028	116.5	23.3	-1.5	-4.3	a	475.2	95	-0.5	-1.1	a	26.3	5.3	-0.7	-1.6	a	56.4	11.3	-0.5	-1.1	a
LC0029	195.3	62.5	0.5	0.6	a	756	225	1.9	2	a						54.7	10.9	-0.6	-1.5	a
LC0030	138.7	34.7	-0.9	-1.9	a	506.9	101.4	-0.2	-0.5	a	26.5	6.6	-0.7	-1.3	a	76.5	42.1	0.9	0.6	c
LC0031	188	103.4	0.3	0.3	c	404.1	222.3	-1.1	-1.1	a	31.6	17.4	0	0	c	105.9	58.2	3	1.4	c
LC0032	154.9	34.1	-0.5	-1.1	a	678	162.7	1.3	1.8	a	19.2	3.8	-1.8	-4.9	a	86.8	19.1	1.7	2.3	a
LC0033	152	38	-0.6	-1.1	a	698.5	174.6	1.4	1.9	a	28.9	7.2	-0.3	-0.6	a	60.2	15	-0.2	-0.4	a
LC0034	152	38	-0.6	-1.1	a	615	154	0.7	1.1	a	31.1	12.4	0	0	a	66.5	20	0.2	0.3	a
LC0035	230.4	92.2	1.5	1.2	c	485.8	194.3	-0.4	-0.5	a	28.1	11.2	-0.5	-0.5	a	122	48.8	4.2	2.4	c
LC0036	110.52	0	-1.7		b	455.07	0	-0.7		b	18.39	0	-1.9		b	45.79	0	-1.3		b
LC0037	253.2	111	2	1.4	c	434.6	191	-0.8	-1	a	21.2	9	-1.5	-2.1	a	74.3	33	0.8	0.6	c
LC0038	144.8	86.9	-0.8	-0.7	c	592.4	355.4	0.5	0.3	c	21.1	12.6	-1.5	-1.6	a	84.4	50.6	1.5	0.8	c

	Ergotaminine					Ergocorninine					Ergocryptinine					Ergocristinine				
Lab code	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C	Result (µg/kg)	U lab (µg/kg)	z-score	Zeta score	C
LC0002	124.4	44.3	-0.9	-1.3	a	84	21.7	-0.9	-1.9	a	48.4	9.9	-0.6	-1.4	a	167.9	43.7	-1.1	-2.2	a
LC0003	152.1	66.9	-0.1	-0.1	a	81.6	35.9	-1.0	-1.3	a	47.9	21.1	-0.7	-0.7	a	187.5	82.5	-0.7	-0.8	a
LC0004	217.9	65.4	1.8	1.8	a	102.1	30.6	-0.2	-0.3	a	119.9	36	5.2	3.5	c	294.1	88.2	1.5	1.6	a
LC0005	142.7	23.8	-0.4	-0.9	a	102.8	17.1	-0.1	-0.3	a	138.7	23.1	6.7	7	a	239	39.8	0.4	0.8	a
LC0006	208.5	16.1	1.5	4.6	b	134.3	8.2	1.2	4.8	b	80.5	4.7	2	7.3	b	303.6	7.9	1.7	7.8	b
LC0007	152.7	45.8	-0.1	-0.1	a	102.5	30.7	-0.2	-0.2	a	122.9	36.8	5.4	3.6	c	160.9	48.2	-1.2	-2.3	a
LC0008	251	25	2.8	6.4	a	128	13	0.9	2.8	a	82.9	8	2.2	5.8	a	312	31	1.9	5	a
LC0009	162	14.6	0.2	0.5	b	109	19.2	0.1	0.3	a	99	9.9	3.5	7.8	a	222	21.8	0	0.1	a
LC0010	145.9	43.7	-0.3	-0.4	a	97.6	29.3	-0.4	-0.6	a	59.3	17.8	0.3	0.4	a	223.5	67	0.1	0.1	a
LC0011	185.9	55.8	0.9	1	a	119.7	35.9	0.6	0.7	a	157.7	47.3	8.3	4.3	c	258.2	77.46	0.8	0.9	a
LC0012	224	0	2		b	113	0	0.3		b	132	0	6.2		b	255	0	0.7		b
LC0013	392	144	6.9	3.3	c	187	77	3.5	2.1	c	163	69	8.7	3.1	c	583	202	7.5	3.6	c
LC0014	155.9	46	0	0	a	97.6	31	-0.4	-0.5	a	118.9	37	5.1	3.4	c	170.1	49	-1	-1.9	a
LC0015	153.8	46	-0.1	-0.1	a	97	29	-0.4	-0.6	a	99.4	30	3.5	2.9	c	239.9	72	0.4	0.5	a
LC0016																				
LC0017	321	0	4.8		b	158	0	2.2		b	137	0	6.6		b	396	0	3.6		b
LC0018	321	80	4.8	4	c	118	18	0.5	1.2	a	312	78	20.8	6.6	c	288	72	1.4	1.8	a
LC0019	174	90	0.5	0.4	c	106	28	0	0	a	114	20	4.7	5.6	a	215	60	-0.1	-0.2	a
LC0020	150.4	30.1	-0.2	-0.3	a	123.4	24.7	0.7	1.3	a	158.6	31.7	8.3	6.4	c	178.8	35.8	-0.9	-2	a
LC0021	159	48	0.1	0.1	a	122	37	0.7	0.8	a	110	60	4.4	1.8	c	230	70	0.2	0.3	a
LC0022	440	37	8.3	14	a	140	39	1.5	1.7	a	130	25	6	5.8	c	390	31	3.5	9.2	a
LC0023	196	20	1.2	3.1	a	141	15	1.5	4.1	a	139	15	6.7	10.6	a	281	30	1.2	3.4	a
LC0024	161.5	80.8	0.2	0.1	c	117.9	59	0.5	0.4	c	112.3	56.2	4.6	2	c	198.6	99.3	-0.5	-0.4	c
LC0025						111.1	16.7	0.2	0.5	a	131.8	19.8	6.2	7.4	a	247.5	37.1	0.6	1.3	a
LC0026	225.28	0	2		b	93.27	0	-0.6		b	64.38	0	0.7		b	157.67	0	-1.3		b
LC0027	151	0	-0.2		b	90	0	-0.7		b	103	0	3.8		b	183	0	-0.8		b
LC0028	141.3	28.3	-0.4	-0.9	a	144.3	28.9	1.6	2.5	a	118.7	23.7	5.1	5.2	a	215	43	-0.1	-0.2	a
LC0029						240	85	5.7	3.1	c	52.3	16.7	-0.3	-0.4	a	134	48	-1.8	-3.3	a
LC0030	198.2	49.6	1.2	1.6	a	115.1	23	0.4	0.7	a	73.1	18.3	1.4	1.8	a	256.5	77	0.7	0.9	a
LC0031	277.1	152.4	3.5	1.6	c	132.3	72.8	1.1	0.7	c	144	79.2	7.1	2.2	c	374.2	205.8	3.2	1.5	c
LC0032	262	62.9	3.1	3.3	a	146.8	32.3	1.7	2.4	a	132.7	29.2	6.2	5.2	c	279.1	67	1.2	1.7	a
LC0033	126.2	31.5	-0.9	-1.7	a	112.5	28.1	0.3	0.4	a	102.9	25.7	3.8	3.6	c	210.2	52.5	-0.2	-0.4	a
LC0034	154	41.3	-0.1	-0.1	a	112	28	0.3	0.4	a	102	25.5	3.7	3.5	c	228	57	0.2	0.2	a
LC0035	311	124.4	4.5	2.5	c	157.2	62.9	2.2	1.6	c	177.4	71	9.9	3.4	c	393.6	157.4	3.6	2.2	c
LC0036	102.28	0	-1.6		b	80.76	0	-1.1		b	84.28	0	2.3		b	161	0	-1.2		b
LC0037	173.4	76	0.5	0.4	c	133.3	59	1.2	0.9	c	94.9	42	3.2	1.8	c	163.9	72	-1.2	-1.5	a
LC0038	218.3	131	1.8	0.9	c	138.6	83.2	1.4	0.8	c	92	55.2	2.9	1.3	c	296.2	177.7	1.6	0.8	c

Annex 9. Summary statistics of the PT for the individual ergot alkaloid epimers

	Units	Ergometrine	Ergosine	Ergotamine	Ergocornine	α-Ergocryptine	Ergocristine	Ergometrinine	Ergosinine	Ergotaminine	Ergocorninine	α-Ergocryptinine	Ergocristinine
No. of participants		37	37	37	37	37	37	37	37	37	37	37	37
No. of laboratories that submitted results		35	36	35	37	37	37	34	35	34	36	36	36
Assigned value	µg/kg	85	178	539	189	175	531	31.3	63.6	156	106.1	56.0	221
Expanded uncertainty of the assigned value (k=2)	µg/kg	10	20	49	12	13	36	3.2	5.6	16	8.4	4.7	20
Robust mean	µg/kg	78	175	542	187	170	576	25.8	74	194	119	112	242

Annex 10. Method details and quantification approaches as reported by the participants

