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# Report on the 2015 Proficiency Test of the European Union Reference Laboratory for Mycotoxins for the Network of National Reference Laboratories

*Determination of Citrinin in Red Yeast Rice*

Ádám Tölgyesi
Joerg Stroka

2016

EUR 27753

Joint Research Centre

# Report on the 2015 Proficiency Test of the European Union Reference Laboratory for Mycotoxins for the Network of National Reference Laboratories

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## Acknowledgements

The organizers of the study would like to thank CODA-CERVA and the colleagues involved in the project for their support. The laboratories participating in this exercise, listed in **Table 1**, are also kindly acknowledged.

**Table 1: Participating laboratories**

| Organisation | Country |
|---|---|
| AGES GmbH | Austria |
| CODA-CERVA | Belgium |
| Federal Agency for the Safety of the Food Chain ( FASFC) | Belgium |
| Bulgarian Food Safety Agency | Bulgaria |
| "Dr. Andrija Stampar" Teaching Institute of Public Health | Croatia |
| State General Laboratory | Cyprus |
| Czech Agriculture and Food Inspection Authority | Czech Republic |
| Central Institute for Supervising and Testing in Agriculture (UKZUZ) | Czech Republic |
| DTU Food | Denmark |
| Agricultural Research Centre | Estonia |
| Finnish Customs Laboratory | Finland |
| Laboratoire SCL de Rennes | France |
| Eurofins WEJ Contaminants GmbH | Germany |
| Federal Inst. for Risk Assessment | Germany |
| General Chemical State laboratory | Greece |
| National Food Chain Safety Office, Food And Feed Safety Directorate, Feed Investigation NRL | Hungary |
| National Food Chain Safety Office, Food And Feed Safety Directorate, Food Toxicological NRL | Hungary |
| Public Analyst's Laboratory, Dublin, Ireland | Ireland |
| ISTITUTO ZOOPROFILATTICO SPERIMENTALE LOMBARDIA EMILIA-ROMAGNA (IZSLER) | Italy |
| Italian National Institute of Health (ISS) | Italy |
| Institute of Food Safety, Animal Health and Environment BIOR | Latvia |
| National Food and Veterinary Risk Assessment Institute | Lithuania |
| Laboratoire National de santé | Luxembourg |
| Public Health Laboratory | Malta |
| RIKILT | Netherlands |
| National Veterinary Research Institute | Poland |
| ASAE | Portugal |
| Sanitary Veterinary and Food Safety Directorate Bucharest | Romania |
| UNS FTNS | Serbia |
| State Veterinary And Food Institute, Veterinary and Food Institute in Košice | Slovakia |
| National Laboratory of Health, Environment and Food | Slovenia |
| National Center For Food (Spanish Consumers Food and Nutrition Agency) | Spain |
| National Food Agency | Sweden |
| Fera Science Ltd. | United Kingdom |
| Glasgow Scientific Services | United Kingdom |
| Tayside Scientific Services | United Kingdom |

## Abstract

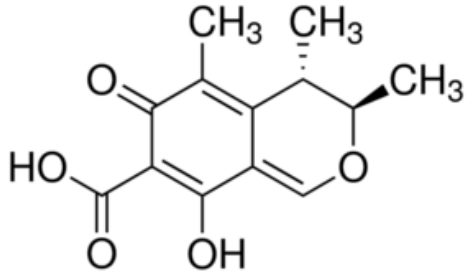
The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the European Union Reference Laboratory (EURL) for Mycotoxins. One of its core tasks is to organise proficiency tests (PTs) among appointed National Reference Laboratories (NRLs). This report presents the results of the PT on the determination of citrinin in red yeast rice samples. The test items were two naturally contaminated samples (Sample A and B). The materials were obtained from CODA-CERVA (Tervuren, Belgium). Samples were processed (milled, homogenized and packed) by the IRMM and dispatched to the participants in July 2015. Each participant received two amber containers filled with approximately 20 g per test material. The analysis of Sample B, which contained a level of citrinin in a region relevant for food law enforcement in the EU, was mandatory, while the determination of citrinin in sample A was optional, as it was intended to assess the measurement capability near the region of the estimated LOQ for many methods in use. Thirty-six participants from twenty-nine countries registered for the exercise and thirty-three sets of results were reported. The assigned values, established by exact-matching double isotope dilution mass spectrometry at the EURL for Mycotoxins, were 13.8 µg/kg (Sample A) and 1142 µg/kg (Sample B) for citrinin. The expanded uncertainties of the respective assigned values were 2.1 and 46 µg/kg. Participants were invited to report the uncertainty of their measurements. This was done by the majority of laboratories. Laboratory results were rated with z-scores and zeta-scores in accordance with ISO 13528:2005 and the IUPAC International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, whereas the zeta-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value. Only z-scores were used for the evaluation of performance. In total 47% of the attributed z-scores were below an absolute value of two, which showed that only half of the participants performed satisfactorily. The evaluation of results suggests a strong correlation between underperformance and the nature and quality of the reference standard used for calibration. Another source for unsatisfactory results may be the amount and composition of extraction solvents used by the participants. However, as the distribution of testing results reported by the participants is skewed to the right (i.e. a significant number of participants overestimated the citrinin content) a calibrant whose nominal concentration deviated from the true value was the most likely cause.

# 1. Introduction

Citrinin is a mycotoxin that occurs mainly in stored grains, fruits, vegetable juices, herbs and spices. It is produced by *Aspergillus*, *Penicillium* and *Monascus* fungi. Citrinin (**Figure 1**) can also be found in *Monascus* fermentation products, described as red mould rice. It is nephrotoxic and considered genotoxic and carcinogenic based on the available data [**1**].

Commission Regulation (EC) No 212/2014 lays down a temporary maximum limit of 2000 µg/kg for citrinin for food supplements based on rice fermented with the red yeast *Monascus purpureus* [**2**]. This maximum level will be reviewed before 1st of January 2016 based on the updated exposure and toxicity data. Rice fermented with *Monascus purpureus* has gained popularity because the yeast produces statins, which have cholesterol lowering abilities.

**Figure 1: Chemical structure of citrinin**



# 2. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [**3**], one of the core duties of the EURL is to organise proficiency tests (PTs) for the benefit of staff of NRLs. The scope of this PT was to test the competence of the appointed NRLs to determine the amount of citrinin in red yeast rice.

The PT design and data processing was in line with the IUPAC International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories [**4**].

The EURL Mycotoxins performed the assessment of the measurement results on the basis of requirements laid down in legislation and followed the administrative and logistic procedures of ISO/IEC 17043:2010 [**5**]. JRC-IRMM is an ISO/IEC 17043:2010 accredited PT provider.

## 2.1 Confidentiality

Confidentiality of the identity of participants and their results towards third parties is guaranteed, with the exception of DG SANTE. Results were reported by laboratories using RingDat software, part of the ProLab software (Quodata, Dresden, DE), which was used for data evaluation. Laboratory specific files with the extension "*.LAB" and "*.LA2", which were generated by the ProLab software, were provided to each laboratory individually (personal files) by email.

## 3. Time frame

The PT was initially announced by e-mail on the 4th of May 2015 and was published on the EURL web page [**6**]. The exercise was opened for registration on 29th of May 2015 (**Annex 2**) and the deadline for registration was 19th of July 2015. The samples were dispatched to the participants on 28th and 29th of July 2015 (**Annex 3**). Reporting deadline was 25th of September 2015.

# 4. Material

## 4.1 Preparation

The test materials used in this study were a blend of naturally contaminated red yeast with blank white rice. The red yeast and the blank rice were obtained from CODA-CERVA (Tervuren, Belgium). The mixture was milled to obtain a particle size of 250 µm using a centrifugal mill (ZM 200, Retsch, Haan, DE) . This material was further homogenised in a tumble mixer and was then packed in amber plastic containers, taking portions from different places of the lot at random; total sample size was ca. 20 g.

## 4.2 Homogeneity

To verify the homogeneity of the test materials 10 units per material (Sample A and Sample B) were selected at random. Two independent determinations per bottle were performed using a liquid chromatography isotope dilution tandem mass spectrometry detection (LC-ID-MS/MS) based method. The order of measurements of the batch was randomised. Homogeneity was evaluated according to ISO 13528:2005 [**7**]. The materials proved to be adequately homogeneous (**Annex 4**).

## 4.3 Stability

The stability study was conducted following an isochronous experimental design [**8**];-70 °C was chosen as reference temperature for sample storage. Stability was evaluated according to the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [**4**]. The materials proved to be adequately stable at 25 °C, 4 °C and -18 °C for the period between dispatch and the deadline for submission of results (**Annex 5**).

## 4.4 Distribution

The test materials were dispatched in polystyrene boxes, containing cooling packs, on 28$^{th}$ and 29$^{th}$ of July 2015. The samples were mostly received within 24 hours after dispatch.

Each participant received:
*a)* two units containing approximately 20 g of each test material,
*b)* an accompanying letter with instructions on sample handling and reporting (**Annex 3**),
*c)* a sample receipt form (**Annex 6**) and
*d)* laboratory specific reporting files with a lab code by email.

The materials were shipped in a way that + 4 °C was not exceeded. Upon arrival storage was required to be at -18 °C until analysis.

## 5. Instructions to participants

The laboratories were asked to report the recovery corrected value as well as their expanded measurement uncertainty in µg/kg (coverage factor k=2).

The concentration range was indicated to the participants (10 – 2000 µg/kg). The analysis of Sample B was mandatory, while the determination of citrinin in Sample A was only optional.

Results were reported by the participants using RingDat software, which is part of the ProLab software. Laboratory specific files generated by the ProLab software were sent to each laboratory individually (personal files) by email. A specific questionnaire was also included. The questionnaire was intended to provide further information on method and laboratory details to allow conclusions on possible individual and common effects observed for possible follow-up procedures. A copy of the questionnaire is in **Annex 7**.

Participants received the information that the materials were shipped in cooling packs and that upon arrival the materials needed to be stored immediately at -18°C until the analysis is performed.

## 6. Reference values and their uncertainties

Assigned values and their uncertainties for the test samples were established by "Exact-Matching Double Isotope Dilution Mass Spectrometry" at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [**9**]. The assigned values were 13.8 µg/kg (Sample A) and 1142 µg/kg (Sample B) for citrinin. The expanded uncertainties (k=2) of the respective assigned values were 2.1 and 46 µg/kg.

# 7. Evaluation of results

## 7.1 General observations

Thirty-six laboratories participated in this PT: NRLs from twenty-eight Member States (two different NRLs for food and feed in two Member States), one expert reference laboratory from a 3rd country and 5 appointed Official Control Laboratories (OCLs) from 4 Member States. Thirty-three sets of results were reported, three laboratories did not send back results.

All laboratories were free to use their method of choice. Two LC-MS/MS methods for citrinin were provided for those laboratories that did not have a method beforehand. These were the method used by the EU-RL and the method of CODA-CERVA (Belgian NRL), which is a candidate method for standardisation.

