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Determination of the acrylamide content in potato chips

Report on proficiency test organised by the EURL-PAH

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Lubomir Karasek

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European Union Reference Laboratory
Polycyclic Aromatic Hydrocarbons

Four marker PAHs in coconut oil

Report on the interlaboratory comparison organised by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

Stefanka Bratinova,
Lubomir Karasek

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Table of content

Acknowledgements

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Austrian Agency for Health and Food Safety	Austria
MA 38 - Lebensmitteluntersuchung Wien	Austria
Laboratorium ECCA NV	Belgium
Scientific Institute of Public Health (WIV-ISP)	Belgium
SGS Belgium NV	Belgium
State General Laboratory	Cyprus
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Laboratory of Public Health, Valencia	Spain
Swedish National Food Agency	Sweden
RIKILT	the Netherlands
Fera Science Ltd	UK

Executive summary

This report presents the results of a proficiency test (PT) organised by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons (EURL-PAHs) in 2017 under its extended scope including process contaminants. The aim of this PT is to evaluate the ability of the European official control laboratories to reliably analyse acrylamide in potato chips.

On request by DG SANTE and in agreement with National Reference Laboratories the test material used in this exercise was a commercial potato chip acquired from a local supermarket, cryogenically ground and homogenised at the EURL-PAH premises. In addition participants received a solution of acrylamide with known content for the verification of their instrument calibration.

Forty officially nominated National Reference Laboratories (NRLs) and Official food Control Laboratories (OCLs) of the EU Member States, Norway and Iceland participated to the study.

The test material was characterised by the EURL-PAH. The assigned value and the corresponding measurement uncertainty were determined from independent replicate measurements on two different days by isotope dilution mass spectrometry.

Participants were free to choose their method of analysis. The performance of the participating laboratories in determining acrylamide in the test material was expressed as z- and zeta-scores.

This PT demonstrated the high competence of the participating laboratories in the analysis of acrylamide. About 90% of the reported test results were assessed as satisfactory based on the z-scores.

List of abbreviations and definitions

AA	Acrylamide
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL-PAH	European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons
ILC	Interlaboratory comparison
ISO	International Organization for Standardization
IUPAC	International Union for Pure and Applied Chemistry
JRC	Joint Research Centre
LOD	Limit of Detection
LOQ	Limit of Quantitation
ML	Maximum level
NIST	National Institute of Standards and Technology, US
NRL	National Reference Laboratory
OCL	Official food control laboratory
PT	Proficiency test

1. Introduction

The European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons in Food (EURL-PAH) is hosted by the Joint Research Centre of the European Commission. One of its core tasks is to organise comparative testing (proficiency tests, PTs) for the National Reference Laboratories (NRLs) [1, 2].

This study, which is part of EURL-PAHs work programme for 2017 falls under the extended scope of the EURL-PAHs which includes other process contaminants - undesired substances that are generated during food processing or that enter food during food processing, in particular PAHs, free forms of MCPD, but also MCPD esters, as well as glycidyl esters, acrylamide, and furan.

EFSA confirmed in its 2015 opinion that acrylamide is a carcinogenic substance with carcinogenic effects at the current levels of dietary exposure. It was found that the levels of consumed acrylamide did not decrease in recent years. The main contributors to the exposure are coffee (and coffee substitutes), potato-based and cereal-based products.

The EFSA opinion triggered a regulatory measure to reduce the presence of acrylamide in food to ensure a high level of human health protection. This measure is intended to be enforceable, feasible and credible for an effective reduction of the presence of acrylamide in food. Based on the Food Hygiene Regulation, this measure requires from all concerned food business operators to reduce the presence of acrylamide in food. The regulatory measures' objective is to achieve a reduction by setting strict levels to be used as a benchmark. The benchmark levels should serve as a measure of the efficacy of the applied mitigation measures. They are proposed to be set at a strict level taking into account the most recent occurrence data from the EFSA database. Complementary after the adoption of the regulatory measure obliging food business operators to apply mitigation measures, it is foreseen in a second phase to initiate the discussion on setting maximum levels for acrylamide in certain foods or food categories, which are placed on the market ready to eat.

This PT aimed to evaluate the measurement capability of EU official control laboratories (OCLs) for the determination of the content of acrylamide in potato chips, in support of the newly envisaged regulatory measures for acrylamide in food. The PT targeted a wide range of OCLs outside the EURL-PAHs network. Since the appointed NRLs for PAHs did not have the determination of acrylamide systematically included in their mandate, they could participate to this PT on a voluntary basis. Alternatively, they were requested to identify suitable laboratories in their country that would be interested in participating. Participants were asked to determine the acrylamide content applying their in-house analysis methods.

2. Scope

This PT aimed to evaluate the equivalence of results reported by EU official food control laboratories (OCLs) when determining the acrylamide content in potato chips.

The appropriateness of the reported measurement uncertainty was also assessed as this parameter is important in the compliance assessment.

The PT was designed and evaluated following the administrative and logistic procedures of the JRC Unit in charge of the EURL-PAH, which is accredited for the organisation of PTs according to ISO/IEC 17043:2010 [10].

3. Setup of the exercise

3.1 Participating Laboratories

Only officially nominated control laboratories (OCLs) of the EU Member States were admitted as participants. Forty-one laboratories registered to the PT, and 40 of them reported results. The list of participants is provided in the Acknowledgment section.

3.2 Time frame

The PT was announced on the JRC public webpage (see ANNEX 1) and invitation letters were sent to the laboratories on March 17, 2017 (see ANNEX 2) with deadline for registration via EUSurvey webpage (see ANNEX 3) by April 17, 2017. Test samples were dispatched to participants on May 2, 2017 and the deadline for reporting of results was set to June 6, 2017. The documents sent to the participants are presented in ANNEX 4-6.

3.3 Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed by non-disclosing the identity of participants to third parties, transmission of data through a dedicated web-based interface and a secure databank hosted by JRC. European Commission rules on data protection were strictly applied. However, the laboratory codes of the National Reference Laboratories could be disclosed to DG SANTE upon request for assessment of their long-term performance.

3.4 Design of the proficiency test

Each participant received an ampoule of an acetonitrile solution (2 mL), with a known content, together with the amber glass vial containing the potato chip test material.

Participants were requested to perform triplicate analysis of the test item and to report the three individual results together with the "final" value for proficiency assessment and the corresponding measurement expanded uncertainty (specifying the coverage factor used). Results had to be corrected for recovery. Only the final value was used for performance assessment.

Furthermore, participants were also requested to report details of the performance of the applied analytical method (see ANNEX 7), together with the estimate of their limits of detection (LOD) and quantification (LOQ) (ANNEX 8).

4. Test materials

4.1 Preparation

The potato chip test item was prepared at the EURL-PAH starting from commercial potato chips, acquired at a local supermarket. In order to avoid the introduction of heterogeneity during the grinding process (due to fatty particles sticking together), all samples were frozen in liquid nitrogen prior to processing and kept at temperatures below −110 ^{0}C. The material was then milled to have particle sizes below 500 μm. Special attention was given to the temperature of the samples during grinding.

The material was filled in portions of approximately 10 g in 20 mL glass vials capped with aluminium caps with silicone/PTFE septa and stored at -20 °C. Each vial was uniquely numbered. The homogeneity and stability of the samples were tested as it is described below.

The acrylamide standard solution (1.99 μg/g) was prepared from a neat substance (SIGMA, > 99 %), and checked against the certified reference materials (ERM-BD273). This solution was ampouled under inert atmosphere and flame sealed in 2 ml amber glass ampoules.

4.2 Homogeneity and stability

The potato chip test material was tested for significant inhomogeneity, according to the IUPAC International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, and for sufficient homogeneity according to ISO 13528:2015 [11]. Isotope dilution high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used for analysis. The method precision complied with the requirements laid down in ISO 13528:2015 [11].

The homogeneity experiment consisted of duplicate analysis of 10 samples randomly selected along the filling sequence among the amber glass vials prepared for dispatch. Duplicate analyses were performed in random order. The test material was rated sufficiently homogenous at a sample intake of 2 g and no trend was observed. Details of the homogeneity tests are given in ANNEX 9.

The stability of the test material was evaluated following the requirements in ISO 13528:2015. Six randomly selected samples were stored at three different conditions for 9 weeks, covering the whole period of the PT exercise - from the dispatch of the test items to the end of the submission of the results.

The first set of 3 samples was stored at room temperature as recommended storage conditions (~ 21 °C). The second set of 3 samples was stored for the whole period of the study in a deep freezer at the reference temperature (~ -80 °C), while the third set was stored at room temperature for one week, mimicking the possible temperature increase during transport. After the deadline for reporting of results had expired, all 9 samples were analysed in duplicate under repeatability conditions. No significant differences of the analyte content of the test samples were found. Hence stability of the test samples over the whole period of the study was assumed, provided that the recommended storage conditions were applied.

4.3 Assigned value, corresponding uncertainty, and standard deviation for proficiency assessment

The assigned values were determined at the EURL-PAH applying isotope dilution mass spectrometry (ID-MS) with bracketing calibration, a method implemented and validated at the EURL-PAH. This method was ring-trial validated in the frame of the CEN mandate to become the European standard EN 16618:2015 [12].

The associated uncertainties (u_{Xpt}) of the assigned values were calculated combining the uncertainty of the characterisation (u_{char}) with the contributions for homogeneity (u_{bb}) and stability (u_{st}) in compliance with ISO/IEC Guide 98 (GUM) [13]:

$$u_{Xpt} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{st}^2} \qquad \text{Eq. 1}$$

The stability study confirmed that the material was stable and the uncertainty contribution due to stability was set to zero ($u_{st} = 0$) for all analytes. The uncertainty contribution deriving from homogeneity (u_{bb}) was calculated using SoftCRM [16].

The standard deviation for proficiency assessment, σ_{pt}, was calculated using the Horwitz equation for analyte concentration ≥ 120 µg/kg:

$$\sigma_p = 0.02 \cdot c^{0.8495} \qquad \text{Eq. 2}$$

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

The assigned value, corresponding uncertainty, and standard deviation for proficiency assessment of the PT are shown in Table 1.