Lab code	Q.4 Matrices	Q.7 Analytical method	Q.8 Bibliographic reference	Q.9 Clean-up	Q.10 Extraction conditions
LC0002	Rye	LC-MS/MS	Internal procedure from BfR	SPE ALUMINA	10 g + 50 ml ethyl acetate, methanol, ammonia hydroxide solution, isopropanol 75+5+7+7 (v+v+v+v), 45 min shaking
LC0003	cereals, baked cereal products (bread)	LC-MS/MS	Unpublished method of the § 64 LFGB working group in Germany	SPE -alumina	5 g sample extracted with 25 ml extraction solvent (ethyl acetate/methanol/ ammonia 25%/Isopropanol) 75/5/7/7 (v/v/v/v) for 45 minutes by shaking on a shaking machine, centrifugation, clean up 1 ml with SPE (alumina), wash with 2 ml extraction solvent, evaporation, resolve in mobile phase
LC0004		LC-MS/MS	In house method	MycoSep 150Ergot, Romer Labs	Extraction solvent: 84 (200 mg/L ammonium carbonate in water) :16 acetonitrile; ratio mass of sample to volume of solvent was 0,1; 2h; shaking
LC0005	Cereals	UPLC-MS/MS	CEN Method: Draft WI00275289 Ergot Alkaloid Method 20160525	SPE-Silica	5g sample, 25mls 84:16 ACN:200 mg Ammonium Carbonate in Water 30mins shaking
LC0006	cereals and cereal flour, bakery products, compound feed	HPLC-FLD, LC-MS/MS	BVL L 15.01./02 -5:2012-01	SPE (alumina) from waters	Extraction solvent composition: mixture of ethyl acetate, methanol, ammonium hydroxide solution - 75, 5 and 7 parts by volume Volume of solvent: 50.0 mL / Mass of sample: 10.0 g Duration of extraction: 45 min SPE (alumina): 5.0 mL, eluate is evaporated under stream of nitrogen residue redissolved in 1 mL acetonitrile / 0.2g/L ammonium carbamate (1:1)
LC0007		LC-MS/MS	RIKILT: Determination of ergot alkaloids-feed-LC-MS/MS	none	2,5g of sample with 25 ml methanol/water 60/40 (v/v); shaken for 30 min on a rotary tumbler, ultracentrifugation
LC0008	grains, flours	LC-MS/MS	in house method	none	Ethyactetat/Methanol/NH4OH (75 + 5 + 7), 8 g, 45 min
LC0009	Cereals (wheat, barley, buckwheat, rye)	LC-MS/MS	TC 275 WI 00275290	Bondesil, PSA bulk sorbent, 40ìm	10g sample, 50 mL extraction solvent (ACN:Ammonium carbonate, 84:16), 30 min shake.
LC0010	none	LC-MS/MS	J. Di Mavungu et al., Food Chemistry 135 (2012) 292-303	dilution 1:4 in water	extraction into acetonitrile using vortex for 3 minutes
LC0011	cereals	HPLC-FLD	§64 LFGB; 15.01/02-5	SPE Alumina B (Waters)	20 g sample, 100 ml solvent (Ethylacetate/methanol/NH3 25%/2-Propanol; 75/5/7/7; v/v/v/v); 45 min stirring
LC0012	cereals	HPLC-FLD	Romer MicoSep 150 Application Note	MicoSep 150	Extraction solvent: ACN/(NH4)2CO3 200 mg/L / Mass sample: 5 g / volume solvent: 20 mL / Extraction time: 30' / Agitation method: orbital
LC0013	Rye flour	LC-OrbitrapMS	Methods created by Romer Labs and sold with the product Mycosep 150 Ergot Push-through format	We used the Mycosep 150 Ergot columns	20 g + 100 mL (84+16) ACN/(NH4)2CO3; 30 min stirring; filter; push through Mycosep 150 Ergot columns and injection
LC0014	cereals	LC-MS/MS	in-house method	no clean-up	Extraction solvent: acetonitrile with 1% formic acid (10mL), water (10mL) Mass of sample: 2 grams / Duration of extraction: 30 min, shaking
LC0015	Not applicable	ULC-MS/MS		Modified QuEChERS	4g sample + 30 mL extraction solvent (acetonitril/ water (84/16) + ammonium carbonate). / Overhead mixer for 45 min. / 2 mL supernatants is filtered through a 0.2um syringe filter and injected directly

LC0016	Flour, animal feed	HPLC-FLD	Development and validation of an analytical method for determination of ergot alkaloids in animal feedingstuffs with high performance liquid chromatography-fluorescence detection, E.Kowalczyk, E.Patyra, A.Grelik, K.Kwiatek, Polish Journal of Veterinary Science Vol. 19, No 3 (2016), 559 - 565	The supernatant was transferred to a polyprophylene tube containing 1g of magnesium sulfate, 0.4g of dispersive sorbent PSA and 0.08g of activated carbon. The tuba was shaken for 20 min., centrifuged, filtered, evaporated to dryness under nitrogen stream. The residue was reconstituted in 0.4mL of mobile phase (acetonitrile:2mM ammonium carbonate 50:50, v/v) and passed through a 0.45um syringe filter and injected to instrumental analysis.		5g of milled feed sample was weighted in a 50mL polypropylene tube. 20mL of dichloromethane, 5mL of acetonitrile, 5mL of 25% ammonia solution and a mixture of QuEChERS salts containing 0.5g of sodium chloride, 4g of magnesium sulphate, 0.25g of sodium hydrogen citrate sesquihydrate and 0.5g of sodium citrate were added. The sample was shaken on horizontal shaker for 30 min.
LC0017	Cereals, feed	LC-HRMS/MS (Q Exactive)	Ivanova et al., 2016 (World Mycotoxin Journal, 2016, 11-16) The published method was used with some modifications	Two steps extraction was combined with low temperature storage and centrifuging steps	2.5 g sample was used for sequantial extraction with acetonitrile:water:formic acid, 80:19.9:0.1, v/v/v (10 ml, 30 min) followed by acetonitrile:water:formic acid, 20:79.9:0.1, v/v/v (10 ml, 30 min)	
LC0018	Cereals, grass	LC-MS/MS	Kokkonen M and Jestoi M. 2010. Determination of ergot alkaloids from grains with UPLC-MS/MS. J. Sep. Sci. 33, 2322-2327.	MycoSep 150 Ergot columns	Extraction solvent: acetonitrile/ammonium carbonate solution (0.2 g of ammonium carbonate per 1 L of deionised water); ratio 84:16, 100 mL; Mass of sample: 20 g / Extraction time: 0,5 hour / Agitation method: linear shaker	
LC0019	Cereals	LC-MS/MS	1. Food Additives and contaminants: Part B, Vol 2, No 1, June 2009, pp. 79-85 2. Mol. Nutr. Food. Res. 2009, 53, pp. 500-507	SPE-alumina (Waters Sep-Pak Alumina B), PSA-Bonded Silica (Supelco)	Extraction solvent (ammonium carbonate 200 mM pH 10 - acetonitrile : 16/84 (v/v)) Solvent Volume: 25 ml, Mass of sample: 5 g, Extraction duration: 1h, Agitation: Rotary shaker	
LC0020	Rye	UHPLC-MS/MS	-	Mycosep 150 Ergot column	Acetonitrile/ammonium carbonate 3 mM pH 9 (84:16 (v/v) 25 mL to 5 g of sample. 30 minutes shake.	
LC0021	grain cereals, flour (rye, wheat ....)	LC-MS/MS	Méthode CEN Doc 602 (S. Mac Donald): extraction and Bondesil clean up standard preparation and Quantification method by MLSA if needed by Mulder	phase Bondesil PSA. (Agilent)	extraction solvent composition: ACN/Ammonium Carbonate 84/16 volume of solvent: 25 mL mass of sample:5 g duration extraction and agitation : 30 minutes rotation agitation then centrifugate 10 minutes at 4000 G	
LC0022	Graind (mostly rye)	LC-MS/MS	Kokkonen M., Jestoi M. (2010): Determination of ergot alkaloids from grains with UPLC-MS/MS, J.Sep.Sci., 33, 2322-2327	SPE: Mycosep 150 Ergot - (Romer Labs)	acetonitrile:ammonium carbonate buffer (84:16; v/v) 20 g sample 100 ml extraction solvent 1 hour shaking in a horizontal shaker	
LC0023	Cereals and products thereof	LC-MS/MS	In-House Method	MycoSep 150 Ergot	ACN/Ammonium Hydroxide/Ammonium Carbonate Buffer. 1:4. 30 minutes	
LC0024	complete feed, silage, cereals,hay	LC-MS/MS	BOLECHOVÁ, M.; ÈÁSLAVSKÝ, J.; POSPÍCHALOVÁ, M.; KOSUBOVÁ, P. UPLC-MS/MS method for determination of selected pyrrolizidine alkaloids in feed. FOOD CHEMISTRY, 2015, s. 265-270. ISSN: 0308- 8146.	QuEChERS	0,1% HCOOH in H2O + ACN (1:1), 2 g of sample, horizontal shaking for 20 min	