Only liquid chromatographic techniques were used for the determination of citrinin: high-performance liquid chromatography (HPLC) with mass spectrometric (70%) or fluorescence detection (FLD) (30%).

## 7.2 Scores and evaluation criteria

Individual laboratory performance was assessed in terms of z and zeta (ζ) scores in accordance with ISO 13528:2005 [**7**] and the IUPAC International Harmonised Protocol [**4**].

$$z = \frac{x_{lab} - X_{ref}}{\sigma_p} \qquad \textbf{Equation 1.}$$

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{lab} + u^2_{ref}}} \qquad \textbf{Equation 2.}$$

where:

$x_{lab}$ is the measurement result reported by a participant
$X_{ref}$ is the reference value (assigned value)
$u_{lab}$ is the standard uncertainty reported by a participant
$u_{ref}$ is the standard uncertainty of the reference value
$\sigma_p$ is the standard deviation for proficiency assessment (target standard deviation)

$\sigma_p$ was calculated using the Horwitz equation, modified by Thompson [**10**] for analyte concentrations < 120 µg/kg:

- for analyte concentration < 120 µg/kg *(Sample A)*

$$\sigma_p = 0.22 \cdot c \qquad \textbf{Equation 3.}$$

- for analyte concentration ≥ 120 µg/kg *(Sample B)*

$$\sigma_p = 0.02 \cdot c^{0.8495} \qquad \textbf{Equation 4.}$$

where:
c = concentration of the measurand (assigned value, $X_{ref,}$ ) expressed as a dimensionless mass ratio, e.g. 1 µg/kg = $10^{-9}$, 1 mg/kg = $10^{-6}$

The z-score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, $\sigma_p$. The z-score is interpreted as:

| | |
|---|---|
| $\|z\| \leq 2$ | satisfactory result |
| $2 < \|z\| \leq 3$ | questionable result |
| $\|z\| > 3$ | unsatisfactory result |

The zeta (ζ)-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value. The ζ-score is the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value, its uncertainty as well as the uncertainty of the assigned values.

The interpretation of the zeta-score is similar to the interpretation of the z-score:

| | |
|---|---|
| $\|\zeta\| \leq 2$ | satisfactory result |
| $2 < \|\zeta\| \leq 3$ | questionable result |
| $\|\zeta\| > 3$ | unsatisfactory result |

An unsatisfactory |ζ|-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory |ζ|-score indicated an uncertainty which is not consistent with the laboratory's deviation from the reference value.

## 7.3 Laboratory results and scoring

Statistical evaluation of the results was performed using the ProLab software [**11**]. The calculation of the robust mean and standard deviation were computed according to Algorithm A of ISO 13528:2005. Isotope dilution mass spectrometry (IDMS) was used to establish the assigned values. Both z-scoring and zeta-scoring were calculated for citrinin, however only unsatisfactory z-scores will result in the request for corrective actions.

Summaries of the statistical evaluation for citrinin in the test samples are presented in **Table 2.** The results as reported by the participants are summarised in **Table 3** together with the z-scores and zeta-scores.

**Table 2: Summary statistics for citrinin**

| | | Sample A | Sample B |
|---|---|---|---|
| Number of results | | 29 | 33 |
| Range of results | µg/kg | 9.0-890.0 | 620-3100 |
| Robust mean of results of participants | µg/kg | 31.8 | 1469 |
| Main mode from kernel density analysis | µg/kg | 20.7 | 1264 |
| Assigned value | µg/kg | **13.8** | **1142** |
| Expanded uncertainty (k=2) of the assigned value | µg/kg | 2.1 | 46 |
| Robust standard deviation | µg/kg | 20.0 | 445 |
| Target standard deviation ($\hat{\sigma}$) (fitness for purpose) | µg/kg | 3.0 | 179 |
| Number (percentage) of results of \|z\| > 2.0 | | 19 (63%) | 14 (42%) |
| Number (percentage) of results of \|ζ\| > 2.0 | | 19 (68%) | 15 (52%) |

**Figures 2** and **3** provide the individual laboratory values and their uncertainty as reported. **Figures 4** and **5 (Annex 1)** show the Kernel density plot of the reported values.

**Table 3: Results of analysis, z-scores and zeta-scores for citrinin**
For those laboratories obtained a z-score or zeta-score higher than 4, a value of 4 is tabulated. Lab 8 and lab 13 did not indicate a measurement uncertainty. Lab 2, 14 and 15 did not report results.
(Colour code: green – satisfactory, yellow – questionable, red – unsatisfactory)

| Lab Code | SAMPLE A | | | SAMPLE B | | |
|---|---|---|---|---|---|---|
| | Result | z- | zeta- | Result | z- | zeta- |
| 1 | 14.1 | 0.1 | 0.2 | 1099.7 | -0.2 | -0.3 |
| 2 | *No result* | | | *No result* | | |
| 3 | 12 | -0.6 | -0.8 | 1250 | 0.6 | 0.8 |
| 4 | 890 | 4 | 4 | 1438 | 1.7 | 2 |
| 5 | 56 | 4 | 4 | 620 | -2.9 | -4 |
| 6 | *No result* | | | 1207 | 0.4 | 0.9 |
| 7 | 11.68 | -0.7 | -0.9 | 1162 | 0.1 | 0.1 |
| 8 | 13.3 | -0.2 | | 1310.2 | 0.9 | |
| 9 | 43 | 4 | 4 | 3100 | 4 | 4 |
| 10 | 421.8 | 4 | 3.9 | 1690 | 3.1 | 1.3 |
| 11 | 8.97 | -1.6 | -2.3 | 1036 | -0.6 | -0.8 |
| 12 | 25.4 | 3.8 | 3.5 | 1710 | 3.2 | 2.6 |
| 13 | 31.4 | 4 | | 1790 | 3.6 | |
| 14 | *No result* | | | *No result* | | |
| 15 | *No result* | | | *No result* | | |
| 16 | 29.7 | 4 | 3.5 | 1147.7 | 0 | 0 |
| 17 | 245 | 4 | 4 | 2434 | 4 | 4 |
| 18 | 36.1 | 4 | 4 | 1625 | 2.7 | 2.4 |
| 19 | 50.29 | 4 | 4 | 2130.5 | 4 | 4 |
| 20 | 31 | 4 | 4 | 1507 | 2 | 4 |
| 21 | < 50 | | | 2300 | 4 | 3.3 |
| 22 | 31 | 4 | 3.6 | 2046.3 | 4 | 2.9 |
| 23 | *No result* | | | 1151.7 | 0.1 | 0.1 |
| 24 | 55 | 4 | 4 | 2100 | 4 | 3 |
| 25 | 55.53 | 4 | 4 | 970.85 | -1 | -3.5 |
| 26 | 23 | 3 | 2.5 | 1540 | 2.2 | 2.9 |
| 27 | 14.73 | 0.3 | 0.5 | 1033.55 | -0.6 | -1.6 |
| 28 | 13.04 | -0.2 | -0.6 | 1038 | -0.6 | -1.4 |
| 29 | 22.3 | 2.8 | 4 | 1509 | 2 | 4 |
| 30 | 17.7 | 1.3 | 0.9 | 1827 | 3.8 | 1.7 |
| 31 | 12.1 | -0.6 | -0.8 | 1423 | 1.6 | 1.2 |
| 32 | 31.7 | 4 | 4 | 1099.6 | -0.2 | -0.6 |
| 33 | 15.42 | 0.5 | 0.9 | 1569 | 2.4 | 2.6 |
| 34 | 41.5 | 4 | 4 | 1410.5 | 1.5 | 4 |
| 35 | 38.8 | 4 | 4 | 1245.2 | 0.6 | 1.1 |
| 36 | *No result* | | | 1223 | 0.5 | 0.5 |

The results are written as reported by the laboratories.

**Figure 2: Citrinin in red yeast rice – Sample A**

Certified value: Xref = 13.8 µg/kg; Uref = 2.1 µg/kg (k=2); σ = 3.0 µg/kg

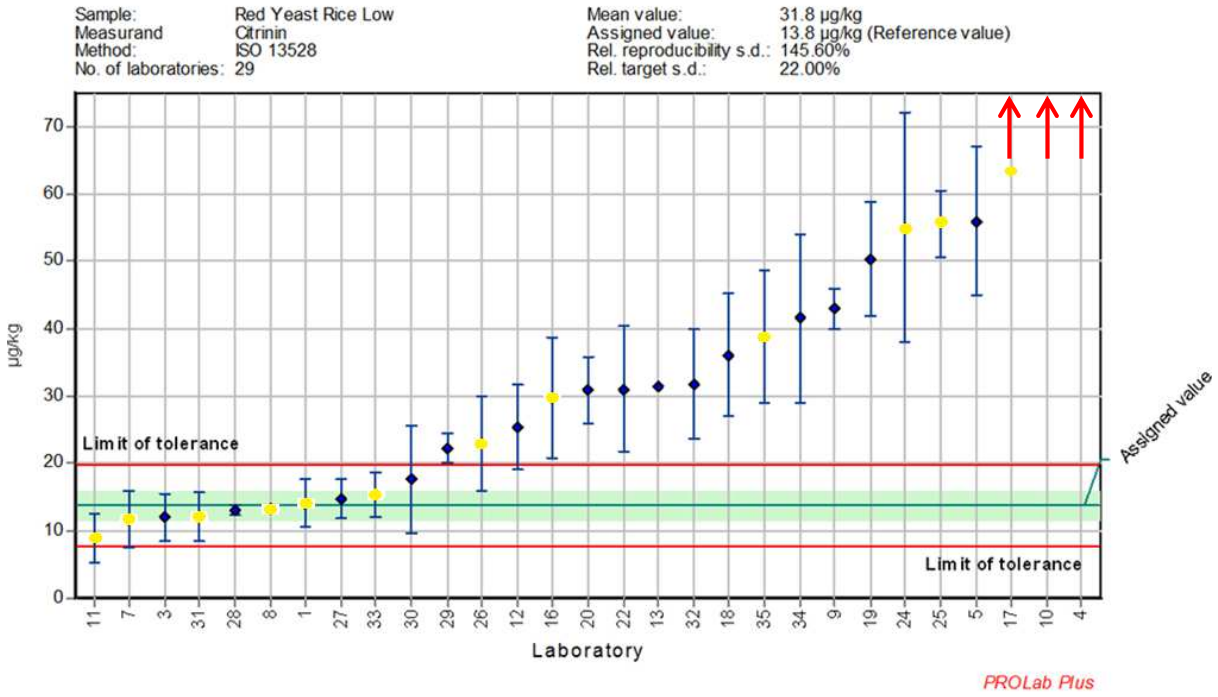
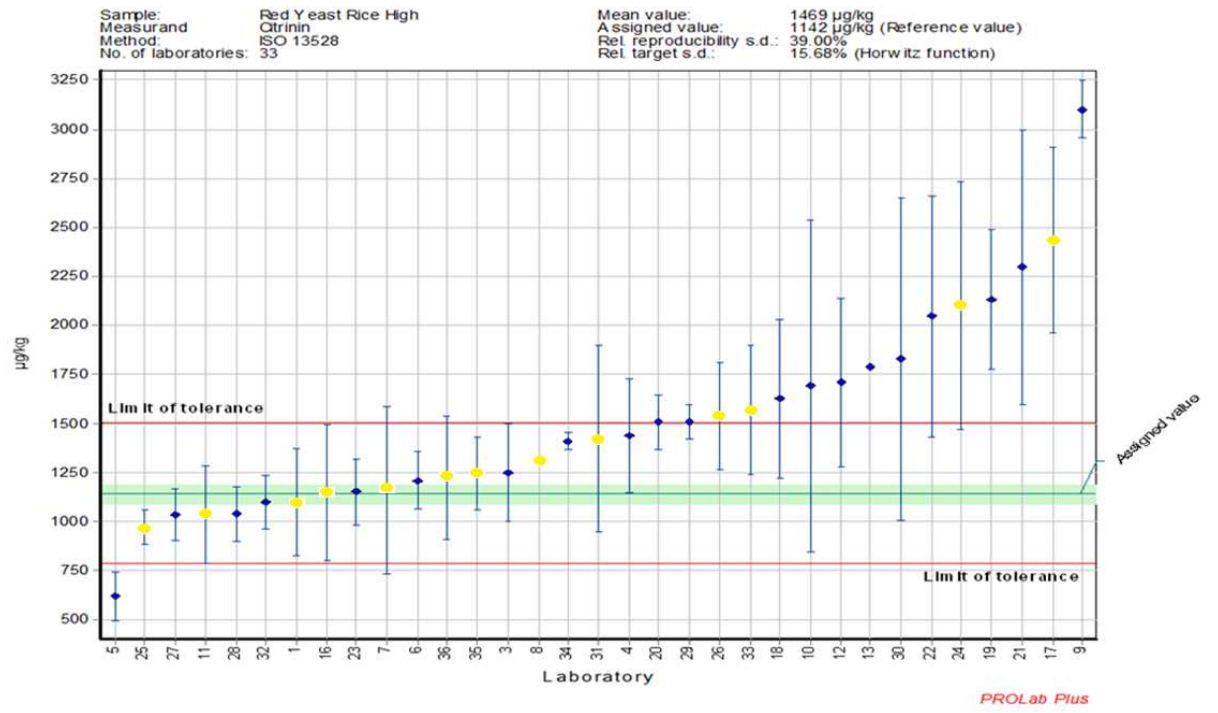