Table 1: Assigned value (X_{pt}), associated expanded uncertainties ($U(x_{pt})$, k=2) and standard deviation for proficiency assessment (σ_{pt}) (for the potato chips test item, expressed based on mass of entire product (on product basis).

Analyte	Analyte short name	x_{pt}	$U(x_{pt})$	σ_{pt} (= u_f)	
		mg/kg	mg/kg	mg/kg	%
Acrylamide	AA	0.673	0.045	0.114	17

5. Evaluation of laboratories

5.1 General

The performance of the laboratories in determining the acrylamide content in potato chips was assessed using z-scores [11]. Zeta-scores were also calculated taking into account the measurement uncertainties reported by the participants.

The results as reported by participants are listed in ANNEX 10.

5.2 Evaluation parameter

z-scores were calculated based on the final values (x_i) as follows:

$$z = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}} \qquad \text{Eq. 3}$$

where x_{pt} is the assigned value, and σ_{pt} the standard deviation for proficiency assessment.

In contrast to z-scores, zeta-scores describe the agreement of the reported ranges ($x_i \pm u(x_i)$) with the respective assigned ranges ($x_{pt} \pm u(x_{pt})$). The following equation applies:

$$zeta = \frac{x_i - x_{pt}}{\sqrt{u(x_i)^2 + u(x_{pt})^2}} \qquad \text{Eq. 4}$$

Whenever participants did not report measurement uncertainties, $u(x_i)$ was set to zero, which increases the zeta-score.

The performance of the laboratories was classified according to ISO/IEC 17043:2010 [10]. The following scheme is applied for the interpretation of both z-scores and zeta scores:

$|score| \leq 2.0$ = satisfactory performance
$2.0 < |score| < 3.0$ = questionable performance
$|score| \geq 3.0$ = unsatisfactory performance

5.3 Evaluation of results

z-scores were attributed only to the "final values". Individual results of replicate analyses were not rated.

Statistical evaluation of the results was performed using the PROLab software [13]. While the ID-MS results provided by the EURL-PAH were set as assigned values, Algorithm A+S of ISO 13528:2015 [11] was applied to compute the robust means and robust standard deviations (as additional information).

The confidence interval of the robust mean calculated from the results reported by the participants (ANNEX 10, Kernel density plot) is in good agreement with the confidence interval

of the assigned value. However, the robust standard deviation of the results was lower than the target standard deviation for proficiency assessment (Table 1).

Figure 1 shows that 90 % and 77 % of the participants obtained satisfactory z- and zeta-scores, respectively (|score| ≤ 2). Only 7.5 % of the results fall into the unsatisfactory performance range (|score| > 3).

Figure 1: Histogram of z- and zeta-scores

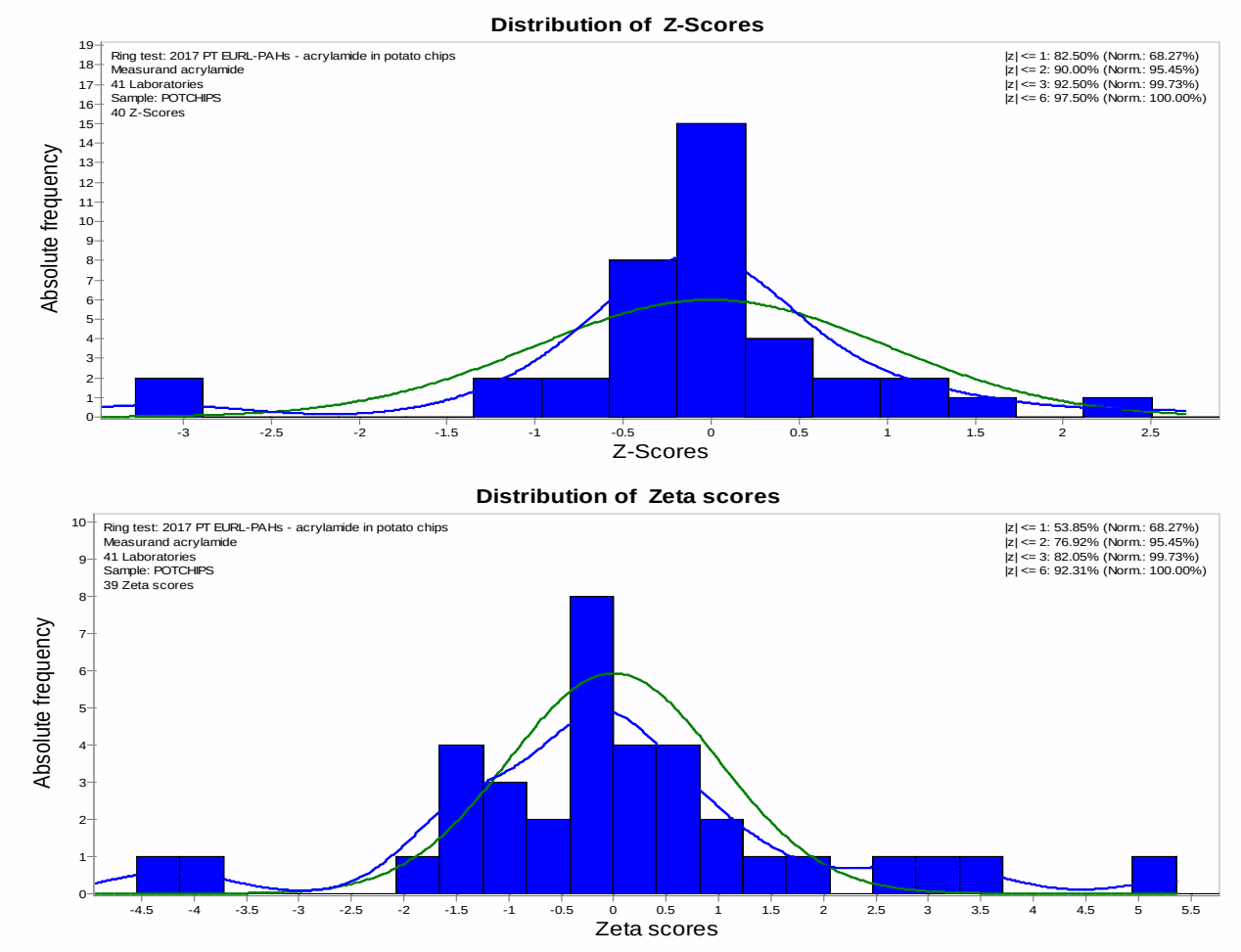
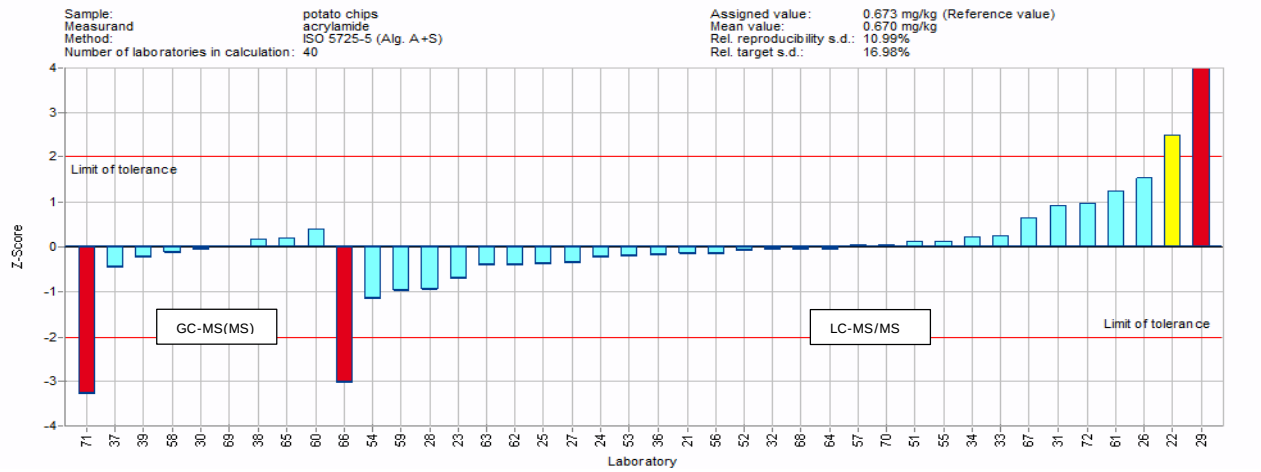


Figure 2: z- scores, grouped by analytical method



Thirty-six participants obtained satisfactory z-scores (blue bars in Figure 2, and green cells in ANNEX 10), while the results of only 3 participants were classified as non-satisfactory (red bars in Figure 2, and red cells in ANNEX 10). One laboratory got a questionable z-score (yellow bar in Figure 2, and yellow cell in ANNEX 10) while one participant did not report results.

Annex 10 presents the reported results and the corresponding evaluation. The numerical values and the calculated z- and zeta-scores are presented in the Table. The figure shows the individual analysis results of the three replicate determinations and the reported uncertainty interval together with respective Kernel density plot.

The results are normally distributed. The major mode is close to the assigned value and to the robust mean calculated from the reported results. This confirms that the measurement of AA in potato chips is under statistical control. No influence from the analytical techniques used (GC-MS(MS) or LC-MS/MS) could be identified.

The plausibility of the uncertainty statements of the laboratories was assessed in the current PT classifying every reported uncertainty into three groups (Annex 10) according to the rules described hereafter.