LC0025	rye bread	HPLC-FLD	Internal laboratory validated method based on Mullller C, Kemmlein S, Klaffke H, Krauthause W, Preiss-Weigert A and Wittkowski R, 2009. A basic tool for risk assessment: a new method for the analysis of ergot alkaloids in rye and selected rye products. Molecular Nutrition & Food Research, 53, 500-507; Storm ID, Rasmussen PH, Strobel BW and Hansen HC, 2008. Ergot alkaloids in rye flour determined by solid-phase cation-exchange and high-pressure liquid chromatography with fluorescence detection. Food Additives & Contaminants. Part A, 25, 338-346.	SPE with MycoSep 150 Ergot Push-through columns of Romer Labs	extraction solvent: mixture of 200mg/l ammonium carbonate (in water) / acetonitrile- 16/84 vol of solvent- 100ml mass of sample- 20g 30 min extraction by shaker
LC0026	cereals, flour, bread	LC-MS/MS (for this PT); HPLC-FLD	§ 64 LFGB - Entwurf der Methode "Untersuchung von Lebensmitteln - Bestimmung von Ergotalkaloiden in Brot und Brötchen" ; Juli 2016	SPE - alumina; waters ; Sep-Pak Plus WATO20205	extraction solvent: Ethylacetat/MeOH/NH3-Lsg(25%)/2-Propanol 75/5/7/7 (v/v/v) ; 100ml Extr.-sov und 20,0 g Sample ; 45 Min. extraction time
LC0027	N/A	LC-MS/MS	CEN method draft 6 'Determination of ergot alkaloids and tropane alkaloids in feed by LCMSMS'	N/A	Extraction solvent is 0.4% formic acid in MeOH/H20 60:40 Volume of solvent: 100ml / Mass of sample: 10g / Duration of the extraction: 30mins / Agitation method: Rotary shaker
LC0028	Cereals, all types (mostly wheat, oats and barley based)	LC-MS/MS	Krska et al (2008). Simultaneous determination of six major ergot alkaloids and their epimers in cereals and foodstuffs by LC-MS/MS. Anal Bioanal Chem 391:563–576 DOI 10.1007/s00216-008-2036-6	Dispersive SPE, Bondesil Primary Secondary amine (PSA)	10g sample extracted with 50ml extraction solution (acetonitrile:ammonium carbonate, 84:16) shaken for 30 minutes at moderate speed.
LC0029	cereals	LC-MS/MS	EFSA Scientific Report 2011	Romer-MycoSep 150 Ergot Multifunctional Columns	10 g sample+50 ml solvent (acetonitrile84-ammonium carbamate16), homogenisation, filtration (MycoSep150 Ergot), evaporation,injection
LC0030	rye	HPLC-FLD	BVL F 0104:2013-04 (Untersuchung von Futtermitteln - Bestimmung von Ergotalkaloiden in Roggen und Weizen - HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase (Übernahme der amtlichen Methode L 15.01/02-5, Januar 2012, Band I (Lebensmittel) der Amtlichen Sammlung)	SPE - alumina (alkaline) Sep-Pak Plus Alumina B Cartidges (Waters, Part No.: WAT020505)	extraction solvent: EtOAc/MeOH/25% NH3/2-propanol (75/5/7/7) extraction solvent volume: 25 mL extraction time: 45 min via shaking sample weight: 5 g
LC0031	Cereals	LC-MS/MS		Non	Acetonitrile:Water:Acetic acid 79:20:1 100 ml, 25 g sample / Shaking 30 min, filtration
LC0032	cereals and cereal based products	LC-MS/MS	HPLC/MS/MS Method for the determination of ergot alkaloids in cereals, R. Krska and C. Crews, October 2007, Food Standards Agency	Not used	84% ACN + 16% 3 mM (NH4)2CO3 aq.; 5 g of sample with 25 ml of extraction solvent; 1 h shaking in rotator
LC0033	feeds: cereal grains (rye, wheat, barley triticale), compound feeds foods: breakfast cereals	LC-MS/MS	RIKILT SOP A1070 (Animal feed - determination of ergot alkaloids and tropane alkaloids - LC-MS/MS)	ultrafiltration over 30 kD filter (Amicon Ultra 4, Millipore)	Extraction with MeOH/H2O/Formic acid 60/40/0.4 v/v/v 4 gram extracted with 40 ml extraction solvent, 30 min rotary tumbler
LC0034	wheat, cereals	LC-MS/MS	Romer Labs sample preparation, with LC-MS/MS method	SPE, Romer Labs Mycosep 150 Ergot Column	84/16 ACN/0.2 g/liter(NH4)2CO3Aqua

LC0035	cereals and bread	LC-MS/MS			15 g of sample are extracted in 60 mL of ethylacetate and 15 mL of ammonium hydroxide solution (25%). The samples are sonicated for 15 min and afterwards shaked for 15 min. Remove 1 mL of the upper phase and evaporate the solvent. Resolve the residue in 1 mL ACN/H2O (v/v 70/30)
LC0036	Cereals anf animal feed	LC-HRMS (Orbitrap) for feed, LC-MS/MS for Cereals	RIKILT SOP 2015	No clean-up	4g of sample; Extraction solvent 40ml of 0,4% formic acid in methanol/ water (60:40) (v/v); 30 minutes in a rotary tumbling machine and centrifugation.
LC0037	We have participated in CEN interlaboratory for EA in food and Feed without prior experience	LC-MS/MS	S. MacDonald (CEN Item 1 Draft WI00275289 - Ergot Alkaloid)	PSA	Extr solvent: acetonitrile:ammonium carbonate (84:16). 50mL extr solvent per 10g sample 30 min shaking
LC0038	feed	LC-MS/MS	BfR SOP 002_82_PV 039-2, modified §64 LFGB L 15.01/02	SPE Sep-Pak Plus Alumina B	20 g sample, extraction with 200 ml ethylacetate/methanol/25% NH4OH/ isopropanol (75/5/7/7, v/v/v/v) for 45 min with an orbital shaker

Lab code	**Q.11 Chromatographic conditions**	**Q.12 Detection conditions (λ, FLD)**	**Q.13 MS conditions**	**Q.14 Calibration approach**	**Q.15 Quantification**
LC0002	Mobile phase: ammonia carbamate buffer: acetonitrile (1:1) 10 µl injection, Phenomenex Gemini C6-Phenyl, 30 ºC		Ergocristine: 610.3 >223.2 Ergotamine: 582.6 > 223.2 Ergocryptine: 576.3 > 268.3 Ergosine: 548.27 > 208 Ergocornine: 562.3 >208 Ergometrine: 326.18 >223 Ergocryptinine: 576.3 >223.3 Ergocorminine: 562.3 >223 Ergometrinine: 326.18 >208 Ergosinine: 548.27 >223	Standards in pure solvent	Supplied calibration solution
LC0003	mobile phase A: water with 10 mmol ammonium acetate mobile phase B: acetonitrile column: gemini C6 phenyl (phenmomenx) 2 x 100 mm, 3 µm injection volumne: 5 µl column temp.: 30 °C		Ergometrin ESI+ m/Z 326> 223 Ergometrinin ESI+ m/Z 326> 208 Ergosin ESI+ m/Z 548> 223 Ergotamin ESI+ m/Z 582> 223 Ergocornin ESI+ m/Z 562> 544 alpha- Ergocryptin ESI+ m/Z 576> 558 Ergocristin ESI+ m/Z 610> 592 Ergosinin ESI+ m/Z 548> 530 Ergotaminin ESI+ m/Z 582> 564 Ergocorninin ESI+ m/Z 562> 544 alpha- Ergocryptinin ESI+ m/Z 576> 558 Ergocristinin ESI+ m/Z 610> 592	Standards in pure solvent	Supplied calibration solution

LC0004	A: 200 mg/L ammonium carbonate in water B: ACN / C18, 20 ul, 40 C	-	Ergometrine 326.2>223.3 ESI+ Ergosine 548.4>223.2 ESI+ Ergotamine 582.2>223.2 ESI+ Ergocornine 562.2>223.3 ESI+ a-Ergocryptine 576.4>223.3 ESI+ Ergocristine 610.4>592.3 ESI+ Ergometrinine 326.2>208.2 ESI+ Ergosinine 548.4>223.1 ESI+ Ergotaminine 582.4>223.1 ESI+ Ergocorninine 562.3>544.3 ESI+ a-Ergocryptinine 576.4>558.4 ESI+ Ergocristinine 610.4>592.4 ESI+	Standards in pure solvent	Supplied calibration solution
LC0005	Mobile Phase A: 200mg Ammonium Carbonate in Water, pH 10 Mobile Phase B: Acetonitrile Column: Thermo Hypersil Gold Injection Volume:		ESI+ Qualification Quantification Ergocornine 562.1 > 207.8 562.1 > 222.9 Ergocorninine 562 > 208.1 562 > 223.2 Ergocryptine 575.9 > 208 575.9 > 223 Ergocryptinine 576 > 223.2 576 > 268.2 Ergometrine 326 > 207.9 326 > 223.2 Ergometrinine 326 > 180 326 > 223.1 Ergosine 548.1 > 208 548.1 > 223 Ergosinine 548.1 > 208 548.1 > 223.1 Ergotamine 582 > 208 582 > 223 Ergotaminine 582.1 > 208 582.1 > 223.2 Erogocristine 610.3 > 223 610.3 > 267.9 Erogocristinine 610.2 > 223 610.2 > 268.2	Standards in pure solvent	Our own standards
LC0006	Mobile phase conditions: acetonitrile / 0.2g/L ammonium carbamate (1:1) Analytical column HPLC-FLD: C6-Phenyl 250 mm x 4,6 mm, 5 µm particle size Analytical column LC-MSMS: C6-Phenyl 150 mm x 2.0 mm, 3 µm particel size Injection volume: 10 µL Column temperature: 30 °C	HPLC-FLD: Wavelength excitation: 330 nm Wavelength emission: 415 nm	Ergometrine - ESI+ m/z 326.1>223.1 (CE 35,0 V) Ergometrinine - ESI+ m/z 326.1>208.1 (CE 35,0 V)	Standards in pure solvent / Matrix-matched calibration	Supplied calibration solution / Our own standards
LC0007	A: 6mM Ammonium hydroxide in water; B: 6mM Ammonium hydroxide in acetonitrile Column Xbridge C18 5*150*3 Inj volume 5 uL T° column 40°C		Ergosine ESI+ m/z 548>208 (CE 40V) Ergosinine ESI+ m/z 548>223 (CE 30V) Ergocriptine ESI+ m/z 576>208 (CE 40V) Ergocriptinine ESI+ m/z 576>223 (CE 30V) Ergocornine ESI+ m/z 562>223 (CE 35V) Ergocorninine ESI+ m/z 562>305 (CE 25V) Ergocristine ESI+ m/z 610>223 (CE 35V) Ergocristinine ESI+ m/z 610>325 (CE 25V) Ergometrine ESI+ m/z 326>207 (CE 20V) Ergometrinine ESI+ m/z 326>222 (CE 22V) Ergotamine ESI+ m/z 582>207 (CE 35V) Ergotaminine ESI+ m/z 582>223 (CE 30V)	Standard addition calibration	Supplied calibration solution
LC0008	Acetonitrile/Buffer Phenylhexyl, 50 ul, ambient	LC-MS/MS		Standards in pure solvent	Our own standards

