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# Report on the inter-laboratory comparison organised by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

*Four marker PAHs in smoked black pepper*

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## Abstract

This report presents the results of the inter-laboratory comparison (ILC) organised as a proficiency test (PT) by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons (EURL PAHs) on the determination of the four EU marker PAHs, benz[a]anthracene (BAA), benzo[a]pyrene (BAP), benzo[b]fluoranthene (BBF) and chrysene (CHR) in smoked black pepper .

The test material used in this exercise was commercial smoked black peppercorns acquired from an on-line store, which were in the EURL PAH laboratories finely ground and homogenised. Participants also received a solution of PAHs in the solvent of their choice (either toluene or acetonitrile) with known PAH content for the verification of their instrument calibration.

Both officially nominated National Reference Laboratories (NRLs) and official food control laboratories (OCLs) of the EU Member States participated in the study. Twenty-nine NRLs and 18 OCLs subscribed for participation.

The test material was characterised at the EURL PAH. The assigned values and their uncertainties were determined from independent replicate measurements on two different days by a primary method of measurement.

Participants were free to choose the method of analysis. The performance of the participating laboratories in the determination of the target PAHs in the test material was expressed by both z-scores and zeta-scores. Additionally, the compliance of reported method performance characteristics was checked against specifications given in legislation.

This PT demonstrated the competence of the participating laboratories in the analysis of regulated PAHs in smoked black pepper. About 67% percent of the reported test results were graded with z-scores below an absolute value of two, indicating acceptable agreement with the independently assigned values of the test material.

The EURL PAH asked participants to assess also compliance of the sample with legislative limits. Seventy one percent of the participants, who answered to this question, assessed the compliance of the test sample with EU legislation correctly.

The EURL-PAH is operated by a JRC Unit, which is an ISO/IEC 17043 accredited PT provider and the respective rules were applied during all phases of this PT.

# 1. Introduction

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

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic substances. The chemical structure of PAHs consists of two or more fused aromatic rings. PAHs may be formed during the incomplete combustion of organic matter and can be found in the environment. In food, PAHs may be formed during industrial food processing and domestic food preparation, such as smoking, drying, roasting, baking, frying, or grilling [3,4].

Of the many hundreds of different PAHs, benzo[a]pyrene is, the most studied which is often used as a marker for PAHs in ambient air and food [5]. The European Commission revised in 2011 legislation on PAHs taking thereby into consideration the conclusions drawn by the European Food Safety Authority (EFSA) on "Polycylic Aromatic Hydrocarbons in Food" [6]. New maximum levels (MLs) for the sum of four substances (PAH4) - benzo[*a*]pyrene (BAP), benz[*a*]anthracene (BAA), benzo[*b*]fluoranthene (BBF) and chrysene (CHR), (Table 1) were introduced whilst a separate maximum level for benzo[*a*]pyrene was maintained [7, 8].

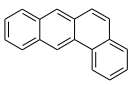
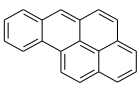
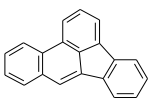
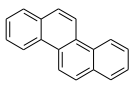
In recent years, high levels of PAHs were found in dried herbs and dried spices which were reasoned by the application of bad drying practices. Consequently, maximum levels were set for PAHs in dried herbs and dried spices, laid down in Commission Regulation (EU) 2015/1933 from 27 October 2015 [9].

Traditional smoking and processing methods applied for the production of smoked paprika and smoked cardamom resulted in high levels of PAHs. However, given that the consumption of these spices is low, and to enable these smoked products to remain on the market, they were exempted from the maximum levels set by the Commission Regulation [9].

Along with salt, pepper is one of the oldest and best-known spices. A small portion of the traded pepper is smoked before sale over hickory wood for achieving a subtle, yet smoky flavour. Smoked black pepper is not exempted from Commission Regulation (EU) 2015/1933 and has therefore to comply with the set maximum levels [9].

In support to the implementation of the Commission Regulation (EU) No 2015/1933 [9], the EURL PAH agreed with NRLs to focus in the 2016 EURL PAH proficiency test (PT) exercise on the determination of PAHs in herbs and spices, in particular in smoked black pepper.

**Table 1:** Names and structures of the four EU marker PAHs.

| | | | | | |
|---|---|---|---|---|---|
| **1** | **Benz[*a*]anthracene (BAA)** |  | **2** | **Benzo[*a*]pyrene (BAP)** |  |
| **3** | **Benzo[*b*]fluoranthene (BBF)** |  | **4** | **Chrysene (CHR)** |  |

# 2. Scope

As specified in Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with food and feed law, animal health and animal welfare rules [2], one of the core duties of EURLs is to organise comparative testing.

This PT aimed to evaluate the comparability of results reported by NRLs and EU official food control laboratories (OCLs) for the four EU marker PAHs in smoked black pepper. The appropriateness of the reported measurement uncertainty was also evaluated as this parameter is important in the compliance assessment of food with EU maximum levels.

The PT was designed and evaluated under the umbrella of the organiser's accreditation according to ISO/IEC 17043:2010 [10].

# 3. Setup of the exercise

## 3.1 Participating Laboratories

Officially nominated NRLs and OCLs of the EU Member States were admitted as participants. The participants are listed in Table 2 and Table 3, respectively.

**Table 2:** List of participating National Reference Laboratories (NRL)

| *Institute* | *Country* |
|---|---|
| AGES GmbH | Austria |
| Scientific Institute of Public Health (WIV-ISP) | Belgium |
| Croatian Veterinary Institute - Branch Veterinary Institute of Split | Croatia |
| State General Laboratory | Cyprus |
| State Veterinary Institute Prague | Czech Republic |
| Bundesamt für Verbraucherschutz und Lebensmittelsicherheit | Germany |
| Danish Food Administration | Denmark |
| Health Board | Estonia |
| Centro Nacional de Alimentación.<br>Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) | Spain |
| Finnish Food Safety Authority Evira | Finland |
| LABERCA - Oniris | France |
| General Chemical State Laboratory | Greece |
| National Food Chain Safety Office, Feed Investigation | Hungary |
| National Food Chain Safety Office, Food and Feed Safety Directorate | Hungary |
| Istituto Superiore di Sanità (ISS) | Italy |
| Public Analyst Laboratory | Ireland |
| National Food and Veterinary Risk Assessment Institute | Lithuania |
| Laboratoire National de Santé | Luxembourg |

| | |
|---|---|
| Institute of Food Safety, Animal Health and Environment "BIOR" | Latvia |
| RIKILT | the Netherlands |
| National Institute of Public Health - National Institute of Hygiene | Poland |
| ASAE - Autoridade de Seguranca Alimentar e Economica | Portugal |
| Swedish National Food Agency | Sweden |
| Institute of Public Health Maribor, Institute of Environmental Protection | Slovenia |
| State Veterinary and Food Institute Dolny Kubin | Slovakia |
| Fera Science Ltd | UK |

From the 28 NRLs, 2 NRLs did not report results.

**Table 3:** List of participating Official Food Control Laboratories (OCL)

| *Institute* | *Country* |
|---|---|
| LVA GmbH Klosterneuburg | Austria |
| Institut für Umwelt und Lebensmittelsicherheit des Landes Vorarlberg | Austria |
| Federal Laboratory for the Safety of Food chain, Tervuren | Belgium |
| SGS Bulgaria Ltd. | Bulgaria |
| Finnish Customs Laboratory | Finland |
| CVUA-MEL | Germany |
| Thüringer Landesamt für Verbrauchrschutz | Germany |
| Landeslabor Berlin-Brandenburg | Germany |
| Bavarian Food Safety Authority - Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit | Germany |
| CVUA Karlsruhe | Germany |
| CVUA-RRW | Germany |
| Landesuntersuchtungsamt für Chemie, Hygiene und Veterinärmedizin | Germany |
| Landesuntersuchungsanstalt für das Gesundheits-und Veterinärwesen Sachsen | Germany |
| LAVES (Lower Saxony State Office for Consumer Protection and Food Safety) | Germany |
| Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche | Italy |
| Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna | Italy |
| NofaLab B.V. | The Netherlands |
| Dr. A. Verwey B.V. | The Netherlands |
| Edinburgh Scientific Services | UK |
| Glasgow Scientific Services | UK |

From the 21 registered OCLs, 1 OCLs did not report results.

## 3.2 Time frame

The PT was announced on the IRMM web page (see ANNEX 1) and invitation letters were sent to the laboratories on 01 February 2016 (see ANNEX 2) with deadline for registration via EUSurvey webpage (see ANNEX 3) until 22 February 2016. Test samples were dispatched (see ANNEX 4) on 15-16 March 2016 and the deadline for reporting of results was set to 22 April 2016. The documents sent to the participants are presented in ANNEX 5.

## 3.3 Confidentiality

The laboratory codes of participants are disclosed only to the participants, unless they were enrolled in the study by a third party, covering the participation fee. In this case the codes of the respective laboratories will be also disclosed to the enrolling third party. In all other cases codes will only be disclosed on a request and upon the written consent of the participant.

## 3.4 Design of the proficiency test

The design of the PT foresaw triplicate analysis of the test items and reporting of individual results of replicate analyses for individual analytes based on the mass of the entire test portion (on product basis). Additionally a "values for proficiency assessment", in the following denoted as "final values", were requested for both the single analytes and the sum of the four PAHs. They had to be expressed on product basis as well. All results had to be reported corrected for recovery; the "final values" had also to be accompanied by the respective expanded measurement uncertainties and the corresponding coverage factors. Only final values were used for performance assessment.

Participants were asked to report besides analysis results also details of the performance of the applied analytical method (see ANNEX 9). Additionally, the EURL PAH asked participants (NRLs and official control laboratories) to assess the compliance of the sample according to the current legislative limits.

Each participant received at least one ampoule of a solution of the target PAHs in the chosen solvent (2 ml), with known content, and one amber glass vial containing the smoked black pepper test material.

# 4. Test materials

## 4.1 Preparation

The test item of this PT was smoked black pepper. Participants also received a solution of the 4 EU markers PAHs either in acetonitrile or in toluene (according to their choice, see ANNEX 5) with known concentrations, which allowed them to check their instrument calibration against an independent reference. Participants received the technical specifications (see ANNEX 6) of the chosen solution together with the test material.

The smoked black pepper powder test item was prepared at the EURL PAH starting from two kilos of smoked black peppercorns, acquired via an on-line shop. The material was ground to a fine powder and homogenized. Aliquots of about 25 g were packed in amber glass screw cap vials and stored in a refrigerator at about 4 °C.

The standard solutions were prepared from neat reference substances checked against certified reference materials (NIST). Single standard stock solutions of each analyte

were produced from neat substances on a microbalance and dissolution in toluene. Mixed standards were prepared gravimetrically from the single standard stock solutions in the respective solvents and further diluted to the concentrations specified in ANNEX 6. The standard solutions were ampouled under inert atmosphere and flame sealed in 2 ml amber glass ampoules.

## 4.2 Homogeneity and stability

The smoked black pepper powder 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]. Homogeneity experiments consisted of sample extraction by pressurized liquid extraction, size-exclusion chromatography followed by solid phase extraction clean-up and gas-chromatography with mass-spectrometric detection. The method precision complied with the requirements laid down in ISO 13528:2015 [11].

Homogeneity experiments included duplicate analysis of 10 samples randomly selected along the filling sequence among the amber glass vials prepared for dispatch. The duplicate analyses were performed in random order. The test material was rated sufficiently homogenous and no trend was observed. Details of the homogeneity tests are given in ANNEX 7.

The stability of the test material was evaluated following the requirements in ISO13528:2015. Six randomly selected samples were stored at two different conditions over the period from the dispatch of the material to the end of the submission of the results.

The first set of 3 samples was stored in a refrigerator at recommended conditions (~ +4 °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). After the deadline for reporting of results had expired, all 6 samples were analysed in duplicate under repeatability conditions.

Significant differences of the analyte contents of the test samples were not found. Hence stability of the test samples can be assumed over the whole period of the study provided that the recommended storage conditions were applied (ANNEX 8)

## 4.3 Assigned value and standard deviation for proficiency assessment

The assigned values were determined at the EURL PAH applying an analytical method based on isotope dilution mass spectrometry (WI-D-0607) [12]. This implied the preparation of standard solutions from two totally independent sources - NIST SRM 2260a and neat certified reference materials BCR® from IRMM. The analytical method was fully validated by collaborative trial and is accredited according to ISO 17025. This method became recently a European standard EN16619:2015. The respective associated uncertainties of the assigned values were calculated based on GUM approach [13].

The assigned value for the sum of 4 PAH was calculated from the individual assigned values, and its corresponding uncertainty was calculated from the uncertainties of the individual assigned values according to law of error propagation considering covariances.

The standard deviation for proficiency assessment, $\sigma_P$, was set for the individual analytes equal to the maximum tolerable uncertainty (Uf), which is calculated according to Equation 1 [8]. A LOD value of 0.30 µg/kg, and $\alpha$ equal to 0.2 were applied for this purpose. The standard deviation for proficiency testing was calculated for the SUM4PAH parameter from the $\sigma_P$ - values of the individual analytes applying the law of error propagation.

Equation 1 $$u_f = \sqrt{(\mathrm{LOD}/2)^2 + (\alpha \mathrm{C})^2}$$ [7]

where $u_f$ relates to the maximum tolerated standard measurement uncertainty, LOD to the limit of detection, α to a numeric factor depending on the concentration C as given in Commission Regulation (EC) No 333/2007, amended by Regulation (EC) 836/2011 [8].

**Table 4:** Assigned values and their associated expanded uncertainties (k=2) for the smoked black pepper test item, expressed based on mass of entire product (on product basis).

| Analyte | Analyte short name | Assigned value | U | $\sigma_P$ | |
|---|---|---|---|---|---|
| | | µg/kg | µg/kg | µg/kg | % |
| Benz[*a*]anthracene | BAA | **34.22** | **2.06** | 6.85 | 20.0 |
| Chysene | CHR | **39.84** | **3.34** | 7.97 | 20.0 |
| Benzo[*b*]fluoranthene | BBF | **17.16** | **1.28** | 3.44 | 20.0 |
| Benzo[*a*]pyrene | BAP | **14.40** | **1.04** | 2.88 | 20.0 |
| Sum of the four marker PAHs | SUM4PAH | **105.62** | **4.26** | 11.42 | 10.8 |

$\sigma_p$ standard deviation for proficiency assessment.

U expanded uncertainty of the assigned value (k=2).

# 5. Evaluation of laboratories

## 5.1 General

The most important evaluation parameter was the performance of the laboratories in the determination of the target PAHs in the test material, which was expressed by z-scores [11]. zeta-Scores were calculated in addition considering the uncertainty of the test results as estimated by each participant.

The compliance with legislation of the performance characteristics of the analytical methods applied by the participants for the analysis of the test sample was evaluated as well.

The results as reported by participants are listed in ANNEX 10. In case the coverage factor k was not reported by the participant, a coverage factor of two was assumed.

## 5.2 Evaluation parameter

**z-Scores**

z-Scores were calculated based on the final values. Equation 2 presents the formula for calculation of z-scores.

Equation 2 $$z = \frac{\left(x_{lab} - X_{assigned}\right)}{\sigma_P}$$ [9]

where z refers to the z-score, $x_{lab}$ to the reported "final value", $X_{assigned}$ to the assigned value, and $\sigma_P$ to the standard deviation for proficiency testing.

**zeta-Scores**

In addition to z-scores, zeta-scores were calculated. In contrast to z-scores, zeta-scores describe the agreement of the reported result with the assigned value within the respective uncertainties. zeta-Scores were calculated according to Equation 3.

Equation 3

$$zeta = \frac{x_{lab} - X_{assigned}}{\sqrt{u_{lab}^2 + u_{assigned}^2}} \quad [9]$$

where zeta refers to the zeta-score, $x_{lab}$ to the reported "final value", $X_{assigned}$ to the assigned value, $u_{lab}$ to the standard measurement uncertainty of the reported result, and $u_{assigned}$ to the standard uncertainty of the assigned value.

Whenever uncertainties of reported results were not provided by the participants, they were set in Equation 3 to zero, which is most unfavourable for zeta score calculation.

Unsatisfactorily large zeta-scores might be caused by underestimated measurement uncertainties, large bias, or a combination of both. Therefore, reported uncertainties were checked against the uncertainties of the reference values. It should be mentioned that some laboratories might have reported absolute uncertainties instead of the requested relative measurement uncertainties, resulting in very low, unrealistic values for that parameter.

On the contrary, satisfactory zeta scores might be obtained even with high bias if the uncertainty is sufficiently high. However, legislation specifies maximum tolerable standard uncertainties. Uncertainties exceeding them are not considered fit-for-purpose. Therefore, the uncertainties reported by the participants for the 4 marker PAHs were checked whether they comply with the threshold values provided by the "fitness-for-purpose" function (Equation 1). The results reported by the participants and the maximum tolerated LOD of 0.30 µg/kg were used for the calculation of the respective threshold values. Reported uncertainties that were non-compliant are highlighted in yellow in Table 6.

**Performance classification scheme**

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". The individual results of replicate analyses were not rated.

Each laboratory had to report a total of 5 results; therefore the expected total number of results of the 49 participants was 245. Three participants did not report results at all and other participants did not report results for all 5 parameters. In total 226 results were received, which equals to 92.2 % of the expected. The results, as reported by participants are presented in ANNEX 10.

Statistical evaluation of the results was performed using PROLab software [13]. Robust mean values and robust standard deviations were calculated according to Algorithm A+S of ISO 13528:2015 [11].

It should be noted that the confidence intervals of the robust means calculated from the participants' results (ANNEX 10, Kernel density plot) overlap well with the confidence intervals of the assigned values. The robust standard deviation (rSD) of the results of participants reported for BaP in smoked black pepper test material were lower than the target SD, however for the rest of the analytes, the rSDs of the result were higher than the target SD.

66.4% of the results reported by the participants obtained satisfactory z-scores ≤ +/-2 while 64.7% of the results had satisfactory zeta-score (Figure 1). In contrast to previous PTs, the difference between the rates of satisfactory z-scores and zeta scores is very small, which indicate a significant improvement of the participants in the estimation of realistic uncertainties.

22.6% of the results fall into the unsatisfactory performance range with z-scores > |3|.

**Figure 1:** Histogram of z- and zeta-scores for the contents of BAA, BAP, BBF, CHR, and the SUM4PAH

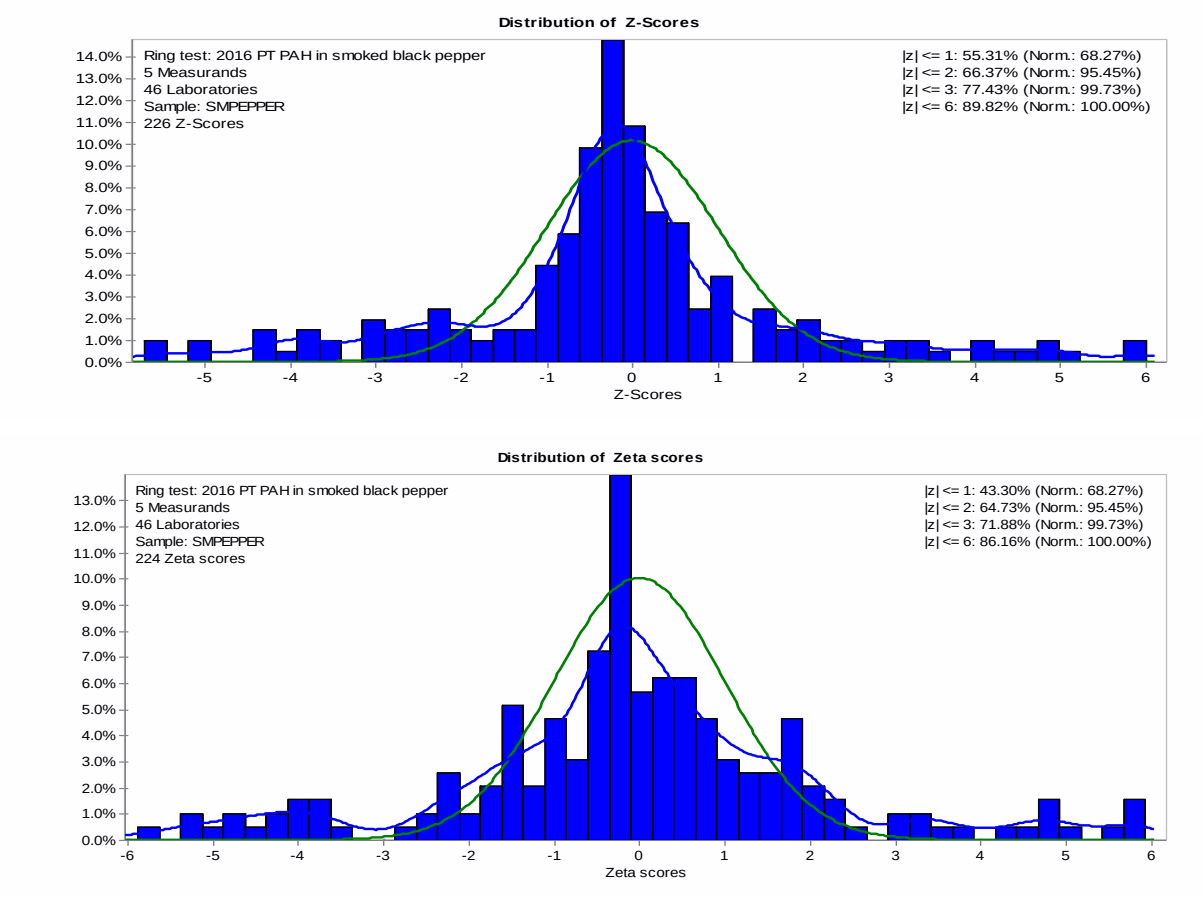
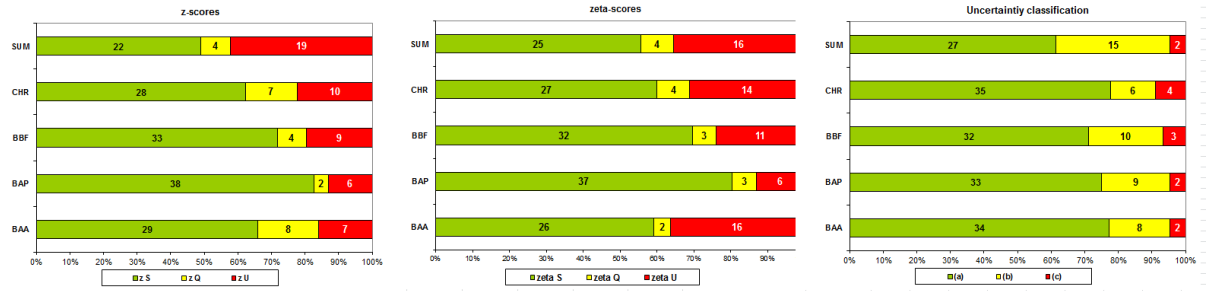


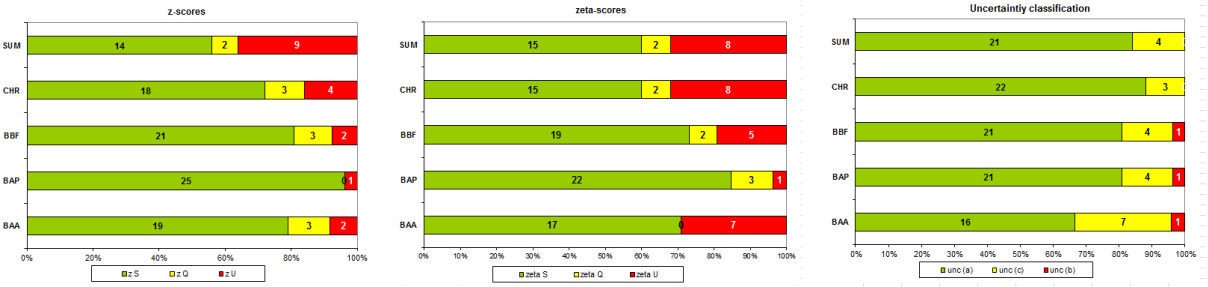

Figure 2 presents the distribution of performance ratings (z- and zeta-scores) for the individual measurands for the whole population (I), NRLs (II) and OCLs (III). It contains also an overview of the compliance of reported uncertainty, according to the criteria explained further on and the classification shown in the tables of the Annex 11.