**Figure 3: Citrinin in red yeast rice – Sample B**

Certified value: Xref = 1142 µg/kg; Uref = 46 µg/kg (k=2); σ = 179 µg/kg




This graph displays reported results and their uncertainties. The uncertainties are shown as bars. The green line corresponds to Xref, the green shadow covers the boundary of the reference interval (Xref ± $u_{ref}$), and the red lines mark the boundary of the target interval (Xref ± 2σ). Laboratories highlighted in yellow used the same source of citrinin stock solution as the EURL Mycotoxin used during this study.

## 7.4 Evaluation of questionnaire

The questionnaire along with a summary of the answers from all 33 laboratories that reported results are presented in the **Annex 8.**

Most of the laboratories (70%) used LC-MS, whereas LC-FLD was used in 30% of cases. Eight participants used immunoaffinity columns for clean-up, while other laboratories used either no clean-up or applied only salt induced phase separation.

Most of the laboratories analyse annually less than 50 samples for citrinin and only two laboratories (6%) are accredited for this type of analysis.

The two most commonly recovery estimation methods were the use of an internal standard or spike surrogates to blank rice sample. Details about the applied methodologies – extraction, clean up, overnight stop, etc. - are presented in **Annex 8 and 9**.

Generally, alcohol based extraction was used as recommended in the scientific opinion on citrinin by the European Food Safety Authority (EFSA) [**1**]. Seventeen laboratories (89%) out of nineteen that used an alcoholic extraction, obtained satisfactory z-scores for Sample B. Those laboratories that used acetonitrile or an acetonitrile – water mixture for extraction failed for Sample B.

Thirteen laboratories (39%) used the same source (manufacturer and batch) of standard stock solution as the EURL. Eight (24%) laboratories purchased dry citrinin powder for the preparation of their own stock solution. The remaining laboratories (37%) used different sources of stock standard solution, mainly material from the same supplier, but a different batch than the stock standard used by the EURL.

Sample A contained 13.8 µg/kg citrinin. Ten laboratories obtained satisfactory results for Sample A. Six participants (60%) out of these used the same source of stock solution as the EURL.

Sample B contained 1142 µg/kg citrinin. Nineteen laboratories obtained satisfactory absolute z-scores < 2 for this sample. Forty-seven percent of satisfactory results (9 laboratories) were obtained using the same calibrant as the EURL. In other words, 69% of participants who used the same stock standard solution as the EURL, passed for Sample B (9 laboratories out of 13).

From the laboratories that purchased powdered citrinin to prepare their stock standard only one obtained a satisfactory z-score for sample B. Remarkable is that this laboratory reported to have checked the concentration of their stock standard solution by photometry according to [**12**].

The above discussed facts strongly suggest that alcohol based extraction is superior to those with acetonitrile. Furthermore, the obtained performance is strongly driven by the quality of the calibrant, more than by any other sample manipulation (methodology) used in the laboratory.

Preliminary results of the PT were presented at the annual EURL/NRL workshop in October 2015. Details on the evaluation of various parameters asked in the questionnaire with respect to the z-scoring will be presented at the next EURL/NRL meeting in 2016.

Two participants out of thirty-three did not find the instructions adequate, however did not address details or suggestions. The registration-reporting interface used in this PT received mostly positive feedback.

# 8. Conclusions

Thirty-three sets of results (Sample A and B) were reported for citrinin.

Half of the participants performed satisfactorily. The consensus values (robust mean) and the values assigned by IDMS were in poor agreement for the low level (Sample A). As this level was thought to be rather challenging, as it was close to the assumed LOQ and far from any currently established regulated levels, no follow-up is thought to be necessary.

However, the evaluation of results from sample B, suggests that the quality of available calibrants that can be purchased as well as inadequate extraction solvents are likely causes for underperformance. Especially the influence and the quality of calibration solutions will be addressed in a follow-up action.

## References


[**1**] [EFSA] European Food Safety Authority. 2012. Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. EFSA J. 10(3):2605. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2605.pdf

[**2**] Commission Regulation (EC) No 212/2014 of 6 March 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminant citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus* http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2014:067:0003:0004:EN:PDF

[**3**] Commission Regulation (EC) No 882/2004 of the European Parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0882:20060525:EN:PDF

[**4**] Thompson, M., Ellison, S.L.R., and Wood, R. 2006. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem. 78(1):145–196. http://media.iupac.org/publications/pac/2006/pdf/7801x0145.pdf

[**5**] ISO/IEC 17043:2010 - Conformity assessment -- General requirements for proficiency testing

[**6**] IRMM. Inter-laboratory Comparisons at the Institute for Reference Materials and Measurements.; Available from: http://irmm.jrc.ec.europa.eu/EURLs/EURL_mycotoxins/interlaboratory_comparisons/Pages/index.aspx

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[**8**] Lamberty, A., Schimmel, H., Pauwels, J. 1998. The study of the stability of reference materials by isochronous measurements, Fresenius J. Anal. Chem. 360:359-361. http://link.springer.com/content/pdf/10.1007/s002160050711.pdf

[**9**] Mackay, L.G., et al. 2003. High accuracy analysis by isotope dilution mass spectrometry using an iterative exact matching technique. Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement 8(5):191-194.

[**10**] Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst 125:385-386.

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

[**12**] Molinie, A., et al. 2005. Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B1: development of a method for simultaneous extraction of ochratoxin A and citrinin, Food Chem. 92:391-400.

## List of abbreviations and definitions

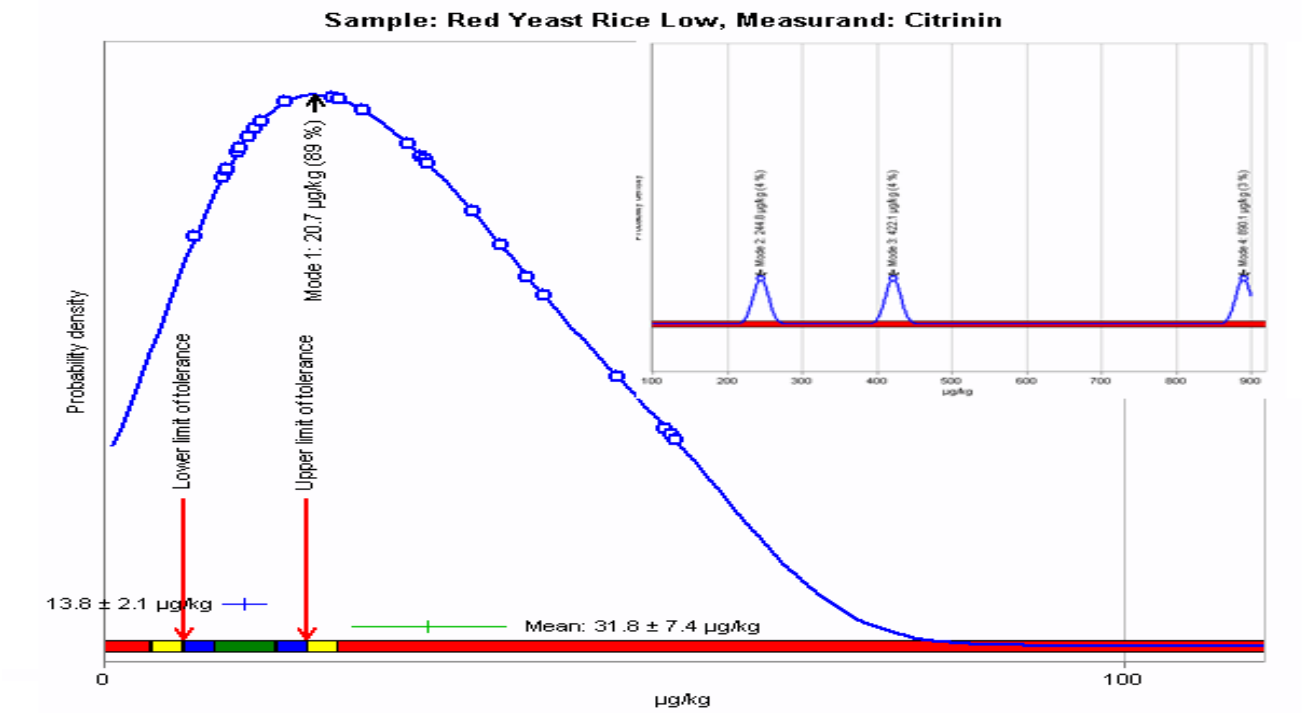
| | |
|---|---|
| EURL | European Reference Laboratory |
| FLD | Fluorescent detection |
| HPLC | High-performance liquid chromatography |
| IAC | Immunoaffinity column |
| IDMS | Isotope Dilution Mass Spectrometry |
| IRMM | Institute for Reference Materials and Measurements |
| ISO | International Organisation for Standardisation |
| IUPAC | International Union for Pure and Applied Chemistry |
| JRC | Joint Research Centre |
| LC-MS | Liquid chromatography mass spectrometry |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| NRL | National Reference Laboratory |
| OCL | Official Control Laboratory |
| PT | Proficiency Test |