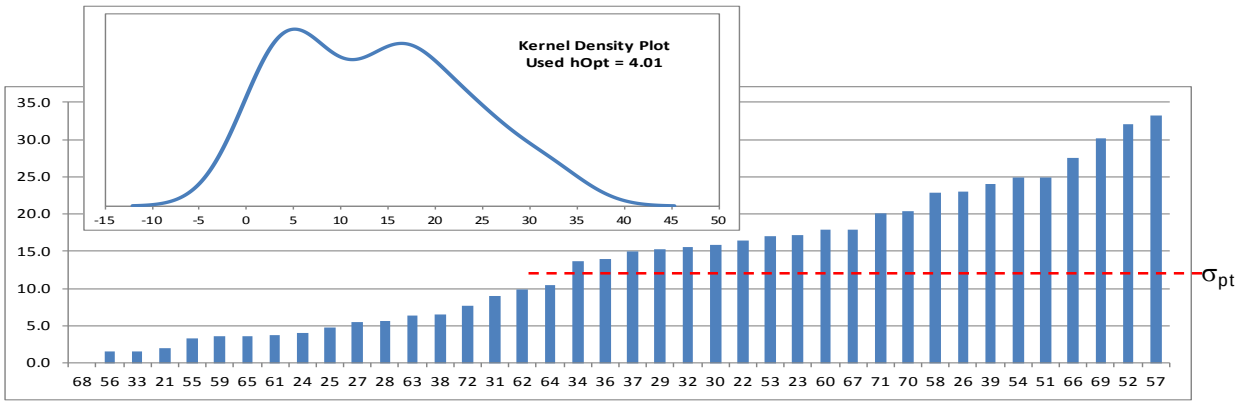
The standard measurement uncertainty from a laboratory ($u(x_i)$) is most likely to fall in a range between a minimum and a maximum uncertainty (case "**a**": $u_{min} \leq u(x_i) \leq u_{max}$). The minimum uncertainty (u_{min}) is set for the respective analyte to the standard uncertainty of the assigned value ($u(x_{pt})$). 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 measurement uncertainty smaller than $u(x_{pt})$. The maximum uncertainty is set to the standard deviation accepted for the assessment of results (σ_{pt}), derived from the Horwitz equation (Eq. 2). Consequently, case "a" becomes: $u(x_{pt}) \leq u(x_i) \leq \sigma_{pt}$.

If $u(x_i)$ is smaller than $u(x_{pt})$ (case "**b**": $u(x_i) < u(x_{pt})$) the laboratory might have underestimated its measurement uncertainty.

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. Both cases may require corrective actions.

As seen from the bar graphs in Figure 3, the reported expanded uncertainty vary from 1.5 % to 33 %. ANNEX 10 shows that 12 laboratories may have underestimated their uncertainties (type "**b"**), based on the above explained criteria. The design of the PT (with 3 replicate analyses) contributes with additional information to this uncertainty evaluation, assessing the repeatability amongst the replicates. Six of the above mentioned laboratories (21, 28, 33, 59, 65, 68) have reported individual values outside their confidence interval (x ± U), confirming that the reported uncertainty is not realistic. For other four labs (25, 27, 56 and 63) with type "**b"** uncertainty however, the precision of their replicated results were very high, meaning that their reported uncertainty could be reasonable. Laboratory 62 has "a" type of uncertainty, however its replicate values still lay outside the confidence interval (x ± U), meaning as well underestimation.

Figure 3: Graphical presentation of the expanded uncertainties (in 5) as reported by the participants



5.5 Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire filled by the participants (ANNEX 8). Data are presented as reported.

Nearly 75% of the participants have more than 5, or even more than 10 years (25%) of experience with the method (Figure 4) and as many as 100-1000 samples analysed for acrylamide in the past years (Figure. 5). Five of the participants were quite new in the fields of acrylamide analysis with less than a year of experience, however they all obtained successful z-scores. Participants with questionable (1) and non-satisfactory (3) z-scores, declared more than 5 years of experience and participation in similar proficiency tests in the previous years.

Concerning the instrumental techniques applied, a strong trend towards replacing the GC-MS(MS) analysis with LC/MS-MS was observed, when comparing with the previous PTs, organised by JRC-Geel in 2007-2009. LC-MS/MS is currently the preferred method with more than 75% of participants applying it (Figure 6).

Most of the participants (35/40) prepared their calibration solutions starting from neat substances. Only 5 used commercial standard mixtures in solvent.

No significant difference was noticed between the results of the different populations.

Five laboratories are not accredited for the determination of acrylamide, while other five laboratories were not accredited for the analytical method they used in this PT (currently in the transition, moving from GC to LC method).

Figure 4: Years of experience with the method

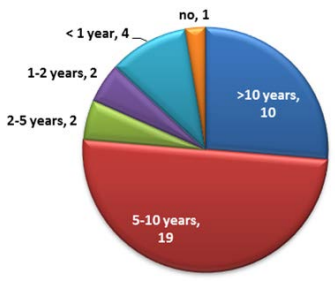


Figure 5. Number of samples analysed for acrylamide in the past few years

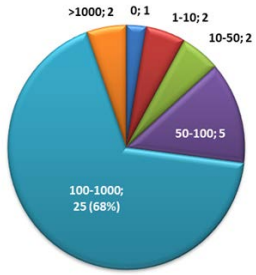


Figure 6: Method of analysis used and the corresponding number of laboratories

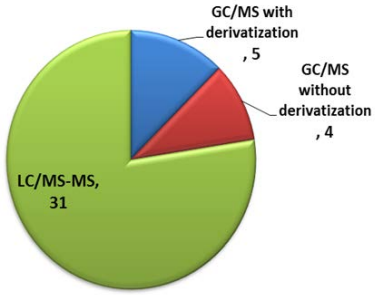
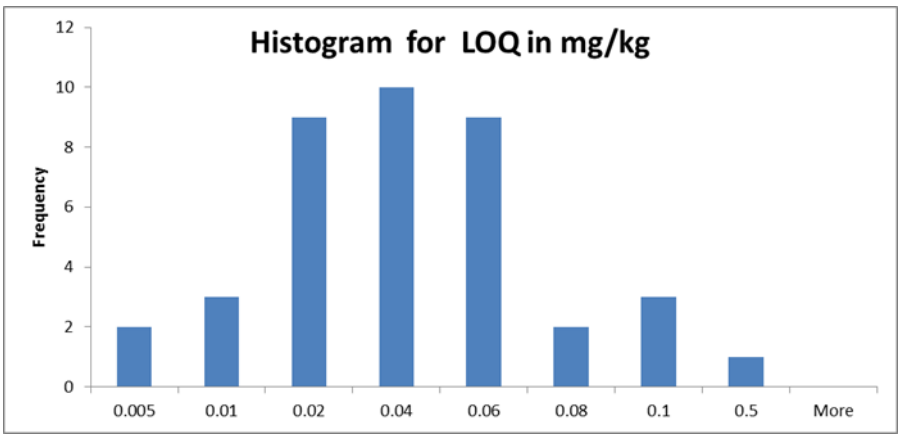


Figure 7: Histogram of the reported LOQ.



Participants were requested to report their LOD/LOQs together with the test results (ANNEX 8). Difference of two orders of magnitude were observed amongst the reported LOQs, despite

existence of the Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food [17] published by the EURL-PAH.

Figure 7 shows that the majority of the participants reported LOQs ranging from 0.02 mg/kg to 0.06 mg/kg, in compliance with the benchmark levels currently under discussion (ML/10 ≈ 0.75 mg/kg).

Conclusion

Forty participants from 20 Member States reported analysis results. More than 90 % of the results obtained satisfactory performance ratings, which prove the high proficiency of the official control laboratories in performing that type of analysis. Laboratories applied analytical methods with sound performance characteristics for that type of matrix.

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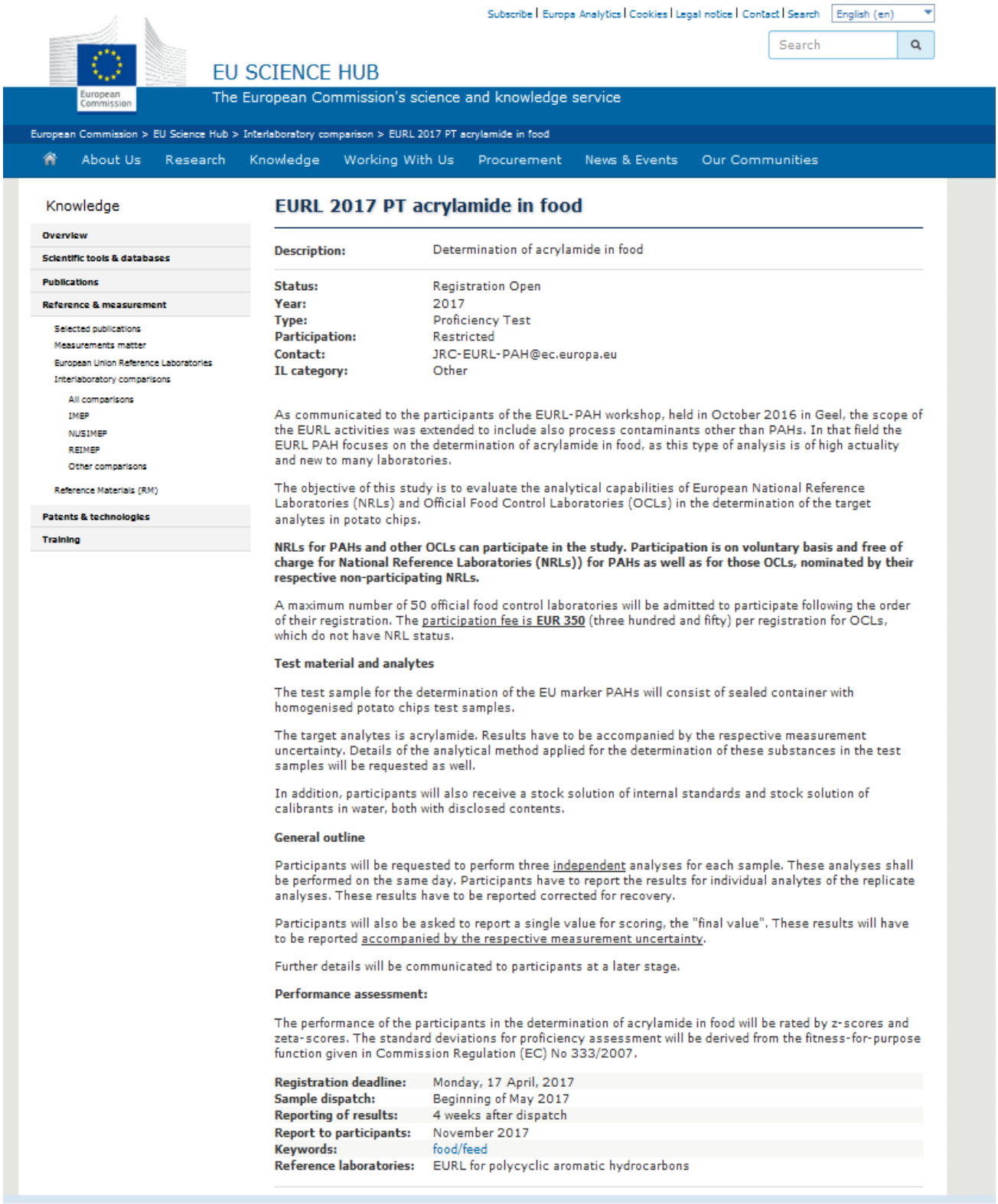
ANNEX 1: Announcement of the PT on the JRC webpage



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    - All comparisons
    - IMEP
    - NUSIMEP
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EURL 2017 PT acrylamide in food

Description: Determination of acrylamide in food

Status: Registration Open
Year: 2017
Type: Proficiency Test
Participation: Restricted
Contact: JRC-EURL-PAH@ec.europa.eu
IL category: Other

As communicated to the participants of the EURL-PAH workshop, held in October 2016 in Geel, the scope of the EURL activities was extended to include also process contaminants other than PAHs. In that field the EURL PAH focuses on the determination of acrylamide in food, as this type of analysis is of high actuality and new to many laboratories.