**Figure 2:** Percentage/number (label on bars) of laboratories with satisfactory (green), questionable (yellow) and unsatisfactory performance (red)

I - all participants




II- only NRLs




III - only OCLs

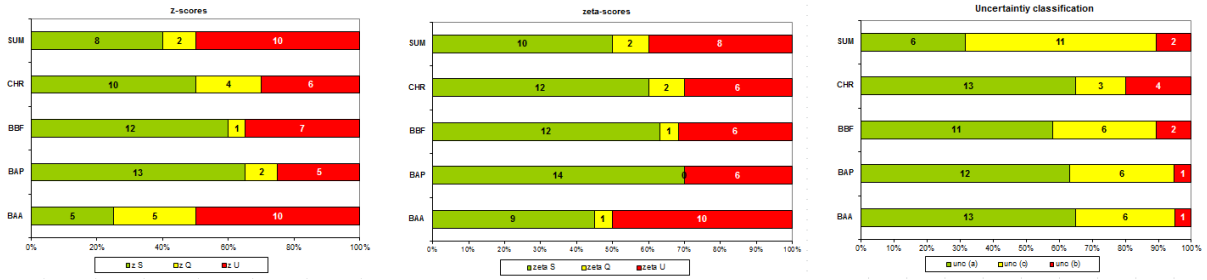


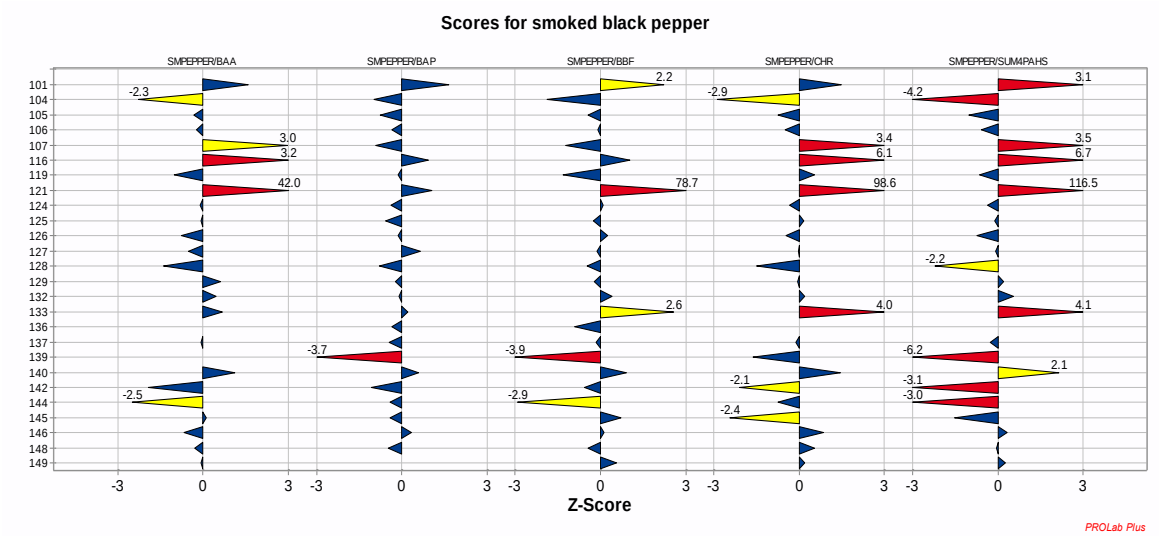

Figure 3 and Figure 4 provide overviews of the individual z-scores and zeta-scores assigned to the results reported for the smoked black pepper test material by NRLs and OCLs respectively. The larger the triangles, the larger were the differences to the assigned values. Yellow triangles represent z-scores in the questionable and red triangle in the non-satisfactory performance range. The corresponding scores were presented next to the triangles.

Twenty-three participants obtained for at least four out of five rated results z-scores in the satisfactory performance range. However, 20 participants were less successful. They reported at maximum two results that were considered satisfactory. Three participants (OCLs) did not report any result that fell into the satisfactory performance range. It should be mentioned that the smoked black pepper test material was highly contaminated with PAHs, which could have caused issues with the working range of methods applied by some participants. Moreover several participants reported chromatographic problems linked to interferences stemming from the matrix or non-target PAHs. Given the complexity of the study, the performance of the NRLs may be summarised as satisfactory with room for improvement. Big differences were noted in the performances of NRLs and OCLs, especially for BAA, which caused for three quarters of the OCLs troubles.

The numerical values of the calculated z (zeta)-scores were compiled in the tables of Annex 10. All scores in the questionable performance range were highlighted in yellow, while scores, indicating unsatisfactory performance are presented with red background.

The distributions of results for the individual analytes are displayed in the figures of ANNEX 10 together with respective Kernel density plots. The figures show for each analyte individual analysis results of the three replicate determinations.

**Figure 3:** Graphical presentation of z- and zeta- scores corresponding to the "final values for proficiency assessment" reported by the NRLs for the contents of BAA, BAP, BBF, CHR, and the SUM4PAH parameter in the smoked black pepper test material.



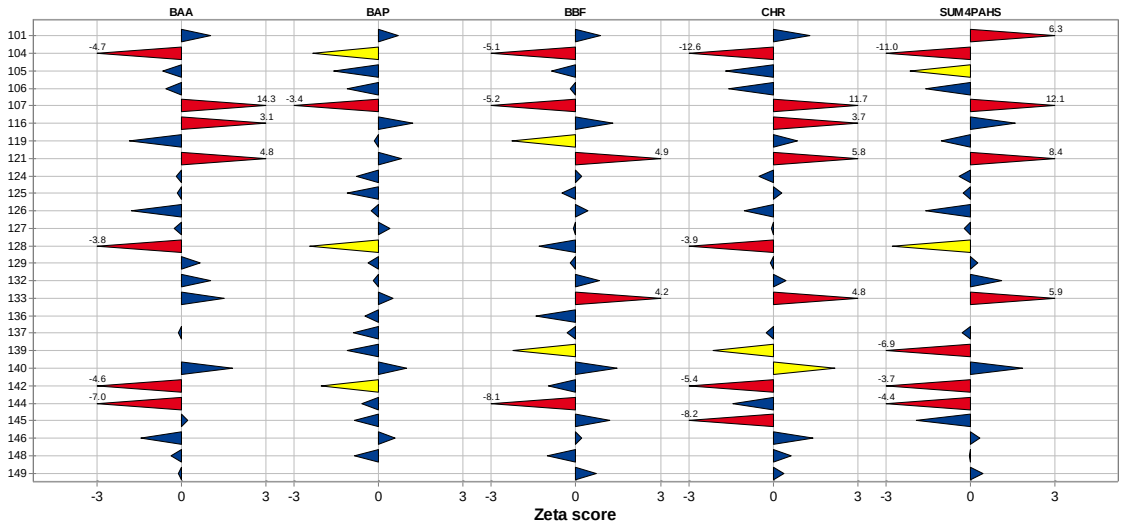
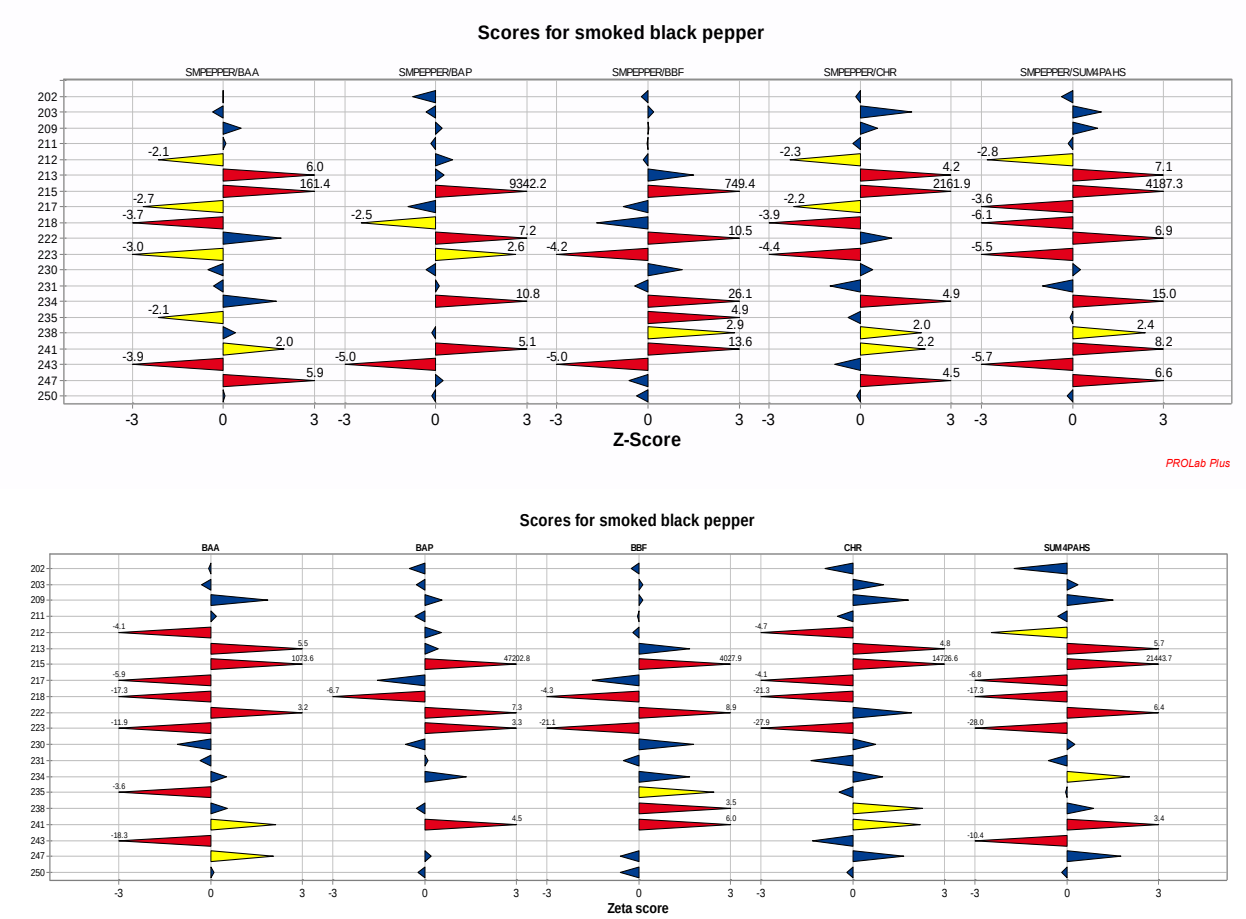



**Figure 4:** Graphical presentation of z and zeta-scores corresponding to the "final values for proficiency assessment" reported by the OCLs for the contents of BAA, BAP, BBF, CHR, and the SUM4PAH parameter in the smoked black pepper test material.




The plausibility of the uncertainty statements of the laboratories was assessed in the current PT classifying every reported uncertainty into three groups (Annex 10 and Figure 2) according to the following rules.

The standard measurement uncertainty from a laboratory ($u_{lab}$) is most likely to fall in a range between a minimum and a maximum uncertainty (case "a": $u_{min} \leq u_{lab} \leq u_{max}$). The minimum uncertainty ($u_{min}$) is set for the respective analyte to the standard uncertainty of the assigned value ($u_{ref}$). This is based on the assumption that it is unlikely that a laboratory carrying out the analysis on a routine basis would determine the measurand with a smaller measurement uncertainty than that achieved in the experiments for the characterisation of the test material, which were based on isotope dilution mass spectrometry applying bracketing calibration. The maximum uncertainty is set to the standard deviation accepted for the assessment of results (σ), in this PT set to the maximum threshold given by the "fitness-for-purpose" function $U_f$. Consequently, case "a" becomes: $u_{ref} \leq u_{lab} \leq \sigma$.

If $u_{lab}$ is smaller than $u_{ref}$ (case "b": $u_{lab} < u_{ref}$) the laboratory might have underestimated its measurement uncertainty.

If $u_{lab}$ is larger than σ (case "c": $u_{lab} > \sigma$) the laboratory might have overestimated its measurement uncertainty, or applied an analytical method that was not fit-for-purpose. Both cases require corrective action!

As can be concluded from Figure 2, the measurement uncertainties estimated by NRLs were more plausible, and to a higher degree compliant with legislation, than those

estimated by OCLs. This is particularly applicable to uncertainty estimates for the sum parameter.

Although the estimation of measurement uncertainties improved over recent PT rounds, the EURL PAH will continue to pay attention to this parameter, in the PTs to come, as measurement uncertainty has major implications on the assessment of compliance of food according to European legislation.

As indicated by the Kernel density plots displayed in Annex 11, the distributions of results are close to the Gaussian distribution. The major modes are close to the assigned (reference) values and the robust means calculated from the results of the participants. This supports the conclusion that the measurement of PAHs in smoked black pepper is from the statistical point of view under control. However, plotting Kernel density distributions in dependence of the applied analytical techniques reveal technique-dependant differences in the distributions of results for BAA and CHR. Systematically higher rSDs were noted for results obtained by HPLC-FD compared to results obtained by GC-MS measurements (Table 5). Stronger matrix effects affecting separation and peak integration might explain this finding.

**Table 5.** Statistical parameters depending on applied analysis technique

| Measurand | Sample | Class of methods | Mean | Rel. repeatability s.d. | Rel. reproducibility s.d. | Number of values | No. of laboratories | Laboratories [%] |
|---|---|---|---|---|---|---|---|---|
| - Sample-measurand combination : smoked black pepper - BAA | | | | | | | | |
| BAA | SMPEPPER | HPLC | 37.67 | 7.16% | 72.07% | 48 | 17 | 38.64% |
| BAA | SMPEPPER | GC-MS(MS) | 35.54 | 3.93% | 26.93% | 67 | 24 | 54.55% |
| BAA | SMPEPPER | GC-HRMS | 35.55 | 1.54% | 17.79% | 9 | 3 | 6.82% |
| | | | | | | 124 | 44 | 100.0 |
| - Sample-measurand combination : smoked black pepper - BAP | | | | | | | | |
| BAP | SMPEPPER | HPLC | 13.86 | 4.32% | 20.99% | 54 | 19 | 42.22% |
| BAP | SMPEPPER | GC-MS(MS) | 14.67 | 6.00% | 16.43% | 63 | 23 | 51.11% |
| BAP | SMPEPPER | GC-HRMS | 14.34 | 4.56% | 12.36% | 9 | 3 | 6.67% |
| | | | | | | 126 | 45 | 100.0 |
| - Sample-measurand combination : smoked black pepper - BBF | | | | | | | | |
| BBF | SMPEPPER | HPLC | 18.17 | 7.37% | 45.07% | 54 | 19 | 42.22% |
| BBF | SMPEPPER | GC-MS(MS) | 18.71 | 4.85% | 31.05% | 64 | 23 | 51.11% |
| BBF | SMPEPPER | GC-HRMS | 18.33 | 1.86% | 11.00% | 9 | 3 | 6.67% |
| | | | | | | 127 | 45 | 100.0 |
| - Sample-measurand combination : smoked black pepper - CHR | | | | | | | | |
| CHR | SMPEPPER | HPLC | 40.06 | 6.95% | 64.72% | 51 | 18 | 40.00% |
| CHR | SMPEPPER | GC-MS(MS) | 44.54 | 2.93% | 27.21% | 65 | 24 | 53.33% |
| CHR | SMPEPPER | GC-HRMS | 46.82 | 2.80% | 23.11% | 9 | 3 | 6.67% |
| | | | | | | 125 | 45 | 100.0 |
| - Sample-measurand combination : smoked black pepper - SUM4PAHS | | | | | | | | |
| SUM4PAHS | SMPEPPER | HPLC | 109.16 | 6.81% | 58.00% | 34 | 12 | 27.27% |
| SUM4PAHS | SMPEPPER | GC-MS(MS) | 113.83 | 2.81% | 19.30% | 40 | 16 | 36.36% |
| SUM4PAHS | SMPEPPER | GC-HRMS | 115.05 | 2.46% | 15.26% | 9 | 3 | 6.82% |
| | | | | | | 83 | 31 | 70.5 |

Inconsistencies were still observed in the number of significant figures of reported measurement results and associated uncertainties. The EURL PAH will address this issue again at the coming workshop as a harmonised way of reporting results makes part of the proper implementation of EU legislation.

In general NRLs performed better than non-NRLs, not only in terms of z- and zeta-scores but also for their reasonable measurement uncertainty statements (Fig.2)

## 5.4 Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire filled in by the participants (ANNEX 9). Data is presented as reported.

36% of the participants did not have yet experience with the determination of PAHs in herbs and spices and especially not with smoked black pepper, as this food category was regulated very recently only. Almost half of the participants used non validated/verified methods.

From the rest of the participants, seventeen were already accredited for the category herbs and spices and used validated method, but half of them did not have experience with black pepper as matrix. Detailed analysis of the individual results reported by this more experienced group of participants showed a significantly higher percentage of satisfactory performance ratings (80 % satisfactory z-scores) compared to less experienced laboratories, reaching 90 % of z-scores less than an absolute value of three.

Nine participants prepared their calibration solutions in the laboratory from neat compounds, while the rest used commercial standard mixtures in solvent. No significant difference was noticed between the results of both populations.

## 5.5 Compliance assessment

As important as the correct analysis of the test sample is the interpretation of results. The assigned analyte contents of the smoked black pepper test material exceeded the maximum level specified for BAP and the sum of four PAHs as laid down in the Commission Regulation (EU) No 2015/1933. The respective maximum levels (ML) for BAP and for the sum of the four PAHs are 10.0 µg/kg and 50.0 µg/kg.

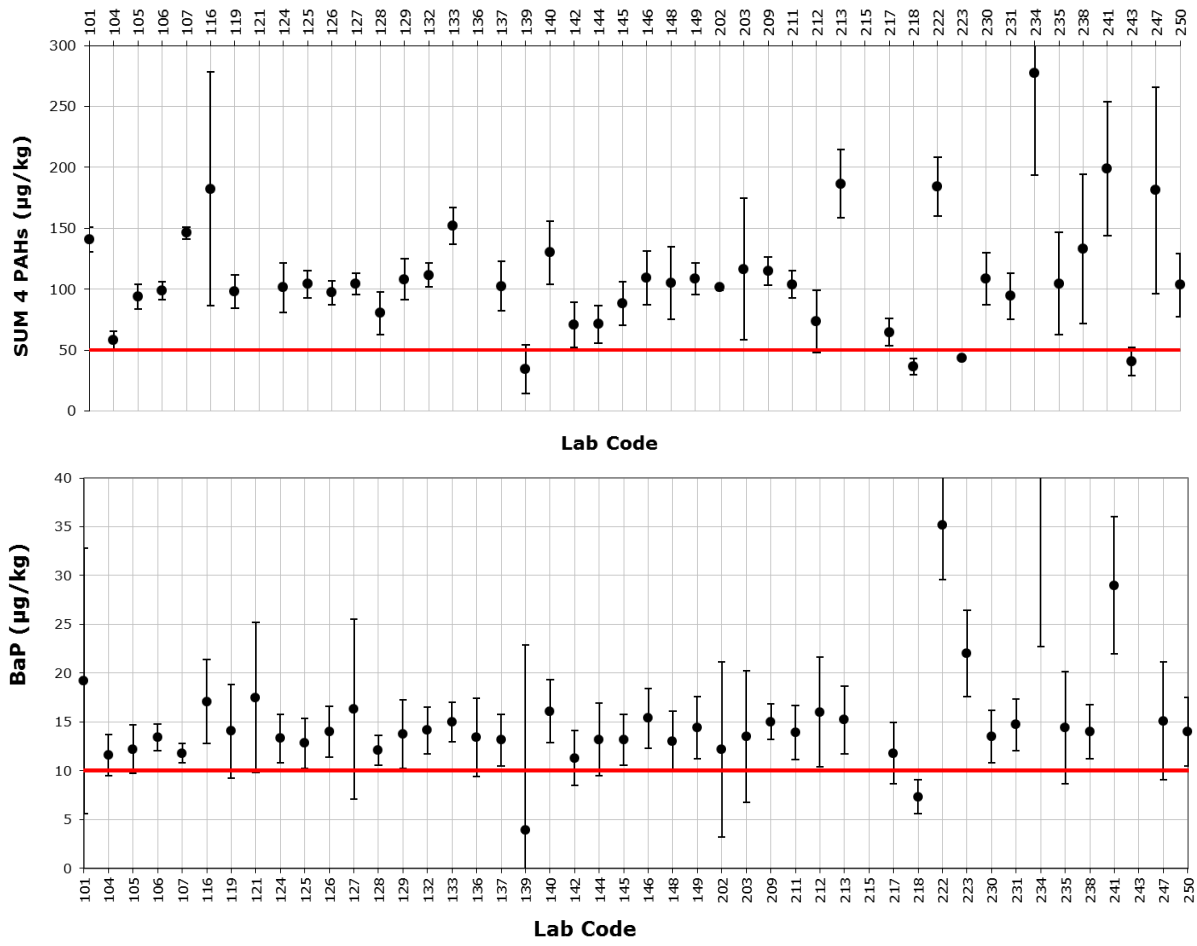
The EURL asked the participants in this study to assess, based on their analysis results, the compliance of the sample with the current legislative limits. Figure 5 presents the distribution of the reported results with associated uncertainties for BaP and the sum of four PAHs in relation to the maximum levels defined in legislation (indicated by red lines).