# 9. Annexes

## 9.1 Figures

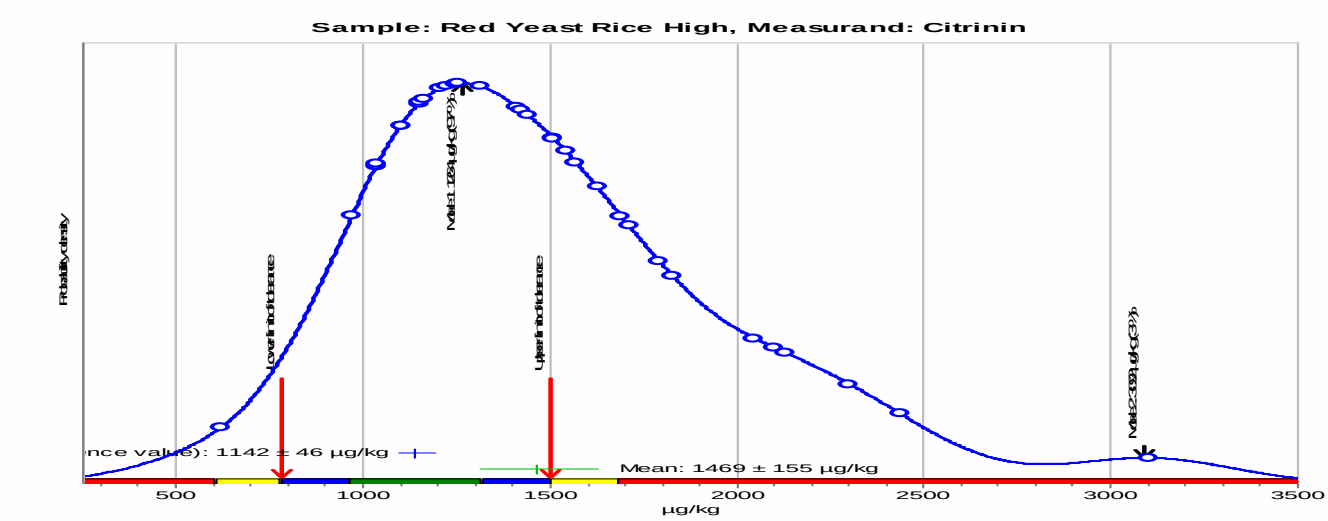
**Figure 4: Citrinin in red yeast rice – Sample A**

Kernel density plot for citrinin content of the red yeast rice.




Kernel density plot of results corrected for recovery. Red arrows show the lower and upper tolerance limits. The blue line between red arrows marks the boundary of the reference interval ($X_{ref} \pm u_{ref}$). The green line indicates the robust mean of results of participants and its uncertainty.

**Figure 5: Citrinin in red yeast rice – Sample B**
Kernel density plot for citrinin content of the red yeast rice.




Kernel density plot of results corrected for recovery. Red arrows show the lower and upper tolerance limits. The blue line between red arrows marks the boundary of the reference interval ($X_{ref} \pm u_{ref}$). The green line indicates the robust mean of results of participants and its uncertainty.

## 9.2 Opening of registration

**TOELGYESI Adam (JRC-GEEL)**

**From:** STROKA Joerg (JRC-GEEL)
**Sent:** 29 May 2015 14:22
**Cc:** TOELGYESI Adam (JRC-GEEL)
**Subject:** NRL annual PT

Dear Colleagues from the NRLs,

On behalf of the EU-RL for Mycotoxins, I have the pleasure to announce the opening for registration of the inter-laboratory comparison for the determination of citrinin in red yeast rice in two test materials both contaminated in the range of 10-2000 µg/kg.
This proficiency test (PT) was initially announced by e-mail on the 29th April 2015. It is obligatory for EU National Reference Laboratories to participate according to Regulation (EC) No 882/2004.

The deadline for registration is 19th June 2015. The samples will be dispatched in July 2015.

In order to register, laboratories must enter the details online by using this link:
https://ec.europa.eu/eusurvey/runner/EURLMYCO2015PTCITRININ

Technical details on the PT design will be communicated upon sample dispatch.

For NRLs the participation is free of charge. The participation fee for official food control laboratories is 270 Euro per participant. The full participation fee is payable upon dispatch of the test samples. Enrolled control laboratories will be contacted for payment details upon registration.

**NOTE for all NRLs**: Please forward this email to those official food control laboratories you wish to participate, so they can register for the PT.

With best regards,

Joerg Stroka and Adam Toelgyesi

Ádám TÖLGYESI, Ph.D.
Post-Doctoral Scientist

**European Commission**
Directorate-General Joint Research Centre
Institute for Reference Materials and Measurements
Standards for Food Bioscience unit
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+32 14 571313
adam.toelgyesi@ec.europa.eu
http://ec.europa.eu/dgs/jrc
http://irmm.jrc.ec.europa.eu

# 9.3 Accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

Ref. Ares(2015)3162485 - 28/07/2015

Geel, 28 July 2015

**2015 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on the determination of citrinin in red yeast rice**

Dear Participant,

Please read the following information carefully before starting any analysis. If there are additional questions, do not hesitate to contact us either by phone or e-mail (see details below).

The 2015 EURL PT aims to assess the citrinin content in two contaminated test samples (marked as "Sample A", "Sample B"). You will be asked to report the recovery corrected value (µg/kg), together with your measurement uncertainty (µg/kg) for a coverage factor of 2 (k=2).

Based on the current maximum level of citrinin (2000 µg/kg) the citrinin content of **sample B** is relevant, therefore the analysis of **sample B is mandatory**. **Sample A** contains lower concentration level of citrinin and its analysis is **optional**. For those laboratories that plan to participate in citrinin CEN method validation study sample A can be used as a pre-trial sample.

Please confirm the parcel's receipt by e-mail immediately by using the **"Materials Receipt Form"**. If any material is damaged, please request new material immediately.

The materials are shipped cooled. After receipt take the samples immediately at -18°C until the analysis is performed.

**Reporting the results**
Data generated by the participants will be collected by using software RingDat, supplementary to ProLab software, used until now for professional data handling and statistical analyses of interlaboratory tests results.
You will receive by email some files for reporting results. You should follow the following instructions:

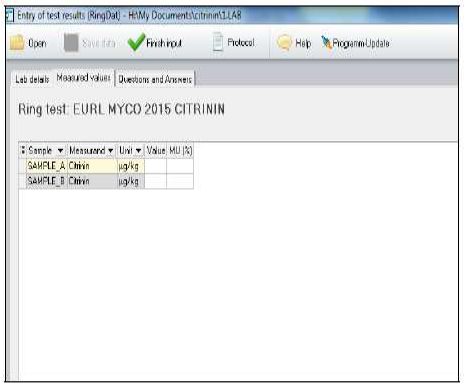
1. Download a simple data entry program RingDat free from the QuoData web page using following link: http://quodata.de/ringdat_en.php
User: *ringdat*
Password: *prolabdata*

2. Save to the same folder the two lab specific files with the extension **"*.Lab"** and **"*.LA2"**, generated by the ProLab software and provided to each laboratory individually (personal files) by this mail.

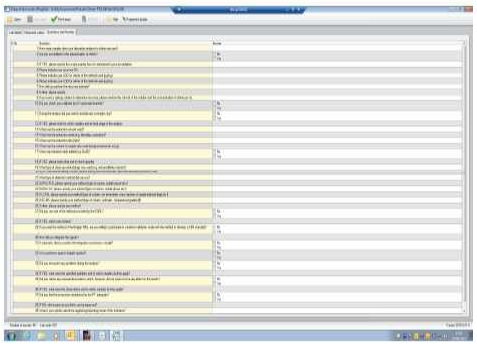
3. Start the RingDat.exe program and open **"*.LAB"** file for reporting the results. A table will appear with cells for every measurand/sample combination



- the name of each laboratory and the samples are codified by the software, so that each participant will receive samples with unique codified numbers (i.e., 058);
- The **"*.LA2"** file contains information about the participant – laboratory name and laboratory code;
- The **"*.LAB"** file is unique to each laboratory (personal) and contains information about the samples and measurand that have to be analysed and reported.
- First tab contains the detailed information for the laboratory
- Second tab contains table for entering the results.




- Third tab contains a general questionnaire.





4. Fill in the result table with your data. On the pictures above, minimum required field to be filled are shown. Please report only **ONE** value per sample together with method uncertainty and recovery rate.

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

6. After finishing the input, save the file using the button on the top menu of the window. You could 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 pushing the "Finish input" button.

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

8. If you want to **correct some of your entries after finishing the input, you should use the original *.LAB file** downloaded from the mail.

Deadline for reporting will be the 25th September 2015.

In case of questions, please do not hesitate to contact us.