The objective of this study is to evaluate the analytical capabilities of European National Reference Laboratories (NRLs) and Official Food Control Laboratories (OCLs) in the determination of the target analytes in potato chips.

NRLs for PAHs and other OCLs can participate in the study. Participation is on voluntary basis and free of charge for National Reference Laboratories (NRLs)) for PAHs as well as for those OCLs, nominated by their respective non-participating NRLs.

A maximum number of 50 official food control laboratories will be admitted to participate following the order of their registration. The participation fee is **EUR 350** (three hundred and fifty) per registration for OCLs, which do not have NRL status.

Test material and analytes

The test sample for the determination of the EU marker PAHs will consist of sealed container with homogenised potato chips test samples.

The target analytes is acrylamide. Results have to be accompanied by the respective measurement uncertainty. Details of the analytical method applied for the determination of these substances in the test samples will be requested as well.

In addition, participants will also receive a stock solution of internal standards and stock solution of calibrants in water, both with disclosed contents.

General outline

Participants will be requested to perform three independent analyses for each sample. These analyses shall be performed on the same day. Participants have to report the results for individual analytes of the replicate analyses. These results have to be reported corrected for recovery.

Participants will also be asked to report a single value for scoring, the "final value". These results will have to be reported accompanied by the respective measurement uncertainty.

Further details will be communicated to participants at a later stage.

Performance assessment:

The performance of the participants in the determination of acrylamide in food will be rated by z-scores and zeta-scores. The standard deviations for proficiency assessment will be derived from the fitness-for-purpose function given in Commission Regulation (EC) No 333/2007.

Registration deadline: Monday, 17 April, 2017
Sample dispatch: Beginning of May 2017
Reporting of results: 4 weeks after dispatch
Report to participants: November 2017
Keywords: food/feed
Reference laboratories: EURL for polycyclic aromatic hydrocarbons

ANNEX 2: Announcement of the PT via e-mail

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials

Geel, 16/03/2017

Inter-laboratory comparison on the determination of acrylamide in food

Dear Madam/Sir,

Registration for participation in the inter-laboratory comparison study organised by the EURL PAH on the determination of acrylamide in food is **open until 17 April 2017**.

Participation is on voluntary base and free of charge for National Reference Laboratories (NRLs) for PAHs or those official food control laboratories (OCLs) delegated by respective NRL, not participating in the PT. **The participation fee for other official food control laboratories is 350 Euro per participation.** Confidentiality of data is granted.

The target analytes is acrylamide in potato chips. Results have to be reported accompanied by the respective measurement uncertainty.

Each participant will be provided with sealed container containing preliminary homogenised 10-12 g potato chips. Participants will also receive internal standards solution and calibrants solution in water with disclosed content;

This inter-laboratory comparison is organised under accreditation to ISO 17043.

Detailed information will soon be available on the EURL website:
http://irmm.jrc.ec.europa.eu/EURLs/EURL_PAHs/interlaboratory_comparisons/Pages/index.aspx

Timing:

- **Deadline for registration: 17 April 2017**
- Dispatch of samples: **beginning of May 2017.** A detailed outline of the study will be included in the parcels. Participants will be asked to return a sample receipt to the organiser
- Deadline for reporting of results: **4 weeks after the dispatch of the samples**.

Registration procedure:

You are invited to register via following link:
https://ec.europa.eu/eusurvey/runner/acrylamide

PT coordinator	Second contact
Stefanka Bratinova	Lubomir Karasek
Fax: 0032-14-571800 e-mail: jrc-eurl-pah@ec.europa.eu	

Participants are invited to indicate any justified requests.

Distribution of information:
The NRLs are kindly requested to distribute as soon as possible this information and the link to the Registration form to the interested OCLs, **and to assist the EURL in identifying other laboratories that are eligible to participate in the study.**

Should you require further clarification, please do not hesitate to contact the EURL team via:
JRC-EURL-PAH@ec.europa.eu

With kind regards,
Stefanka Bratinova

Cc: H.Emons, P.Dehouck, L.Karasek

ANNEX 3: Registration form

EURL PAH 2017 Proficiency Test on the determination of acrylamide in food

Fields marked with * are mandatory.

European Union Reference Laboratory
Polycyclic Aromatic Hydrocarbons

EURL PAH 2017 PT - acrylamide in food - Registration

As communicated to the participants of the EURL-PAH Workshop, held in October 2016 in Geel, EURL-PAH has to organise the second PT in 2017 on acrylamide in food. The dispatch is foreseen for the first week of May. Before that, during the last week of April, one day hands on training will be organised for the interested NRLs in the premises of the EURL.

We would not expecte from laboratories, that were appointed NRLs for PAHs, to cover topics outside the scope of their appointment. However, we offer the possibility to participate in activities organised by the EURL PAHs on process contaminants other than PAHs on voluntary basis. In case you decided to not participate, you are kindly requested to identify laboratory in your country that would be more suitable /interested in participation to the foreseen PT on acrylamide determination free of charge. Please send the questionnair/registration to other interested parties and fill it in by 24th March 2017 and .

* Organisation

Department

* Address (for DHL shipment)

1

* City

* Postal code

* Country

* Name of the contact person

* Email

* Telephone (DHL requirement)

* Are you going to participate in the PT on acrylamide in food (potato chips), organised by EURL-PAHs. The dispatch is foreseen for the begining of May.

- ○ Yes
- ○ No

Which instrumental method you are going to apply

- ☐ GC/MS
- ☐ LC/MS-MS

* Prefered solvent for the standard solution

- ☐ acetonitrile
- ☐ toluene

* NRL or OCL

- ○ NRL
- ○ OCL

Any comment or request (not more than 100 characters)

2

ANNEX 4: INSTRUCTIONS TO THE PARTICIPANTS

Ref. Ares(2017)2272698 - 03/05/2017

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

Geel, 28 April 2017

EURL-PAH 2017 PT- acrylamide in potato chips

Dear Madame/Sir,

The inter-laboratory comparison study organised by the EU-RL PAHs on the determination of the content of the acrylamide in potato chips starts with the dispatch of the samples.

The target analyte is the acrylamide in the final product. Each participant is provided with amber glass vials containing a portion of potato chips, naturally contaminated with acrylamide, a known standard solution of AA and D_3-AA in water for checking of their instrument calibration against an external reference.

Outline of the study.

The participating laboratories shall apply for the analyses a method of their choice.

The laboratories shall report the results by 6th June 2017 at the latest following the instructions provided further on in this document.

The participants are requested to report the results obtained from three replicate analyses. They also have to report a final value for proficiency assessment. Results have to be reported corrected for recovery and the results for proficiency assessment ("final values") have to be accompanied by the respective measurement uncertainty.

Participants are also requested to report together with the results details of the applied analysis method and some method performance characteristics.

Test material and analytes

1. One 50 ml amber vial, labelled as *"EU-RL PAHs PT 2017 Interlaboratory comparison, acrylamide in potato chips"* containing approximately 10 g of a naturally contaminated homogenised potato chips. The analyte content shall be determined in triplicate. The participants have to report to the EU-RL besides the individual results of the replicate analyses also one value, on which they would like their performance to be assessed. This value is called on the reporting file "final value".

   If not analysed immediately after receiving, please store the potato chips sample in freezer (-18°C).

2. Two ampoules, labelled as "acrylamide", or "deuterated acrylamide", with about 1 ml of a solution of **acrylamide in water**. The analyte concentration is given in the attached document. The solutions may be used by the participants to check their instrument calibration against an independent reference. Participants do not have to report results for this solution.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783. E-mail: jrc-eurl-pah@ec.europa.eu

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 mail some files for reporting results. You should follow the following instructions:

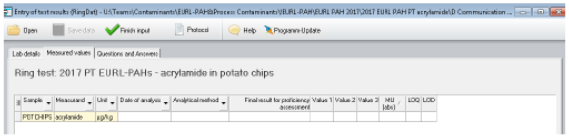
1. If not available already, please download the data entry program RingDat free from the QuoData web page using following link: http://quodata.de/ringdat_en.php

User: *ringdat*
Password: *prolabdata*

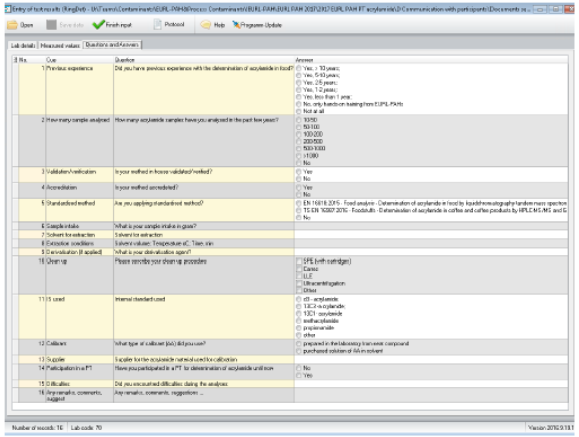
2. Save to the same folder the two lab specific files with the extension **"*.LAB"** and **"*.LA2"**, generated by the ProLab software and provided to each laboratory individually (personal files) by mail.