The decision criterion for non-compliance is specified in Commission Regulation (EC) No 333/2007 [7]. A lot or sub-lot shall be rejected if the content value of this lot or sub-lot is beyond reasonable doubt above the respective maximum level given in legislation, taking into account the expanded measurement uncertainty and correction for recovery. This translates in a content value that is derived from the measured and recovery corrected content value by subtraction of the expanded uncertainty (k=2). This situation is provided in Figure 6 if the lower end of the error bar (representing the expanded measurement uncertainty) associated with the reported result (black dot) is above the red line.

Thirty laboratories out of 42 assessing compliance with legislative limits, classified the test sample correctly as non-compliant. Twelve laboratories classified the sample as compliant. Amongst them were 3 laboratories (139, 215, 243), for which the conclusion was technically correct, due to their biased results. Five participants (101, 129, 133, 140, 142) did not reply to the questionnaire and another two (215, 243) classified the sample as compliant without having value for one or the two regulated parameters (BaP and the SUM4PAHs).

Due to the high analyte contents of the test sample, which exceeded the MLs significantly, around 71 % of the participants, who replied to the questionnaire, assessed the compliance of the test sample with EU legislation correctly. However the conclusions drawn by four participants (136, 144, 212 and 238) lack scientifically a solid basis. As a consequence, a lot of attention will be paid in future to the interpretation of the analytical results.

**Figure 5**. Distribution of the results reported by the participants and the associated expanded measurement uncertainties for BaP and the SUM PAHs in relation to the MLs.




*The solid red lines represent the current maximum levels (MLs) of 10.0 µg/kg for BAP and 50.0 µg/kg for the sum of four PAHs respectively.*

## 6. Follow-up actions for underperforming laboratories

All laboratories that got "questionable" or "non-satisfactory" performance ratings (z-scores) are urged to perform root cause analysis, and to implement corrective actions.

The EURL will set up follow-up measures in due time for all NRLs that received for at least one of the four PAHs (BAA, BAP, BBF, and CHR) z-scores > |3| as required by Regulation (EC) 882/2004, and by the "Protocol for management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with European Union Reference Laboratories (EURLs) activities". These laboratories shall perform as an immediate action root-cause-analysis, and shall report to the EURL PAH in writing the identified cause for their underperformance as well as the corrective actions that they are going to take. A repetition of this PT is envisaged for the near future.

## Conclusion

Forty six participants reported analysis results. The performance of most participants was satisfactory. More than 66 % of the results reported by NRLs and OCLs respectively obtained satisfactory performance ratings. The, compared to previous PTs, rather lower rate of successful performance might be attributed to the complexity of the matrix, and the fact that more than a half of the participants did not have prior experience with it.

The great majority of participants in this inter-laboratory comparison applied analytical methods which, with regard to performance characteristics, were compliant with EU legislation. However, some participants are urged to improve in this respect.

## References


1 EU, Regulation (EC) No 776/2006 the European Parliament and of the Council of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community reference laboratories. Official Journal of the European Union, 2006. **L 136**: p. 3-8. Available from: http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006: 136:0003:008:EN: PDF

2 EU, 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. Official Journal of the European Communities, 2004. **L191**: p. 1-52. Available from:

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7 EU, Commission Regulation (EC) No 835/2011 of 19 August 2011, amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 2006. **L 215: p. 4-8**. Available from: http://eur-lex.europa.eu/ LexUriServ/LexUriServ.do?uri=OJ:L:2011 :215: 0004:0008:EN:PDF

8 EU, Regulation (EU) No 836/2011 the European Parliament and of the Council of 19 August 2011 amending Regulation (EC) No 333/2007, laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium,

mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs: Official Journal of the European Union, 2011. **L 215**: p. 9-16 Available from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215: 0009:0016:EN:PDF

9 Commission Regulation (EU) 2015/1933 of 27 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in cocoa fibre, banana chips, food supplements, dried herbs and dried spices http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R1933&from=EN

10 ISO/IEC 17043:2010. "Conformity assessment - General requirements for proficiency testing providers". Geneva, Switzerland

11 ISO 13528:2015 "Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons", issued by International Organisation for Standardisation, Geneva, Switzerland

12 WI-D-0607 Determination of 4 EU target PAHs in fatty food matrices by pressurized liquid extraction, size-exclusion chromatography followed by solid phase extraction clean-up and gas-chromatography with mass-spectrometric detection, EURL PAH

13 PROLab, Software for PT programs and collaborative studies; http://quodata.de/en/software/for-interlaboratory-tests.html

14 IUPAC International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. http://www.iupac.org/publications/pac/2006/pdf/7801x0145.pdf

15 Evaluation of measurement data – Guide to the expression of uncertainty in measurement JCGM 100:2008 (GUM 1995 with minor corrections)

## List of abbreviations and definitions

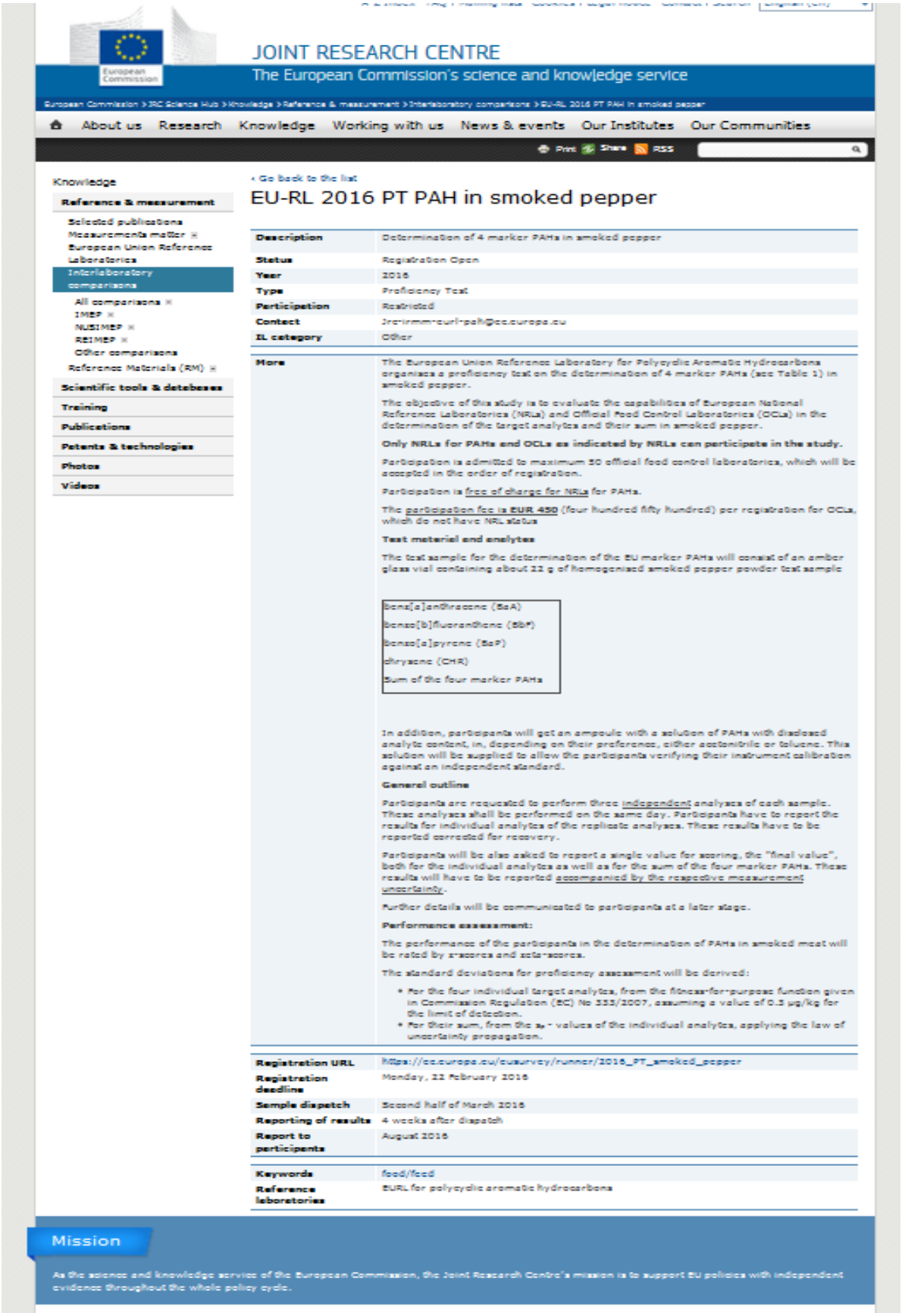
| | |
|---|---|
| BAA - | Benz[*a*]anthracene |
| BAP - | Benzo[*a*]pyrene |
| BBF - | Benzo[*b*]fluoranthene |
| CHR - | Chrysene |
| EC - | European Commission |
| EFSA - | European Food Safety Authority |
| EU – | European Union |
| EURL PAH - | European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons |
| ILC – | Inter-laboratory comparison |
| IRMM - | Institute for Reference Materials and Measurements |
| ISO | International Organisation for Standardisation |
| 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 |
| NRL - | National Reference Laboratory |
| OCL - | Official food control laboratory |
| PAHs - | Polycyclic aromatic hydrocarbons |
| PT - | Proficiency test |
| SUM4PAH- | Sum of the four markers PAHs |

## List of figures

## List of tables

# ANNEXES

## ANNEX 1: Announcement of the PT on the IRMM webpage



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- Publications
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# EU-RL 2016 PT PAH in smoked pepper

| | |
|---|---|
| **Description** | Determination of 4 marker PAHs in smoked pepper |
| **Status** | Registration Open |
| **Year** | 2016 |
| **Type** | Proficiency Test |
| **Participation** | Restricted |
| **Contact** | jrc-irmm-eurl-pah@ec.europa.eu |
| **IL category** | Other |

**More**

The European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons organises a proficiency test on the determination of 4 marker PAHs (see Table 1) in smoked pepper.

The objective of this study is to evaluate the capabilities of European National Reference Laboratories (NRLs) and Official Food Control Laboratories (OCLs) in the determination of the target analytes and their sum in smoked pepper.

**Only NRLs for PAHs and OCLs as indicated by NRLs can participate in the study.**

Participation is admitted to maximum 30 official food control laboratories, which will be accepted in the order of registration.

Participation is free of charge for NRLs for PAHs.

The participation fee is **EUR 450** (four hundred fifty hundred) 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 an amber glass vial containing about 22 g of homogenised smoked pepper powder test sample

| |
|---|
| benz[a]anthracene (BaA) |
| benzo[b]fluoranthene (BbF) |
| benzo[a]pyrene (BaP) |
| chrysene (CHR) |
| Sum of the four marker PAHs |

In addition, participants will get an ampoule with a solution of PAHs with disclosed analyte content, in, depending on their preference, either acetonitrile or toluene. This solution will be supplied to allow the participants verifying their instrument calibration against an independent standard.

**General outline**

Participants are requested to perform three independent analyses of 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 be also asked to report a single value for scoring, the "final value", both for the individual analytes as well as for the sum of the four marker PAHs. 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 PAHs in smoked meat will be rated by z-scores and zeta-scores.

The standard deviations for proficiency assessment will be derived:

- For the four individual target analytes, from the fitness-for-purpose function given in Commission Regulation (EC) No 333/2007, assuming a value of 0.5 µg/kg for the limit of detection.
- For their sum, from the $s_R$ - values of the individual analytes, applying the law of uncertainty propagation.

| | |
|---|---|
| **Registration URL** | https://ec.europa.eu/eusurvey/runner/2016_PT_smoked_pepper |
| **Registration deadline** | Monday, 22 February 2016 |
| **Sample dispatch** | Second half of March 2016 |
| **Reporting of results** | 4 weeks after dispatch |
| **Report to participants** | August 2016 |
| **Keywords** | food/feed |
| **Reference laboratories** | EURL for polycyclic aromatic hydrocarbons |

Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

## ANNEX 2: Announcement of the PT via e-mail

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

Geel, 01/02/2016
Ref. Ares(2016) 637851

**Inter-laboratory comparison on the determination of four EU marker PAHs in smoked pepper**

Dear Madam/Sir,

Registration for participation in the inter-laboratory comparison study organised by the EURL PAH on the determination of the 4 marker PAHs in smoked pepper is **open until 22th February 2016.**

Participation is mandatory and free of charge for National Reference Laboratories (NRLs) for PAHs. Confidentiality of data is granted.

In support to the NRLs, and to facilitate fulfilling their tasks as defined in Regulation (EC) No 882/2004, EU Official Food Control Laboratories (OCLs) falling under the responsibility of the NRLs may participate in the study. **The participation fee for official food control laboratories is 450 Euro per participation.**

The target analytes are listed in the following Table.

| |
|---|
| benz[*a*]anthracene (BaA) |
| benzo[*b*]fluoranthene (BbF) |
| benzo[*a*]pyrene (BaP) |
| chrysene (CHR) |
| SUM of the 4 marker PAHs |

Results have to be reported corrected for recovery and accompanied by the respective measurement uncertainty for both the individual PAHs and the sum of the four marker PAHs. **Additionally participants will be asked to perform compliance assessment according to the corresponding legislative limits**

Each participant will be provided with an amber glass vial containing approximately 22 g of smoked pepper test sample

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

Participants will also receive a standard solution in either acetonitrile or toluene with disclosed content; which may be used for verification of instrument calibration.

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: 22th February 2016**
- Dispatch of samples: **second half of March.** 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/2016_PT_smoked_pepper

| **PT coordinator** | **Second contact** |
|---|---|
| Stefanka Bratinova<br><br>Fax: 0032-14-571783<br>e-mail: jrc-irmm-eurl-pah@ec.europa.eu | Thomas Wenzl |

Participants are invited to indicate the preferred solvent type of the standard solution (either toluene or acetonitrile) in the Registration Form as well as any justify additional 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 OCLs under their responsibility, and to assist the EURL in identifying laboratories that are eligible to participate in the study.

**Access of NRLs to performance data of official food control laboratories:**
**Two options:**

1) *NRL enrols OCLs and covers participation fee.*
   The NRL submits to the EURL a list of participants including name and address of laboratory, and details of the contact person (name, address - no post box! - email and telephone number). The coverage of the participation fees must be confirmed and details for invoicing (e.g. order number) have to be provided. It shall be made clear, that the full participation fee is payable upon dispatch of the test samples. In return,

2) The *OCL (identified as such by the respective NRL) enrols itself* in the inter-laboratory comparison *and covers the participation fee*.
The NRL will get access to performance data of the OCL only upon providing to the EU-RL for PAHs a letter of consent.

Should you require further clarification, please do not hesitate to contact the EURL team via:

JRC-IRMM-EURL-PAH@ec.europa.eu

With kind regards,

Stefanka Bratinova

Cc: Thomas Wenzl, Beatriz de la Calle, Franz Ulberth

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-irmm-eurl-pah@ec.europa.eu

Web site: http://irmm.jrc.ec.europa.eu

# ANNEX 3: Registration form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

Geel, 01/02/2016
Ref. Ares(2016) 637851

**Inter-laboratory comparison on the determination of four EU marker PAHs in smoked pepper**

Dear Madam/Sir,

Registration for participation in the inter-laboratory comparison study organised by the EURL PAH on the determination of the 4 marker PAHs in smoked pepper is **open until 22th February 2016.**

Participation is mandatory and free of charge for National Reference Laboratories (NRLs) for PAHs. Confidentiality of data is granted.

In support to the NRLs, and to facilitate fulfilling their tasks as defined in Regulation (EC) No 882/2004, EU Official Food Control Laboratories (OCLs) falling under the responsibility of the NRLs may participate in the study. **The participation fee for official food control laboratories is 450 Euro per participation.**

The target analytes are listed in the following Table.

| |
|---|
| benz[*a*]anthracene (BaA) |
| benzo[*b*]fluoranthene (BbF) |
| benzo[*a*]pyrene (BaP) |
| chrysene (CHR) |
| SUM of the 4 marker PAHs |

Results have to be reported corrected for recovery and accompanied by the respective measurement uncertainty for both the individual PAHs and the sum of the four marker PAHs. **Additionally participants will be asked to perform compliance assessment according to the corresponding legislative limits**

Each participant will be provided with an amber glass vial containing approximately 22 g of smoked pepper test sample



Participants will also receive a standard solution in either acetonitrile or toluene with disclosed content; which may be used for verification of instrument calibration.

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: 22th February 2016**
- Dispatch of samples: **second half of March.** 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/2016_PT_smoked_pepper

| **PT coordinator** | **Second contact** |
|---|---|
| Stefanka Bratinova | Thomas Wenzl |
| Fax: 0032-14-571783<br>e-mail: jrc-irmm-eurl-pah@ec.europa.eu | |

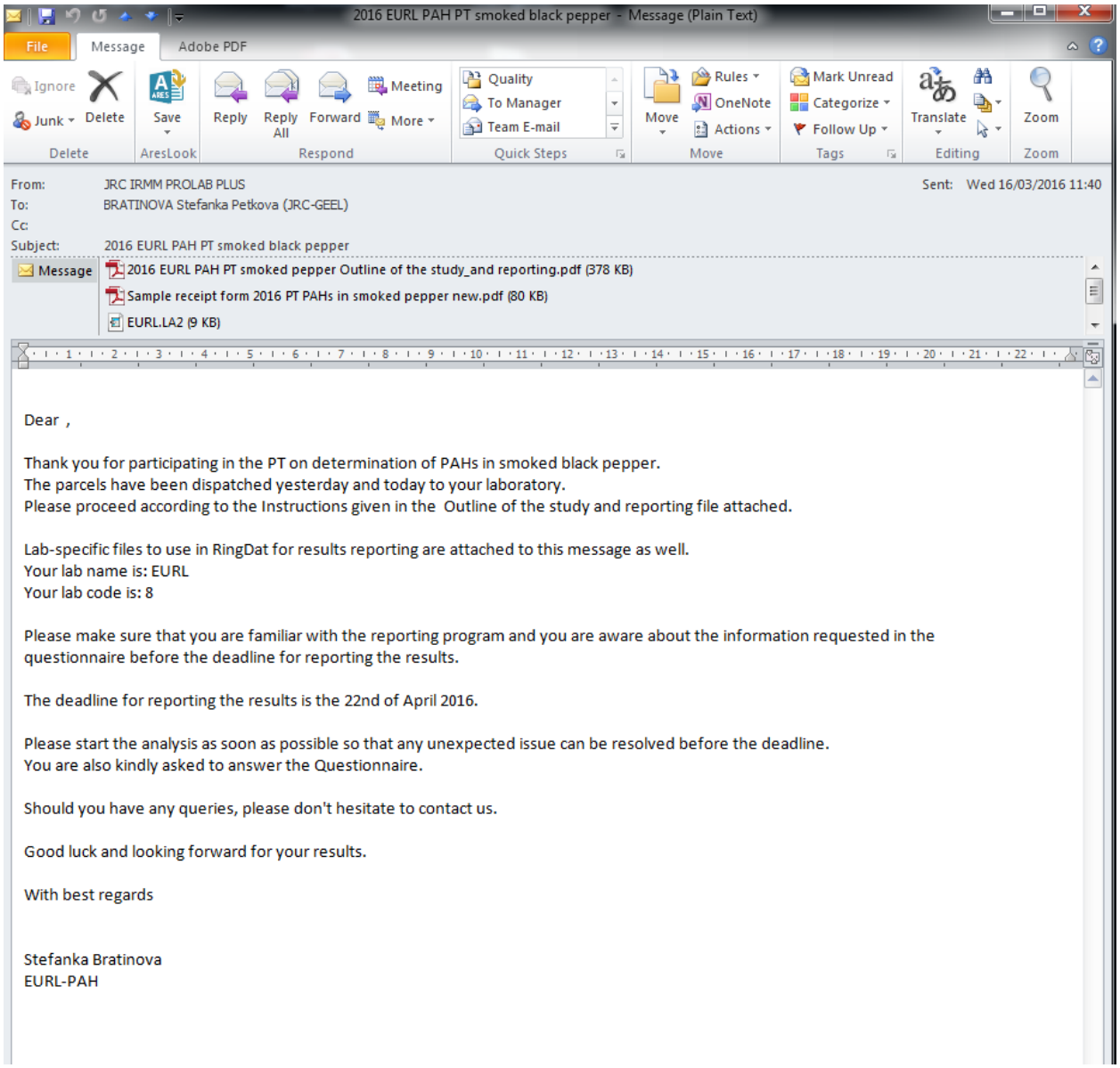
Participants are invited to indicate the preferred solvent type of the standard solution (either toluene or acetonitrile) in the Registration Form as well as any justify additional requests.

**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 OCLs under their responsibility, and to assist the EURL in identifying laboratories that are eligible to participate in the study.