Tel: +32-14-571313
FAX: +32-14-571 783
E-mail: JRC-IRMM-EURL-MYCOTOX@ec.europa.eu

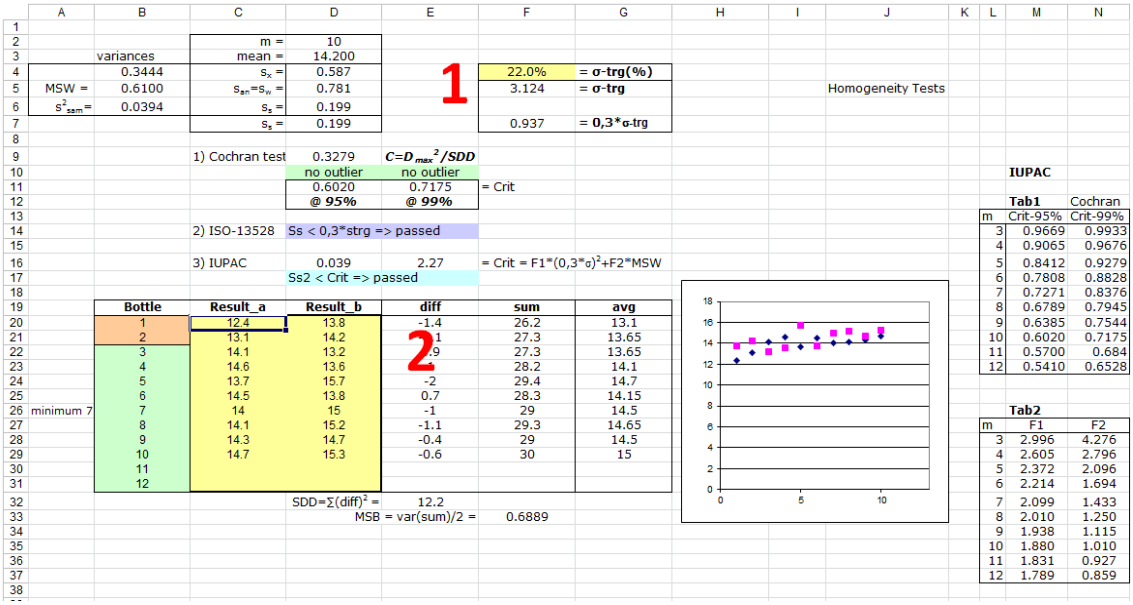
With kind regards,

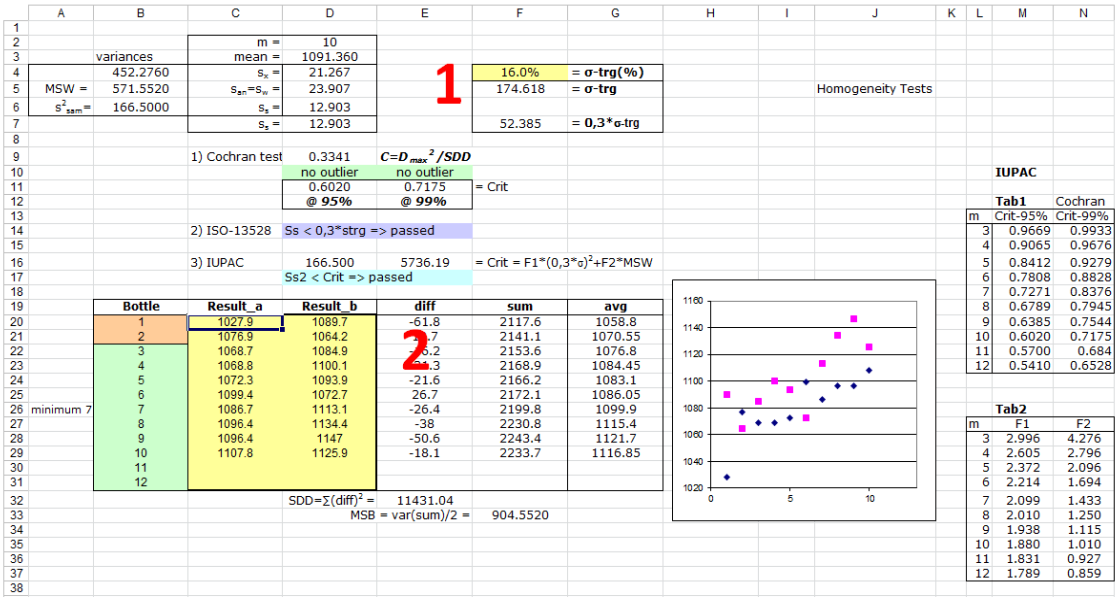
Ádám Tölgyesi
(on behalf of the Operating Manager of the EU-RL Mycotoxins)

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

## 9.4 Homogeneity test

| Homogeneity according to ISO 13528:2005 [7] | Sample A | Sample B |
|---|---|---|
| | Citrinin | Citrinin |
| Mean | 14.2 | 1091 |
| $\hat{\sigma}$ | 3.124 (22%) | 175 (16%) |
| 0.3 $\hat{\sigma}$ (critical value) | 0.937 | 52 |
| $S_X$ (standard deviation of sample averages) | 0.587 | 21 |
| $S_W$ (within-sample standard deviation) | 0.781 | 24 |
| $S_S$ (between-sample standard deviation) | 0.199 | 13 |
| **$S_S$ < 0.3 $\hat{\sigma}$** | **Passed** | **Passed** |



## 9.5 Stability study

**Stability study – Sample A**

| Date | Time | - 70 °C (Reference) | | - 18 °C | | + 4 °C | | + 25 °C | |
|---|---|---|---|---|---|---|---|---|---|
| 30/07/2015 | 3 days | | | | | 15.4 | 14.5 | 17.2 | 16.6 |
| 24/08/2015 | 4 weeks | | | | | 15.0 | 14.1 | 17.0 | 16.6 |
| 21/09/2018 | 8 weeks | 13.5 | 13.1 | 13.6 | 13.5 | 15.2 | 14.3 | 17.2 | 17.0 |
| **Slope of linear regression significantly <> 0 (95 %)** | | | | **No** | | **No** | | **No** | |

**Stability study – Sample B**

| Date | Time | - 70 °C (Reference) | | - 18 °C | | + 4 °C | | t stat | + 25 °C | |
|---|---|---|---|---|---|---|---|---|---|---|
| 30/07/2015 | 3 days | | | | | 1165 | 1170 | 0.6 | 1009 | 1067 |
| 24/08/2015 | 4 weeks | | | | | 1163 | 1171 | 0.59 | 1004 | 1034 |
| 21/09/2018 | 8 weeks | 1190 | 1164 | 1189 | 1218 | 1145 | 1151 | 1.81 | 1045 | 1072 |
| **Slope of linear regression significantly <> 0 (95 %)** | | | | **No** | | **Yes** | | **t stat < t crit two-tail** | **No** | |

## 9.6 Acknowledgement of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 28 July 2015

### PROFICIENCY TESTING MATERIALS RECEIPT FORM

| |
|---|
| **Name:** |
| **Institute:** |
| **Address:** |
| **Member State:** |

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

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

| | |
|---|---|
| Date of receipt | |
| Samples' numbers | |
| All items have been received undamaged | YES / NO |
| *If NO, please list damaged items:* | |

**Contents of the parcel:**

a) A bag including 2 test materials for analysis:
- Sample A
- Sample B

b) A bag containing the following documents:
- This materials receipt form
- Copy of instructions

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

Ádám TÖLGYESI

E-mail: JRC-IRMM-CRL-MYCOTOX@ec.europa.eu

**Your Signature / Stamp here:**

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

## 9.7 Questionnaire

| No. | Cue | Question | Answers |
|---|---|---|---|
| - | Ring test : EURL MYCO 2015 CITRININ (42 questions, 829 answers) | | |
| 1 | Number of samples per year | How many samples does your laboratory analyse for citrinin per year? | 28 Answers |
| 2 | Accreditation | Are you accredited for the determination of citrinin? | 33 Answers |
| 3 | Scope of the accreditation | IF YES, please specify the scope exactly how it is mentioned in your accreditation | 2 Answers |
| 4 | Red yeast rice classification | As what type of product is red yeast rice classified in your country (food, pharmaceutical..). Please specify | 26 Answers |
| 5 | Recovery | Please indicate your recovery (%) | 31 Answers |
| 6 | LOD | Please indicate your LOD for citrinin of the method used (µg/kg) | 27 Answers |
| 7 | LOQ | Please indicate your LOQ for citrinin of the method used (µg/kg) | 28 Answers |
| 8 | Recovery estimation | How did you perform the recovery estimate? | 31 Answers |
| 9 | Other recovery estimation | If other, please specify | 21 Answers |
| 10 | Spiking solution details | If you used a spiking solution to determine recovery, please mention the solvent of the solution and the concentration of citrinin per mL | 26 Answers |
| 11 | Calibrant check | Do you check your calibrant to verify the nominal concentration? | 32 Answers |
| 12 | If YES, describe | If YES, please describe how | 5 Answers |
| 13 | Overnight stop | During the analysis did you need to include any overnight stop? | 33 Answers |
| 14 | IF YES, describe | IF YES, please state for which samples and at what stage of the analysis | 2 Answers |
| 15 | Extraction solvent | What was the extraction solvent used? | 33 Answers |
| 16 | Extraction mode | What was the extraction mode (e.g. blending, sonication)? | 32 Answers |
| 17 | Extraction time | What was the extraction time (min)? | 33 Answers |
| 18 | Solvent to sample ratio | What was the solvent to sample ratio used during extraction (in mL/g) | 30 Answers |
| 19 | Used method given by the EURL | Did you use any of the methods provided by the EURL? | 33 Answers |
| 20 | If YES, which? | If YES, which one of them? | 12 Answers |
| 21 | Participation in a MVS | If you used the method of the Belgian NRL, are you willing to participate in a method validation study with this method to develop a CEN standard? | 18 Answers |
| 22 | Deviation from the method | If you deviated from the method used, please give a description | 10 Answers |
| 23 | If other methods, describe | If you didn't use these methods please reply to questions 24-31 | |
| 24 | Clean-up methodology | What type of clean-up methodology was used (e.g. immunoaffinity column)? | 18 Answers |
| 25 | Immunoaffnity column | If you used immunoaffinity column, please specify the manufacturer and product code (if available) | 9 Answers |
| 26 | Detection method | What type of detection method did you use? | 21 Answers |
| 27 | If HPLC-FLD, describe | If HPLC-FLD, please specify your method (type of column, mobile phase, detector settings, etc.) | 10 Answers |
| 28 | If HPLC-UV, describe | If HPLC-UV, please specify your method (type of column, mobile phase, detector settings, etc.) | 1 Answer |
| 29 | If LC-MS, describe | If LC-MS, please specify your method (type of column, ion information, mass fraction of sample injected [mg] etc.) | 13 Answers |
| 30 | If GC-MS, describe | If GC-MS, please specify your method (type of column, split ratio, temperature [gradient]) | |
| 31 | If other, specify | If other, please specify your method | |
| 32 | Integration method | How did you integrate the signals? | 29 Answers |
| 33 | Confirmation of integration | If automatic, did you confirm the integration correctness visually? | 27 Answers |
| 34 | Protection against daylight | Was protection against daylight applied? | 32 Answers |
| 35 | Any problems? | Did you encounter any problems during the analysis? | 33 Answers |
| 36 | IF YES, describe the problem | IF YES, what were the specified problems and to which samples do they apply? | 5 Answers |
| 37 | Unusual observations | Did you notice any unusual observations which, however, did not seem to have any effect on the results? | 33 Answers |
| 38 | IF YES, describe | IF YES, what were the observations and to which samples do they apply? | |
| 39 | Instrucitons adequate? | Did you find the instructions distributed for this PT adequate? | 33 Answers |
| 40 | IF NO, describe | IF NO, which parts do you think can be improved? | |
| 41 | Opinion about interface | What is your opinion about the registering/reporting format of this interface? | 26 Answers |
| 42 | Any other comments | Any other comments you wish to address? | 13 Answers |