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 is codified by the software,
   - 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 measurands, that have to be analysed and reported.
   - First tab contains the detailed information for the laboratory
   - Second tab contains table for entering the results. You could filter the entries by sample or by measurand. The cells marked with red are mandatory to be filled
   - Third tab contains a general questionnaire.

4. Fill in the result table with your data.



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-eurl-pah@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.

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

With kind regards,

Stefanka Bratinova
EURL-PAHs

ANNEX 5. SAMPLE RECEIPT form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

PROFICIENCY TESTING MATERIAL RECEIPT FORM

2017 PT- acrylamide in potato chips

Contact person	
Affiliation	
City, Country	

Content of the two parcels:

1. One 50 ml amber glass vial containing about 10 g of potato chips
2. One glass ampule, containing about 1 ml of acrylamide in water
3. One glass ampule, containing about 1 ml of D_3-acrylamide in water
4. One sample receipt form (= this form), which is e-mailed as well to be filed and send electronically

TEST SAMPLE SHOULD BE STORED IN A FREESER AT -20°C
ACRYLAMIDE SOLUTIONS SHOULD BE STORED IN A REFRIGERATOR AT 4 °C

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

Date of the receipt of the test materials	
Date of the receipt of the standard solutions of AA	
All items have been received undamaged	YES ☐ / NO ☐
If NO, please list damaged items	

Please return the completed form to

Stefanka Bratinova

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 800. http://jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 3.
E- mail: jrc-EURL-PAH@ec.europa.eu

ANNEX 6: Technical specifications of the calibration solutions

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

Geel, 02/05/2017

Standard solution specification sheet	Acrylamide in water
Date of production: 27/04/2017	Total volume: 1 mL
Expiry date: *October 2017*	

Standard solution composition:

	Product name	CAS	Conc.* (ng/g)	Conc.* (ng/mL)	U** ± %
1	Acrylamide	[79-06-1]	**1990**	**1984**	0.3

** The concentrations were calculated taking into account the purity statements of the single products. The concentration values are based on the gravimetrical preparation data.*

*** U is the expanded uncertainty calculated by multiplying the combined standard uncertainty with the coverage factor 2 (corresponding to a confidence level of 95%). The standard uncertainty is equal to the square root of the sum of the squares of the uncertainties associated with each single operation involved in the preparation of this standard solution.*

Solvent: water

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783.

E-mail: jrc-eurl-pah@ec.europa.eu
Web site: http://ec.europa.eu/jrc

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

Geel, 02/05/2017

Standard solution specification sheet	D_3-acrylamide in water
Date of production: 27/04/2017	Total volume: 1 mL
Expiry date: *October 2017*	

Standard solution composition:

	Product name	CAS	Conc.* (ng/g)	Conc.* (ng/mL)	U** ± %
1	D_3-Acrylamide	[122775-19-3]	**1008**	**1005**	0.6

** The concentrations were calculated taking into account the purity statements of the single products. The concentration values are based on the gravimetrical preparation data.*

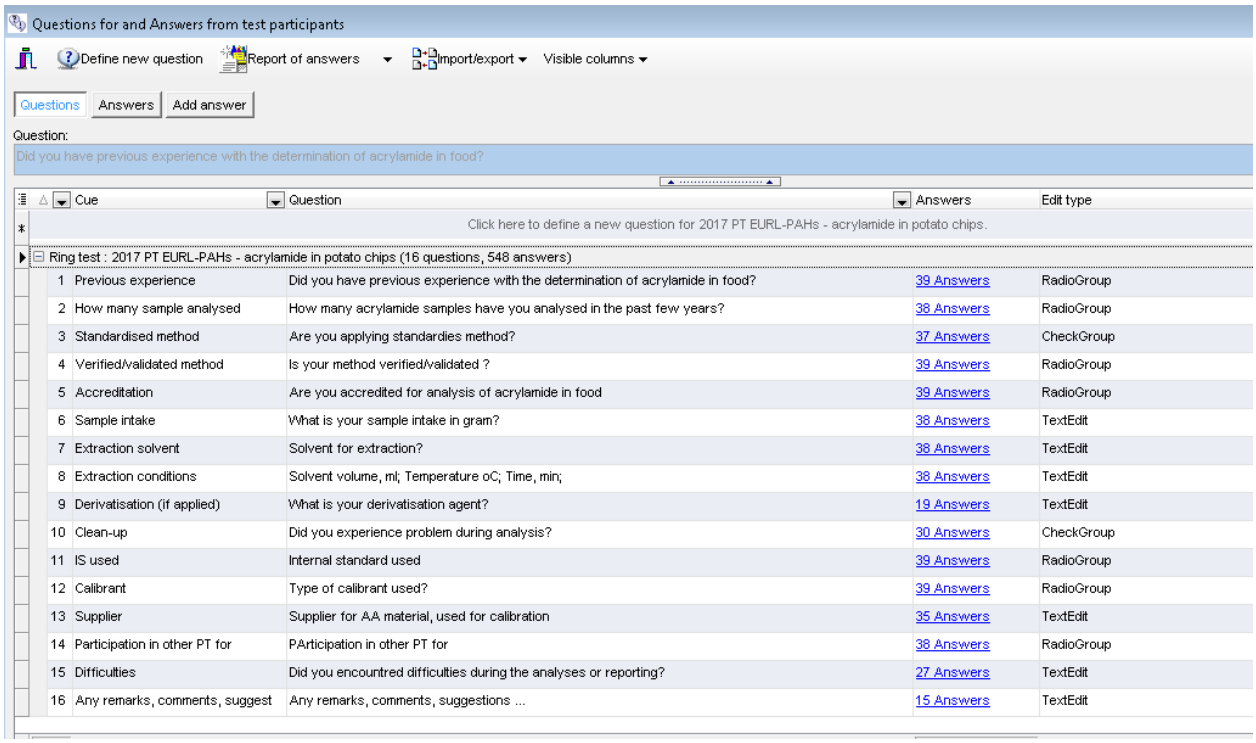
*** U is the expanded uncertainty calculated by multiplying the combined standard uncertainty with the coverage factor 2 (corresponding to a confidence level of 95%). The standard uncertainty is equal to the square root of the sum of the squares of the uncertainties associated with each single operation involved in the preparation of this standard solution.*

Solvent: water

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783.

E-mail: jrc-eurl-pah@ec.europa.eu
Web site: http://ec.europa.eu/jrc

ANNEX 7. Questionnaire & Answers from participants



Questions for and Answers from test participants

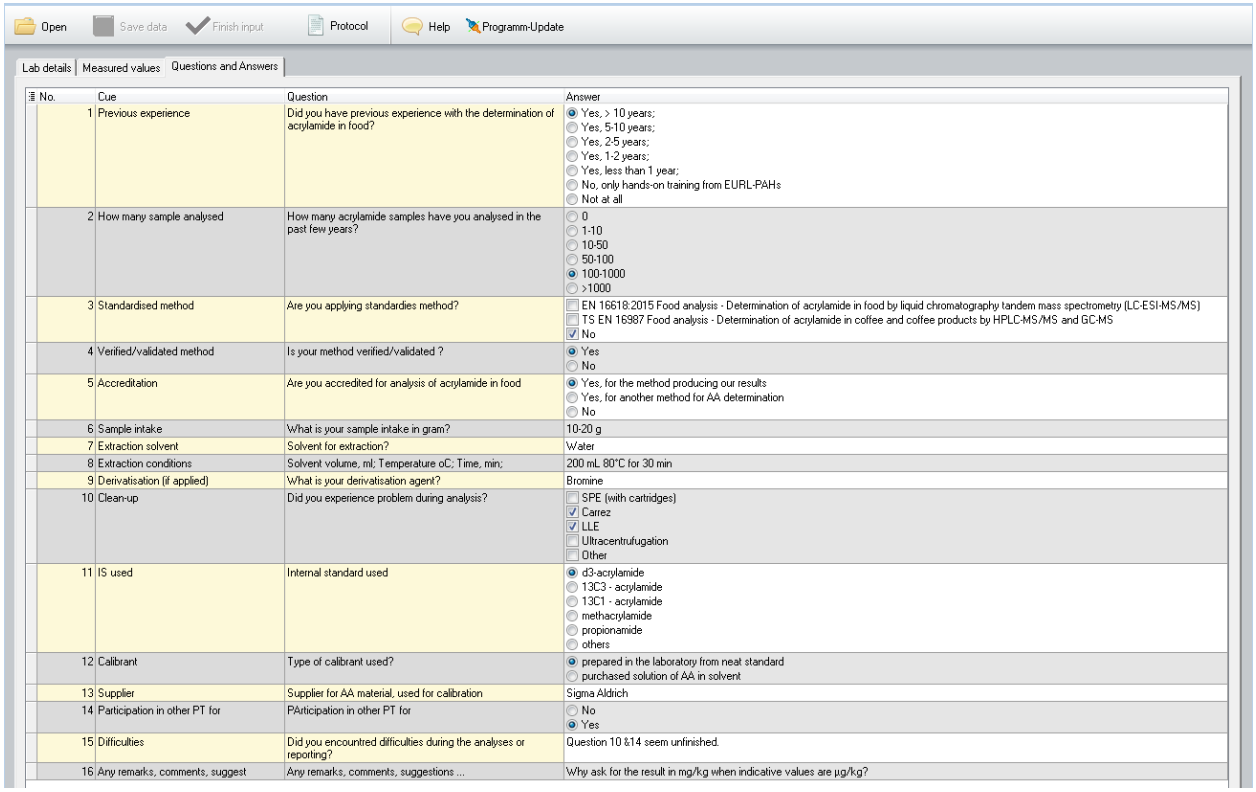
Define new question | Report of answers | Import/export | Visible columns

Questions | Answers | Add answer

Question:

Did you have previous experience with the determination of acrylamide in food?