**Access of NRLs to performance data of official food control laboratories:**
**Two options:**

1) *NRL enrols OCLs and covers participation fee.*
   The NRL submits to the EURL a list of participants including name and address of laboratory, and details of the contact person (name, address - no post box! - email and telephone number). The coverage of the participation fees must be confirmed and details for invoicing (e.g. order number) have to be provided. It shall be made clear, that the full participation fee is payable upon dispatch of the test samples. In return,

# ANNEX 4: Announcement of material dispatch



2016 EURL PAH PT smoked black pepper - Message (Plain Text)

File | Message | Adobe PDF

Ignore | Junk | Delete | Save | Reply | Reply All | Forward | Meeting | More | Quality | To Manager | Team E-mail | Move | Rules | OneNote | Actions | Mark Unread | Categorize | Follow Up | Translate | Zoom

Delete | AresLook | Respond | Quick Steps | Move | Tags | Editing | Zoom

From: JRC IRMM PROLAB PLUS
Sent: Wed 16/03/2016 11:40
To: BRATINOVA Stefanka Petkova (JRC-GEEL)
Cc:
Subject: 2016 EURL PAH PT smoked black pepper

Message
2016 EURL PAH PT smoked pepper Outline of the study_and reporting.pdf (378 KB)
Sample receipt form 2016 PT PAHs in smoked pepper new.pdf (80 KB)
EURL.LA2 (9 KB)

Dear ,

Thank you for participating in the PT on determination of PAHs in smoked black pepper.
The parcels have been dispatched yesterday and today to your laboratory.
Please proceed according to the Instructions given in the Outline of the study and reporting file attached.

Lab-specific files to use in RingDat for results reporting are attached to this message as well.
Your lab name is: EURL
Your lab code is: 8

Please make sure that you are familiar with the reporting program and you are aware about the information requested in the questionnaire before the deadline for reporting the results.

The deadline for reporting the results is the 22nd of April 2016.

Please start the analysis as soon as possible so that any unexpected issue can be resolved before the deadline.
You are also kindly asked to answer the Questionnaire.

Should you have any queries, please don't hesitate to contact us.

Good luck and looking forward for your results.

With best regards

Stefanka Bratinova
EURL-PAH

# ANNEX 5: Documents sent to participants - OUTLINE and REPORTING INSTRUCTIONS

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

Geel, 04 March 2016

## EURL-PAH 2016 PT- PAHs in smoked pepper

Dear Madame/Sir,

The inter-laboratory comparison study organised by the EU-RL PAHs on the determination of four EU marker PAHs in smoked pepper starts with the dispatch of the samples.

The target analytes are the four EU marker PAHs (benzo[*a*]pyrene, benzo[*b*]fluoranthene, benz[*a*]anthracene, chrysene) and their sum. The participants are requested to report results on all of them.

Each participant is provided with amber glass vials containing a portion of smoked pepper, naturally contaminated with PAHs and a known standard solution in either toluene or acetonitrile for checking of the 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 22nd April 2016 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 (also for the sum parameter).

**Additionally participants are asked to perform compliance assessment according to the CURRENT legislative limits.**

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 60 ml amber vial, labelled as *"EU-RL PAHs PT 2016 Interlaboratory comparison, 4 EU PAHs in smoked black pepper"* containing > 20 g of a naturally contaminated homogenised smoked black pepper. 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".

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Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-pah@ec.europa.eu



**If not analysed immediately after receiving, please store the smoked black pepper sample in the refrigerator, protected of light.**

2. Depending on your preference, one ampoule, labelled as "PAH4 in acetonitrile", or "PAH4 in toluene", with about 1 ml of a solution of **4 EU priority PAHs in acetonitrile, respectively toluene**. The analyte concentration of your preferred solution 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.

Please bear in mind that the solutions **do not contain any internal standard**. The standard solution in acetonitrile contains small amounts of toluene, which stem from the preparation of stock solution from neat materials.

### 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:

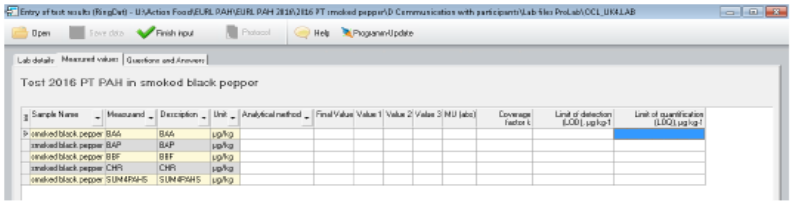
1. If not available already, please download the data entry program RingDat free from the QuoData web page using following link: http://quodata.de/fileadmin/RingDat/ringdat4_en.exe

User: *ringdat*
Password: *prolabdata*

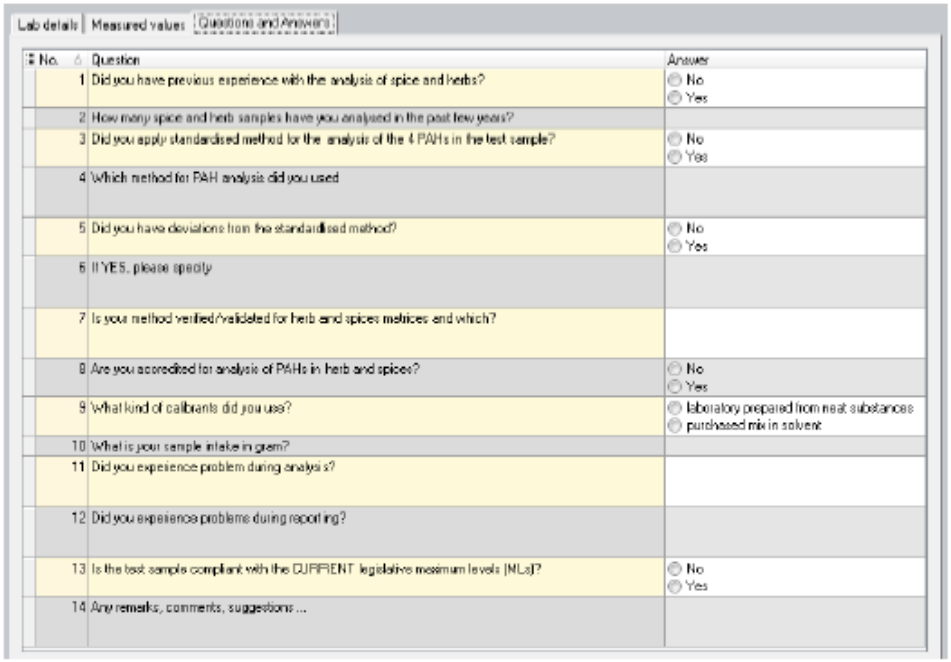
2. Save to the same folder the two lab specific files with the extension **"*.LAB"** and **"*.LA2"**, generated by the ProLab software and provided to each laboratory individually (personal files) by mail.

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.


Lab details | Measured values | Questions and Answers

| No. | Question | Answer |
|---|---|---|
| 1 | Did you have previous experience with the analysis of spice and herbs? | ○ No ○ Yes |
| 2 | How many spice and herb samples have you analysed in the past few years? | |
| 3 | Did you apply standardised method for the analysis of the 4 PAHs in the test sample? | ○ No ○ Yes |
| 4 | Which method for PAH analysis did you used | |
| 5 | Did you have deviations from the standardised method? | ○ No ○ Yes |
| 6 | If YES, please specify | |
| 7 | Is your method verified/validated for herb and spices matrices and which? | |
| 8 | Are you accredited for analysis of PAHs in herb and spices? | ○ No ○ Yes |
| 9 | What kind of calibrants did you use? | ○ laboratory prepared from neat substances ○ purchased mix in solvent |
| 10 | What is your sample intake in gram? | |
| 11 | Did you experience problem during analysis? | |
| 12 | Did you experience problems during reporting? | |
| 13 | Is the test sample compliant with the CURRENT legislative maximum levels (MLs)? | ○ No ○ Yes |
| 14 | Any remarks, comments, suggestions ... | |

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-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

SAMPLE RECEIPT

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

## PROFICIENCY TESTING MATERIAL RECEIPT FORM

## 2016 PT- PAHs in smoked black pepper

| | |
|---|---|
| **Contact person** | |
| **Affiliation** | |
| **City, Country** | |

**Content of the parcel**

1. One 60 ml amber glass vial containing about 20 g of smoked black pepper
2. One 2 ml amber glass ampule, containing about 1 ml of 4 markers PAHs in solvent
3. PAH standard solution specification sheet
4. Solvent safety data sheet
5. One sample receipt form (= this form), which is e-mailed as well to be filed and send electronically

**IF NOT ANALYSED IMMEDIATLY AFTER RECEIVING THE PARCEL, PLEASE PUT THE TEST SAMPLES IN THE REFRIGERATOR.**

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 | |
| 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) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 3. E-mail: jrc-irmm-eurl-PAH@ec.europa.eu

# ANNEX 6: Technical specifications of the calibration solutions

## ACETONITRILE SOLUTION

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

Geel, 24/02/2016

| **Standard solution specification sheet** | **PAH4 in ACETONITRILE** |
|---|---|
| Date of production: 18/02/2016 | Total volume: 1 mL |
| Expiry date: *August 2016* | |

**Standard solution composition:**

| | Product name | CAS | Conc.* (ng/g) | Conc.* (ng/mL) | U** ± % |
|---|---|---|---|---|---|
| 1 | Benz[*a*]anthracene | 56-55-3 | **64.3** | **50.6** | 0.3 |
| 2 | Benzo[*a*]pyrene | 50-32-8 | **64.2** | **50.5** | 0.4 |
| 3 | Benzo[*b*]fluoranthene | 205-99-2 | **64.0** | **50.3** | 0.5 |
| 4 | Chrysene | 218-01-9 | **64.9** | **51.1** | 0.4 |
| 5 | SUM PAH4 | | **257.4** | **202.4** | 0.9 |

** 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: Acetonitrile:Toluene (m/m 99.4:0.6)**

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Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783.

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Web site: http://irmm.jrc.ec.europa.eu



## TOLUENE SOLUTION

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons**

Geel, 24/02/2016

| **Standard solution specification sheet** | **PAH4 in TOLUENE** |
|---|---|
| Date of production: 18/02/2016 | Total volume: 1 mL |
| Expiry date: *August 2016* | |

**Standard solution composition:**

| | Product name | CAS | Conc.* (ng/g) | Conc.* (ng/mL) | U** ± % |
|---|---|---|---|---|---|
| 1 | Benz[*a*]anthracene | 56-55-3 | **58.0** | **50.3** | 0.3 |
| 2 | Benzo[*a*]pyrene | 50-32-8 | **57.8** | **50.1** | 0.4 |
| 3 | Benzo[*b*]fluoranthene | 205-99-2 | **57.6** | **50.0** | 0.5 |
| 4 | Chrysene | 218-01-9 | **58.5** | **50.7** | 0.4 |
| 5 | SUM PAH4 | | **232.0** | **201.1** | 0.9 |

** 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: Toluene**

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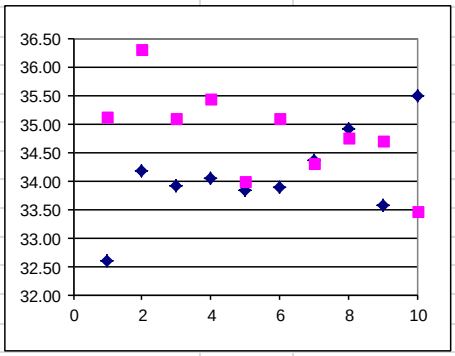
Web site: http://irmm.jrc.ec.europa.eu

# ANNEX 7: Homogeneity of the smoked black pepper test material

**BaA**

| | | | | | |
|---|---|---|---|---|---|
| | **n =** | 10 | | | |
| | **mean =** | 34.4572 | 20% | **= σ-trg(%)** | |
| 0.1797822 | **$s_x$ =** | 0.42401 | 6.89143 | **= σ-trg** | |
| √MSW = | **$s_w$ =** | 1.02808 | | | |
| | **$s_s$ =** | 0.59051 | 2.06743 | **= 0,3*σ** | 1.713738009 |
| | ISO-13528 | **passed** | | | |
| | F = | 0.34019 | 3.02038 | = Fcrit | |
| | | **passed** | | | |
| | IUPAC | | | | |
| | (MSB-MSW)/2 | -0.3487 | 9.10315 | = F1*(0,3*σ)²+F2*MSW | |
| | | **passed** | | | |

| Bottle | Result a | Result b | diff | sum | avg |
|---|---|---|---|---|---|
| Ampoule 08 | 32.60 | 35.13 | -2.52 | 67.73 | 33.87 |
| Ampoule 14 | 34.18 | 36.31 | -2.13 | 70.49 | 35.25 |
| Ampoule 22 | 33.92 | 35.09 | -1.17 | 69.01 | 34.51 |
| Ampoule 39 | 34.04 | 35.44 | -1.40 | 69.48 | 34.74 |
| Ampoule 43 | 33.84 | 33.99 | -0.15 | 67.82 | 33.91 |
| Ampoule 54 | 33.90 | 35.11 | -1.21 | 69.00 | 34.50 |
| Ampoule 67 | 34.36 | 34.32 | 0.04 | 68.68 | 34.34 |
| Ampoule 73 | 34.93 | 34.75 | 0.18 | 69.68 | 34.84 |
| Ampoule 87 | 33.58 | 34.70 | -1.12 | 68.28 | 34.14 |
| Ampoule 95 | 35.50 | 33.46 | 2.03 | 68.96 | 34.48 |

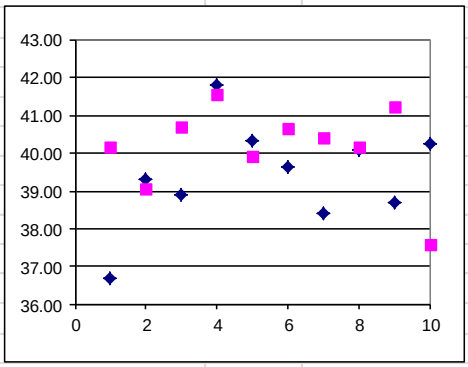



| | | |
|---|---|---|
| **Σ(diff)² =** | 21.1392 | |
| var(sum)/2 = | 0.35956 | =MSB |

**CHR**

| | | | | | |
|---|---|---|---|---|---|
| | **n =** | 10 | | | |
| | **mean =** | 39.7815 | 20% | **= σ-trg(%)** | |
| 0.78537 | **$s_x$ =** | 0.88621 | 7.9563 | **= σ-trg** | |
| √MSW = | **$s_w$ =** | 1.3061 | | | |
| | **$s_s$ =** | 0.25994 | 2.38689 | **= 0,3*σ** | 0.653420757 |
| | ISO-13528 | **passed** | | | |
| | F = | 0.92078 | 3.02038 | = Fcrit | |
| | | **passed** | | | |
| | IUPAC | | | | |
| | (MSB-MSW)/2 | -0.0676 | 12.4338 | = F1*(0,3*σ)²+F2*MSW | |
| | | **passed** | | | |

| Bottle | Result a | Result b | diff | sum | avg |
|---|---|---|---|---|---|
| Ampoule 08 | 36.70 | 40.16 | -3.46 | 76.85 | 38.43 |
| Ampoule 14 | 39.32 | 39.08 | 0.23 | 78.40 | 39.20 |
| Ampoule 22 | 38.88 | 40.69 | -1.81 | 79.57 | 39.79 |
| Ampoule 39 | 41.79 | 41.57 | 0.22 | 83.37 | 41.68 |
| Ampoule 43 | 40.33 | 39.94 | 0.39 | 80.27 | 40.13 |
| Ampoule 54 | 39.63 | 40.67 | -1.04 | 80.29 | 40.15 |
| Ampoule 67 | 38.40 | 40.42 | -2.02 | 78.81 | 39.41 |
| Ampoule 73 | 40.09 | 40.17 | -0.08 | 80.26 | 40.13 |
| Ampoule 87 | 38.71 | 41.23 | -2.52 | 79.94 | 39.97 |
| Ampoule 95 | 40.26 | 37.59 | 2.67 | 77.86 | 38.93 |

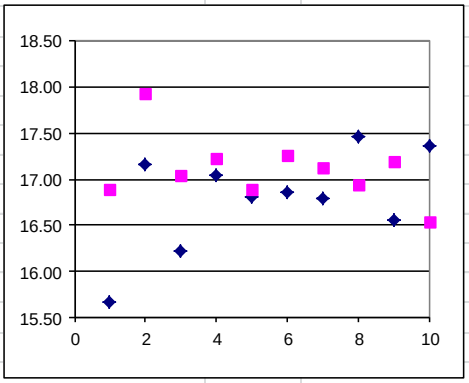



| | | |
|---|---|---|
| **Σ(diff)² =** | 34.1177 | |
| var(sum)/2 = | 1.57075 | =MSB |

## BbF

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **n =** | 10 | | | | | |
| | **mean =** | 16.9483 | 20% | **= σ-trg(%)** | | | |
| 0.11272 | **$s_x$ =** | 0.33574 | 3.38967 | **= σ-trg** | | | |
| √MSW = | **$s_w$ =** | 0.47155 | | | | | |
| | **$s_s$ =** | 0.03931 | 1.0169 | **= 0,3*σ** | | 0.23191101 | |
| | ISO-13528 | **passed** | | | | | |
| | F = | 1.0139 | 3.02038 | = Fcrit | | | |
| | | **passed** | | | | | |
| | IUPAC | | | | | | |
| | (MSB-MSW)/2 | 0.00154 | 2.16866 | = F1*(0,3*σ)$^2$+F2*MSW | | | |
| | | **passed** | | | | | |

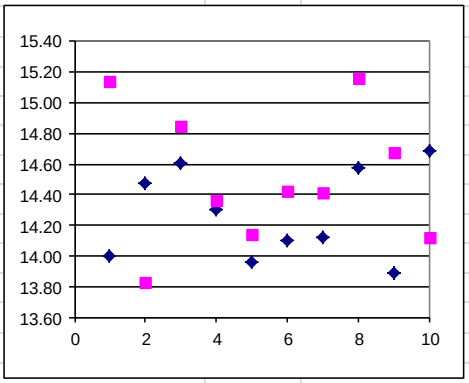
| Bottle | Result a | Result b | diff | sum | avg |
|---|---|---|---|---|---|
| Ampoule 08 | 15.67 | 16.90 | -1.22 | 32.57 | 16.29 |
| Ampoule 14 | 17.15 | 17.94 | -0.78 | 35.09 | 17.54 |
| Ampoule 22 | 16.22 | 17.04 | -0.82 | 33.27 | 16.63 |
| Ampoule 39 | 17.03 | 17.22 | -0.19 | 34.26 | 17.13 |
| Ampoule 43 | 16.80 | 16.89 | -0.09 | 33.69 | 16.85 |
| Ampoule 54 | 16.85 | 17.26 | -0.41 | 34.11 | 17.06 |
| Ampoule 67 | 16.80 | 17.13 | -0.33 | 33.93 | 16.96 |
| Ampoule 73 | 17.45 | 16.94 | 0.51 | 34.39 | 17.20 |
| Ampoule 87 | 16.55 | 17.20 | -0.65 | 33.75 | 16.87 |
| Ampoule 95 | 17.36 | 16.55 | 0.81 | 33.91 | 16.95 |
| | | **Σ(diff)$^2$ =** | 4.44711 | | |
| | | var(sum)/2 = | | 0.22545 | =MSB |




## BaP

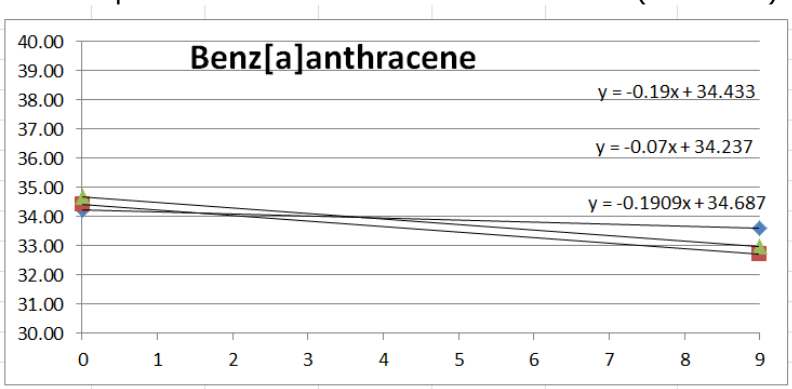
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **n =** | 10 | | | | | |
| | **mean =** | 14.3934 | 20% | **= σ-trg(%)** | | | |
| 0.06553 | **$s_x$ =** | 0.25598 | 2.87869 | **= σ-trg** | | | |
| √MSW = | **$s_w$ =** | 0.40227 | | | | | |
| | **$s_s$ =** | 0.12404 | 0.86361 | **= 0,3*σ** | | 0.861762153 | |
| | ISO-13528 | **passed** | | | | | |
| | F = | 0.80985 | 3.02038 | = Fcrit | | | |
| | | **passed** | | | | | |
| | IUPAC | | | | | | |
| | (MSB-MSW)/2 | -0.0154 | 1.56557 | = F1*(0,3*σ)$^2$+F2*MSW | | | |
| | | **passed** | | | | | |

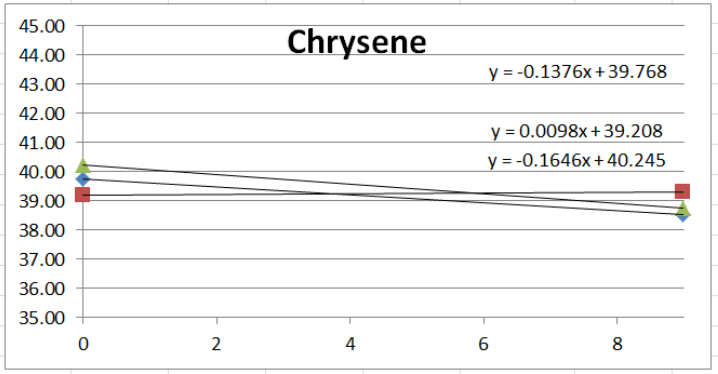
| Bottle | Result a | Result b | diff | sum | avg |
|---|---|---|---|---|---|
| Ampoule 08 | 14.01 | 15.14 | -1.13 | 29.15 | 14.57 |
| Ampoule 14 | 14.47 | 13.84 | 0.64 | 28.31 | 14.16 |
| Ampoule 22 | 14.61 | 14.85 | -0.24 | 29.46 | 14.73 |
| Ampoule 39 | 14.31 | 14.36 | -0.06 | 28.67 | 14.34 |
| Ampoule 43 | 13.96 | 14.14 | -0.19 | 28.10 | 14.05 |
| Ampoule 54 | 14.11 | 14.42 | -0.32 | 28.53 | 14.26 |
| Ampoule 67 | 14.13 | 14.42 | -0.29 | 28.54 | 14.27 |
| Ampoule 73 | 14.57 | 15.16 | -0.59 | 29.73 | 14.87 |
| Ampoule 87 | 13.89 | 14.67 | -0.78 | 28.56 | 14.28 |
| Ampoule 95 | 14.69 | 14.12 | 0.56 | 28.81 | 14.40 |
| | | **Σ(diff)$^2$ =** | 3.23648 | | |
| | | var(sum)/2 = | | 0.13105 | =MSB |

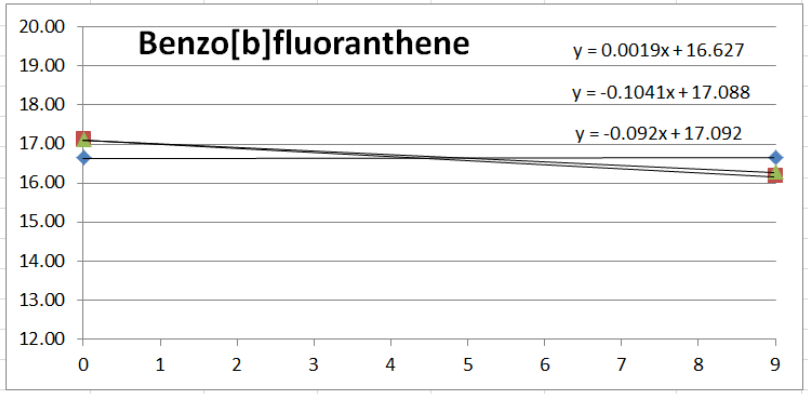


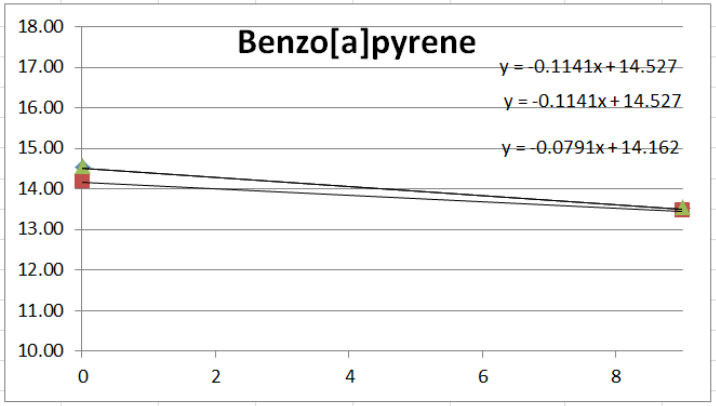
## ANNEX 8. Stability of the smoked black pepper test material for the period of the study

- in a refrigerator at recommended conditions (~ -4 °C).
- in a deep freezer at the reference conditions - (~ -80 °C).