LC0009	ACN:Ammonium carbonate (200mg/L) Gradient. injectin volume 4ìl, Waters UPLC BEH C18 100x2.1, 1.7ìm. 40 C column temperature		Ergometrine and ergometrinine - ESI+ m/z 326>208, 326>223, Ergosine and ergosinine - ESI+ m/z 548>208, 548>223, Ergocornine and ergocorninine - ESI+ m/z 562>208, 562>223, Ergocryptine and ergocryptinine - ESI+ m/z 576>223, 576>268, Ergotamine and ergotaminine - ESI+ m/z 582>208, 582>223, Ergocristine and ergocristinine - ESI+ m/z 610>223, 326>268	Standards in pure solvent	Supplied calibration solution
LC0010	H2O + 0.1 % formic acid / acetonitrile + 0.1 % formic acid. Column temperature: 40°C. Type of column: HSS T3; injection volume: 5 ul		Ergocristine: ESI+ m/z 592.4 > 223.3 (CV 30V; CE 30V) Ergotamine: ESI+ m/z 582.5 > 208.2 (CV 22V; CE 25V) Ergocryptine: ESI+ m/z 576.3 > 208.1 (CV 21V; CE 25V) Ergocornine: ESI+ m/z 562.5 > 223.3 (CV 20V; CE 25V) Ergosine: ESI+ m/z 548.3 > 223.2 (CV 20V; CE 33V) Ergometrine: ESI+ m/z 326.3 > 208.2 (CV 30V; CE 30V)	Standards in pure solvent	Supplied calibration solution
LC0011	acetonitrile/ammonium carbamate 0,2g/L; 50/50; v/v; Zorbax Eclipse XDB-C18, 5µ, 250 x 4,6 mm; 50µl; 30°C	Ex 330 nm; Em 415		Standards in pure solvent	Our own standards
LC0012	Mobile phase A: ACN Mobile phase B: (NH4)2CO3 200 mg/L Column type: Kinetex C18 EVO 100*2.1 2.6 µ Injection volume: 5 µL temperature column: 40°C	lambda adsorption = 415nm lambda excitation = 330 nm		Standards in pure solvent	Supplied calibration solution / Our own standards
LC0013	Mobile phase: gradient of A: 3,03 mM (NH4)2CO3 and B: ACN Column: Phenomenex Kinetex C18 XB 50x2,1 mm, 1,7 µm 100A Injection volume: 5 µL Temperature: 25 °C	/	We used an LC-Orbitrap with ESI+ ionisation and determination of the exact mass of each alkaloids	Standards in pure solvent	Supplied calibration solution
LC0014	Mobile phase A: 3mM NaHCO3 Mobile phase B: acetonitrile Column: Supelco Discovery C18 (150x2.1mm, 5um) Temperature of column: 30C Injection volume: 5uL		Ergocornin ESI+ 562/268, 562/223, 562/305 Ergocorninin ESI+ 562/277, 562/223, 562/305 Ergocristin ESI+ 610/223, 610/268, 610/208 Ergocristinin ESI+ 610/223, 610/305, 610/325 Ergocryptin ESI+ 576/223, 576/205, 576/268 Ergocryptinin ESI+ 576/223, 576/305, 576/291 Ergometrin ESI+ 326/223, 326/208, 326/197 Ergometrinin ESI+ 326/223, 326/208, 326/180 Ergosin ESI+ 548/223, 548/277, 548/263 Ergosinin ESI+ 548/223, 548/268, 548/208 Ergotamin ESI+ 582/223, 582/208, 582/268 Ergotaminin ESI+ 582/223, 582/277, 582/297	Matrix-matched calibration	Supplied calibration solution

LC0015	Water (ammonium hydroxide/ammonium acetate buffer) +acetonitrile Phenomenex Kinetex EVO column 1.7um, 100*2.1mm at 40°C 1ul injection volume		DIHYDRO-ergoCRISTINE 612.10>270.13 30 ESI+ ergoCRISTINE 610.4>223.10 35 ESI+ ergoCRISTININE 610.4>223.10 35 ESI+ ergoTAMINE 582.29>223.13 35 ESI+ ergoTAMININE 582.29>223.13 35 ESI+ alfa ergoKRYPTININE 576.4>223.2 35 ESI+ alfa ergoKRYPTINE 576.4>223.1 35 ESI+ ergoCORNININE 562.40>277.10 25 ESI+ ergoCORNINE 562.40>268.10 25 ESI+ ergoSININE 548.30>223.1 30 ESI+ ergoSINE 548.30>223.1 30 ESI+ ergoMETRININE 326.20>208.1 30 ESI+ ergoMETRINE 326.20>208.1 30 ESI+	Standard addition calibration	Our own standards
LC0016	Column Luna C18, 250 nm x 4.6mm, 5 um, Phenomenex. Temp. of the column 25 °C Injection volume 100 uL Mobile phase A: 2mM ammonium carbonate Mobile phase B: acetonitrile Gradient mode	Excitation wavelenght 330nm Emission wavelenght 420nm		Standards in pure solvent	Supplied calibration solution
LC0017	Eluent A was water and eluent B was 98% methanol (both containing 5 mM ammonium acetate and 0.1% acetic acid). Compounds were separated on Kinetex 2.6 µm, F5 100 Å, 150 x 2.1 mm column. The injection volume was 1 µl and the temperature of column was 30 C.			Matrix-matched calibration	Supplied calibration solution
LC0018	Mobile phase: component A - deionised water containing 0.2 g of ammonium carbonate per litre, component B - acetonitrile containing 1 mL of formic acid per litre Column: Ascentis Express Phenyl-hexyl column, 2.7 um, 10x2.1 cm (Supelco) /Injection volume: 10uL Column temperature: 30oC	N.A.	Ergometrin ESI+ 326,223 > 223,17 Ergometrinin ESI+ 326,202 > 180,183 Ergozin ESI+548,351 > 208,055 Ergozinin ESI+548,415 > 223,161 Ergokornin ESI+ 562,351 > 208,047 Ergokorninin ESI+ 562,351 > 223,086 Ergokriptin ESI+ 576,351 > 223,092 Ergokriptinin ESI+ 576,351 > 223,092 Ergotamin ESI+ 582,351 > 208,041 Ergotaminin ESI+ 582,351 > 223,08 Ergokristin ESI+ 610,351 > 208,050 Ergokristinin ESI+ 610,351 > 305,174	Standard addition calibration	Supplied calibration solution