	Cue	Question	Answers	Edit type
*		Click here to define a new question for 2017 PT EURL-PAHs - acrylamide in potato chips.		
	Ring test : 2017 PT EURL-PAHs - acrylamide in potato chips (16 questions, 548 answers)			
1	Previous experience	Did you have previous experience with the determination of acrylamide in food?	39 Answers	RadioGroup
2	How many sample analysed	How many acrylamide samples have you analysed in the past few years?	38 Answers	RadioGroup
3	Standardised method	Are you applying standardies method?	37 Answers	CheckGroup
4	Verified/validated method	Is your method verified/validated ?	39 Answers	RadioGroup
5	Accreditation	Are you accredited for analysis of acrylamide in food	39 Answers	RadioGroup
6	Sample intake	What is your sample intake in gram?	38 Answers	TextEdit
7	Extraction solvent	Solvent for extraction?	38 Answers	TextEdit
8	Extraction conditions	Solvent volume, ml; Temperature oC; Time, min;	38 Answers	TextEdit
9	Derivatisation (if applied)	What is your derivatisation agent?	19 Answers	TextEdit
10	Clean-up	Did you experience problem during analysis?	30 Answers	CheckGroup
11	IS used	Internal standard used	39 Answers	RadioGroup
12	Calibrant	Type of calibrant used?	39 Answers	RadioGroup
13	Supplier	Supplier for AA material, used for calibration	35 Answers	TextEdit
14	Participation in other PT for	PArticipation in other PT for	38 Answers	RadioGroup
15	Difficulties	Did you encountred difficulties during the analyses or reporting?	27 Answers	TextEdit
16	Any remarks, comments, suggest	Any remarks, comments, suggestions ...	15 Answers	TextEdit



Open | Save data | Finish input | Protocol | Help | Programm-Update

Lab details | Measured values | Questions and Answers

No.	Cue	Question	Answer
1	Previous experience	Did you have previous experience with the determination of acrylamide in food?	(•) Yes, > 10 years; ( ) Yes, 5-10 years; ( ) Yes, 2-5 years; ( ) Yes, 1-2 years; ( ) Yes, less than 1 year; ( ) No, only hands-on training from EURL-PAHs ( ) Not at all
2	How many sample analysed	How many acrylamide samples have you analysed in the past few years?	( ) 0 ( ) 1-10 ( ) 10-50 ( ) 50-100 (•) 100-1000 ( ) >1000
3	Standardised method	Are you applying standardies method?	[ ] EN 16618:2015 Food analysis - Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS) [ ] TS EN 16987 Food analysis - Determination of acrylamide in coffee and coffee products by HPLC-MS/MS and GC-MS [x] No
4	Verified/validated method	Is your method verified/validated ?	(•) Yes ( ) No
5	Accreditation	Are you accredited for analysis of acrylamide in food	(•) Yes, for the method producing our results ( ) Yes, for another method for AA determination ( ) No
6	Sample intake	What is your sample intake in gram?	10-20 g
7	Extraction solvent	Solvent for extraction?	Water
8	Extraction conditions	Solvent volume, ml; Temperature oC; Time, min;	200 mL 80°C for 30 min
9	Derivatisation (if applied)	What is your derivatisation agent?	Bromine
10	Clean-up	Did you experience problem during analysis?	[ ] SPE (with cartridges) [x] Carrez [x] LLE [ ] Ultracentrufugation [ ] Other
11	IS used	Internal standard used	(•) d3-acrylamide ( ) 13C3 - acrylamide ( ) 13C1 - acrylamide ( ) methacrylamide ( ) propionamide ( ) others
12	Calibrant	Type of calibrant used?	(•) prepared in the laboratory from neat standard ( ) purchased solution of AA in solvent
13	Supplier	Supplier for AA material, used for calibration	Sigma Aldrich
14	Participation in other PT for	PArticipation in other PT for	( ) No (•) Yes
15	Difficulties	Did you encountred difficulties during the analyses or reporting?	Question 10 &14 seem unfinished.
16	Any remarks, comments, suggest	Any remarks, comments, suggestions ...	Why ask for the result in mg/kg when indicative values are µg/kg?

Lab Code	1. Previous experience	2. Sample analysed	3. Standardised method	4.Validated method	5. Accreditation	6. Sample intake
21	Not at all	0	No	No	No	1
22	Yes, 2-5 years;	100-1000	EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	Yes	No	2
23	Yes, less than 1 year;	100-1000	EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	No	No	2
24	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	1.5 for crisps, 3 for general samples
25	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
26	Yes, 5-10 years;	50-100		Yes	Yes, for the method producing our results	2
27	Yes, 2-5 years;	50-100	No	Yes	Yes, for the method producing our results	
28	Yes, 5-10 years;	10-50	No	Yes	Yes, for the method producing our results	2-6g depending on matrix, 2 g for potato chips
29	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
30	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	10-20 g
31	Yes, less than 1 year;	1-10	No	Yes	Yes, for another method for AA determination	2
32	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	2 g
33	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
34	Yes, less than 1	50-100	No	No	No	2
36	Yes, 1-2 years;	10-50	No	Yes	Yes, for the method producing our results	2
37	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
38	Yes, > 10 years;	> 1000	No	Yes	Yes, for the method producing our results	5
39	Yes, 5-10 years;	1-10	No	Yes	No	2
51	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	10
52	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	2
53	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	0.5 gram
54	Yes, > 10 years;	> 1000	No	Yes	Yes, for the method producing our results	2
55	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	about 1 g
56	Yes, 2-5 years;	100-1000	No	Yes	Yes, for another method for AA	1 gram
57	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	1 -2 (normally 2, for this PT 1), ambient temperature
58	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	2.5 (values 1 und 2), 1.25 (value 3)
59	Yes, 5-10 years;	100-1000	EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	No	Yes, for another method for AA determination	2,0 g
60	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
61	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	1
62	Yes, 5-10 years;	> 1000	EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	Yes	Yes, for another method for AA determination	2,0
63	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	2
64	Yes, less than 1 year;	50-100	EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	Yes	Yes, for another method for AA determination	2
65	Yes, 5-10 years;	100-1000		Yes	Yes, for the method producing our results	1.5 - 2 g
66	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	usually 20 g, in this PT only 2 g
67	Yes, 5-10 years;	50-100	No	Yes	Yes, for the method producing our results	0.45
69	Yes, 5-10 years;	100-1000	No	Yes	Yes, for the method producing our results	5
70	Yes, 1-2 years;		EN 16618:2015 Food Analysis - Determination of acrylamide in food by liquidchromatography tandem mass spectrometry (LC-ESI-MS/MS)	Yes	Yes, for the method producing our results	5g
71	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	3 g
72	Yes, > 10 years;	100-1000	No	Yes	Yes, for the method producing our results	3

Lab Code	7. Extraction solvent	8. Extraction conditions	9. Derivatisation (if applied)	10. Clean-up	11. IS used
21	water	10 ml, Ambient temperature, 15min mechanical agitation	none	SPE (with cartridges)	d3-acrylamide
22	water	40 ml, RT, 60 min		SPE (with cartridges)	d3-acrylamide
23	Water	40 mL, 40°C, 60 min	-	SPE (with cartridges)	d3-acrylamide
24	water	ultra-turrax 2 min using 30 mL water	no	SPE (with cartridges) / UI	d3-acrylamide
25	H20	30, 25, 2		SPE (with cartridges) / UI	d3-acrylamide
26	actonitrilie/water	10/10 ml, ambient, 30 min		Other	d3-acrylamide
27					d3-acrylamide
28	water	30 ml, 21 oC, 60 min	-	SPE (with cartridges)	d3-acrylamide
29	water, hexane	10ml water, 2ml hexane		SPE (with cartridges)	d3-acrylamide
30	Water	200 mL 80°C for 30 min	Bromine	Carrez / LLE	d3-acrylamide
31	Water	15, 70, 5		Carrez / LLE	d3-acrylamide
32	Water	15 ml water, shake 30 min, room temperature		SPE (with cartridges)	d3-acrylamide
33	acetonitrile	10	no	LLE	d3-acrylamide
34	Methanol water 5:1	18ml, room temperature, overnight	-	LLE	d3-acrylamide
36	water	40, roomtemp, 60			d3-acrylamide
37	ethylacetate	2x10	KBr	LLE / UI	d3-acrylamide
38	Water	Boiling water, 100 ml shaken for 1 hour	Bromine	Carrez	13C3-acrylamide
39	Water	20 ml, room temp., 30 min	bromine	Carrez / UI	d3-acrylamide
51	water	100 ml, room temp., 30 min		Carrez	d3-acrylamide
52	LCMS water	10 ml,25°C,1min	no	Other	d3-acrylamide
53	95:5 water:methanol	10 ml, room temperature, 30 min			d3-acrylamide
54	methanol/water	50 mL, 20°C, 30 min	no		d3-acrylamide
55	Water	40 ml , 40 °C , 45 min			d3-acrylamide
56	2 mol NaCl in Water	15 mL Solvent, minimum: 20 min, Roomtemp.	none		d3-acrylamide
57	Water	40 ml, 2h		SPE (with cartridges)	d3-acrylamide
58	water	50 ml, 70 oC, 30 min	bromine	SPE (with cartridges) / Carrez	d3-acrylamide
59	water	40 ml, 25°C, 60 min		SPE (with cartridges)	d3-acrylamide
60	water/hexane/acetonitrile	10/10/10, ambient, < 1min	none	Other	d3-acrylamide
61	water	38 ml, 40 °C, 10 min		SPE (with cartridges) / Carrez	d3-acrylamide
62	Water	40 , Ambient , 60	none	SPE (with cartridges)	d3-acrylamide
63	water	100 ml, 40°C, 15 min ultrasonic		SPE (with cartridges) / Carrez	d3-acrylamide
64	Water	40, 20, 60		SPE (with cartridges)	d3-acrylamide
65	water	20 ml, ultrasonic bath at 50 °C for 30 min	none		d3-acrylamide
66	1-Propanol	usually 50 ml 1-Propanol, extraction 30 min RT and 30 min 50-60°C			d3-acrylamide
67	Water	4.9 ml, ambient, 30 min		SPE (with cartridges) / Carrez / UI	13C3-acrylamide
69	Acetate Ethyl	Extraction 1 h with water. Centrifugation. Extraction Liq/Liq with heptane. The aqueous phase is kept. The acrylamide is extracted from the Extrelut phase (column) with ethyl acetate. The eluate is evaporated to dryness and then taken up in 500 µl of ethyl acetate.	none		d3-acrylamide
70	acétonitrile	10ml , 10 min		SPE (with cartridges)	13C1-acrylamide
71	water	50 ml, ambient temperatur, 30 min		LLE	d3-acrylamide
72	water	30 ml, 60oC, 30 min	No	SPE (with cartridges) / Carrez / UI	d3-acrylamide