# ANNEX 9. Questionnaire and answers from the participants

| No. | Cue | Question | Answers | Global no. | Edit type |
|---|---|---|---|---|---|
| * | | | Click here to define a new question for 2016 PT PAH in smoked black pepper. | | |
| | Ring test : 2016 PT PAH in smoked black pepper (14 questions, 497 answers) | | | | |
| 1 | previous experience | Did you have previous experience with the analysis of spice and herbs? | 41 Answers | 1 | RadioGroup |
| 2 | how many sample analysed | How many spice and herb samples have you analysed in the past few years? | 41 Answers | 2 | ComboBox |
| 3 | standardised method | Did you apply standardised method for the analysis of the 4 PAHs in the test sample? | 40 Answers | 3 | RadioGroup |
| 4 | method for PAH analysis | Which method for PAH analysis did you used (name of the standard method or laboratory method)? | 37 Answers | 7 | Memo |
| 5 | deviations from the method | Did you have deviations from the standardised method? | 35 Answers | 4 | RadioGroup |
| 6 | If YES, please specify | If YES, please specify | 17 Answers | 5 | Memo |
| 7 | verified/validated method | Is your method verified/validated for herb and spices matrices and which? | 38 Answers | 9 | Memo |
| 8 | Accreditation | Are you accredited for analysis of PAHs in herb and spices? | 40 Answers | 6 | RadioGroup |
| 9 | type of calibrants | What kind of calibrants did you use? | 41 Answers | 8 | RadioGroup |
| 10 | Sample intake | What is your sample intake in gram? | 40 Answers | 14 | TextEdit |
| 11 | problems during analysis | Did you experience problem during analysis? | 37 Answers | 10 | Memo |
| 12 | problems during reporting | Did you experience problems during reporting? | 36 Answers | 11 | Memo |
| 13 | sample compliant with MLs | Is the test sample compliant with the CURRENT legislative maximum levels (MLs)? | 41 Answers | 12 | RadioGroup |
| 14 | Any remarks, comments, suggest | Any remarks, comments, suggestions ... | 13 Answers | 13 | Memo |

Lab details | Measured values | Questions and Answers

| No. | Question | Answer |
|---|---|---|
| 1 | Did you have previous experience with the analysis of spice and herbs? | ○ No ○ Yes |
| 2 | How many spice and herb samples have you analysed in the past few years? | |
| 3 | Did you apply standardised method for the analysis of the 4 PAHs in the test sample? | ○ No ○ Yes |
| 4 | Which method for PAH analysis did you used | |
| 5 | Did you have deviations from the standardised method? | ○ No ○ Yes |
| 6 | If YES, please specify | |
| 7 | Is your method verified/validated for herb and spices matrices and which? | |
| 8 | Are you accredited for analysis of PAHs in herb and spices? | ○ No ○ Yes |
| 9 | What kind of calibrants did you use? | ○ laboratory prepared from neat substances ○ purchased mix in solvent |
| 10 | What is your sample intake in gram? | |
| 11 | Did you experience problem during analysis? | |
| 12 | Did you experience problems during reporting? | |
| 13 | Is the test sample compliant with the CURRENT legislative maximum levels (MLs)? | ○ No ○ Yes |
| 14 | Any remarks, comments, suggestions ... | |

| Lab Code | 1. Previous experience | 2. How many sample analysed | 3. Standardised method | 4. Method for PAH analysis |
|---|---|---|---|---|
| 101 | | | | |
| 104 | No | 0 | No | |
| 105 | No | 0 | No | in-house method |
| 106 | Yes | <10 | Yes | SLV-m097.f |
| 107 | No | <10 | Yes | FC102.1 |
| 116 | No | 0 | Yes | EN 16619:2015 |
| 119 | No | 0 | Yes | HPLC-FLD |
| 121 | No | 0 | No | In house method (QMI 132) |
| 124 | No | 0 | No | Laboratory method |
| 125 | Yes | <50 | No | 03-02 Determination of PAHs in food by using HPLC/FLD or GC/IDMS-SIM |
| 126 | No | 0 | No | |
| 127 | Yes | <10 | Yes | GC/MS with GPC cleanup |
| 128 | Yes | <10 | Yes | Laboratory procedure: Soxhlet, GPC, SPE silica |
| 129 | | | | |
| 132 | Yes | <50 | No | FSG410 |
| 133 | | | | |
| 136 | No | 0 | No | laboratory method (PLE/GPC-HPLC/FLD) |
| 137 | No | 0 | No | laboratory method |
| 139 | No | 0 | Yes | HPLC/FLD |
| 140 | | | | |
| 142 | | | | |
| 144 | No | <10 | No | in-house method |
| 145 | No | <10 | No | In-house laboratory method |
| 146 | No | <10 | No | Laboratory method |
| 148 | Yes | <100 | No | Analysis of PAH with pressurized liquid extraction, clean up via SPE (Silica and Styrene/Divinylbenzene stationary phases), quantification with GC-MS (laboratory method) |
| 149 | Yes | <100 | Yes | SOP PALC0075 Determination of PAHs in food by GC-MS |
| 202 | Yes | <10 | No | QuEChERS extraction with acetonitrile and a C18 clean-up (900 mg MgSO4 + 150 mg PSA + 150 mg C18), followed by evaporation under a stream of nitrogen and reconstitution in acetonitrile |
| 203 | No | <10 | Yes | Determination of PAHs in foodstuffs with GCMS |
| 209 | No | <1000 | No | Extraction with PLE (cyclohexane)<br />1st Clean-Up with GPC (Bio-beads S-X3)<br />2nd Clean-Up with SPE (SiOH)<br />GC-MS (Column: Varian PAH Select (30m x 0,25mm x 0,15µm)) |
| 211 | yes | 100 | | laboratory method |
| 212 | Yes | <50 | No | HPLC-FLD after Saponification and GPC |
| 213 | Yes | <50 | No | QMP_504_VW_402 in-house method pretreatment and ISO 22959 detection |
| 215 | Yes | <50 | Yes | in-house method using SampliQ QUECHERS AOAC kit |
| 217 | Yes | <10 | Yes | §64 LFGB L 07.00-40 (slightly modified) |
| 218 | Yes | <50 | Yes | DGF CIII-17a<br />1997-08 |
| 222 | Yes | <50 | Yes | Laboratory method: reflux sample with KOH, extraction with hexane and purified with silica |
| 223 | Yes | <10 | No | Laboratory method |
| 230 | Yes | <100 | No | |
| 231 | Yes | <10 | Yes | ONR CEN TS 16621 2014 06 01 |
| 234 | No | 0 | No | laboratory method |
| 235 | Yes (Tea samples) | 10 | Yes | laboraty method |
| 238 | No | 0 | No | The determination of benz(a)anthracene, chrysene, benzo(b&j)fluoranthene, benzo(a)pyrene and PAH4 total by GCMSMS |
| 241 | No | 0 | No | |
| 243 | No | 0 | Yes | QUECHERS EXTRACTION - GC-MSMS (QQQ) ANALYSIS |
| 247 | Yes | <100 | Yes | Sample preparation:NPR-CEN/TS 16621, Food analysis-Determination of benzo(a)pyrene, benzo(a)anthracene, chrysene and benzo(b)fluoranthene in foodstuffs by HPLC-FD <br />Analysis: ISO22959 |
| 250 | Yes | <100 | Yes | DIN CEN/TS 16621 |

| Lab Code | 5. Deviations from the method | 6. If YES, please specify | 7. Verified/validated method | 8. Accreditation |
|---|---|---|---|---|
| 101 | | | | |
| 104 | No | | No | No |
| 105 | No | | no | No |
| 106 | No | | yes, herb tea | Yes |
| 107 | Yes | There seemed to be a lot of fat/dirt in this sample,<br />which partly was avoided by a low sample weight of 0.5 g and partly by an extra dilution (x10) before injection on the GC-MS system. | No | No |
| 116 | Yes | no labelled standard used and also different diameter of GPC column. Quantification was done using standard adding method of nature PAHs to tested sample of black pepper. | No | No |
| 119 | Yes | lower sample weight for pepper 2g instead of 5g | no | No |
| 121 | | | No | No |
| 124 | No | | NO | No |
| 125 | No | | Yes, Determination of PAHs in food by using HPLC/FLD or GC/IDMS-SIM. | Yes |
| 126 | No | laboratory internal method | No | No |
| 127 | No | | | No |
| 128 | Yes | Additional cean-up with MIP columns | Yes | Yes |
| 129 | | | | |
| 132 | No | | Yes, both herbs and spices | Yes |
| 133 | | | | |
| 136 | | | only for herbs | |
| 137 | Yes | extraction:<br />-addition of isotopic labelled internal standard<br />-addition of cyclohexane/acetone (50/50)<br />-mix with ultra turrax<br />purification:<br />-Liq./Liq. with water<br />-elimination of the aqueous phase<br />-organic phase on silica SPE<br />-eluat purified on preparative HPLC (DACC)<br />-dry evaporation and reconstitution with cyclohexane<br />injection:<br />GC-MSMS with agilent select PAH 30m column<br /> | No | No |
| 139 | Yes | / | No | No |
| 140 | | | | |
| 142 | | | | |
| 144 | Yes | - Use of less sample amount as normally.<br />- Incubation overnight in acetic acid in order to destroy the pepper oil | no | No |
| 145 | No | | No | No |
| 146 | Yes | Soxleth extraction, followed by GPC, and then SPE on Silica. | No | No |
| 148 | | | validated with dried herbs, verified with curry powder (participation in PT) | Yes |
| 149 | No | | Yes. Validated with spiking stidied on a range of herbs and spices including turmeric, rosemary, chili, cinnamon, ginger, parsley, oregano, black pepper, clove, ground coriander, cardomum and ground cumin. | Yes |
| 202 | No | | Curry spice powder | Yes |
| 203 | Yes | Small changes in the sample preparation part. | No | No |
| 209 | | | verified for curry powder, validated for a mixture of herbs | Yes |
| 211 | | | yes | yes |
| 212 | Yes | less sample intake | Yes (Rosemary) | Yes |
| 213 | No | | BAP - Mixture of different herbs and spices; Analysis of BAP in herbs and spices is accredited | No |
| 215 | No | | no | No |
| 217 | Yes | Before the cyclohexanextract was loaded on the SPE column it was dried with Na2SO4. The residue is dissolved in methanol instet of acetonitrile for chromatographic analysis (HPLC-FLD) | | Yes |
| 218 | Yes | saponification under reflux and higher temperature<br />extend the method for more matrices and more analytes than benzo(a)pyrene | yes, validated for herbs and spices | Yes |
| 222 | No | | Yes, matrix spanish pepper | No |
| 223 | No | | | Yes |
| 230 | No | | yes | Yes |
| 231 | Yes | Removing fat ans oils with GPC | no | No |
| 234 | | | no | Yes |
| 235 | No | | Yes (validated for Tea) | Yes |
| 238 | No | | No | No |
| 241 | Yes | internal method | no | No |
| 243 | Yes | WEIGHED ONLY 1 GRAM | NO | No |
| 247 | No | | Validated for food (solid products) but not specific herbs and spices | Yes |
| 250 | Yes | with internal standards (benz(k)fluoranthene-d12, benzo(b)chrysene), extraction by PLE (Cyclohexan/Ethylacetat), SEC with BioBeads S-X3 (32x2.5 cm) | validated for herbs (matrix: origanum majorana) and spices (matrix: cumin) | Yes |

| Lab Code | 9. Type of calibrants | 10. Sample intake | 11. Problems during analysis |
|---|---|---|---|
| 101 | | | |
| 104 | laboratory prepared from neat substances | 2g | Very difficult matrix with too many chromatographic interferences. A very extreme cleanup procedure was necessary. |
| 105 | purchased mix in solvent | 2.5g | blank sample not available |
| 106 | purchased mix in solvent | usually 10 g, this time 4g because of the low sample amount we received | no |
| 107 | purchased mix in solvent | 0.5 g | There seemed to be a lot of fat/dirt in this sample, which partly was avoided by a low sample weight of 0.5 g and partly by an extra dilution (x10) before injection on the GC-MS system. |
| 116 | purchased mix in solvent | 1 | Yes. We usually use HPLC/FLD approach but due to observe huge interferenties for BAA and CHR we had to establish GC/MS method very quick. No all parameters from EN 16619:2015 could not met due to no time for it and also no enough sample for testing. |
| 119 | purchased mix in solvent | 2 g | no |
| 121 | laboratory prepared from neat substances | 2 | Yes |
| 124 | purchased mix in solvent | 1 | |
| 125 | purchased mix in solvent | 2 | Interference in chrysene by using HPLC/FLD, which could not be removed. In GC-MS only extreme clean-up led to purified peaks of all 4 PAHs. Benzo(a)anthracene and Benzo(b)fluoranthene showed unexpected for black pepper interference in spectrum identification in HPLC/FLD, which could be removed by a more advanced clean-up. All problems related to interference have been solved by using GC-MS and an extreme clean-up. |
| 126 | purchased mix in solvent | 0.5 g | No |
| 127 | laboratory prepared from neat substances | 2,5 | no |
| 128 | purchased mix in solvent | 2 | Difficult with interpretation of chromatogram |
| 129 | | | |
| 132 | purchased mix in solvent | 1 to 5 g dependant on type | No |
| 133 | | | |
| 136 | purchased mix in solvent | 2 | separation problems (BAA, CHR) |
| 137 | purchased mix in solvent | 1 | #NAME? |
| 139 | purchased mix in solvent | | Clean up |
| 140 | | | |
| 142 | | | |
| 144 | purchased mix in solvent | 0.5 | yes. Many interfering peaks, and internal standard with very high RSD |
| 145 | laboratory prepared from neat substances | 15 | We have got the mismatch of BAP declared concentration in 4PAH solution in acetonitrile You have sent 24/02/2016 (Date of production: 18/02/2016). We have used fresh prepared BAP solutions from 1. SUPELCO, 2. ULTRA SCIENTIFIC standards. |
| 146 | purchased mix in solvent | 2.5 | Matrix interferences for Chr and BaA |
| 148 | laboratory prepared from neat substances | 2 | recovery of internal standard for BbF in one analysis (BbF value 1) below 50%, this result not included in final value. |
| 149 | purchased mix in solvent | 5 | No |
| 202 | laboratory prepared from neat substances | 2 | distortion of chromatographic performance through coextracted materials. samples were further diluted |
| 203 | purchased mix in solvent | 2,5-5 | No |
| 209 | purchased mix in solvent | 5 | |
| 211 | solvent calibrant with 13C labeled internal standards | 1-2 g | no |
| 212 | laboratory prepared from neat substances | 0,2 | Interferences in the chromatograms |
| 213 | purchased mix in solvent | 0.5 - 1.0 | Interference from volatile oil regarding detection. |
| 215 | purchased mix in solvent | 2 | no |
| 217 | purchased mix in solvent | 2-20 g (here: 2 -2,5 g) | |
| 218 | laboratory prepared from neat substances | 2,50 g | no |
| 222 | purchased mix in solvent | 5 | No |
| 223 | purchased mix in solvent | 5 | |
| 230 | purchased mix in solvent | 2 g | BAA: matrix effects in the chromatogram |
| 231 | purchased mix in solvent | 2 | Internal standard benzo(a)anthracene D12 was coeluating with any matrix peak, so we changed to benzo(b)chrysene as internal standard. |
| 234 | purchased mix in solvent | 1 | a lot of matrix noise, a higher amount of intake let to a precipation |
| 235 | external calibration | 1,5 g | Yes, the HPLC analysis resulted to unclear peaks (BAA and CRY could not be sufficiently separated from fat). Not enough sample material to optimize the cleaning conditions. |
| 238 | purchased mix in solvent | 2 | No |
| 241 | purchased mix in solvent | 1.5 g | yes, unexpected loose of the labelled internal standards.<br />Low and unreproducible recoveries of labelled internal standards |
| 243 | laboratory prepared from neat substances | 1 GRAM | SOLVENT CONTAMINATION |
| 247 | purchased mix in solvent | 3 | No |
| 250 | purchased mix in solvent | 2-5 g (depending on matrix and expected content) | no |

| Lab Code | 12. Problems during reporting | 13. Sample compliant with MLs | 14. Any remarks, comments, suggest |
|---|---|---|---|
| 101 | | | |
| 104 | No | No | |
| 105 | | No | |
| 106 | no | No | we will like to receive more amount of sample. we usually need at least 10g and if we have to do a triplicat we need at least 30 g from you. In this case we managed as the leveld were high. |
| 107 | Yes. IT issues due to security problems | Yes | |
| 116 | No | No | To lack of sending problematic matrixv for PT without any previous control in EURL! |
| 119 | no | No | |
| 121 | No | No | We weren't able to unmark line 13 |
| 124 | | No | |
| 125 | No | No | The MLs apply only if the assumed food item was placed on the market later than 01.04.2016 |
| 126 | Yes, some parameters were missing or the system did not allow filling them. | No | |
| 127 | no | No | |
| 128 | No | No | |
| 129 | | | |
| 132 | No | No | |
| 133 | | | |
| 136 | | Yes | ad compliance: only related to BAP<br />do not mix herbs and spices in the questionaire<br />only BAP and BBF could be analysed! |
| 137 | No | No | No |
| 139 | Yes | Yes | No expirience |
| 140 | | | |
| 142 | | | |
| 144 | no | Yes | |
| 145 | No | No | |
| 146 | No | No | |
| 148 | no | No | |
| 149 | No | No | |
| 202 | wasn´t able to fill in the method for the "sum of PAH4" | No | |
| 203 | No | No | |
| 209 | No | No | |
| 211 | no | yes, the pepper is compliant with the current MLs, because the pepper is placed on the market prior to No April Yes0No6, but they exceed the MLs which are established since April Yes0No6. | |
| 212 | no | Yes | |
| 213 | No | No | EU 2015/1933 |
| 215 | no | Yes | Although there are no maximum levels set for PAH residues in spices, I would consider that the food does not comply with the food safety requirements of Regulation 178/2002, Article 14 in that it is unsafe by reason of being unfit for human consumption due to the probable cumulative toxic effects of consumption. |
| 217 | | No | |
| 218 | it is not possible to fill in the analytical method and LOQ/LOD for the SUM4PAHs | Yes | |
| 222 | No | No | |
| 223 | No | No | |
| 230 | no | No | Question 13: ML valid from 1 April 2016 |
| 231 | Not til now. | No | |
| 234 | no | Yes | |
| 235 | No | until 3No.03.Yes0No6: no, after this time (0No.04.Yes0No6): yes - VO (EG) Yes0No5/ No933 Yes7. Oktober Yes0No5) | |
| 238 | No | Yes | A suitable blank matrix would have been helpful for evaluation of recovery. |
| 241 | no | Yes | we are not aware of regulation setting limits for PAHs in spices |
| 243 | | Yes | |
| 247 | No | No | NA |
| 250 | I had problems downloading the software as our IT does not allow downloading .exe-files for safety reasons. Sending a .zip-file or a link for a zip-file would be easier. | No | |