LC0019	Gradient of ammonium carbonate 200 mM pH 10 (Mobile phase A), acetonitrile (Mobile phase B) Column: Phenomenex Gemini - NX 5ìm 150 mm X 2 mm Temperature: 30 deg C Injection volume: 10 ìL	N/A	Mode: ESI + Ergometrine-1 - Transition : 326.1/223.0 amu, - CE= 39 Ergometrinine-1 - Transition : 326.2/207.9 amu, - CE= 37 Ergosine-1 - Transition : 548.2/223.0 amu, - CE= 37 Ergotamine-1 - Transition : 582.2/223.1 amu, - CE= 37 Ergocornine-1 - Transition : 562.2/223.1 amu, - CE= 37 a-Ergocryptine-1 - Transition : 576.2/268.1 amu, - CE= 37 Ergocristine-1 - Transition : 610.2/268.1 amu, - CE= 37 Ergosinine-1 - Transition : 548.2/223.0 amu, - CE= 43 Ergotaminine-1 - Transition : 582.2/223.1 amu, - CE= 43 Ergocorninine-1 - Transition : 562.2/543.9 amu, - CE= 43 a-Ergocryptinine-1 - Transition : 576.2/558.2 amu, - CE= 43 Ergocristinine-1 - Transition : 610.2/591.9 amu, - CE= 43	Standards in pure solvent	Supplied calibration solution
LC0020	A: Ammonium carbonate 3 mM pH 9 / B: Acetonitrile, Gradient Acquity UPLC BEH column 1.7 um, 100x2.1 mm, 65 degrees C Vi = 10 uL	-	Ergot alkaloid Quant SRM Qual SRM Cone CE Ergometrin[M+H]+ 326,1 > 223,0 326,1 > 208,0 50 25/30 Ergosin [M+H]+ 548,2 > 223,0 548,2 > 208,0 45 30/43 Ergotamin [M+H]+ 582,1 > 223,0 582,1 > 208,0 45 35/42 Ergocornin[M+H]+ 562,2 > 223,0 562,2 > 208,0 40 38/45 Ergocryptin[M+H]+ 576,2 > 208,0 562,2 > 223,0 45 45/27 Ergocristin[M+H]+ 610,2 > 223,0 61,2 > 208,0 45 37/47 Meloxikam (IS) [M+H]+ 352,0 > 115,0 - 25 20	Standards in pure solvent	Supplied calibration solution
LC0021	Mobile phase composition: A: ACN and B: carbonate ammonium 200mg/L gradient A: 10 to 80% type of analytical column: X Bridge C18 5 µm 150x3mm injection volume: 20 µL temperature column: 40°C	/	ESI+ m/z Transition-1 m/z Transition-2 Ergométrine 326>223 326>208 Ergométrinine 326>223 326>208 Ergosine 548>223 548>208 Ergosinine 548>223 548>208 Ergotamine 582>223 582>208 Ergotaminine 582>223 582>208 Ergocornine 562>223 562>268 Ergocorninine 562>223 562>268 Ergocryptine 576>223 576>268 Ergocryptinine 576>223 576>268 Ergocristine 610>223 610>208 Ergocristinine 610>223 610>208	Standard addition calibration	Supplied calibration solution
LC0022	Eluent A: ammonium carbonate buffer (200 mg/l, pH ~ 8,9) Eluent B: Acetonitrile Column: Waters BEH C18 2,1 x 100 mm(1,7 µm) Injection volume: 10 µl Temperature: 30 C		ergometrine - ESI+ m/z 326 >223 (CE 22V) ergometrinine - ESI+ m/z 326 >208 (CE 28V) ergosine - ESI+ m/z 548 >208 (CE 42V) ergosinine - ESI+ m/z 548 >268 (CE 35V) ergotamine - ESI+ m/z 582 >208 (CE 44V) ergotaminine - ESI+ m/z 582 >223 (CE 32V) ergocornine - ESI+ m/z 562 >268 (CE 25V) ergocorninine - ESI+ m/z 562 >277 (CE 26V) ergocryptine - ESI+ m/z 576 >208 (CE 40V) ergocryptinine - ESI+ m/z 576 >223 (CE 34V) ergocristine - ESI+ m/z 610 >208 (CE 44V) ergocristinine - ESI+ m/z 610 >305 (CE 30V)	Matrix-matched calibration	Supplied calibration solution
LC0023	ACN/Ammonium Hydroxide/ Ammonium Carbonate Buffer C18, 10ul, 40°C		Confidential	Matrix-matched calibration	Supplied calibration solution

LC0024	A=0,1% HCOOH in H2O, B=0,1% HCOOH + 1mM HCOONH4 in MeOH, C18 column, inj = 2 uL		All ESI+. E-metrine 326>208, E-metrinine 326>208, E-sine 548.3>223.2, E-sinine 548.3>223.2, E-tamine 582>268, E-taminine 564>223.2, E-cornine 562.5>223.3, E-corninine 544.4>223.3, E-cryptine 576.3>223.3, E-cryptinine 576.3>223, E-crystine 610.4>223.3, E-crystinine 610.4>223.3	Matrix-matched calibration	Supplied calibration solution
LC0025	phase A: 200mg/l ammonium carbonate in water / phase B: acetonitrile with gradient C18 analytical column 4.6x150mm, 5-Micron injection volume- 20 microlitres temperature of the column- 30°C	Excitation- 250nm Emission- 410nm		Standards in pure solvent	Supplied calibration solution
LC0026	Eluent A: ammonium carbamate buffer 0,2 g/L ; Eluent B: ACN ; 0 Min. 55%A/45%B ; 1 Min. dto ; 15 Min. 20%A/80%B ; LC-Column: Supelco Phenyl-Hexyl Ascentis Express 100 x 4.6 , 2.7 µm ; Injection Volume je 5µl ; column temperature 35 °C	No	Ergometrin -ESI+ m/z 326.1>223.0 (CE33) ; Ergometrinin -ESI+ m/z 326.1>208.0 (CE39) ; Ergosin -ESI+ m/z 548.1>223.0 (CE44) ; Ergosinin -ESI+ m/z 548.1>223.0 (CE43) ; Ergocornin -ESI+ m/z 562.1>268.0 (CE35) ; Ergocorninin -ESI+ m/z 562.1>223.1 (CE45) ; a-Ergokryptin -ESI+ m/z 576.1>268.1 (CE35) ; a-Ergokryptinin -ESI+ m/z 576.1>223.0 (CE47) ; Ergotamin -ESI+ m/z 582.2>223.0 (CE47) ; Ergotaminin -ESI+ m/z 582.2>223.1 (CE43) ; Ergocristin -ESI+ m/z 610.2>268.0 (CE37) ; Ergocristinin -ESI+ m/z 610.2>305.0 (CE39) ;	Standards in pure solvent	Supplied calibration solution
LC0027	Mobile Phase A: 6mM ammonium hydroxide in H20 Mobile Phase B: 6mM ammonium hydroxide in MeOH, gradient elution Column: Xbridge C18, 5um, 150 x 3 mm / Injection volume: 5ul Column temp: 40C	N/A	ESI + Precursor Ion Product ion 1, ion 2, ion 3 Ergocornine 562.4 223.1 305.2 268.1 Ergocorninine 562.4 223.1 305.2 277.1 Ergocristine 610.4 223.1 305.2 268.1 Ergocristinine 610.4 223.1 305.2 325.1 α-Ergocryptine 576.4 208.1 223.1 268.1 α-Ergocryptinine 576.4 208.1 223.1 305.2 Ergometrine 326.2 208.1 223.1 180.1 Ergometrinine 326.2 208.1 223.1 180.1 Ergosine 548.4 208.1 223.1 268.1 Ergosinine 548.4 208.1 223.1 277.1 Ergotamine 582.4 208.1 223.1 268.1 Ergotaminine 582.4 208.1 2	Standard addition calibration	Supplied calibration solution

LC0028	UPLC column Waters BEH (ethylene bridged hybrid), C18, 130A, 1.7um (100 x 2.1mm) Mobile phase gradient: mobile phase A: acetonitrile, mobile phase B: 200mg/l ammonium carbonate solution. Column temperature 40+/- 5C. Inj vol 2ul UHPLC Gradient Conditions. Time (minutes) A% B% Flow (mL min-1) Curve 0.00 5.0 95.0 0.500 Initial 1.50 45.0 55.0 0.500 6 3.5 50.0 50.0 0.500 6 6.0 70.0 30.0 0.500 6 9.0 99.0 1.0 0.500 1 12.0 5.0 95.0 0.500 1		Compound Rt (min) MRM quantitation ion. MRM confirmation ion Ergometrine 1.65 326 => 223 326 => 208 Ergometrinine 2.05 326 => 208 326 => 223 Ergosine 2.70 548 => 223 548 => 208 Ergosinine 4.20 548 => 223 548 => 208 Ergotamine 2.85 582 => 223 582 => 208 Ergotaminine 4.60 582 => 564 582 => 223 Ergocornine 3.30 562 => 268 562 => 223 Ergocorninine 5.00 562 => 544 562 => 223 Ergocryptine 3.70 576 => 268 576 => 223 Ergocryptinine 5.45 576 => 558 576 => 223 Ergoscristine 3.85 610 => 223 610 => 208 Ergocristinine 5.65 610 => 592 610 => 223	Standards in pure solvent	Supplied calibration solution
LC0029	phase A:445ml wather,50 mlmethanol,5ml acetic acid,0,192g amonium acetate phase B:495ml methanol, 5acetic acid, 0,192g amonium acetate column :Pursuit 5 C18 150x 4mm injection volum 15 ul temperature 40 Celsius		ergoSINE-ESI+548.3>223.3 ergoSININE-ESI+530.4>223 ergoCORNINE-ESI+ 562.5>223.3 ergoCORNININE-ESI+ 544.4>277.5 ergoCRIPTINE-ESI+ 576.3>268.4 ergoCRIPTININE-ESI+ 558.5>305.3 ergoCRISTINE-ESI+ 610.4>268.4 ergoCRISTININE-ESI+ 592.4>305	Standards in pure solvent	Supplied calibration solution
LC0030	eluent A: 0,2 g/L ammonium carbamat in water eluent B: ACN column: Phenomenex Gemini 3u C6-Phenyl 110A (150 x 2.00 mm, 3u) / injection volume: 15 uL column temp.: 20 °C	excitation: 330 nm emission: 415 nm		Standards in pure solvent	Supplied calibration solution
LC0031	Methanol, water, formic acid, ammonia, C18, 5 µl, 45 °C		ergocornine ESI+ 562>208 CE50 V ergocorninie ESI+ 544>223 CE37 V ergocristine ESI+ 610>223 CE35 V ergocristinine ESI+ 592>223 CE35 V ergocryptine ESI+ 576>223 CE35 V ergocryptinine ESI+ 558>223 CE35 V ergometrine ESI+ 326>223 CE23 V ergometrinine ESI+ 326>208 CE25 V ergosine ESI+ 548>223 CE33 V ergosinine ESI+ 530>223 CE28 V ergotamine ESI+ 582>223 CE35 V ergotaminine ESI+ 564>223 CE32 V	Matrix-matched calibration	Supplied calibration solution