Lab Code	12. Calibrant	13. Supplier	14. Participation in other PT for AA
21	purchased solution of AA in solvent	Dr Ehrenstorfer	No
22	prepared in the laboratory from neatstandard	LGC, Dr Ehrenstorfer	Yes
23	prepared in the laboratory from neatstandard	Sigma Aldrich	Yes
24	prepared in the laboratory from neatstandard	Sigma Aldrich	Yes
25	prepared in the laboratory from neatstandard	Sigma-Aldrich, Fluka and Cambridge, Isotope Lab (d3-acrylamide)	Yes
26	prepared in the laboratory from neatstandard	Sigma Aldrich	Yes
27	purchased solution of AA in solvent		Yes
28	prepared in the laboratory from neatstandard	Sigma-Aldrich for AA, Polymer Source Inc for d3-AA	Yes
29	prepared in the laboratory from neatstandard	ACROS Organics	Yes
30	prepared in the laboratory from neatstandard	Sigma Aldrich	Yes
31	prepared in the laboratory from neatstandard	chem service	No
32	prepared in the laboratory from neatstandard	Dr.Ehrenstorfer, CIL USA	Yes
33	prepared in the laboratory from neatstandard	Sigma-Aldrich	Yes
34	prepared in the laboratory from neatstandard	Sigma-Alldrich	No
36	prepared in the laboratory from neatstandard	Merck	Yes
37	prepared in the laboratory from neatstandard	Sigma	Yes
38	prepared in the laboratory from neatstandard	Sigma	Yes
39	purchased solution of AA in solvent	Sigma-Aldrich	No
51	purchased solution of AA in solvent	Ultra Scientific	Yes
52	prepared in the laboratory from neatstandard		Yes
53	prepared in the laboratory from neatstandard	Sigma	Yes
54	prepared in the laboratory from neatstandard		Yes
55	purchased solution of AA in solvent	Campro Scientific (d3-acrylamide), LGC (acrylamide)	Yes
56	prepared in the laboratory from neatstandard	Dr. Ehrenstorfer	Yes
57	prepared in the laboratory from neatstandard	Ultra Scientific, Sigma-Aldrich	Yes
58	purchased solution of AA in solvent	Ultra scientific	Yes
59	prepared in the laboratory from neatstandard	Sigma Aldrich, Fluka	Yes
60	prepared in the laboratory from neatstandard	LGC	Yes
61	prepared in the laboratory from neatstandard	Merck	Yes
62	prepared in the laboratory from neatstandard	Sigma	Yes
63	prepared in the laboratory from neatstandard	Sigma Aldrich, Cambridge Isotope	
64	prepared in the laboratory from neatstandard	Sigma-Aldrich	Yes
65	prepared in the laboratory from neatstandard	Alfa Aesar GmbH (AA), Promochem (d3-AA)	Yes
66	prepared in the laboratory from neatstandard	Fluka	Yes
67	prepared in the laboratory from neatstandard	Sigma	Yes
69	prepared in the laboratory from neatstandard	Merck (Acrylamide) et CDN Isotope (Acrylamide d3)	Yes
70	purchased solution of AA in solvent		Yes
71	prepared in the laboratory from neatstandard	Fluka	Yes
72	prepared in the laboratory from neatstandard	Fluka	Yes

Lab Code	15. Difficulties	16. Any remarks, comments, suggest
21	Yes, difficulties to separate interferences, high level of noise background leading to high LOQ/LOD	We made this method just for this PT, we have no control chart, our uncertainty has been calculated by taking the RSD on the triplicate . We have no enough data to calculate it properly.
22		
23	No	-
24	We moved to a new addres so everything we did was the first time in the new lab, using the balance, the SPE cleanup, new and different brand LC-MS/MS	one of our recovery on acrisp was too low, however the recovery on then EURL sample was fine so we report.
25		
26	no	stanadard NMKL 185
27		
28	no	
29	matrix effects	the result was further confirmed by standard additions technique
30	Question 10 &14 seem unfinished.	Why ask for the result in mg/kg when indicative values are µg/kg?
31		
32	no	
33	No	
34	no	We often observe low recoveriesof the internal standrads for the analysis of acrylamide. We are very curious of this is generally observed.
36	No	
37	No	
38		
39	No	No
51	a scaled-down version of our method resulted in severe chromatographic problems, which we have never experienced for other samples so far	due to limited sample amount only 2 g sample were used, due to difficulties in sample preparation (see question 15) the extraction volume was kept constant and not reduced
52	no	
53	no	
54	No	
55		
56	no	
57		
58		Value 3 is not used in calculation of final value, because sample intake of 1.25 g for this replicate is not used in routine, We assumed, that question 10 asked about the used purification steps and answer that. Problems were not experienced.
59	no	
60	no	Differences between our lab-standard and the EURL standard
61	no	
62		Uncertainty is standard deviation
63		
64		data in mg/kg !!!
65	none	The standardised method used is a modified (GC-MS with PCI) German §64-Method L46.00-5, which is based on DIN 10785:2013. This is a prior version to EN 16987.
66	yes, due to small portion of test material we had to reduce our usual test portion down to 2 g and therefore we also reduced extraction solvent proportionally, as a consequence the slurry obtained was very stiff, firm and was difficult to solubilize	
67	No	
69	none	The calibrants are purchased in powder form and the solutions are then prepared by the lab
70	non	
71		
72	No	

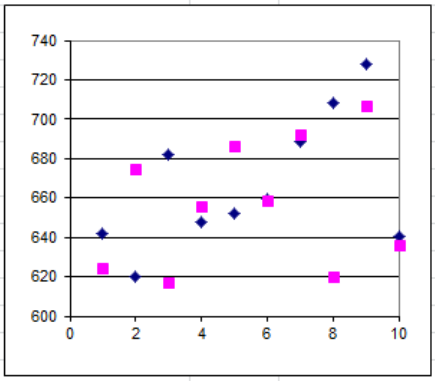
Annex 8. Method performance LOD and LOQ as reported, μg/kg

Lab	LOD	LOQ	Analytical method
21	0.25	0.5	LC-MS/MS
22	0.01	0.032	LC-MS/MS
23	0.008	0.027	LC-MS/MS
24	0.01	0.02	LC-MS/MS
25		0.004	LC-MS/MS
26	0.015	0.05	LC-MS/MS
27	0.001	0.05	LC-MS`/MS
28	0.024	0.075	LC-MS/MS
29	0.03	0.09	LC-MS/MS
30	0.006	0.02	GC-MS(MS)
31	0.003	0.01	LC-MS/MS
32	0.01	0.03	LC-MS/MS
33	0.015	0.05	LC-MS/MS
34	0.023	0.046	LC-MS/MS
39	0.01	0.02	GC-MS(MS)
35			
36		0.005	LC-MS/MS
37	0.003	0.01	GC-MS(MS)
38	0.01	0.03	GC-MS(MS)
51		0.05	LC-MS/MS
52	0.015	0.03	LC-MS/MS
53	0.01	0.02	LC-MS/MS
54	0.01	0.02	LC-MS/MS
55	0.015	0.06	LC-MS/MS
56	0.01	0.03	LC-MS/MS
57	0.05	0.1	LC-MS/MS
58	0.02	0.05	GC-MS(MS)
59	0.025	0.05	LC-MS/MS
60	0.032	0.01	GC-MS(MS)
61	0.006	0.019	LC-MS/MS
62	0.01	0.025	LC-MS/MS
63	0.01	0.03	LC-MS/MS
64	0.01	0.03	LC-MS/MS
65	0.02	0.067	GC-MS(MS)
66	0.002	0.011	LC-MS/MS
67		0.05	LC-MS/MS
68			LC-MS/MS
69	0.006	0.02	GC-MS(MS)
70	0.01	0.02	LC-MS/MS
71	0.03	0.1	GC-MS(MS)
72	0.003	0.025	LC-MS/MS

ANNEX 9: Homogeneity of the potato chips test material

	n =	10		
	mean =	662.0323	22%	= **σ-trg(%)**
648.2555	s_x =	25.46086	145.647	= **σ-trg**
√MSW =	s_w =	29.13267		
sbb=ss	s_s =	14.96326	43.6941	= **0,3*σ**
	ISO-13528	passed		
	F =	1.527621	3.02038	= Fcrit
		passed		
	IUPAC			
	(MSB-MSW)/2	223.8991	4446.45	= F1*(0,3*σ)²+F2*MSW
		passed		

Bottle	Result a	Result b	diff	sum	avg
bottle 01	642	624	17.47	1265.76	632.88
bottle 13	620	675	-54.80	1295.18	647.59
bottle 20	682	617	64.68	1298.70	649.35
bottle 22	648	656	-8.05	1303.62	651.81
bottle 31	652	686	-34.46	1338.04	669.02
bottle 34	659	659	0.32	1317.86	658.93
bottle 43	689	692	-3.29	1380.88	690.44
bottle 46	708	620	88.02	1328.52	664.26
bottle 50	728	707	21.32	1435.15	717.58
bottle 55	640	636	4.04	1276.94	638.47
		Σ(diff)² =	16974.3		
		var(sum)/2 =		1296.51	=MSB