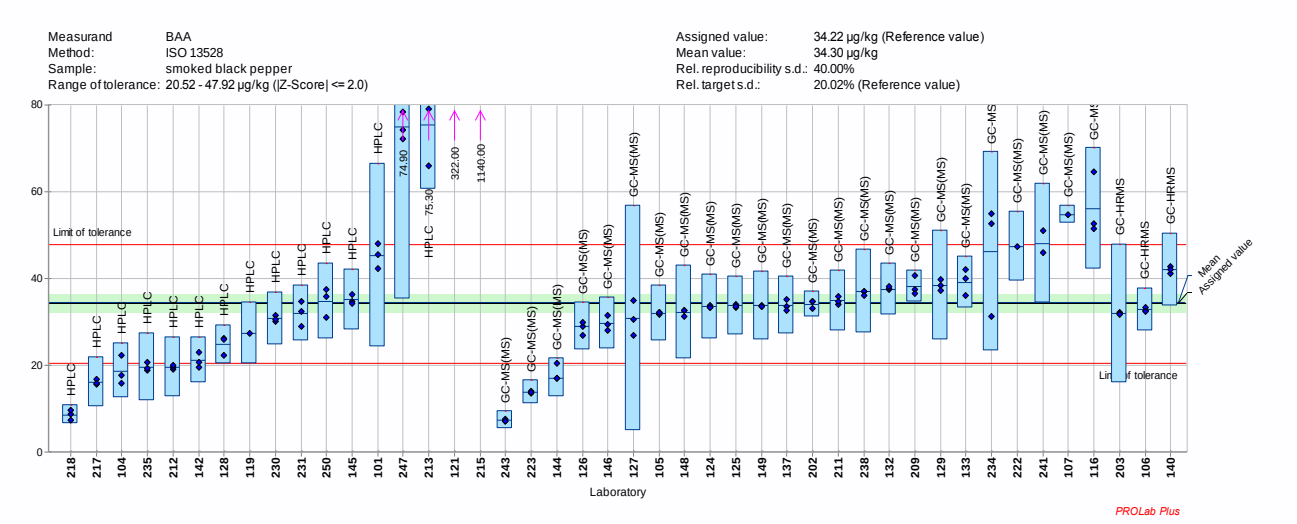
## Annex 10. Method performance LOD and LOQ as reported

| Column | BAA | Column | BAP | Column | BBF | Column | CHR | Column |
|---|---|---|---|---|---|---|---|---|
| Lab code | LOD [µg/kg] | LOQ [µg/kg] | LOD [µg/kg] | LOQ [µg/kg] | LOD [µg/kg] | LOQ [µg/kg] | LOD [µg/kg] | LOQ [µg/kg] |
| 101 | 0.03 | 0.9 | 0.02 | 0.9 | 0.09 | 0.89 | 0.04 | 0.89 |
| 104 | 1.5 | 0.5 | 2 | 0.6 | 1.6 | 0.5 | 3 | 0.9 |
| 105 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 |
| 106 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 |
| 107 | 0.2 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 |
| 116 | 0.45 | 0.15 | 0.6 | 0.2 | 0.6 | 0.2 | 0.45 | 0.15 |
| 119 | 0.05 | 0.03 | 0.05 | 0.03 | 0.1 | 0.05 | 0.05 | 0.03 |
| 121 | 0.25 | 0.08 | 0.25 | 0.08 | 0.25 | 0.08 | 0.25 | 0.08 |
| 124 | 0.09 | 0.03 | 0.09 | 0.03 | 0.09 | 0.03 | 0.09 | 0.03 |
| 125 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 126 | 0.8 | 0.29 | 0.8 | 0.29 | 0.8 | 0.29 | 0.8 | 0.29 |
| 127 | 0.5 | 0.3 | 0.5 | 0.3 | 0.5 | 0.3 | 0.5 | 0.3 |
| 128 | 0.5 | 0.25 | 0.16 | 0.08 | 0.4 | 0.2 | 0.5 | 0.25 |
| 129 | 0.25 | 0.07 | 0.28 | 0.08 | 0.33 | 0.09 | 0.44 | 0.12 |
| 132 | 0.03 | 0.03 | 0.12 | 0.12 | 0.09 | 0.09 | 0.1 | 0.1 |
| 133 | 0.3 | 0.7 | 0.3 | 0.8 | 0.2 | 0.5 | 0.6 | 1.3 |
| 136 | | | 0.2 | 0.06 | 0.3 | 0.1 | | |
| 137 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 139 | 0.25 | 0.16 | 0.25 | 0.07 | 0.25 | 0.07 | 0.25 | 0.05 |
| 140 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 |
| 142 | 1 | 0.2 | 0.5 | 0.1 | 0.5 | 0.1 | 1 | 0.2 |
| 144 | 0.2 | 0.1 | 0.65 | 0.35 | 0.4 | 0.2 | 0.2 | 0.1 |
| 145 | 0.2 | 0.06 | 0.2 | 0.06 | 0.2 | 0.06 | 0.5 | 0.2 |
| 146 | 0.06 | 0.02 | 0.09 | 0.03 | 0.09 | 0.03 | 0.06 | 0.02 |
| 148 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 149 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 202 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 203 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 209 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 |
| 211 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 | 0.1 |
| 212 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 213 | 2 | 0.7 | 0.5 | 0.2 | 0.5 | 0.2 | 2 | 0.7 |
| 215 | 1 | 0.1 | 1 | 0.1 | 1 | 0.1 | 1 | 0.1 |
| 217 | 0.7 | 0.2 | 0.7 | 0.2 | 0.7 | 0.2 | 0.7 | 0.2 |
| 218 | 0.15 | 0.03 | 0.07 | 0.01 | 0.15 | 0.03 | 0.16 | 0.03 |
| 222 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 223 | 0.5 | 0.2 | 0.5 | 0.2 | 0.5 | 0.2 | 0.5 | 0.2 |
| 230 | 0.9 | 0.2 | 0.3 | 0.1 | 0.6 | 0.2 | 0.3 | 0.1 |
| 231 | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 |
| 234 | 11 | 3.8 | 12 | 4.1 | 6 | 3 | 7 | 3 |
| 235 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 |
| 238 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 |
| 241 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 | 0.9 | 0.3 |
| 243 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 247 | 0.1 | 0.02 | 0.1 | 0.01 | 0.1 | 0.04 | 0.1 | 0.04 |
| 250 | 0.06 | 0.21 | 0.11 | 0.41 | 0.11 | 0.38 | 0.09 | 0.31 |

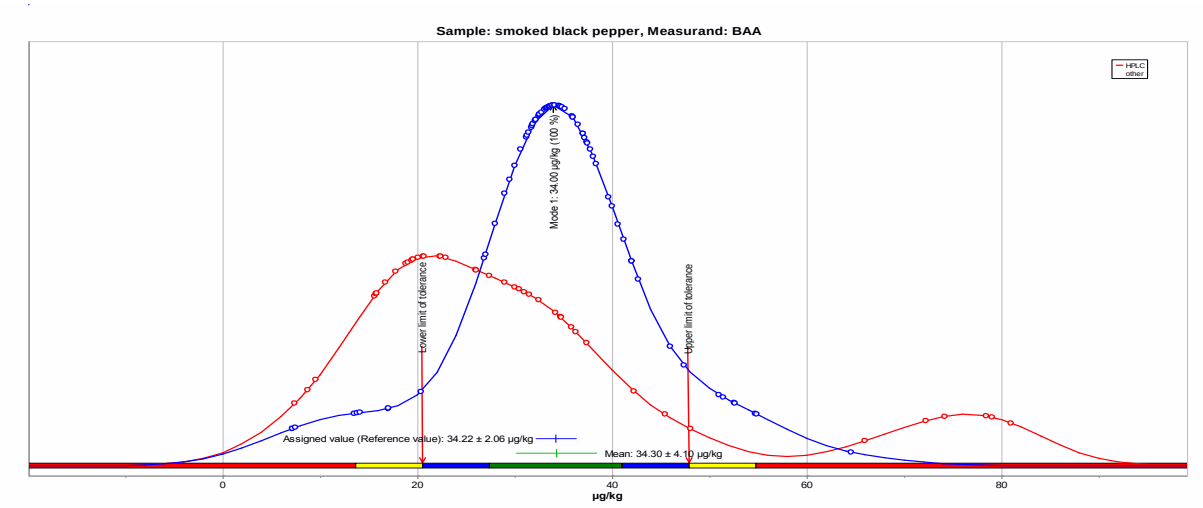
# ANNEX 11: Data reported by participants

The data reported by the participants are compiled in the following tables. 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. Very slight differences in the mean values on both graphs below are possible, as on the Kernel density plot the mean values are calculated based on the "final values" reported by the participants while on the distribution of the individual results graphs, they are calculated based on the three replicate results.

**Distribution of individual results of replicate determinations reported for the benz[*a*]anthracene (BAA) content of the smoked black pepper 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 benz[*a*]anthracene (BAA) content of the smoked black pepper test sample**
*Red dots and line - HPLC results; blue dots and lines - GC-mass spectrometry results*



## Results, as reported by the participants and scoring, for the content of benz[*a*]anthracene (BAA) of the smoked black pepper test sample.

*Due to a software problem, the reported significant zeros after the comas are missing*

| Lab code | Measurand name | Value 1 | Value 2 | Value 3 | Final value | U lab | k | Analytical method | u lab | Z-Score | Zeta score | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | BAA | 48.04 | 42.28 | 45.43 | 45.25 | 21.2 | 2 | HPLC | 10.6 | 1.6 | 1.0 | c |
| 104 | BAA | 22.4 | 15.8 | 17.8 | 18.7 | 6.31 | 2 | HPLC | 3.16 | -2.3 | -4.7 | a |
| 105 | BAA | 32.2 | 31.8 | 32.1 | 32 | 6.39 | 2 | GC-MS(MS) | 3.2 | -0.3 | -0.7 | a |
| 106 | BAA | 32.56 | 33.25 | 32.46 | 32.76 | 4.91 | 2 | GC-HRMS | 2.46 | -0.2 | -0.5 | a |
| 107 | BAA | 54.7 | | | 54.7 | 2 | 2 | GC-MS(MS) | 1 | 3.0 | 14.3 | b |
| 116 | BAA | 51.45 | 52.55 | 64.55 | 56.18 | 14 | 2 | GC-MS(MS) | 7 | 3.2 | 3.1 | c |
| 119 | BAA | 27.38 | | | 27.38 | 7.12 | 2 | HPLC | 3.56 | -1.0 | -1.8 | a |
| 121 | BAA | 310 | 334 | 321 | 322 | 121.13 | 2 | HPLC | 60.56 | 42.0 | 4.8 | c |
| 124 | BAA | 33.4 | 33.8 | 33.3 | 33.5 | 7.5 | 2 | GC-MS(MS) | 3.75 | -0.1 | -0.2 | a |
| 125 | BAA | 33.4 | 34.1 | 33.5 | 33.7 | 6.71 | 2 | GC-MS(MS) | 3.35 | -0.1 | -0.1 | a |
| 126 | BAA | 29 | 27 | 30 | 29 | 5.56 | 2 | GC-MS(MS) | 2.78 | -0.8 | -1.8 | a |
| 127 | BAA | 30.64 | 34.88 | 26.88 | 30.8 | 26 | 2 | GC-MS(MS) | 13 | -0.5 | -0.3 | c |
| 128 | BAA | 26.1 | 26 | 22.3 | 24.8 | 4.5 | 2 | HPLC | 2.25 | -1.4 | -3.8 | a |
| 129 | BAA | 39.66 | 38.38 | 37.21 | 38.42 | 12.6 | 2 | GC-MS(MS) | 6.3 | 0.6 | 0.7 | a |
| 132 | BAA | 37.49 | 37.77 | 38.07 | 37.49 | 6 | 2 | GC-MS(MS) | 3 | 0.5 | 1.0 | c |
| 133 | BAA | 40 | 36 | 42 | 39 | 5.95 | 2 | GC-MS(MS) | 2.97 | 0.7 | 1.5 | a |
| 136 | BAA | | | | | | | | | | | |
| 137 | BAA | 33.64 | 35.16 | 32.58 | 33.8 | 6.76 | 2 | GC-MS(MS) | 3.38 | -0.1 | -0.1 | a |
| 139 | BAA | < 0.16 | < 0.16 | < 0.16 | | | 2 | HPLC | | | | |
| 140 | BAA | 41.2 | 42.7 | 42 | 42 | 8.4 | 2 | GC-HRMS | 4.2 | 1.1 | 1.8 | c |
| 142 | BAA | 22.9 | 19.6 | 20.7 | 21.1 | 5.31 | 2 | HPLC | 2.65 | -1.9 | -4.6 | a |
| 144 | BAA | 17.06 | 20.36 | 17.01 | 17.1 | 4.43 | 2 | GC-MS(MS) | 2.21 | -2.5 | -7.0 | a |
| 145 | BAA | 34.8 | 34.2 | 36.3 | 35.1 | 7 | 2 | HPLC | 3.5 | 0.1 | 0.2 | a |
| 146 | BAA | 28.01 | 29.49 | 31.44 | 29.65 | 5.93 | 2 | GC-MS(MS) | 2.96 | -0.7 | -1.5 | c |
| 148 | BAA | 32.74 | 32.56 | 31.29 | 32.2 | 10.9 | 2 | GC-MS(MS) | 5.45 | -0.3 | -0.4 | a |
| 149 | BAA | 33.6 | 33.9 | 33.6 | 33.7 | 8 | 2 | GC-MS(MS) | 4 | -0.1 | -0.1 | a |
| 202 | BAA | 33.1 | 34.6 | 34.6 | 34.1 | 3 | 2 | GC-MS(MS) | 1.5 | 0.0 | -0.1 | a |
| 203 | BAA | 32.2 | 31.9 | 31.7 | 31.9 | 15.95 | 2 | GC-HRMS | 7.97 | -0.3 | -0.3 | c |
| 209 | BAA | 40.64 | 37.42 | 36.49 | 38.18 | 3.67 | 2 | GC-MS(MS) | 1.83 | 0.6 | 1.9 | a |
| 211 | BAA | 35.9 | 34.1 | 34.7 | 34.9 | 7 | 2 | GC-MS(MS) | 3.5 | 0.1 | 0.2 | a |
| 212 | BAA | 19.04 | 19.59 | 20.07 | 19.6 | 6.91 | 2 | HPLC | 3.46 | -2.1 | -4.1 | a |
| 213 | BAA | 79 | 81 | 66 | 75.3 | 14.79 | 2 | HPLC | 7.4 | 6.0 | 5.5 | c |
| 215 | BAA | 970 | 1310 | | 1140 | 0 | 2 | HPLC | 0 | 161.4 | 1073.6 | b |
| 217 | BAA | 16.74 | 15.6 | 15.85 | 16.06 | 5.82 | 2 | HPLC | 2.91 | -2.7 | -5.9 | a |
| 218 | BAA | 8.74 | 7.45 | 9.57 | 8.59 | 2.13 | 2 | HPLC | 1.07 | -3.7 | -17.3 | a |
| 222 | BAA | 47.4 | | | 47.4 | 8.1 | 2 | GC-MS(MS) | 4.05 | 1.9 | 3.2 | a |
| 223 | BAA | 14.1 | 13.5 | 13.8 | 13.8 | 2.76 | 2 | GC-MS(MS) | 1.38 | -3.0 | -11.9 | a |
| 230 | BAA | 30.01 | 31.49 | 30.49 | 30.7 | 6.11 | 2 | HPLC | 3.05 | -0.5 | -1.1 | c |
| 231 | BAA | 34.68 | 32.48 | 28.97 | 32.04 | 6.41 | 1 | HPLC | 6.41 | -0.3 | -0.3 | a |
| 234 | BAA | 52.63 | 54.89 | 31.19 | 46.2 | 23.08 | 1 | GC-MS(MS) | 23.08 | 1.7 | 0.5 | c |
| 235 | BAA | 18.8 | 20.6 | 19.4 | 19.5 | 7.8 | 2 | HPLC | 3.9 | -2.1 | -3.6 | c |
| 238 | BAA | 37 | 37 | 36 | 37 | 9.69 | 2 | GC-MS(MS) | 4.84 | 0.4 | 0.6 | a |
| 241 | BAA | 51 | 46 | | 48 | 13.86 | 2.16 | GC-MS(MS) | 6.41 | 2.0 | 2.1 | a |
| 243 | BAA | 7.5 | 7.2 | 7.2 | 7.3 | 2.1 | 2 | GC-MS(MS) | 1.05 | -3.9 | -18.3 | a |
| 247 | BAA | 74.2 | 72.2 | 78.4 | 74.9 | 39.7 | 2 | HPLC | 19.85 | 5.9 | 2.0 | c |
| 250 | BAA | 37.4 | 35.8 | 31 | 34.7 | 8.69 | 2 | HPLC | 4.35 | 0.1 | 0.1 | a |

**Satisfactory**, **Questionable**, **Unsatisfactory**

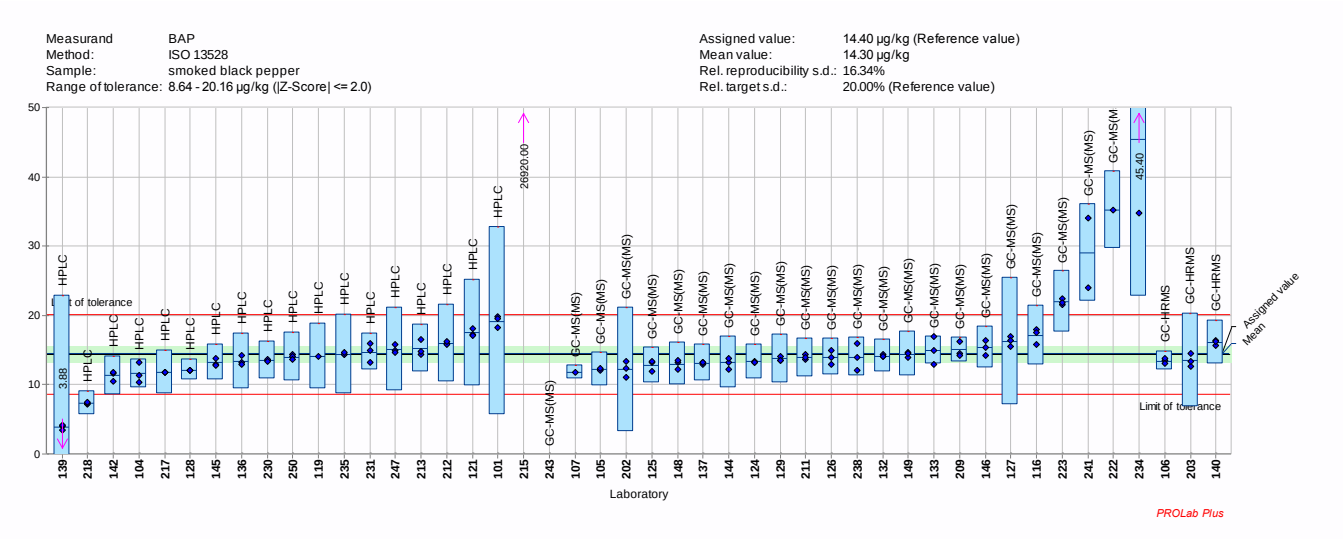
***a*** : $u_{ref} \leq u_{lab} \leq u_{max}$ $(\sigma_p)$;

**b** : $u_{lab} < u_{ref}$;

**c** : $u_{lab} > u_{max}$ $(\sigma_p)$

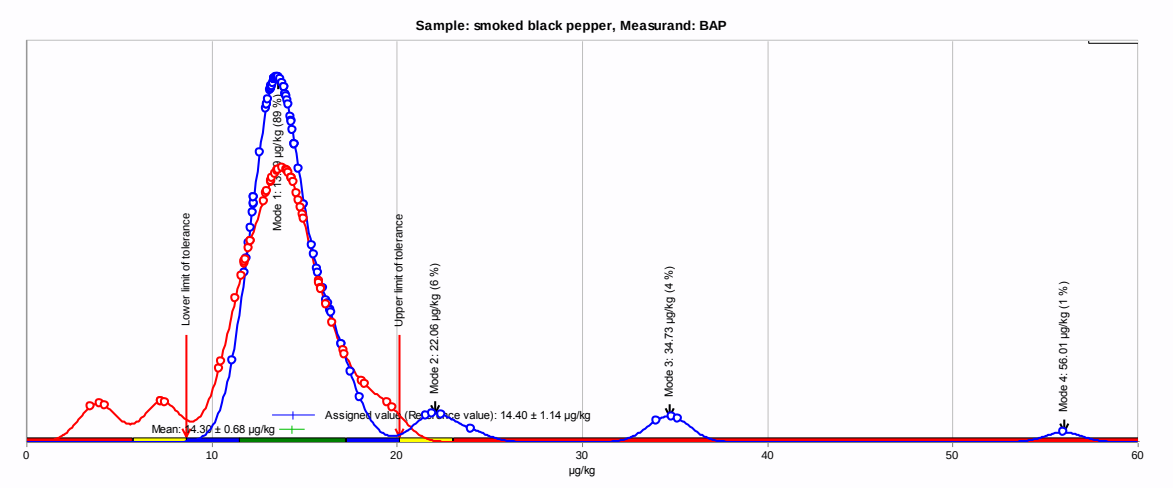
**Distribution of individual results of replicate determinations reported for the benzo[*a*] pyrene (BAP) content of the smoked black pepper test sample**

blue triangles: individual results of replicate determinations, blue box: reported expanded measurement uncertainty (k=2), blue horizontal line in blue box: average of replicate determinations, green dotted 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;




**Kernel density plot of the reported values for proficiency assessment for the benzo[*a*]pyrene (BAP) content of the smoked black pepper test sample**

*Red dots and line - HPLC results; blue dots and lines - GC-mass spectrometry results*



## Results, as reported by the participants, for the content of benzo[*a*]pyrene (BAP) of the smoked black pepper test sample.