LC0032	A: ACN; B: 3 mM (NH4)2CO3 aq.; gradient programme: 0 min 5% A, 3 min 45% A, 16 min 55% A, 18 min 80% A, 22 min 80% A, 23 min 5% A, total time 27 min; HPLC column: Waters XBridge BEH C18 2,1 mm x 100 mm, 1.7 um; injection volume: 10 ul; Tcol.=40C	Not used	all ESI+ ergometrine (CE 20) & ergometrinine (CE 15): 326.2>223; ergosine (CE 35) & ergosinine (CE 25): 548,3>223; ergotamine (CE 40) & ergotaminine (CE 30): 582.6>223; ergocornine (CE 40) & ergocorninine (CE 30): 562.3>223; ergocriptine (CE 32) & ergocriptinine (CE 18): 576.5>223; ergocristine (CE 35) & ergocristinine (CE 20): 610.3>223	Standards in pure solvent	Supplied calibration solution
LC0033	Mobile phase A: 10 mM ammonium carbonate in water pH 9; Mobile phase B: acetonitrile Column: Waters Acquity BEH C18, 150 x 2.1 mm, 1.7 um inject: 2 ul, temperature: 50oC		ESI positive ergometrine: 326.2>223.1 (25); 326.2>208.1 (30) ergometrinine: 326.2>208.1 (30); 326.2>223.1 (25) ergosine: 548.4>223.1 (30); 548.4>208 (40) ergosinine: 548.4>223.1 (30); 548.4>208 (40) ergocornine: 562.4>268.1 (25); 562.4>223.1 (35) ergocorninine: 562.4>223.1 (35); 562.4>305.2 (25) a-ergocryptine: 576.4>223.1 (35); 576.4>268.1 (25) a-ergocryptinine: 576.4>223.1 (35); 576.4>208.1 (40) ergotamine: 582.4>223.1 (35); 582.4>208.1 (40) ergotaminine: 582.4>277.1 (25); 582.4>223.1 (35) ergocristine: 610.4>223.1 (35); 610.4>268.1 (25) ergocristinine: 610.4>223.1 (35); 610.4>3	Standard addition calibration	Supplied calibration solution / Our own standards
LC0034	NH4Acaq/ACN gradient		ESI+	Standards in pure solvent	Our own standards
LC0035	column: Macherey Nagel Nucleodur PFP 125/3, 5 µm injection volume: 30 µL oven temperature: 30 °C mobile phase A: H2O + 200 mg ammonium carbamate mobile phase B: ACN/H2O (v/v 90/10)		ergometrine/inine - ESI+ m/z 326.1>223.2 (CE 33V) ergosine/inine - ESI+ m/z 548.2>223.2 (CE 33V) ergotamine/inine - ESI+ m/z 582.3>223.2 (CE 45V) ergocornine/inine - ESI+ m/z 562.2>268.2 (CE 35V) alpha-ergocryptine/inine - ESI+ m/z 576.3.1>268.2 (CE 34V) ergocristine/inine - ESI+ m/z 610.5>268.2 (CE 33V)	Standards in pure solvent	Our own standards
LC0036	Mobile phase A:10 mM ammonium acetate 0.05% Ammonium acetate 25% Mobile Phase B: Acetonitrile Column:Kinetex 2.6um EVO C18 (100x2.1 mm) Injection volume: 3ul Tª: 50 ºC		Ergometrine-ESI+ m/z 326.22>223.15 Ergometrinine-ESI+ m/z 326.22>208.15 Ergosine-ESI+ m/z 548.32>223.18 Ergosinine-ESI+ m/z 548.32>223.18 Ergocornine-ESI+ m/z 562.35>223.18 Ergocorninine-ESI+ m/z 562.35>277.13 α-Ergocryptine-ESI+ m/z 576.35>268.15 α-Ergocryptinine-ESI+ m/z 576.35>223.18 Ergotamine-ESI+ m/z 576.35>223.18 Ergotaminine-ESI+ m/z 576.35>305.18	Matrix-matched calibration / Standard addition calibration	Our own standards

LC0037	HPLC column used: Acquity BEH C18 1.7?m, 2.1x50 mm Mobile phase: MPA: (NH4)2CO3 10mM, pH10; MPB: 100% AcCN Flow rate: 0.4 ml/min Column temperature: 40 °C Injection volume: 10 ul	No	ESI+ Ergometrine: 326,2 >208,0 (CE 35) Ergometrinine: 326,2>223,0 (CE 35) Ergosine: 548,3>208,0 (CE 40) Ergosinine: 548,3>223,0 (CE 40) Ergotamine: 582,6>208,0 (CE 45) Ergotaminine: 582,6>223,0 (CE 45) Ergocornine: 562,3>208,0 (CE 35) Ergocorninine: 562,3>223,0 (CE 35) α-Ergocryptine: 576,5>223,0 (CE 35) α-Ergocryptinine: 576,5>268,0 (CE 35) Ergocristine: 610,3>223,0 (CE 40) Ergocristinine: 610,3>268,0 (CE 40)	Standards in pure solvent	Supplied calibration solution
LC0038	A: 0,2 g/l ammonium carbamate (2,6 mmol); B: acetonitrile Gemini C6-Phenyl 2*150 mm, 3 µm, Phenomenex, 30 °C, 10 µl injection		ESI+: ergometrine 326,1>223,1, CE34; ergosine 548,5>223,2, CE20; a-ergokryptine 576,3>208,2, CE18; ergotamine 582,2>223,2, CE 18; ergocristine 610,3> 223,2, CE18; ergometrinine 326,1>223,1, CE18; ergosinine 548,6>530,3, CE13 ergocorninine 562,2> 544,4, CE 12; a-ergokryptinine 576,3>558,4, CE10; ergotaminine 582,2>562,2, CE 14; ergocristinine 610,4>592,4, CE10; ergocorninine 562,3>268,1, CE 18; LSD 324,1>207,1, CE 10 (internal standard for injection)	Standards in pure solvent	Supplied calibration solution

Lab code	Q.16 Areas in FLD	Q.17 Approach method uncertainty	Q.18 Recovery estimation	Q.19 Recovery correction	Q.20 Supplier of standards	Q.21 Overnight stop
LC0002			YES	Corrected for recoveries	Biopure (used for recovery experiments)	No
LC0003		top down principle, uncertainty = 2 x standard deviation (n = > 20)	spiking blank material (rye)	Corrected for recoveries	LGC (not used for calibration)	No
LC0004		From validation parameters.	By using spiked sample.	Corrected for recoveries		
LC0005			Spiked Samples	Corrected for recoveries	Biopure- Romer Labs	No
LC0006	Estimated based upon mean value of peak area Ergosine: 1.640.00 Ergotamine: 4.770.000 Ergotamine 1/5: 900.000 Ergosinine: 450.000 Ergotaminine: 1.180.000 Ergocornine: 1.800.000 Ergocorninine: 985.000 Ergokrytine: 1.155.000 Ergokryptinine: 597.000 Ergocristine: 2.426.000 Ergocristine 1/5: 460.000	10 times measurement of reference material (rye flour)	10 times measurement of reference material (rye flour)	Not corrected	Sigma Aldrich, Biopure Coring	NO

	Ergocristinine: 1.896.000					
LC0007		no	standard addition	Corrected for recoveries		No
LC0008		10%	standard addition	Not corrected	Coring	No
LC0009		GUM	Spiked samples	Corrected for recoveries		No
LC0010		repeated analyses using k = 2	spiking of sample at 50 ug/kg	Corrected for recoveries		No
LC0011	Ergometrine 3,47; Ergosine 7,47; Ergotamine 15,6; Ergocornine 2,26; α-Ergocryptine 2,86; Ergocristine 7,8; Ergometrinine 0,75; Ergosinine 3,24; Ergotaminine 4,59; Ergocorninine 1,26; α-Ergocryptinine 2,73; Ergocristinine 5,29	MU (expanded; k =2) is estimated with different samples within the lab under repeatability and reproducibility conditions. The calculation program is homemade.	spiked samples	Not corrected		
LC0012		no	by spiking -ine alkaloids at 200 µg/Kg and -inine at 100 µg/Kg	Corrected for recoveries	Romer	No
LC0013		Yes, with Horwitz approach	With standard addition at a blank matrix	Not corrected	(Romer Labs)	No
LC0014		Horwitz equation	spiked sample	Corrected for recoveries		No
LC0015		Yes: 40%	spikes	Not corrected	Biopure	No
LC0016	Ergometrine 2731,2901 Ergotamine 9196, 9470 Ergocornine 3785, 3904 Ergocriptine 2232, 2216 Ergocristine 10410, 10671	Uncertainty was calculated on the basis of reproducibility, assuming that it is doubling.	Blank feed samples spiked at concentrations levels 25, 150 and 400ug/kg (six replicates for each concentration) were used to evaluate the method's recovery. The samples were analysed with the same instrument and the same operator.	Not corrected		No
LC0017			Blank material was spiked at 25 ng/ml	Not corrected		After extraction, supernatants were stored at 4 C for 18-20 h
LC0018	N.A.	Standard deviation, coverage factor 2	Analysing spiked samples	Corrected for recoveries	N.A.	No
LC0019	N/A	Yes	From spiked rye samples	Corrected for recoveries	N/A	No
LC0020		Validation study, spiked samples	Spiked blank rye sample	Corrected for recoveries	biopure	No
LC0021		by recovery rates	by spiking	Not corrected	We used the standard provided by the EURL. Our own standard are from BIOPURE .cf comment	No
LC0022		Using validation results and quality control samples' results (intra- and inter-day repeatability, bias)	With spiked matrix samples	Corrected for recoveries		No