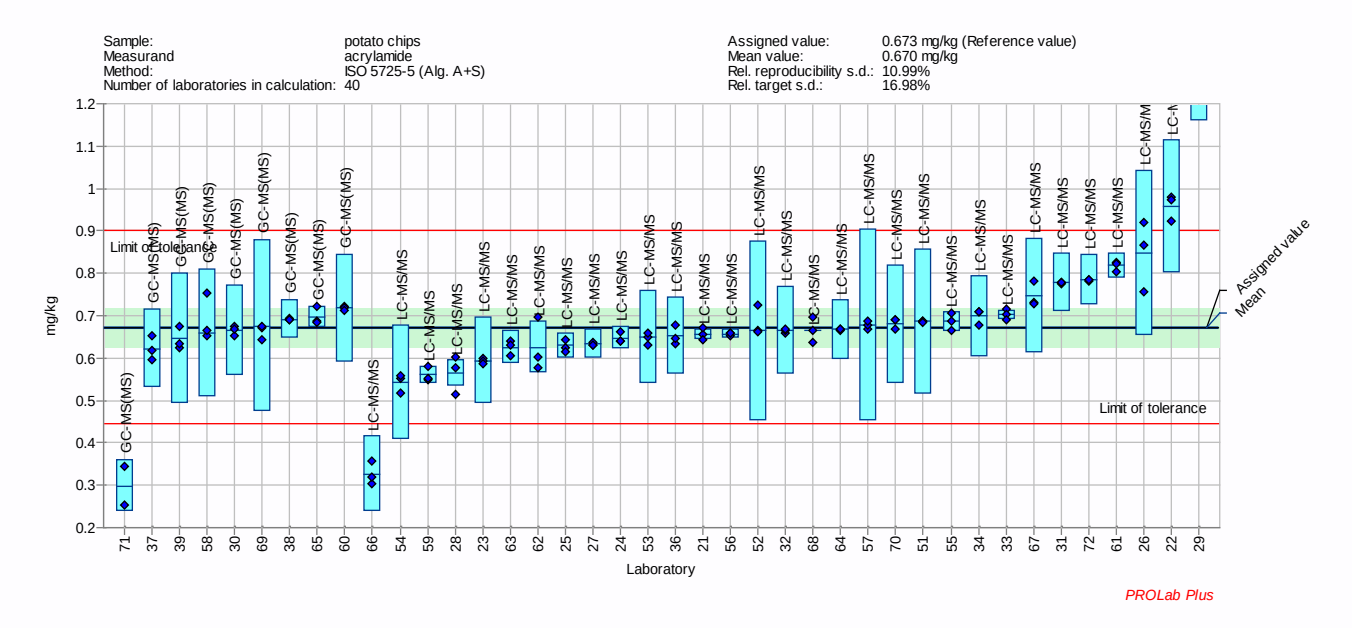


ANNEX 10: Data reported by participants

The data reported by the participants are compiled and presented in the following graphs and table. The results of replicate analyses together with the expanded measurement uncertainty (k=2) reported for the value for proficiency assessment are depicted in the graphs. Red lines indicate the thresholds for satisfactory z-scores. "Mean values" and "Rel. reproducibility s.d." represent the robust mean values and the robust relative standard deviations of the participants data, calculated according to the ISO 13528 algorithm (ISO 5725-5, Algorithm A+S).

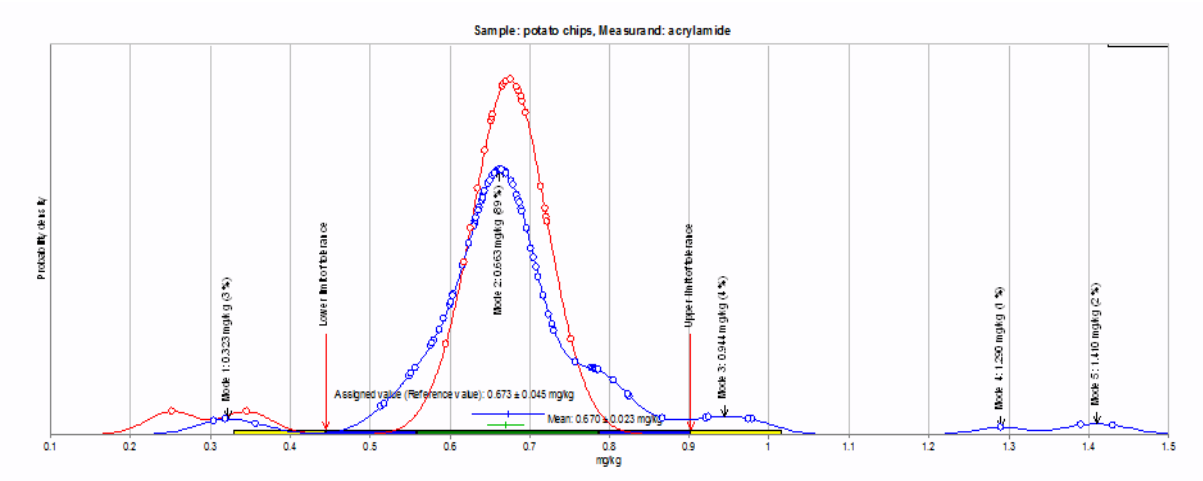
Distribution of individual results of replicate determinations reported for the acrylamide (AA) content in potato chips test sample

blue rombus: individual results of replicate determinations, blue box: reported expanded measurement uncertainty (k=2), blue horizontal line in blue box: average of replicate determinations, green line: assigned value, green area around assigned value: expanded uncertainty of the assigned value (k=2), red lines: lower and upper limit of satisfactory z-score range; green band: confidence interval of the assigned value



Kernel density plot of the reported values for proficiency assessment for the acrylamide (AA) content in the potato chips test sample

Blue dots and line - HPLC results; red dots and lines - GC-mass spectrometry results



Results, as reported by the participants and scoring, for the content of AA in potato chips test sample.

Due to a software problem, the reported significant zeros after the comas are missing
Assigned value: xpt ± U(xpt) (k=2) = 0.673 ± 0.045 mg/kg; $\square_{pt}$ = 0.114 mg/kg ; $\square_{pt\%}$ = 17 %

Lab code	M 1	M 2	M 3	X lab	U lab	k	Analytical method	u lab	Z-Score	Zeta score	Classification
21	0.656	0.671	0.644	0.657	0.013	2	LC-MS/MS	0.006	-0.1	-0.7	b
22	0.979	0.924	0.974	0.959	0.157	2	LC-MS/MS	0.079	2.5	3.5	a
23	0.6	0.593	0.587	0.593	0.102	2	LC-MS/MS	0.051	-0.7	-1.4	a
24	0.64	0.641	0.663	0.648	0.026	2	LC-MS/MS	0.013	-0.2	-1	b
25	0.624	0.642	0.616	0.63	0.03	2	LC-MS/MS	0.015	-0.4	-1.6	b
26	0.866	0.757	0.921	0.848	0.195	2	LC-MS/MS	0.098	1.5	1.7	a
27	0.631	0.636	0.631	0.633	0.035	2	LC-MS/MS	0.018	-0.4	-1.4	b
28	0.514	0.576	0.601	0.564	0.032	2	LC-MS/MS	0.016	-1	-3.9	b
29	1.39	1.29	1.43	1.37	0.21	2	LC-MS/MS	0.105	6.1	6.5	a
30	0.653	0.676	0.668	0.666	0.106	2	GC-MS(MS)	0.053	-0.1	-0.1	a
31	0.777	0.78	0.779	0.779	0.07	2	LC-MS/MS	0.035	0.9	2.5	a
32	0.658	0.665	0.67	0.666	0.104	2	LC-MS/MS	0.052	-0.1	-0.1	a
33	0.717	0.702	0.689	0.703	0.011	2	LC-MS/MS	0.006	0.3	1.3	b
34	0.679	0.708	0.711	0.699	0.096	2	LC-MS/MS	0.048	0.2	0.5	a
36	0.677	0.633	0.648	0.653	0.091	2	LC-MS/MS	0.046	-0.2	-0.4	a
37	0.595	0.654	0.618	0.622	0.093	2	GC-MS(MS)	0.046	-0.4	-1	a
38	0.695	0.691	0.689	0.692	0.045	2	GC-MS(MS)	0.023	0.2	0.6	a
39	0.676	0.626	0.635	0.646	0.155	2	GC-MS(MS)	0.078	-0.2	-0.3	a
51	0.687	0.686	0.684	0.686	0.171	2	LC-MS/MS	0.086	0.1	0.1	a
52	0.664	0.663	0.724	0.664	0.213	2	LC-MS/MS	0.106	-0.1	-0.1	a
53	0.65	0.63	0.66	0.65	0.111	2	LC-MS/MS	0.055	-0.2	-0.4	a
54	0.552	0.557	0.518	0.542	0.135	2	LC-MS/MS	0.067	-1.1	-1.8	a
55	0.665	0.705	0.687	0.686	0.023	2	LC-MS/MS	0.012	0.1	0.5	b
56	0.66	0.653	0.658	0.657	0.01	2	LC-MS/MS	0.005	-0.1	-0.7	b
57	0.669	0.679	0.686	0.678	0.225	2	LC-MS/MS	0.113	0	0	a
58	0.652	0.666	0.752	0.659	0.151	2	GC-MS(MS)	0.075	-0.1	-0.2	a
59	0.549	0.552	0.58	0.561	0.02	2	LC-MS/MS	0.01	-1	-4.5	b
60	0.721	0.719	0.714	0.718	0.128	2	GC-MS(MS)	0.064	0.4	0.7	a
61	0.805	0.825	0.823	0.818	0.03	2	LC-MS/MS	0.015	1.3	5.4	b
62	0.604	0.578	0.696	0.626	0.062	2	LC-MS/MS	0.031	-0.4	-1.2	a
63	0.64	0.632	0.605	0.626	0.04	2	LC-MS/MS	0.02	-0.4	-1.6	b
64	0.665	0.669	0.667	0.667	0.07	2	LC-MS/MS	0.035	-0.1	-0.1	a
65	0.686	0.722	0.684	0.697	0.025	2	GC-MS(MS)	0.012	0.2	0.9	b
66	0.357	0.305	0.319	0.327	0.09	2	LC-MS/MS	0.045	-3	-6.9	a
67	0.731	0.782	0.728	0.747	0.134	2	LC-MS/MS	0.067	0.6	1	a
68	0.696	0.664	0.637	0.666	0	2	LC-MS/MS	0	-0.1	-0.8	b
69	0.644	0.671	0.676	0.676	0.204	2	GC-MS(MS)	0.102	0	0	a
70	0.69	0.69	0.67	0.68	0.139	2	LC-MS/MS	0.07	0.1	0.1	a
71	0.346	0.252		0.299	0.06	2	GC-MS(MS)	0.03	-3.3	-10	a
72	0.783	0.784	0.786	0.784	0.06	2	LC-MS/MS	0.03	1	3	a

Satisfactory, Questionable, **Unsatisfactory**
a : $u_{ref} \leq u_{lab} \leq u_{max}$ (σ_p);
b : $u_{lab} < u_{ref}$;
c : $u_{lab} > u_{max}$ (σ_p)

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