*Due to a software problem, the reported significant zeros after the comas are missing*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | BAP | 19.77 | | 18.26 | | 19.47 | | 19.17 | 13.60 | 2.00 | HPLC | 6.80 | 1.66 | 0.70 | c |
| 104 | BAP | 13.20 | | 10.40 | | 11.30 | | 11.60 | 2.09 | 2.00 | HPLC | 1.05 | -0.97 | -2.35 | a |
| 105 | BAP | 12.20 | | 12.10 | | 12.30 | | 12.20 | 2.50 | 2.00 | GC-MS(MS) | 1.25 | -0.76 | -1.60 | a |
| 106 | BAP | 13.45 | | 13.73 | | 13.06 | | 13.41 | 1.34 | 2.00 | GC-HRMS | 0.67 | -0.34 | -1.13 | a |
| 107 | BAP | 11.80 | | | | | | 11.80 | 1.00 | 2.00 | GC-MS(MS) | 0.50 | -0.90 | -3.43 | b |
| 116 | BAP | 15.75 | | 17.99 | | 17.49 | | 17.08 | 4.30 | 2.00 | GC-MS(MS) | 2.15 | 0.93 | 1.20 | a |
| 119 | BAP | 14.04 | | | | | | 14.04 | 4.77 | 2.00 | HPLC | 2.38 | -0.13 | -0.15 | a |
| 121 | BAP | 17.20 | | 17.10 | | 18.10 | | 17.50 | 7.71 | 2.00 | HPLC | 3.86 | 1.08 | 0.80 | c |
| 124 | BAP | 13.20 | | 13.40 | | 13.20 | | 13.30 | 2.51 | 2.00 | GC-MS(MS) | 1.25 | -0.38 | -0.80 | a |
| 125 | BAP | 13.20 | | 13.40 | | 11.90 | | 12.80 | 2.59 | 2.00 | GC-MS(MS) | 1.30 | -0.56 | -1.13 | a |
| 126 | BAP | 14.00 | | 13.00 | | 15.00 | | 14.00 | 2.60 | 2.00 | GC-MS(MS) | 1.30 | -0.14 | -0.28 | a |
| 127 | BAP | 15.51 | | 17.00 | | 16.38 | | 16.30 | 9.20 | 2.00 | GC-MS(MS) | 4.60 | 0.66 | 0.41 | c |
| 128 | BAP | 12.10 | | 12.10 | | 12.00 | | 12.10 | 1.50 | 2.00 | HPLC | 0.75 | -0.80 | -2.44 | a |
| 129 | BAP | 13.57 | | 14.08 | | 13.52 | | 13.73 | 3.51 | 2.00 | GC-MS(MS) | 1.76 | -0.23 | -0.36 | a |
| 132 | BAP | 14.13 | | 14.37 | | 14.03 | | 14.13 | 2.40 | 2.00 | GC-MS(MS) | 1.20 | -0.09 | -0.20 | a |
| 133 | BAP | 15.00 | | 13.00 | | 17.00 | | 15.00 | 2.00 | 2.00 | GC-MS(MS) | 1.00 | 0.21 | 0.52 | a |
| 136 | BAP | 13.21 | | 14.16 | | 12.95 | | 13.40 | 4.02 | 2.00 | HPLC | 2.01 | -0.35 | -0.48 | a |
| 137 | BAP | 12.96 | | 13.19 | | 13.24 | | 13.13 | 2.63 | 2.00 | GC-MS(MS) | 1.31 | -0.44 | -0.89 | a |
| 139 | BAP | 3.97 | | 4.22 | | 3.46 | | 3.88 | 18.98 | 2.00 | HPLC | 9.49 | -3.65 | -1.11 | c |
| 140 | BAP | 15.70 | | 16.40 | | 16.20 | | 16.10 | 3.22 | 2.00 | GC-HRMS | 1.61 | 0.59 | 1.00 | a |
| 142 | BAP | 11.60 | | 10.50 | | 11.80 | | 11.30 | 2.80 | 2.00 | HPLC | 1.40 | -1.08 | -2.05 | a |
| 144 | BAP | 13.18 | | 13.83 | | 12.25 | | 13.20 | 3.73 | 2.00 | GC-MS(MS) | 1.87 | -0.42 | -0.62 | a |
| 145 | BAP | 13.00 | | 12.80 | | 13.80 | | 13.20 | 2.60 | 2.00 | HPLC | 1.30 | -0.42 | -0.85 | a |
| 146 | BAP | 14.25 | | 15.41 | | 16.43 | | 15.36 | 3.07 | 2.00 | GC-MS(MS) | 1.54 | 0.33 | 0.59 | a |
| 148 | BAP | 13.24 | | 12.26 | | 13.50 | | 13.00 | 3.10 | 2.00 | GC-MS(MS) | 1.55 | -0.49 | -0.85 | a |
| 149 | BAP | 14.70 | | 13.90 | | 14.50 | | 14.40 | 3.21 | 2.00 | GC-MS(MS) | 1.60 | 0.00 | 0.00 | a |
| 202 | BAP | 11.10 | | 12.30 | | 13.30 | | 12.20 | 8.98 | 2.00 | GC-MS(MS) | 4.49 | -0.76 | -0.49 | c |
| 203 | BAP | 12.60 | | 14.50 | | 13.40 | | 13.50 | 6.75 | 2.00 | GC-HRMS | 3.38 | -0.31 | -0.26 | c |
| 209 | BAP | 16.27 | | 14.50 | | 14.29 | | 15.02 | 1.83 | 2.00 | GC-MS(MS) | 0.91 | 0.22 | 0.58 | a |
| 211 | BAP | 14.30 | | 13.60 | | 13.90 | | 13.90 | 2.79 | 2.00 | GC-MS(MS) | 1.40 | -0.17 | -0.33 | a |
| 212 | BAP | 15.92 | | 15.82 | | 16.17 | | 16.00 | 5.61 | 2.00 | HPLC | 2.81 | 0.56 | 0.56 | a |
| 213 | BAP | 14.30 | | 14.80 | | 16.50 | | 15.20 | 3.45 | 2.00 | HPLC | 1.72 | 0.28 | 0.44 | a |
| 215 | BAP | 26,950 | | 26,890 | | | | 26,920 | 0.00 | 2.00 | HPLC | 0.00 | 9,342.22 | 47,202.81 | b |
| 217 | BAP | 11.80 | | 11.83 | | 11.76 | | 11.80 | 3.15 | 2.00 | HPLC | 1.58 | -0.90 | -1.55 | a |
| 218 | BAP | 7.25 | | 7.26 | | 7.49 | | 7.33 | 1.76 | 2.00 | HPLC | 0.88 | -2.45 | -6.75 | a |
| 222 | BAP | 35.20 | | | | | | 35.20 | 5.60 | 2.00 | GC-MS(MS) | 2.80 | 7.22 | 7.28 | a |
| 223 | BAP | 21.60 | | 22.40 | | 21.90 | | 22.00 | 4.41 | 2.00 | GC-MS(MS) | 2.20 | 2.64 | 3.34 | a |
| 230 | BAP | 13.52 | | 13.58 | | 13.41 | | 13.50 | 2.70 | 2.00 | HPLC | 1.35 | -0.31 | -0.61 | a |
| 231 | BAP | 15.90 | | 14.96 | | 13.29 | | 14.72 | 2.66 | 1.00 | HPLC | 2.66 | 0.11 | 0.12 | a |
| 234 | BAP | 55.99 | | 34.82 | | | | 45.40 | 22.70 | 1.00 | GC-MS(MS) | 22.70 | 10.76 | 1.37 | c |
| 235 | BAP | 14.30 | | 14.40 | | 14.60 | | 14.40 | 5.76 | 2.00 | HPLC | 2.88 | 0.00 | 0.00 | c |
| 238 | BAP | 12.00 | | 16.00 | | 14.00 | | 14.00 | 2.80 | 2.00 | GC-MS(MS) | 1.40 | -0.14 | -0.26 | a |
| 241 | BAP | 34.00 | | 24.00 | | | | 29.00 | 7.00 | 2.18 | GC-MS(MS) | 3.21 | 5.07 | 4.48 | c |
| 243 | BAP | < 2.00 | NB | < 2.00 | NB | < 2.00 | NB | | | 2.00 | GC-MS(MS) | | -5.00 | -999.00 | |
| 247 | BAP | 14.90 | | 15.80 | | 14.70 | | 15.10 | 6.04 | 2.00 | HPLC | 3.02 | 0.24 | 0.23 | c |
| 250 | BAP | 14.30 | | 14.10 | | 13.60 | | 14.00 | 3.50 | 2.00 | HPLC | 1.75 | -0.14 | -0.22 | a |

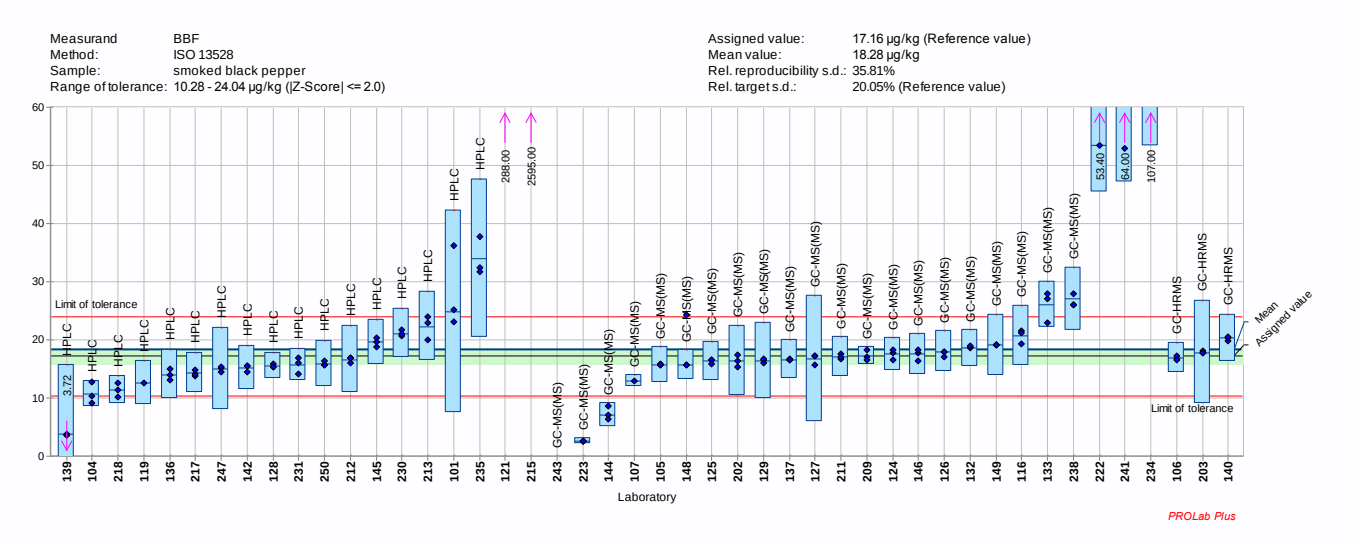
**Satisfactory**, Questionable, **Unsatisfactory**

***a*** : $u_{ref} \leq u_{lab} \leq u_{max}$ $(\sigma_p)$;

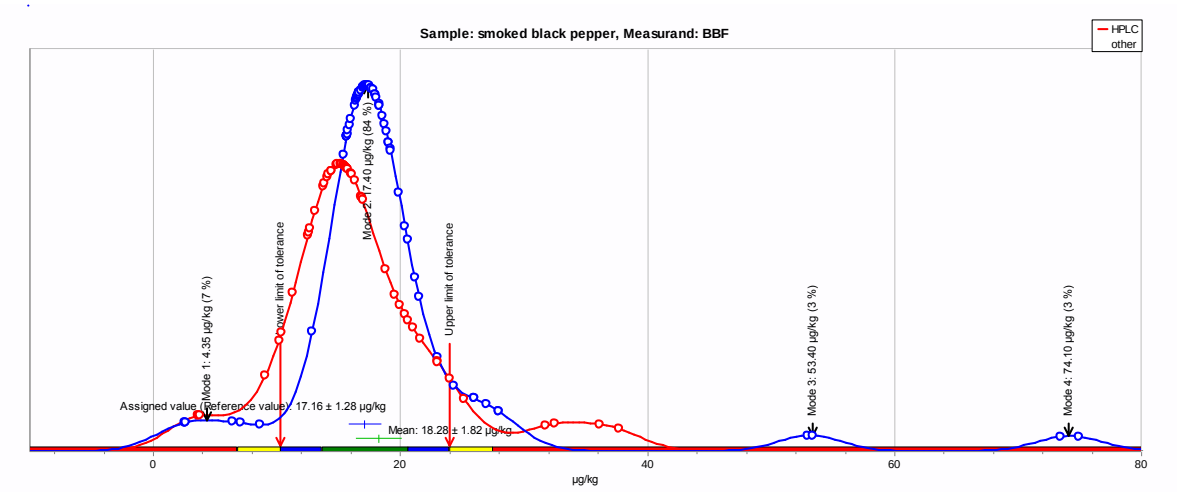
**b** : $u_{lab} < u_{ref}$;

**c** : $u_{lab} > u_{max}$ $(\sigma_p)$

**Distribution of individual results of replicate determinations reported for the benzo[*b*]fluoranthene (BBF) content of the smoked black pepper test sample**
blue triangles: individual results of replicate determinations, blue box: reported expanded measurement uncertainty (k=2), blue horizontal line in blue box: average of replicate determinations, green dotted 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;




**Kernel density plot of the reported values for proficiency assessment for the benzo[*b*]fluoranthene (BBF) content of the smoked black pepper test sample**
*Red dots and line - HPLC results; blue dots and lines - GC-mass spectrometry results*



**Results, as reported by the participants, for the content of benzo[*b*]-fluoranthene (BBF) of the smoked black pepper test sample.**

*Due to a software problem, the reported significant zeros after the comas are missing*

| Lab code | Measu-rand | Value 1 | S 1 | Value 2 | S 2 | Value 3 | S 3 | X lab | U lab | k | Analytical method | u lab | Z-Score | Zeta score | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | BBF | 36.16 | | 23.06 | | 25.2 | | 24.8 | 17.45 | 2 | HPLC | 8.72 | 2.2 | 0.9 | c |
| 104 | BBF | 12.7 | | 9.1 | | 10.4 | | 10.7 | 2.19 | 2 | HPLC | 1.1 | -1.9 | -5.1 | a |
| 105 | BBF | 15.8 | | 15.7 | | 15.7 | | 15.7 | 3.09 | 2 | GC-MS(MS) | 1.55 | -0.4 | -0.9 | a |
| 106 | BBF | 16.71 | | 17.28 | | 16.61 | | 16.87 | 2.53 | 2 | GC-HRMS | 1.27 | -0.1 | -0.2 | a |
| 107 | BBF | 12.9 | | | | | | 12.9 | 1 | 2 | GC-MS(MS) | 0.5 | -1.2 | -5.2 | b |
| 116 | BBF | 19.25 | | 21.56 | | 21.21 | | 20.68 | 5.2 | 2 | GC-MS(MS) | 2.6 | 1.0 | 1.3 | a |
| 119 | BBF | 12.63 | | | | | | 12.63 | 3.79 | 2 | HPLC | 1.9 | -1.3 | -2.3 | a |
| 121 | BBF | 294 | | 284 | | 286 | | 288 | 110 | 2 | HPLC | 55 | 78.7 | 4.9 | c |
| 124 | BBF | 17.7 | | 18.3 | | 16.5 | | 17.5 | 2.9 | 2 | GC-MS(MS) | 1.45 | 0.1 | 0.2 | a |
| 125 | BBF | 16.6 | | 16.5 | | 15.9 | | 16.3 | 3.29 | 2 | GC-MS(MS) | 1.65 | -0.3 | -0.5 | a |
| 126 | BBF | 18 | | 17 | | 18 | | 18 | 3.57 | 2 | GC-MS(MS) | 1.78 | 0.2 | 0.4 | a |
| 127 | BBF | 15.69 | | 17.32 | | 17.18 | | 16.73 | 10.8 | 2 | GC-MS(MS) | 5.4 | -0.1 | -0.1 | c |
| 128 | BBF | 15.8 | | 15.4 | | 15.4 | | 15.5 | 2.2 | 2 | HPLC | 1.1 | -0.5 | -1.3 | a |
| 129 | BBF | 16.54 | | 16.01 | | 16.71 | | 16.42 | 6.55 | 2 | GC-MS(MS) | 3.28 | -0.2 | -0.2 | a |
| 132 | BBF | 18.59 | | 18.76 | | 18.91 | | 18.59 | 3.16 | 2 | GC-MS(MS) | 1.58 | 0.4 | 0.8 | a |
| 133 | BBF | 23 | | 27 | | 28 | | 26 | 4 | 2 | GC-MS(MS) | 2 | 2.6 | 4.2 | a |
| 136 | BBF | 14.97 | | 13.89 | | 13.11 | | 14 | 4.2 | 2 | HPLC | 2.1 | -0.9 | -1.4 | a |
| 137 | BBF | 16.6 | | 16.65 | | 16.63 | | 16.63 | 3.32 | 2 | GC-MS(MS) | 1.66 | -0.2 | -0.3 | a |
| 139 | BBF | 3.65 | | 3.81 | | 3.7 | | 3.72 | 12 | 2 | HPLC | 6 | -3.9 | -2.2 | c |
| 140 | BBF | 19.9 | | 20.4 | | 20.6 | | 20.3 | 4.06 | 2 | GC-HRMS | 2.03 | 0.9 | 1.5 | a |
| 142 | BBF | 14.4 | | 15.6 | | 15.5 | | 15.2 | 3.81 | 2 | HPLC | 1.9 | -0.6 | -1.0 | a |
| 144 | BBF | 7.06 | | 8.65 | | 6.41 | | 7.1 | 2.12 | 2 | GC-MS(MS) | 1.06 | -2.9 | -8.1 | a |
| 145 | BBF | 18.8 | | 20.4 | | 19.6 | | 19.6 | 3.9 | 2 | HPLC | 1.95 | 0.7 | 1.2 | a |
| 146 | BBF | 17.81 | | 16.45 | | 18.36 | | 17.54 | 3.51 | 2 | GC-MS(MS) | 1.75 | 0.1 | 0.2 | a |
| 148 | BBF | 24.36 | | 15.7 | | 15.74 | | 15.7 | 2.62 | 2 | GC-MS(MS) | 1.31 | -0.4 | -1.0 | a |
| 149 | BBF | 19.1 | | 19.1 | | 19.2 | | 19.1 | 5.29 | 2 | GC-MS(MS) | 2.65 | 0.6 | 0.7 | a |
| 202 | BBF | 15.4 | | 17.4 | | 16.3 | | 16.4 | 6.01 | 2 | GC-MS(MS) | 3.01 | -0.2 | -0.2 | a |
| 203 | BBF | 18.1 | | 17.7 | | 17.7 | | 17.8 | 8.9 | 2 | GC-HRMS | 4.45 | 0.2 | 0.1 | c |
| 209 | BBF | 18.34 | | 17 | | 16.59 | | 17.31 | 1.55 | 2 | GC-MS(MS) | 0.78 | 0.0 | 0.1 | a |
| 211 | BBF | 17.5 | | 16.8 | | 17.1 | | 17.1 | 3.39 | 2 | GC-MS(MS) | 1.7 | 0.0 | 0.0 | a |
| 212 | BBF | 16.06 | | 16.89 | | 16.86 | | 16.6 | 5.8 | 2 | HPLC | 2.9 | -0.2 | -0.2 | a |
| 213 | BBF | 20 | | 24 | | 23 | | 22.3 | 5.99 | 2 | HPLC | 3 | 1.5 | 1.7 | a |
| 215 | BBF | 2610 | | 2580 | | | | 2595 | 0 | 2 | HPLC | 0 | 749.4 | 4027.9 | b |
| 217 | BBF | 13.78 | | 14.22 | | 14.89 | | 14.3 | 3.52 | 2 | HPLC | 1.76 | -0.8 | -1.5 | a |
| 218 | BBF | 11.33 | | 10.2 | | 12.56 | | 11.36 | 2.37 | 2 | HPLC | 1.18 | -1.7 | -4.3 | a |
| 222 | BBF | 53.4 | | | | | | 53.4 | 8 | 2 | GC-MS(MS) | 4 | 10.5 | 8.9 | c |
| 223 | BBF | 2.64 | | 2.53 | | 2.6 | | 2.59 | 0.52 | 2 | GC-MS(MS) | 0.26 | -4.2 | -21.1 | b |
| 230 | BBF | 20.67 | | 21.64 | | 21.03 | | 21.1 | 4.2 | 2 | HPLC | 2.1 | 1.1 | 1.8 | a |
| 231 | BBF | 16.96 | | 16.03 | | 14.09 | | 15.69 | 2.82 | 1 | HPLC | 2.82 | -0.4 | -0.5 | a |
| 234 | BBF | 152 | | 93.96 | | 73.47 | | 107 | 53.76 | 1 | GC-MS(MS) | 53.76 | 26.1 | 1.7 | c |
| 235 | BBF | 37.7 | | 32.5 | | 31.8 | | 34 | 13.6 | 2 | HPLC | 6.8 | 4.9 | 2.5 | c |
| 238 | BBF | 26 | | 28 | | 26 | | 27 | 5.47 | 2 | GC-MS(MS) | 2.73 | 2.9 | 3.5 | a |
| 241 | BBF | 53 | | 75 | | | | 64 | 17 | 2.2 | GC-MS(MS) | 7.73 | 13.6 | 6.0 | c |
| 243 | BBF | < 2.00 | NB | < 2.00 | NB | < 2.00 | NB | | | 2 | GC-MS(MS) | | -5.0 | | |
| 247 | BBF | 15.2 | | 14.4 | | 15.3 | | 15 | 7.05 | 2 | HPLC | 3.52 | -0.6 | -0.6 | c |
| 250 | BBF | 15.7 | | 16.3 | | 15.7 | | 15.9 | 4 | 2 | HPLC | 2 | -0.4 | -0.6 | a |

**Satisfactory**, **Questionable**, **Unsatisfactory**

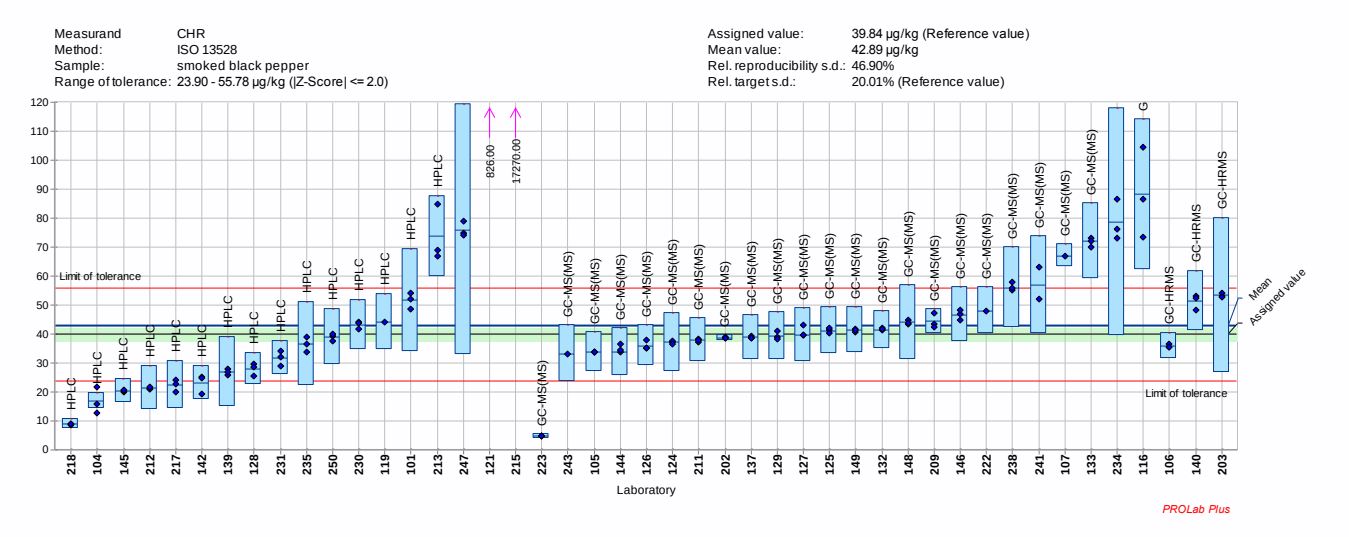
***a*** : $u_{ref} \leq u_{lab} \leq u_{max}$ $(\sigma_p)$;