## 9.8 Experimental details

**Results and method performance characteristics for citrinin**

| Lab Code | Technique | Sample A | | Sample B | | Coverage factor | Recovery [%] | LOD [µg/kg] | LOQ [µg/kg] |
|---|---|---|---|---|---|---|---|---|---|
| | | Result [µg/kg] | Uncertainty [µg/kg] | Result [µg/kg] | Uncertainty [µg/kg] | | | | |
| 1 | LC-MS | 14.1 | 3.5 | 1099.7 | 274.9 | 2 | 100 | 2 | 10 |
| 3 | LC-MS | 12 | 3.6 | 1250 | 250 | 2 | 85 | 1 | 5 |
| 4 | HPLC-FLD | 890 | 178 | 1438 | 288 | 2 | 95 | | |
| 5 | LC-MS | 56 | 11 | 620 | 124 | 2 | 50 | 7 | 20 |
| 6 | HPLC-FLD | | | 1207 | 145 | 2 | 78 | 75 | 100 |
| 7 | LC-MS | 11.68 | 4.29 | 1162 | 427 | 2 | 88 | 1 | 3 |
| 8 | LC-MS | 13.3 | | 1310.2 | | | 95 | | 2.5 |
| 9 | LC-MS | 43 | 3 | 3100 | 145 | 2 | 83 | 5 | 10 |
| 10 | LC-MS | 421.8 | 210.9 | 1690 | 845 | 2 | 95 | 5 | 20 |
| 11 | LC-MS | 8.97 | 3.64 | 1036 | 248 | 2 | 95.5 | 0.6 | 1.2 |
| 12 | HPLC-FLD | 25.4 | 6.4 | 1710 | 430 | 2 | 78.24 | 5 | 10 |
| 13 | HPLC-FLD | 31.4 | | 1790 | | | | 10 | 20 |
| 16 | LC-MS | 29.7 | 8.9 | 1147.7 | 344.3 | 2 | 86 | 7.5 | 25 |
| 17 | LC-MS | 245 | 68 | 2434 | 476 | 2 | 74 | 1.5 | 5 |
| 18 | LC-MS | 36.1 | 9.1 | 1625 | 406 | 2 | 103 | 10 | 25 |
| 19 | LC-MS | 50.29 | 8.38 | 2130.5 | 355.2 | 2 | 101.67 | 0.5 | 2.5 |
| 20 | LC-MS | 31 | 5.58 | 1507 | 136 | 2 | 80 | | |
| 21 | HPLC-FLD | < 50 | | 2300 | 700 | 2 | 75 | 200 | 600 |
| 22 | LC-MS | 31 | 9.3 | 2046.3 | 613.9 | 2 | 94 | | 2.5 |
| 23 | LC-MS | | 0 | 1151.7 | 168.3 | 2 | 65.7 | 40 | 100 |
| 24 | LC-MS | 55 | 17 | 2100 | 630 | 2 | 93 | 12 | 34 |
| 25 | LC-MS | 55.53 | 4.88 | 970.85 | 85.24 | 2 | 71.17 | 1.54 | 5.13 |
| 26 | HPLC-FLD | 23 | 7 | 1540 | 274 | 2 | 97 | 7 | 20 |
| 27 | HPLC-FLD | 14.73 | 2.95 | 1033.55 | 131.3 | 2 | 65.3 | 15 | |
| 28 | LC-MS | 13.04 | 0.79 | 1038 | 137 | 2 | 86 | 0.5 | 1 |
| 29 | HPLC-FLD | 22.3 | 2.1 | 1509 | 86 | 2 | 90 | 4.2 | 12.6 |
| 30 | LC-MS | 17.7 | 8 | 1827 | 822 | 2 | 68.1 | 4 | 7 |
| 31 | LC-MS | 12.1 | 3.6 | 1423 | 478 | 2 | 87 | | |
| 32 | LC-MS | 31.7 | 8.1 | 1099.6 | 136.4 | 2 | 82 | | |
| 33 | HPLC-FLD | 15.42 | 3.23 | 1569 | 329 | 2 | 88 | 2 | 6 |
| 34 | LC-MS | 41.5 | 12.5 | 1410.5 | 42.3 | 2 | 90 | 5 | 10 |
| 35 | LC-MS | 38.8 | 9.8 | 1245.2 | 186.9 | 2 | | 12 | 36 |
| 36 | HPLC-FLD | | | 1223 | 313 | 2 | 102 | 90 | 150 |

The results are written as reported by the laboratories.

## 9.9 Questionnaire

**How many samples does your laboratory analyse for citrinin per year?**
**Are you accredited for the determination of citrinin?**
**Please specify the scope exactly how it is mentioned in your accreditation.**
**As what type of product is red yeast rice classified in your country (food, pharmaceutical..). Please specify.**

| Lab Code | Samples annually | Accredited? | Scope of accreditation | How is red yeast rice classified? |
|---|---|---|---|---|
| 1 | < 50 samples per year | No | | |
| 3 | < 50 samples per year | No | | |
| 4 | | No | | Food supplement |
| 5 | < 50 samples per year | Yes | Cereals | Food |
| 6 | < 50 samples per year | No | | Food supplement sold in health shops |
| 7 | < 50 samples per year | No | | Food supplement |
| 8 | | | | Food |
| 9 | | No | | Food supplement |
| 10 | < 50 samples per year | No | | Food, supplementary food |
| 11 | < 50 samples per year | No | | Pharmaceutical |
| 12 | < 50 samples per year | No | | Not sure about this |
| 13 | < 50 samples per year | No | | Unknown |
| 16 | < 50 samples per year | No | | Food |
| 17 | < 50 samples per year | No | | Food |
| 18 | < 50 samples per year | No | | |
| 19 | 50-250 samples per year | No | | |
| 20 | < 50 samples per year | No | | |
| 21 | < 50 samples per year | No | | Dietary supplement |
| 22 | 50-250 samples per year | No | | Food (supplement) |
| 23 | < 50 samples per year | No | | Food |
| 24 | < 50 samples per year | No | | In general as food, also possible as pharmaceutical depending on the content of monacolin-K. |
| 25 | < 50 samples per year | No | | Food |
| 26 | < 50 samples per year | No | | |
| 27 | < 50 samples | Yes | Citrinin in | Food |

| Lab Code | Samples annually | Accredited? | Scope of accreditation | How is red yeast rice classified? |
|---|---|---|---|---|
| | per year | | cereals and feed by ELISA | |
| 28 | < 50 samples per year | No | | |
| 29 | < 50 samples per year | No | | Food additive, not approved |
| 30 | < 50 samples per year | No | | Food |
| 31 | | No | | |
| 32 | | No | | Food |
| 33 | < 50 samples per year | No | | Food |
| 34 | < 50 samples per year | No | | Food |
| 35 | < 50 samples per year | No | | Food |
| 36 | < 50 samples per year | No | | Food |

**Please indicate your recovery (%)**
**How did you perform the recovery estimate, please specify?**
**Please indicate your LOD for citrinin of the method used (µg/kg)**
**Please indicate your LOQ for citrinin of the method used (µg/kg)**

| Lab Code | Recovery% | Recovery estimation | LOD (µg/kg) | LOQ (µg/kg) |
|---|---|---|---|---|
| 1 | 100 | Internal Standard to Extract. Matrix matched calibration | 2 | 10 |
| 3 | 85 | Internal Standard to Extract | 1 | 5 |
| 4 | 95 | Standard addition | | |
| 5 | 50 | Using a CRM | 7 | 20 |
| 6 | 78 | Internal Standard to Sample | 75 | 100 |
| 7 | 88 | Isotope dilution. Also spiked with citrinin at 1000 µg/kg | 1 | 3 |
| 8 | 95 | Spiked sample | | 2.5 |
| 9 | 83 | Spiking solution | 5 | 10 |
| 10 | 95 | Internal Standard to Sample | 5 | 20 |
| 11 | 95.5 | Citrinin standard to sample A | 0.6 | 1.2 |
| 12 | 78.24 | Internal Standard to Sample | 5 | 10 |
| 13 | | | 10 | 20 |
| 16 | 86 | Internal Standard to Sample | 7.5 | 25 |
| 17 | 74 | Spiked sample of rice | 1.5 | 5 |
| 18 | 103 | Matrix matched calibration | 10 | 25 |
| 19 | 101.67 | Spiking and isotope addition to extract | 0.5 | 2.5 |
| 20 | 80 | | | |
| 21 | 75 | Samples are spiked before the extraction | 200 | 600 |
| 22 | 94 | Based on the validation data | | 2.5 |
| 23 | 65.7 | Absolute method recovery (with isotopic IS) | 40 | 100 |
| 24 | 93 | Internal Standard to Sample | 12 | 34 |
| 25 | 71.17 | Spiking of blank sample | 1.54 | 5.13 |
| 26 | 97 | Spike a clear sample | 7 | 20 |
| 27 | 65.3 | Standard addition to blank sample | 15 | |
| 28 | 86 | Internal Standard to Extract | 0.5 | 1 |
| 29 | 90 | Addition of 15 µL spiking solution of Citrinin to 1 g of sample | 4.2 | 12.6 |
| 30 | 68.1 | Standard addition to Sample A | 4 | 7 |
| 31 | 87 | Spiked samples | | |
| 32 | 82 | Spiking | | |
| 33 | 88 | Spiking to sample | 2 | 6 |
| 34 | 90 | Internal Standard to Sample | 5 | 10 |
| 35 | | Isotope dilution | 12 | 36 |
| 36 | 102 | Spiking procedure | 90 | 150 |