LC0023		yes	Spiking	Corrected for recoveries		No
LC0024		validation procedure	CRM	Not corrected	Romer Labs / Biopure	No
LC0025	peak areas in LU*s: Ergosine- 136.5 Ergocornine- 77.2 a-Ergocryptine- 46.8 Ergocristine- 247.2 Ergosinine- 45.8 Ergocorninine- 97.9 Ergocryptinine- 120.8 Ergocristinine- 192.8	calculation of the expanded uncertainty during the validation of the method	by spiked blank matrix	Not corrected	Biopure	No
LC0026	No	multiple analysis of contaminated samples, calculation with z-scores and validation data (not yet finished)	multiple analysis of spiked samples for different matrices (n=5)	Not corrected	BioPure	No
LC0027	N/A	No	Spiked sample with known concentration	Corrected for recoveries	N/A	Yes, before centrifugation the samples were left in the fridge
LC0028		Yes		Corrected for recoveries		No, just overnight LC-MS/MS run
LC0029			calibration solution in blank sample	Corrected for recoveries	I used the standards provided by the EURL	No
LC0030	Ergometrine: 10 Ergometrinine: 2 Ergosine: 7 Ergosinine: 5 Ergocristine: 20 Ergocristinine: 18 Ergotamine: 24 Ergotaminine: 13 Ergocornine: 8 Ergocorninine: 10 Ergokryptine: 5 Ergokryptinine: 6	With every sample batch, a self-mixed reference material was analyzed (n = 13). The repeated standard deviation was determined and multiplied by the appropriate t-intervall (2,18). The result/measurement uncertainty was rounded, e.g. 18% was rounded to 20%.	The sample was spiked with a standard solution, resulting in an added amount of 20 ug/kg.The final concentration was calculated and the concentration of the non-spiked sample was subtracted. The result was divided by 20 and multiplied by 100.	Not corrected		No
LC0031		Validation ergots and uncertainty for other mycotoxins in the same method (recovery, double samples, PT results, reference materials)	Spiking in blank sample	Corrected for recoveries	Biopure	Yes After sample preparation, before MS-analysis
LC0032		estimation of standard deviation	spiking on three levels (50/25; 100/50; 200/100)	Corrected for recoveries		No
LC0033		estimated MU = 25%, based on performance in PT trials	standard addition (500 ng/g) before extraction vs spike to extract (50 ng/ml)	Corrected for recoveries	Alfarma (Czech Rep), Phytolab (Germany), Fluka (Germany)	No
LC0034		Nordtest method	From control sample	Not corrected	Romer Labs	No
LC0035		reproducibility (comparison of different materials)	Fapas material	Corrected for recoveries	Biopure	No
LC0036		Not yet	Not Yet	Not corrected	Bioser/Sigma	No

LC0037	No	No, we have applied Horwitz 44%	We failed in assessing recoveries since we spiked the PT sample to a level that resulted too low in comparison with the level of the sample, so useless for recovery calculations (spk done at 5-10 ug/kg, while sample levels resulted around 100 ug/kg)	Not corrected	No	No
LC0038		estimated from repeated measurements of the sample	spiking of wheat, extraction	Not corrected		No

Annex 11. Method validation data as reported by the participants

	Ergometrine					Ergometrinine					Ergosine					Ergosinine				
Lab	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**
LC0002	23.4	69.5	7	1.4	0.41	24.1	92	7.2	3.7	1.1	25.3	91.5	7.8	0.15	0.05	23.1	113	7.6	5.4	1.6
LC0003	44.1	91.7	47	2	0.7	43.9	92.4	96	2	0.7	44.0	87.4	94	2	0.7	44.0	95.7	93	2	0.7
LC0004	30.0	78		20	5	30.0	88.6		20	5	30.0	66.1		20	5	29.9	81.3		20	5
LC0005	16.6	94.0		5	1	16.7	115.6		5	1	16.7	87.0		5	1	16.7	75.9		5	1
LC0006	18.6	66.4	77	38.7	12.9	19.0	52.5	104.3	9.1	3.03	7.8	107	101.8	28.5	12.8	8.6	88.9	88.9	16.4	5.5
LC0007	30.0	113		1	0.5	29.7	112		1	0.5	30.0	50		1	0.5	29.8	78		1	0.5
LC0008	9.9	90		10	5	11.2	90		10	5	10.0	91		10	5	10.4	90		10	5
LC0009	14.2	99		0.36	0.11	14.6	107.5		0.29	0.09	10.5	107.9		0.34	0.1	21.3	117.2		0.29	0.09
LC0010	30.0	94		5	1	31.0	94		5	1	30.0	121		5	1	30.0	121		5	1
LC0011	30.0	94	89.6	30	10	30.1	104	107	30	10	30.0	94	111	30	10	30.0	115	99.2	30	10
LC0012		101		1	1	0	119		1	1	0.0	128		1	1	0	122		1	1
LC0013	48.5	76		25	12.5	53.1	92		12.5	6.3	42.2	96		25	12.5	42.9	71		12.5	6.3
LC0014	34.7	78	89	5	1	41.7	69	85	2	0.5	29.1	86	94	5	1	34.8	96	83	2	0.5
LC0015	29.9	100	30	2.5	1	30.3	100	30	2.5	1	29.9	100	30	2.5	1	29.8	100	30	2.5	1
LC0016	28.9	94.8		11.0	8.79															
LC0017		112		183	55	0					0	87		44	13					
LC0018	70.0	100		10	3	30.0	107		10	3	25.0	105		10	3	29.7	109		10	3
LC0019	17.7	100.1		2.1	0.7	17.6	104.7		0.7	0.2	20.2	101		6.4	2	24.0	116.7		2.6	0.8
LC0020	20.0	82	88	20	2	20.1	88				20.0	90	90	20	2	19.9	87	-		
LC0021	50.0	78	20	10	3	52.2	88	100	10	3	31.6	95	95	10	3	50.7	116	108	10	3
LC0022	52.9	115		1	10	221.1	96		0.05	0.5	38.0	75		0.05	0.5	27.3	123		0.5	5
LC0023	10.2	83.7		10	1	10.9	83.0		10	1	10.6	85		10	1	11.4	100		10	1
LC0024	50.0	84		5	3	49.8	88		5	3	50.0	96		5	3	50.1	89		5	3
LC0025											15.0	95.5	101.4	2.5	0.8	15.0	97.6	101.4	2.5	0.8
LC0026	0	101	3.14	1	0.4	0	130	2.1	1	0.4	0	86	3.1	1	0.4	0	105	1.52	1	0.4
LC0027	0	97				0	96				0	110				0	77			
LC0028	20.0	79		0.25	0.003	20.2	94		0.125	0.001	20.0	97		0.25	0.003	20.0	148		0.125	0.001
LC0029				10	5				10	5	20.9	49		10	5	19.9	75		10	5

LC0030	15.0	98.2		16.2	5	24.9	96.2		6	1.8	25.0	106.9		8.1	2.4	55.0	100.9		7.7	2.3
LC0031	55.0	84		2	0.5	55.1	92.3		2	0.5	55.0	92.6		2	0.5	55.0	80.4		2	0.5
LC0032	20.1	69.1		10	5	19.8	91.3		10	5	22.0	65		10	5	22.0	81		20	10
LC0033	25.0	83.4		0.5		24.9	87.5		0.5		25.0	66.9		0.5		24.9	74.2		0.5	
LC0034	25.0	95	100.3	5	1	39.9	95	96.7	5	1	25.0	95	102.1	5	1	30.1	95	94	5	1
LC0035	40.0	100	115	3	1	39.9	100	114	3	1	40.0	67	149	3	1	40.0	100	103	3	1
LC0036	0			5		0			5		0		67	5		0		75	5	
LC0037	44.0			2.6		42.5	73		1.3		44.1			2.5		44.4			1.3	0
LC0038	60.0	73		15.6	9.3	59.7	81		15.6	9.3	60.0	69		15.6	9.3	60.0	106		15.6	9.3

MU – method uncertainty (%), Rec – recovery (%), Std Comp – comparison of standards (%), LOD – limit of detection (μg/kg), LOQ - limit of quantification (μg/kg)