**b** : $u_{lab} < u_{ref}$;

**c** : $u_{lab} > u_{max}$ $(\sigma_p)$

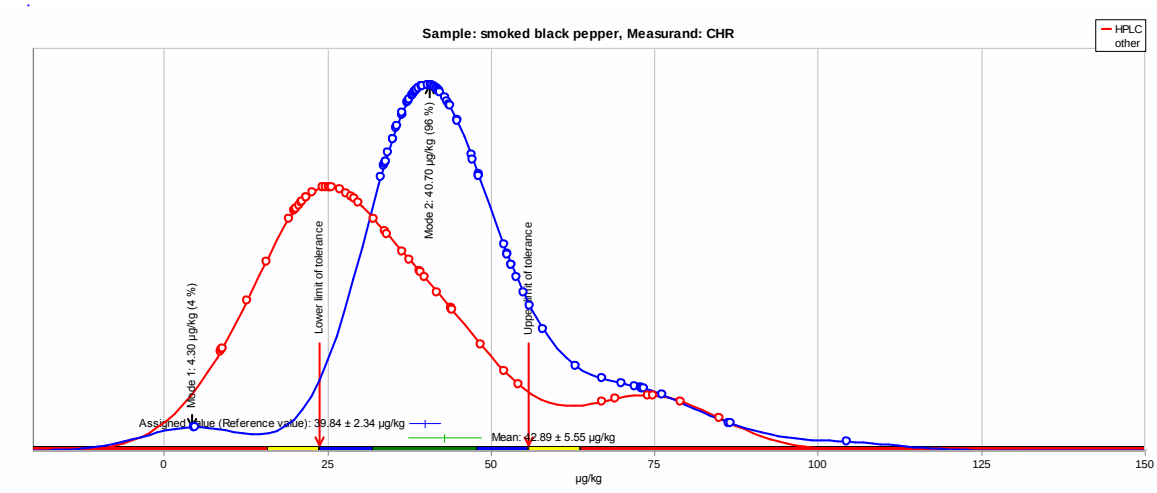
**Distribution of individual results of replicate determinations reported for the chrysene (CHR) content of the smoked black pepper test sample**

blue triangles: individual results of replicate determinations, blue box: reported expanded measurement uncertainty (k=2), blue horizontal line in blue box: average of replicate determinations, green dotted 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;




**Kernel density plot of the reported values for proficiency assessment for the chrysene (CHR) content of the smoked black pepper test sample**

*Red dots and line - HPLC results; blue dots and lines - GC-mass spectrometry results*



**Results, as reported by the participants, for the content of chrysene (CHR) of the smoked black pepper test sample.**

*Due to a software problem, the reported significant zeros after the comas are missing*

| Lab code | Measurand | Value 1 | S 1 | Value 2 | S 2 | Value 3 | S 3 | X lab | U lab | k | Analytical method | u lab | Z-Score | Zeta score | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | CHR | 54.24 | | 48.49 | | 52.03 | | 51.6 | 17.7 | 2 | HPLC | 8.85 | 1.5 | 1.3 | c |
| 104 | CHR | 21.8 | | 12.8 | | 15.7 | | 16.8 | 2.81 | 2 | HPLC | 1.4 | -2.9 | -12.6 | a |
| 105 | CHR | 33.9 | | 33.7 | | 33.9 | | 33.8 | 6.79 | 2 | GC-MS(MS) | 3.4 | -0.8 | -1.7 | a |
| 106 | CHR | 35.58 | | 36.39 | | 35.68 | | 35.9 | 4.49 | 2 | GC-HRMS | 2.24 | -0.5 | -1.6 | a |
| 107 | CHR | 67 | | | | | | 67 | 4 | 2 | GC-MS(MS) | 2 | 3.4 | 11.7 | a |
| 116 | CHR | 73.35 | | 86.66 | | 104.51 | | 88.2 | 26 | 2 | GC-MS(MS) | 13 | 6.1 | 3.7 | c |
| 119 | CHR | 44.13 | | | | | | 44.1 | 9.71 | 2 | HPLC | 4.86 | 0.5 | 0.9 | a |
| 121 | CHR | 820 | | 849 | | 810 | | 826 | 269.89 | 2 | HPLC | 134.95 | 98.6 | 5.8 | c |
| 124 | CHR | 37.3 | | 37.6 | | 36.4 | | 37.1 | 10.2 | 2 | GC-MS(MS) | 5.1 | -0.3 | -0.5 | a |
| 125 | CHR | 41.3 | | 42.1 | | 40.3 | | 41.2 | 8.19 | 2 | GC-MS(MS) | 4.1 | 0.2 | 0.3 | a |
| 126 | CHR | 35 | | 35 | | 38 | | 36 | 7 | 2 | GC-MS(MS) | 3.5 | -0.5 | -1.0 | a |
| 127 | CHR | 42.98 | | 39.5 | | 39.6 | | 39.6 | 9.34 | 2 | GC-MS(MS) | 4.67 | 0.0 | 0.0 | a |
| 128 | CHR | 29.7 | | 28.7 | | 25.4 | | 27.9 | 5.59 | 2 | HPLC | 2.8 | -1.5 | -3.9 | a |
| 129 | CHR | 39.02 | | 40.91 | | 38.12 | | 39.4 | 8.32 | 2 | GC-MS(MS) | 4.16 | -0.1 | -0.1 | a |
| 132 | CHR | 41.41 | | 42.14 | | 41.69 | | 41.4 | 6.63 | 2 | GC-MS(MS) | 3.31 | 0.2 | 0.4 | a |
| 133 | CHR | 70 | | 73 | | 72 | | 72 | 13.06 | 2 | GC-MS(MS) | 6.53 | 4.0 | 4.8 | a |
| 136 | CHR | | | | | | | | | 2 | | | | | |
| 137 | CHR | 39.39 | | 38.55 | | 38.69 | | 38.9 | 7.78 | 2 | GC-MS(MS) | 3.89 | -0.1 | -0.2 | a |
| 139 | CHR | 25.77 | | 27.85 | | 26.94 | | 26.9 | 12 | 2 | HPLC | 6 | -1.6 | -2.1 | a |
| 140 | CHR | 48.2 | | 53.2 | | 52.5 | | 51.3 | 10.26 | 2 | GC-HRMS | 5.13 | 1.4 | 2.2 | a |
| 142 | CHR | 25.3 | | 19.2 | | 24.8 | | 23.1 | 5.8 | 2 | HPLC | 2.9 | -2.1 | -5.4 | a |
| 144 | CHR | 33.81 | | 36.53 | | 34.33 | | 33.8 | 8.14 | 2 | GC-MS(MS) | 4.07 | -0.8 | -1.4 | a |
| 145 | CHR | 20 | | 20.3 | | 20.7 | | 20.4 | 4.11 | 2 | HPLC | 2.06 | -2.4 | -8.2 | a |
| 146 | CHR | 44.92 | | 47.01 | | 48.19 | | 46.7 | 9.34 | 2 | GC-MS(MS) | 4.67 | 0.9 | 1.4 | a |
| 148 | CHR | 44.86 | | 43.36 | | 43.77 | | 44 | 13 | 2 | GC-MS(MS) | 6.5 | 0.5 | 0.6 | a |
| 149 | CHR | 41.8 | | 40.8 | | 41.5 | | 41.4 | 7.81 | 2 | GC-MS(MS) | 3.9 | 0.2 | 0.4 | a |
| 202 | CHR | 38.9 | | 38.6 | | 38.5 | | 38.7 | 1 | 2 | GC-MS(MS) | 0.5 | -0.1 | -0.9 | b |
| 203 | CHR | 53.2 | | 54 | | 52.6 | | 53.3 | 26.65 | 2 | GC-HRMS | 13.32 | 1.7 | 1.0 | c |
| 209 | CHR | 47.29 | | 43.61 | | 42.28 | | 44.4 | 4.38 | 2 | GC-MS(MS) | 2.19 | 0.6 | 1.8 | a |
| 211 | CHR | 38.3 | | 37.4 | | 38 | | 37.9 | 7.6 | 2 | GC-MS(MS) | 3.8 | -0.2 | -0.5 | a |
| 212 | CHR | 21.12 | | 21.08 | | 21.85 | | 21.4 | 7.52 | 2 | HPLC | 3.76 | -2.3 | -4.7 | a |
| 213 | CHR | 85 | | 67 | | 69 | | 73.7 | 14.01 | 2 | HPLC | 7 | 4.2 | 4.8 | a |
| 215 | CHR | 17380 | | 17160 | | | | 17270 | 0 | 2 | HPLC | 0 | 2161.9 | 14726.6 | b |
| 217 | CHR | 24.26 | | 22.69 | | 20.13 | | 22.4 | 8.29 | 2 | HPLC | 4.14 | -2.2 | -4.1 | a |
| 218 | CHR | 8.73 | | 8.88 | | 9.04 | | 8.9 | 1.72 | 2 | HPLC | 0.86 | -3.9 | -21.3 | b |
| 222 | CHR | 48.1 | | | | | | 48.1 | 8.2 | 2 | GC-MS(MS) | 4.1 | 1.0 | 1.9 | a |
| 223 | CHR | 4.71 | | 4.74 | | 4.7 | | 4.7 | 0.94 | 2 | GC-MS(MS) | 0.47 | -4.4 | -27.9 | b |
| 230 | CHR | 41.82 | | 43.99 | | 43.9 | | 43.2 | 8.59 | 2 | HPLC | 4.3 | 0.4 | 0.8 | a |
| 231 | CHR | 34.16 | | 32.12 | | 29.06 | | 31.8 | 5.81 | 1 | HPLC | 5.81 | -1.0 | -1.4 | a |
| 234 | CHR | 76.2 | | 86.4 | | 73.1 | | 78.6 | 39.32 | 1 | GC-MS(MS) | 39.32 | 4.9 | 1.0 | c |
| 235 | CHR | 39.1 | | 33.8 | | 36.5 | | 36.5 | 14.6 | 2 | HPLC | 7.3 | -0.4 | -0.5 | a |
| 238 | CHR | 55 | | 58 | | 56 | | 56 | 13.92 | 2 | GC-MS(MS) | 6.96 | 2.0 | 2.3 | a |
| 241 | CHR | 52 | | 63 | | | | 57 | 16.85 | 2.2 | GC-MS(MS) | 7.66 | 2.2 | 2.2 | a |
| 243 | CHR | 33.2 | | | | | | 33.2 | 9.9 | 2 | GC-MS(MS) | 4.95 | -0.8 | -1.3 | a |
| 247 | CHR | 74.9 | | 74.1 | | 79 | | 76 | 43.32 | 2 | HPLC | 21.66 | 4.5 | 1.7 | c |
| 250 | CHR | 39.2 | | 39.9 | | 37.6 | | 38.9 | 9.7 | 2 | HPLC | 4.85 | -0.1 | -0.2 | a |

**Satisfactory**, Questionable, **Unsatisfactory**

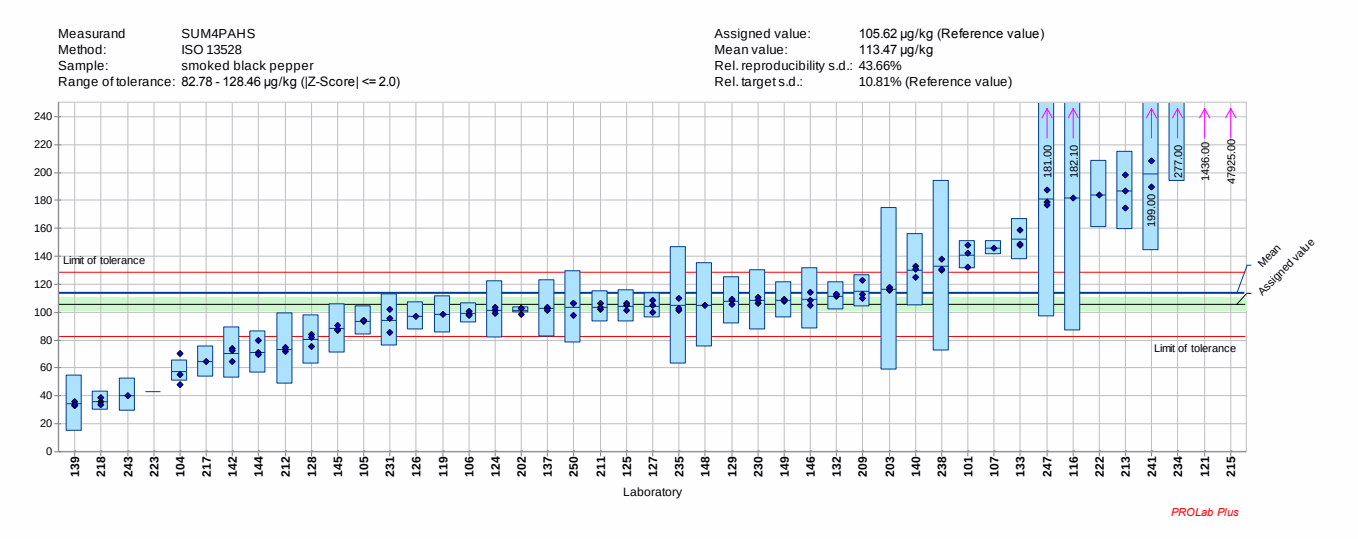
***a*** : $u_{ref} \leq u_{lab} \leq u_{max}$ $(\sigma_p)$;

**b** : $u_{lab} < u_{ref}$;

**c** : $u_{lab} > u_{max}$ $(\sigma_p)$

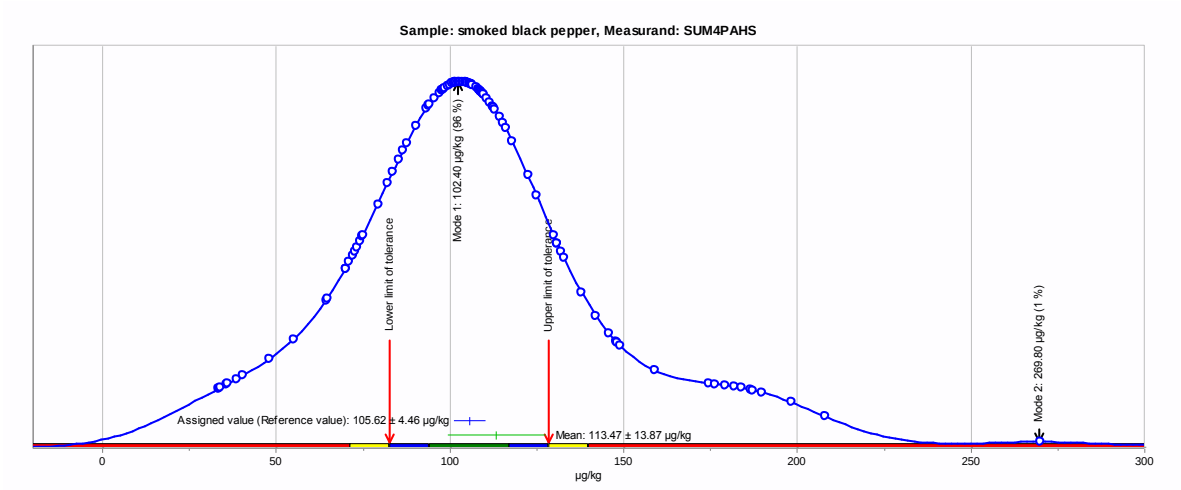
**Distribution of individual results of replicate determinations reported for the sum of the four markers PAHs (SUM4PAH) content of the smoked black pepper test sample**

blue triangles: individual results of replicate determinations, blue box: reported expanded measurement uncertainty (k=2), blue horizontal line in blue box: average of replicate determinations, green dotted 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;




**Kernel density plot of the reported values for proficiency assessment for the SUM4PAH content of the smoked black pepper test sample**

*Red dots and line - HPLC results; blue dots and lines - GC-mass spectrometry results*



**Results, as reported by the participants, for the sum of the four markers PAHs (SUM4PAH) of the smoked black pepper test sample.**

*Due to a software problem, the reported significant zeros after the comas are missing*

| Lab code | X lab | U lab | k | Analytical method | u lab | Z-Score | Zeta score | Classification |
|---|---|---|---|---|---|---|---|---|
| 101 | 140.81 | 10.2 | 2 | HPLC | 5.1 | 3.1 | 6.3 | a |
| 104 | 57.8 | 7.5 | 2 | HPLC | 3.75 | -4.2 | -11 | a |
| 105 | 93.7 | 10.19 | 2 | | 5.09 | -1 | -2.1 | a |
| 106 | 98.92 | 7.24 | 2 | GC-HRMS | 3.62 | -0.6 | -1.6 | a |
| 107 | 146 | 5 | 2 | | 2.5 | 3.5 | 12.1 | a |
| 116 | 182.1 | 96 | 2 | GC-MS(MS) | 48 | 6.7 | 1.6 | c |
| 119 | 98.18 | 13.49 | 2 | | 6.75 | -0.7 | -1 | a |
| 121 | 1436 | 315 | 2 | HPLC | 157.5 | 116.5 | 8.4 | c |
| 124 | 101.4 | 20.41 | 2 | GC-MS(MS) | 10.2 | -0.4 | -0.4 | a |
| 125 | 104.1 | 11.4 | 2 | GC-MS(MS) | 5.7 | -0.1 | -0.2 | a |
| 126 | 97 | 9.9 | 2 | GC-MS(MS) | 4.95 | -0.8 | -1.6 | a |
| 127 | 104.52 | 8.68 | 2 | GC-MS(MS) | 4.34 | -0.1 | -0.2 | a |
| 128 | 80.3 | 17.69 | 2 | HPLC | 8.85 | -2.2 | -2.8 | a |
| 129 | 107.91 | 16.83 | 2 | GC-MS(MS) | 8.41 | 0.2 | 0.3 | a |
| 132 | 111.62 | 10.05 | 2 | GC-MS(MS) | 5.02 | 0.5 | 1.1 | a |
| 133 | 152 | 15 | 2 | GC-MS(MS) | 7.5 | 4.1 | 5.9 | a |
| 136 | | | | | | | | |
| 137 | 102.43 | 20.48 | 2 | GC-MS(MS) | 10.24 | -0.3 | -0.3 | a |
| 139 | 34.46 | 20 | 2 | HPLC | 10 | -6.2 | -6.9 | a |
| 140 | 130 | 26 | 2 | GC-HRMS | 13 | 2.1 | 1.8 | c |
| 142 | 70.7 | 18.22 | 2 | | 9.11 | -3.1 | -3.7 | a |
| 144 | 71.1 | 15.19 | 2 | GC-MS(MS) | 7.59 | -3 | -4.4 | a |
| 145 | 88.2 | 17.59 | 2 | HPLC | 8.8 | -1.5 | -1.9 | a |
| 146 | 109.26 | 21.85 | 2 | GC-MS(MS) | 10.93 | 0.3 | 0.3 | a |
| 148 | 104.9 | 30 | 2 | GC-MS(MS) | 15 | -0.1 | 0 | c |
| 149 | 108.6 | 12.8 | 2 | GC-MS(MS) | 6.4 | 0.3 | 0.4 | a |
| 202 | 101.4 | 2 | 2 | | 1 | -0.4 | -1.7 | b |
| 203 | 116.5 | 58.25 | 2 | GC-HRMS | 29.13 | 1 | 0.4 | c |
| 209 | 114.91 | 11.41 | 2 | GC-MS(MS) | 5.71 | 0.8 | 1.5 | a |
| 211 | 103.8 | 11.19 | 2 | | 5.59 | -0.2 | -0.3 | a |
| 212 | 73.5 | 25.7 | 2 | HPLC | 12.85 | -2.8 | -2.5 | c |
| 213 | 186.5 | 27.99 | 2 | HPLC | 14 | 7.1 | 5.7 | c |
| 215 | 47925 | 0 | 2 | | 0 | 4187.3 | 21443.7 | b |
| 217 | 64.52 | 11.18 | 2 | HPLC | 5.59 | -3.6 | -6.8 | a |
| 218 | 36.17 | 6.65 | 2 | | 3.33 | -6.1 | -17.3 | a |
| 222 | 184.1 | 24 | 2 | GC-MS(MS) | 12 | 6.9 | 6.4 | c |
| 223 | 43.1 | | 2 | | | -5.5 | -28 | |
| 230 | 108.5 | 21.68 | 2 | | 10.84 | 0.3 | 0.3 | a |
| 231 | 94.23 | 18.85 | 1 | HPLC | 18.85 | -1 | -0.6 | c |
| 234 | 277 | 83.42 | 1 | | 83.42 | 15 | 2.1 | c |
| 235 | 104.6 | 41.84 | 2 | HPLC | 20.92 | -0.1 | 0 | c |
| 238 | 133 | 61.2 | 2 | GC-MS(MS) | 30.6 | 2.4 | 0.9 | c |
| 241 | 199 | 55 | 2 | | 27.5 | 8.2 | 3.4 | c |
| 243 | 40.5 | 11.7 | 2 | | 5.85 | -5.7 | -10.4 | a |
| 247 | 181 | 85.07 | 2 | HPLC | 42.53 | 6.6 | 1.8 | c |
| 250 | 103.5 | 25.89 | 2 | | 12.95 | -0.2 | -0.2 | c |

**Satisfactory**, Questionable, **Unsatisfactory**

***a*** : $u_{ref} \leq u_{lab} \leq u_{max}$ $(\sigma_p)$;

**b** : $u_{lab} < u_{ref}$;

**c** : $u_{lab} > u_{max}$ $(\sigma_p)$

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