**What was the type of the calibrant that you purchased?**
**If you used a spiking solution to determine recovery, please mention the solvent of the solution and the concentration of citrinin per mL.**
**Do you check your calibrant to verify the nominal concentration?**
**If YES, please describe how?**

| Lab Code | Calibrant type | Spiking solvent and concentration (Citrinin per mL) | Calibrant check? | How? |
|---|---|---|---|---|
| 1 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | | No | |
| 3 | 100.1 µg/mL in acetonitrile | | No | |
| 4 | | | No | |
| 5 | Powder | | Yes | Comparison of two diluted stock solutions |
| 6 | | 7.74µg/ml | Yes | Measured absorption curve of calibrant with spectrophotometer using methanol as solvent |
| 7 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Citrinin at 101.9µg/ml in acetonitrile and 13C citrinin at 1µg/ml in acetontrile | No | |
| 8 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Citrinin 1 µg/ml MeOH/H2O/acetic acid | | |
| 9 | Powder | Two levels (100 ug/kg in methanol/water (80:20) at 2% acetic acid and 2000 ug/kg) in | No | |
| 10 | Powder | ACN, 1000 ng/mL | No | |
| 11 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Methanol, 10000ng/ml and 100 | No | |
| 12 | 100.1 µg/mL in acetonitrile | acetonitrile, 100 µg/ml | No | |
| 13 | Powder | | No | |
| 16 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | 1 ug/ml | No | |
| 17 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Acetonitrile, 3ug/mL | No | |
| 18 | 100.2 µg/mL in acetonitrile | | No | |
| 19 | 100.1 µg/mL in acetonitrile | 0.1 ml Citrinin 1000ug/l in Dilution Solution(Methanol/H2O/ AcOH (80/18/2, v/v/v) | No | |
| 20 | | | Yes | |
| 21 | Powder | 2.5 µg citrinin per ml in methanol | Yes | Measurement of absorbance at 325 nm epsilon: 4700 in methanol |
| 22 | Powder | Check extraction recovery per sequence on blanc RYR-FS sample: 80 micoL of a 1 ppm solution of CIT in | No | |

| Lab Code | Calibrant type | Spiking solvent and concentration (Citrinin per mL) | Calibrant check? | How? |
|---|---|---|---|---|
| | | MeOH/H2O/AcOH (80:18:2, v/v/v) | | |
| 23 | 100 µg/mL in acetonitrile | water/acetonitrile 90/10 0.1% formic acid | No | |
| 24 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | 4000 ng/ml (MeOH-water-AceticAcid) | No | |
| 25 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | 10 µg/ml in acetonitrile | No | |
| 26 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Acetonitril 10 ug/ml | Yes | Check with independent standard preparation |
| 27 | 102.1 µg/mL in acetonitrile | 100 ppm (ug/ml) in ACN | No | |
| 28 | | MeOH | No | |
| 29 | 100.1 µg/mL in acetonitrile | 0.1 g/L in acetonitrile | No | |
| 30 | Powder | 25.75 µg/ml acetonitrile | No | |
| 31 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | MeOH, 100ìg/ml | No | |
| 32 | Powder | Methanol; 19,7 µg/ml | Yes | Food Chemistry 92, 2005, 391 - 400 |
| 33 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Methanol, 0.05 ug/ml | No | |
| 34 | 100.1 µg/mL in acetonitrile | 70%MeOH; 0.01ug/ml | No | |
| 35 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Solvent methanol/water 70/30, 100-200-300-400-500-600 ug/ml | No | |
| 36 | Same as the EURL purchased (101.9 µg/mL in acetonitrile) | Citrinin 101,9 ug/ml solvent acetonitrile | No | |

**What was the extraction solvent used?**
**What was the extraction mode (e.g. blending, sonication)?**
**What was the extraction time (min)?**
**What was the solvent to sample ratio used during extraction (in mL/g)?**
**During the analysis did you need to include any overnight stop?**
**IF YES, please state for which samples and at what stage of the analysis**

| Lab Code | Extraction solvent | Extraction mode | Extraction time | Solvent to sample ratio | Overnight stop | Which sample/stage of analysis |
|---|---|---|---|---|---|---|
| 1 | MeOH (70%) : H2O (30%) | Shaking | 60 | 5 | No | |
| 3 | MEOH:WATHER 70:30 | Sonication and blending | 60 | 2/50 | No | |
| 4 | methanol-water | Shaking | 45 | | No | |
| 5 | Acetontitril, water | Shaking | 30 | | No | |
| 6 | 70% Ethanol | Shaking - sonication - shaking | 120 | 60 | No | |
| 7 | Methanol : water, 75 :25 | Shaking | 30 | 25 ml / 2.5 g (x10) | No | |
| 8 | Toluene/acetonitrile/acetic acid and ethyl acetate/acetonitrile/acetic acid | Blending | 60 | 5ml/g | No | |
| 9 | Acetonitrile 1% acetic acid | Head-over-head shaking | 60 | 5/1 | No | |
| 10 | 0,1% HCOOH in waters + ACN | Shaking | 20 | 5 | No | |
| 11 | Methanol | Blending | 50 | 6/1 | No | |
| 12 | MeOH/H3PO4 0,1% (70/30 v/v) | Blending + centrifugation | 3 | 100 ml/20g | No | |
| 13 | 75% Methanol | Blending | 2 | 100/20 | No | |
| 16 | ethyl acetate/acetonitrile/acetic acid 75:24:1 | Blending | 60 | 20ml solvent to 4g sample | No | |
| 17 | Acetonitril/Water/Formic acid | Shaking | 30 | 10/1 | No | |

| Lab Code | Extraction solvent | Extraction mode | Extraction time | Solvent to sample ratio | Overnight stop | Which sample/stage of analysis |
|---|---|---|---|---|---|---|
| 18 | Acetonitrile | Mixing | 20 | 10 | No | |
| 19 | Aqueous Solution (AcOH/H2O (1:99, v/v) + 10% (m/v) NaCl + 1.6 % HCl and Extraction solution: EtAc/ACN/AcOH (75:24:1 v/v/v) | Orbital Shaker | 60 | | No | |
| 20 | methanol: Water | Blending | 45 | | No | |
| 21 | methanol | Vortex and heating at 70°C | 30 | 20 | No | |
| 22 | 1.6% HCl in 10% (m/v) NaCl H20/AcOH (99:1, v/v) and EtAc/ACN/AcOH (75:24:1, v/v/v) | Overhead shaking | 60 | 30/4 | No | |
| 23 | methanol/water 70/30% | vortex + shaking on orbital shaker | 45 | 6 | Yes | After extraction and before injection into UPLC |
| 24 | Ethylacetate/Acetonitrile /AceticAcid | Vortex and shaking | 45 | 5 | Yes | For both samples, before LC-MS/MS |
| 25 | Methanol | Sonication at elevated temperature (70 C) | 30 | 10 | No | |
| 26 | MeOH: Water (70:30) | Shake at 65ºC | 15 | 20/1 | No | |
| 27 | 70:30 (MeOH:H2O) | Shaking | 20 | in 25 mL/5g | No | |
| 28 | Methanol - water (70/30, v/v) mixture | Vortex-mix it for 10 s to obtain a homogeneous suspension, and then shake it for | 45 | 12:2 | No | |

| Lab Code | Extraction solvent | Extraction mode | Extraction time | Solvent to sample ratio | Overnight stop | Which sample/stage of analysis |
|---|---|---|---|---|---|---|
| | | 45 min at room temperature at 600 1/min speed using a hand shaker | | | | |
| 29 | methanol/water 70/30 v/v | Shaking at 65°C | 10 | 20 | No | |
| 30 | 70 % methanol | Shaking | 45 | 6 | No | |
| 31 | 70:30 MeOH:H2O | Shaking | 45 | 6 | No | |
| 32 | Methanl - Water (70/30, v/v) | Blending | 45 | 6 | No | |
| 33 | methanol/water = 7/3 | Sonication with heating 65°C and shaking | 60 | 10 mL/g | No | |
| 34 | 70%MeOH | Sonication | 40 | 2g/15ml | No | |
| 35 | methanol/water 70/30 | Shaking | 45 | 6 | No | |
| 36 | Methanol 100% | Blending, sonication | 75 | 25/1 | No | |

**Did you use any of the methods provided by the EURL?**
**If YES, which one of them?**
**If you used the method of the Belgian NRL, are you willing to participate in a method validation study with this method to develop a CEN standard?**
**If you deviated from the method used, please give a description.**

| Lab Code | Provided method | Which method | Deviations from the method | Participation in validation |
|---|---|---|---|---|
| 1 | Yes | EURL method | | No |
| 3 | Yes | EURL method | solvent to sample ratio | |
| 4 | No | | | Yes |
| 5 | No | | | |
| 6 | No | | | |
| 7 | No | | | |
| 8 | Yes | Belgian NRL method | I don't understand if the solvent extraction of the method was toluene or ethyl acetate, and I used both with similar results. The principle of method told about toluene but the solutions point told about EtAc. | No |
| 9 | No | | Extraction solvent, use of 13C13-citrinin to correct for matrix suppression in LC-MSMS apparatus | Yes |
| 10 | No | | QuEChERS | |
| 11 | No | | | Yes |
| 12 | No | | | No |
| 13 | No | | | |
| 16 | Yes | Belgian NRL method | we didn't use internal standard citrinin 13C | No |
| 17 | No | | | No |
| 18 | Yes | EURL method | extraction with water and acetonitrile | No |
| 19 | Yes | Belgian NRL method | We used the M+H ion and 10ul injection volume | Yes |
| 20 | Yes | EURL method | | No |
| 21 | No | | | |
| 22 | No | | | Yes |
| 23 | Yes | EURL method | we only used the extraction part, UPLC-MS/MS part was in-house | |
| 24 | No | | | No |
| 25 | No | | | Yes |
| 26 | No | | | |
| 27 | No | | | |
| 28 | Yes | EURL method | | |
| 29 | No | | | |
| 30 | Yes | EURL method | Used the sample preparation of this method, but another LC-MS/MS-method and no isotope-internal standard. | |
| 31 | Yes | EURL method | | No |
| 32 | Yes | EURL method | A filtration step (paper filter) was added | |

| Lab Code | Provided method | Which method | Deviations from the method | Participation in validation |
|---|---|---|---|---|
| 33 | No | | | No |
| 34 | Yes | EURL method | | |
| 35 | Yes | EURL method | | No |
| 36 | No | | | No |

**What type of clean-up methodology was used (e.g. immunoaffinity column)?**
**If you used immunoaffinity column, please specify the manufacturer and product code (if available)**
**What type of detection method did you use?**
**Please specify your method (type of column, mobile phase, detector settings, etc.)**
**How did you integrate the signals?**
**If automatic, did you confirm the integration correctness visually?**
**Was protection against daylight applied?**

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| 1 | No Clean - up step | | LC-MS | Acquity UPLC BEH C18 1.7µm, 2.1x100mm column. 281.1>249.1, 281.1>205.2 for IS 294>217.05. Injection 10µl. Column temperature 30. | Automatic | Yes | No |
| 3 | | | LC-MS | | Automatic | Yes | No |
| 4 | Immunoaffinity column | VICAM | HPLC-FLD | Column: Waters Sunfire C18 4,6x50mm, 2,5um; ex 350 nm, em 500nm, Temp. of column 25 C. | | Yes | Yes |
| 5 | | | LC-MS | C18 150x3 mm , 5 µm, 251/205.1, 251/233.2, 1.66 mg | Automatic | Yes | No |
| 6 | No Clean-up | | HPLC-FLD | C18; 25cm; 4.6mm i.d.; 5µm. Mobile Phase 50% Acetonitrile 50% pH2.5 Water; Excitation 331nm and Emission 500nm | Automatic | Yes | Yes |
| 7 | Immunoaffinity column | R-Biopharm Rhone | LC-MS | Column: Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm). | Manual | | No |