	Ergotamine					**Ergotaminine**					**Ergocornine**					**Ergocorninine**				
	MU %	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**
LC0002	18.9	86.5	5.4	0.21	0.06	35.6	112.7	11.8	2.8	0.84	22.9	102.8	6.8	5.3	1.6	25.8	96	7.3	2.9	0.87
LC0003	44.0	86.2	99	2	0.7	44.0	94.5	96	2	0.7	44.0	86.8	96	2	0.7	44.0	99.3	95	2	0.7
LC0004	30.0	62.3		20	5	30.0	62.3		20	5	30.0	79.9		20	5	30.0	89.9		20	5
LC0005	16.7	65.9		5	1	16.7	104.9		5	1	16.7	131.9		5	1	16.6	122.1		5	1
LC0006	6.6	92.7	100.1	57.2	19.1	7.7	92.3	89.5	21.8	7.3	7.4	92.2	95.5	27.5	9.2	6.1	87.9	85.4	11.4	3.8
LC0007	30.0	71		1	0.5	30.0	90		1	0.5	30.0	50		1	0.5	30.0	50		1	0.5
LC0008	10.0	90		10	5	10.0	89		10	5	9.9	92		10	5	10.2	91		10	5
LC0009	11.5	106.7		0.36	0.11	9.0	116.5		0.31	0.09	10.4	110.7		0.35	0.11	17.6	98.6		0.2	0.06
LC0010	30.0	107		5	1	30.0	107		5	1	30.0	96		5	1	30.0	96		5	1
LC0011	30.0	93	79.1	30	10	30.0	115	93.2	30	10	30.0	113	97	30	10	30.0	98	91.6	30	10
LC0012	0	115		1	1	0	119		1	1	0	115		1	1	0	124		1	1
LC0013	36.7	90		25	12.5	36.7	73		25	12.5	42.1	97		25	12.5	41.2	120		75	37.5
LC0014	24.4	79	113	5	1	29.5	78	62	2	0.5	29.0	101	64	5	1	31.8	65	98	2	0.5
LC0015	30.0	100	30	2.5	1	29.9	100	30	2.5	1	29.9	100	30	2.5	1	29.9	100	30	2.5	1
LC0016	3.2	99.9		6.6	3.23						6.1	102.4		8.1	5.38					
LC0017	0	88		131	40	0					0	86		40	12	0				
LC0018	26.0	112		10	3	24.9	112		10	3	50.0	124		10	3	15.3	116		10	3
LC0019	17.8	104.9		8.6	2.6	51.7	110		3.8	1.1	14.7	116.9		5.5	1.7	26.4	87.5		0.2	0.1

LC0020	20.0	86	89	20	2	20.0	77	-			20.0	84	87	20	2	20.0	83	-		
LC0021	30.5	95	93	10	3	30.2	121	108	10	3	33.3	94	96	10	3	30.3	121	92	10	3
LC0022	13.5	96		0.05	0.5	8.4	101		0.05	0.5	77.1	48		0.05	0.5	27.9	152		0.05	0.5
LC0023	10.0	91.0		10	1	10.2	93.5		10	1	10.0	100		10	1	10.6	72.5		10	1
LC0024	50.0	74		5	3	50.0	105		5	3	50.0	105		5	3	50.0	101		5	3
LC0025											15.0	96.3	101.4	2.5	0.8	15.0	99.3	101.5	2.5	0.8
LC0026	0	93	3.72	1	0.4	0	88	2.29	1	0.4	0	89	2.91	1	0.4	0	94	2.34	1	0.4
LC0027	0	119				0	77				0	88				0	93			
LC0028	20.0	102		0.25	0.003	20.0	153		0.125	0.001	20.0	110		0.25	0.003	20.0	82		0.125	0.001
LC0029				10	5				10	5	38.3	44		10	5	35.4	40		10	5
LC0030	20.0	66.9		9.9	3	25.0	78.1		8.2	2.4	20.0	87.8		8.3	2.5	20.0	95.7		9.5	2.9
LC0031	55.0	97.1		2	0.5	55.0	84.1		2	0.5	55.0	111.7		2	0.5	55.0	86.8		2	0.5
LC0032	24.0	81.6		10	5	24.0	77.9		20	10	22.0	86.1		10	5	22.0	86.3		20	10
LC0033	25.0	63.5		0.5		25.0	76.7		1		25.0	66.9		0.5		25.0	69.9		0.5	
LC0034	25.0	95	101.8	5	1	26.8	95	95.7	5	1	25.0	95	100.4	5	1	25.0	95	99	5	1
LC0035	40.0	100	123	3	1	40.0	100	110	3	1	40.0	100	127	3	1	40.0	100	119	3	1
LC0036	0			5		0			5		0		104	5		0		95	5	
LC0037	43.9			2.5		43.8			1.3		44.2			2.5		44.3			1.3	
LC0038	60.0	81		15.6	9.3	60.0	112		15.6	9.3	60.0	96.5		15.6	9.3	60.0	124		15.6	9.3

	α-Ergocryptine					**α-Ergocryptinine**					**Ergocristine**					**Ergocristinine**				
	MU %	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**	**MU %**	**Rec %**	**Std Comp**	**LOQ**	**LOD**
LC0002	28.2	99.6	8.7	4.7	1.4	20.5	98	6.1	2.8	0.86	25.5	98.9	7.5	6.3	1.9	26.0	103.1	8.4	9.3	2.8
LC0003	44.0	85.3	93	2	0.7	44.1	94	95	2	0.7	44.0	75.3	96	2	0.7	44.0	97.8	95	2	0.7
LC0004	30.0	95.5		20	5	30.0	85.8		20	5	30.0	58.6		20	5	30.0	58.6		20	5
LC0005	16.7	131.3		5	1	16.7	99.6		5	1	16.7	59.0		5	1	16.7	104.1		5	1
LC0006	6.5	98.8	97	22.9	7.6	5.8	97.4	82.4	10.5	3.5	4.1	97.1	96	40.2	13.4	2.6	82.7	83.2	9.3	3.1
LC0007	30.0	62		1	0.5	29.9	98		1	0.5	30.0	63		1	0.5	30.0	72		1	0.5
LC0008	9.8	87		10	5	9.7	90		10	5	10.0	90		10	5	9.9	90		10	5
LC0009	11.0	108.7		0.15	0.05	10.0	111.7		0.29	0.09	10.2	116.1		0.39	0.12	9.8	109.6		0.38	0.11

LC0010	30.0	112		5	1	30.0	112		5	1	30.0	123		5	1	30.0	123		5	1
LC0011	30.0	110	99	30	10	30.0	104	82.3	30	10	30.0	109	98.5	30	10	30.0	102	95	30	10
LC0012	0	116		1	1	0	115		1	1	0	115		1	1	0	114		1	1
LC0013	39.6	113		25	12.5	42.3	110		12.5	6.3	35.8	87		25	12.5	34.6	100		75	37.5
LC0014	27.3	86	69	5	1	31.1	74	96	2	0.5	24.6	107	61	5	1	28.8	92	99	2	0.5
LC0015	30.3	100	30	2.5	1	30.2	100	30	2.5	1	30.1	100	30	2.5	1	30.0	100	30	2.5	1
LC0016	7.7	89.7		7.7	5.58						2.4	100.4		13.1	11.78					
LC0017	0	123				0					0	78		80	24	0				
LC0018	55.2	122		10	3	25.0	118		10	3	70.0	119		10	3	25.0	103		10	3
LC0019	17.2	111		7	2.1	17.5	93.3		2.3	0.7	25.7	107.5		0.7	0.2	27.9	99.5		1.6	0.5
LC0020	20.0	95	98	20	2	20.0	74				20.0	82	61	20	2	20.0	82			
LC0021	30.3	94	100	10	3	54.5	94	96	10	3	31.7	88	101	10	3	30.4	113	99	10	3
LC0022	33.6	49		0.05	0.5	19.2	145		0.05	0.5	11.0	37		0.05	0.5	7.9	141		0.05	0.5
LC0023	10.3	77.5		10	1	10.8	100.0		10	1	10.2	100		10	1	10.7	70		10	1
LC0024	50.0	200	58	5	3	50.0	100		5	3	50.0	110		5	3	50.0	96		5	3
LC0025	15.0	101.5	101.6	2.5	0.8	15.0	96.7	101.4	2.5	0.8	15.0	100.8	101.1	2.5	0.8	15.0	98.1	101.4	2.5	0.8
LC0026	0	93	3.51	1	0.4	0	93	5.55	1	0.4	0	85	3.17	1	0.4	0	101	3.56	1	0.4
LC0027	0	81				0	90				0	83				0	94			
LC0028	20.0	123		0.25	0.003	20.0	115		0.125	0.001	20.0	117		0.25	0.003	20.0	129		0.125	0.001
LC0029	32.0	53		10	5	31.9	114		10	5	29.8	52		10	5	35.8	120		10	5
LC0030	25.0	82.7		8.2	2.4	25.0	96.7		11.9	3.6	20.0	80.1		7.9	2.3	30.0	108.9		5.3	1.6
LC0031	55.0	109.8		2	0.5	55.0	88.5		2	0.5	55.0	119.4		2	0.5	55.0	80.5		2	0.5
LC0032	22.0	98.7		10	5	22.0	84.1		20	10	24.0	96.4		10	5	24.0	96.4		20	10
LC0033	25.0	66.4		0.5		25.0	71.3		1		25.0	60.8		1		25.0	77.5		1	
LC0034	25.0	95	96.6	5	1	25.0	95	94.9	5	1	25.0	95	98	5	1	25.0	95	98.8	5	1
LC0035	40.0	51	238	3	1	40.0	100	119	3	1	40.0	100	133	3	1	40.0	100	101	3	1
LC0036	0		89	5		0		85	5		0		110	5		0		86	5	
LC0037	43.8			2.5		44.3			1.3		43.9			2.5		43.9			1.3	
LC0038	60.0	94		15.6	9.3	60.0	127		15.6	9.3	60.0	85		15.6	9.3	60.0	123		15.6	9.3

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