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| | | | | Mobile phase A: 0.1 % formic acid in water. Mobile phase B: 0.1 % formic acid in 1:1 (v/v) methanol:acetonitrile. Injection volume: 8 µL. Mass fraction injected: 0.2 µg (0.008 % of sample). Ionisation mode: ES+. Ions monitored: 251>233, 251>205 and 251>91. | | | |
| 8 | | | LC-MS | Phenomenex Luna C18 3µM 2 X 100mm 281,2>204,9 281,2>249,2 | Automatic | Yes | No |
| 9 | Phase separation induced by salts | | LC-MS | Analytical column: Aquity UPLC HSS T3 100 x 2 mm 1.8 um, 40C, injection 5uL, flow 0.4 ul/min. Ions: Citrinin 281.2>177.1, 281.2>205.2, 281.2>245.2, 13C13-CIT 294.2>262.2 | Automatic | Yes | |
| 10 | QuEChERS | | LC-MS | C18, 251>233,0.25 mg | Automatic | Yes | Yes |
| 11 | Syringe filters | | LC-MS | column:Eclipse Plus C18 2.1 x150mm, 1.8 um, precursor ion 280.9, products ion 248.6and 174.5, | Manual | | Yes |

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| | | | | collision energy 19 and 24, injection volume 10ul, flow rate 0.1ml/min | | | |
| 12 | Immunoaffinity column | Vicam (G1070) | HPLC-FLD | Column: Kinetex 2,6 µm, C18; MF: ACN/0,1% H3PO4 (35/65 v/v); detector: ex: 350 nm, em.: 500 nm | Manual | Yes | Yes |
| 13 | Immuno affinity column | R-Biopharm P126 | HPLC-FLD | Gemini C18, 10mM H3PO4 (pH 2.5): Acetonitrile (50:50), Exc 330nm and Exc 500 nm | Automatic | Yes | Yes |
| 16 | | | LC-MS | | Manual | | Yes |
| 17 | No clean-up | | LC-MS | Ascentis Express C18 (10cm x 2,1 mm, 2,7µm); transitions 251,0931 - > 233,0817; 251,0931 - > 205,0863; retention time 7 min; mass fraction of sample injected 1mg | Automatic | Yes | Yes |
| 18 | | | LC-MS | | Automatic | Yes | No |
| 19 | | | LC-MS | HSS T3 2.1 x 100mm 1.9um 251.4> 205.3, 251.4>233.3 264.4>246.4 | | Yes | Yes |
| 20 | | | LC-MS | Column: Gemini 100 x2 mm,Ionisation mode: ESI neg, 281> 249 m/z | Automatic | Yes | No |
| 21 | Only centrifugation | | HPLC-FLD | Column Atlantis T3, mobile phase acetonittile/water | Automatic | Yes | Yes |

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| | | | | with 1% formic acid gradient, exc=330 nm, em=500nm, matrix-matched calibration | | | |
| 22 | | | LC-MS | | Automatic | Yes | No |
| 23 | None | | LC-MS | BEH Acquity 1.7 um, 5 cm 2.1 mm diameter | Automatic | Yes | No |
| 24 | | | LC-MS | | Automatic | Yes | No |
| 25 | Without clean up step | | LC-MS | Hypersil GOLDTM (50 mm x 2.1 mm i.d., 1.9 um); Precursor ion 251.013; Products ions 205.100/233.100: 1mg mass fraction of sample, | Manual | | Yes |
| 26 | Immunoaffinity column | VICAM Citritest_TM | HPLC-FLD | COLUMN-ProntoSil, UHC-446(33X4,6), 3 um, C18, DETECTOR-747 WATERS Excitation 350 nm and Emission 500 nm Mobile Fase Solution A-0,1% phosforic acid, solution B Acetonitrile | | Yes | Yes |
| 27 | Immunoaffinity column | VICAM (Dr Wéber Consulting Kft.); CT114 | HPLC-FLD | Poroshell 120 EC-C18 4,5x50 mm 2,7-Micron; 0,1% phosphoric acid: ACN (60:40);flow rate 1,0 mL/min;350 nm excitation and 500 nm emission | Automatic | Yes | Yes |
| 28 | | | LC-MS | | Automatic | Yes | No |

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| 29 | Immunoaffinity column | VICAM CitriTest HPLC G1070 | HPLC-FLD | column: Phenomenex Gemini C18 150x4.6 mm 5 µm 110 A, gradient elution using acetontrile (A) and water with 0.085% phosphoric acid (B), FLD detection at 350 nm (exc) and 500 nm (em) | Automatic | Yes | Yes |
| 30 | | | LC-MS | Column C18 2.1x50 mm, 1.8 µm, Pos mode 251>233, 0.83 mg | Automatic | Yes | Yes |
| 31 | | | LC-MS | | Automatic | Yes | No |
| 32 | | | LC-MS | Acquity UPLC HSS T3 2,1 x 100 mm 1,8 µm Waters; 281.1 > 205.0, 281.1 > 249.1; 0.625 mg | Automatic | Yes | Yes |
| 33 | Immunoaffinity column | CitriTest (VICAM) - G1070 | HPLC-FLD | Eclipse Plus C18, 4.6x 50 mm, 1.8 um, Mobile phase: 0.1% Phosphoric acid/Acetonitrile, exc.350 nm, em. 500 nm, flow rate 1.0 mL/min, column temperature = 40°C | Manual | | No |
| 34 | | | LC-MS | | Automatic | Yes | No |
| 35 | | | LC-MS | | Automatic | Yes | Yes |
| 36 | No | No | HPLC-FLD | Column:Phenomenex, Kinetex EVO C18, Core-Shell tecnology (150 x 4,6 mm ID, 5 ?m). gradient elution | | No | Yes |

| Lab Code | Clean-up | Manufacturer of IAC | Determination technique | Specification of the method used | Peak integration type | Visual check? | Protection against daylight |
|---|---|---|---|---|---|---|---|
| | | | | water/acetonitrile, ex = 350nm; em = 500 nm; | | | |

**Did you encounter any problems during the analysis?**
**IF YES, what were the specified problems and to which samples do they apply?**
**Did you notice any unusual observations which, however, did not seem to have any effect on the results?**
**IF YES, what were the observations and to which samples do they apply?**

| Lab Code | Problems | Problem description | Unusual observations |
|---|---|---|---|
| 1 | No | | No |
| 3 | No | | No |
| 4 | No | | No |
| 5 | No | | No |
| 6 | No | | No |
| 7 | No | | No |
| 8 | No | | No |
| 9 | No | | No |
| 10 | No | | No |
| 11 | No | | No |
| 12 | No | | No |
| 13 | No | | No |
| 16 | Yes | We have used LC MS Ion Trap technique for detection and we haven't had a good reproducibility , especially for sample B. | No |
| 17 | No | | No |
| 18 | No | | No |
| 19 | No | | No |
| 20 | No | | No |
| 21 | No | | No |
| 22 | No | | No |
| 23 | Yes | Difficult to find MRMs (we used formic acid in our mobile phase) | No |
| 24 | Yes | MS/MS signals were not very repeatable because of Injection volume of 2 microliters (loop 10 microliter) and solvent was ethylacetate. | No |
| 25 | No | | No |
| 26 | No | | No |
| 27 | No | | No |
| 28 | No | | No |
| 29 | No | | No |
| 30 | No | | No |
| 31 | No | | No |
| 32 | No | | No |
| 33 | No | | No |
| 34 | Yes | We analyse this analyte for the first time and because we don't have any CRM and experience we don't know what is the real efficiency of extraction; we noticed different response from "Belgian" method extraction and EURL | No |
| 35 | No and Yes | Strong matrix effect observed. | No |
| 36 | No | | No |

**Did you find the instructions distributed for this PT adequate?**
**IF NO, which parts do you think can be improved?**
**What is your opinion about the registering/reporting format of this interface?**
**Any other comments you wish to address?**

| Lab Code | Instructions adequate? | Improvement | Registering/reporting format | Any Other comments |
|---|---|---|---|---|
| 1 | Yes | | I think that you will receive not enough information about the methods used for sample preparation. But in general such reporting form is acceptable. | For calibration we used white rice. |
| 3 | Yes | | | |
| 4 | Yes | | | |
| 5 | Yes | | | |
| 6 | Yes | | Had some difficulty opening program | None |
| 7 | Yes | | It's OK | |
| 8 | Yes | | Good format | |
| 9 | Yes | | | |
| 10 | Yes | | OK | In this case (citrinin is not routinely monitored), just one sample with given amount of citrinin would be very helpful. |
| 11 | Yes | | | |
| 12 | Yes | | Easy for the prefilled fields | |
| 13 | Yes | | Good | |
| 16 | Yes | | Very good | No |
| 17 | Yes | | Satisfy | We would appreciate blank matrix sample provided by EURL together with Sample A and Sample B |
| 18 | Yes | | (reporting) It is useful prepared answers where you can choose one you need | |
| 19 | Yes | | We have used it many times and its perfectly satisfactory | |
| 20 | No | No suggestion reported | | |
| 21 | Yes | | Globally OK but not very convenient to write comments | This is a new subject for our lab, the method used is not validated. Citrinin content in sample A around 40 µg/kg LOD observed variable from one sample to another, from 20 µg/kg (sample A) to 200 µg/kg |

| Lab Code | Instructions adequate? | Improvement | Registering/reporting format | Any Other comments |
|---|---|---|---|---|
| | | | | (sample B) but it could be more as we have seen in our routine samples. Method used by our lab is "Incidence of citrinin in red yeast rice and various commercial Monascus products" liao Chia-Ding 2014. |
| 22 | Yes | | Easy | |
| 23 | Yes | | Very good | |
| 24 | Yes | | Reporting format was new, difficult to know what is happening. Recovery was not mentioned in the result table? | |
| 25 | Yes | | Good way of reporting of results | |
| 26 | Yes | | -- | We use the standards provided by JCR, because this is the first time we performed this analysis |
| 27 | Yes | | It was appropriate. | We are accredited for the determination of Citrinin. The accredited method is an ELISA method (LOD 15 ug/kg). But the samples were analysed by HPLC method. |
| 28 | Yes | | | We have analysed sample with both methods (EURL and Belgian method). Both methods give significant different results. We have decided to send EURL method results |
| 29 | No | No suggestion reported | OK | The analysis was conducted using standard addition (sample B) which provides intrinsic correction of recovery. Thus, the estimated recovery of 90% (see above) was not used for recovery correction of the values reported. The recovery was determined by spiking experiments (see above). According Commission Regulation 401/2006 the correction for recovery is not necessary in case the recovery rate is between 90-110%. Level of |

| Lab Code | Instructions adequate? | Improvement | Registering/reporting format | Any Other comments |
|---|---|---|---|---|
| | | | | sample A (low level) was determined by external calibration |
| 30 | Yes | | Too many free text squares | |
| 31 | Yes | | Ok | This was the first time that we analysed citrinin in our lab. We didn't have enough standards (only the one provided by EURL) to develop the method. |
| 32 | Yes | | Adequate | |
| 33 | Yes | | Simple and really quick | none |
| 34 | Yes | | Registering ok...reporting a little complicated | |
| 35 | No and Yes | No suggestion reported | Useful | 1. Due to strong matrix effect observed, a standard addition method was applied, so the recovery is included to the result. 2. For the sample A a concentration about at the level of LOQ was measured. 3. The uncertainty reported is at ug/kg and not as % |
| 36 | Yes | | The "question and answers" window is not easy to fill. | |

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