HOW DIFFICULT IS IT TO DETERMINE TOTAL CADMIUM, LEAD, ARSENIC, MERCURY AND INORGANIC ARSENIC IN MUSHROOMS? THE OUTCOME OF IMEP-116 AND IMEP-39

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ABSTRACT

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate General of the European Commission, operates the International Measurement Evaluation Program (IMEP). IMEP organises interlaboratory comparisons (ILCs) in support to European Union (EU) policies. This paper presents the results of two PTs: IMEP-116 and IMEP-39, organised for the determination of total Cd, Pb, As, Hg and inorganic As (iAs) in mushrooms. Participation in IMEP-116 was restricted to National Reference Laboratories (NRLs), while IMEP-39 was open to all other laboratories wishing to participate. Both PTs were organised in support to Regulation (EC) No 1881/2006 which sets the maximum levels for certain contaminants in food.

The test item used in both PTs was a blend of mushrooms of the variety shiitake (*Lentinula edodes*). Five laboratories, with demonstrated measurement capability in the field, provided results to establish the assigned values (X_{ref}). The standard uncertainties associated to the assigned values (u_{ref}) were calculated by combining the uncertainty of the characterisation (u_{char}) with a contribution for homogeneity (u_{bb}) and for stability (u_{st}), whilst u_{char} was calculated following ISO 13528. Laboratory results were rated with z- and zeta (ζ)-scores in accordance with ISO 13528. The standard deviation for proficiency assessment, σ_p, for total Pb (20 %) and iAs (19 %) was calculated using the Horwitz equation modified by Thompson. For the other measurands σ_p was set by the advisory board of the two PTs to 15 % for total As and Hg and to 10 % for total Cd, on the basis of previous performance on similar measurands. Thirty-seven participants from 25 countries reported results in IMEP-116, and 62 laboratories from 36 countries reported for the IMEP-39 study.

The percentage of satisfactory z-scores ranged from 81 % (iAs) to 97 % (total Cd) in IMEP-116 and from 64 % (iAs) to 84 % (total Hg) in IMEP-39.

INTRODUCTION

Asian countries have a long tradition in using mushrooms for their therapeutical properties, for instance to prevent hypertension, hypercholesterolemia and cancer [1, 2]. From a nutritional point of view mushrooms are low in energy and fat but high in protein, carbohydrate and dietary fibre vitamins and minerals [3]. However, edible mushrooms, especially those widely grown, may contain metals such as cadmium, lead, and mercury at levels considerably higher than those in other food commodities [4]. The levels of heavy metals in cultivated mushrooms are normally lower than in wild ones most likely due to the soil composition and contamination and to the age of mycelium (part of the mushroom which grows under the ground surface) which may be several years in nature in a wild mushroom compared to few months in the cultivated ones [4]. The usual content, expressed as mg kg^{-1} in dry matter, of heavy metals in mushrooms from unpolluted areas and accumulating species are: 0.5 to 5 mg kg^{-1} for As, 1 to 5 mg kg^{-1} for Cd and below 5 mg kg^{-1} for Pb and below 0.5 to 5 mg kg^{-1} for Hg [5].

Not much information is available in the literature for metal speciation in mushrooms. The review published by Falandysz and Borovička [6] indicates that bioaccumulation of methylmercury by mushrooms varies between studies and that both, in wild and cultivated mushrooms, methylmercury is less abundant than the inorganic Hg (between 2 and 60 % of total Hg) although the proportions vary depending on the concentration and the analytical method used. Regarding As the main species found in many mushrooms are arsenobetaine, arsenate and arsenite although the type of mushroom has a strong influence [4]. Arsenocholine, trimethylarsonium ion and some unidentified arsenic compounds were also detected [7].

To overcome problems with a high metal content, maximum levels for heavy metals in mushrooms are set by Regulation (EC) No 1881/2006 [8]: 0.050 mg kg^{-1} for Cd and 0.30 mg kg^{-1} for Pb based on wet weight. In the case of Pb maximum levels apply only to common mushroom, oyster mushroom and shiitake mushroom, while the maximum level for Cd applies to funghi in general. No maximum levels have been set yet for iAs and methylmercury.

Since the mushroom consumption increased considerably in the last years due to their nutritional properties, the Directorate for Health and Consumers (DG SANCO) of the European Commission requested the EURL-HM to test the analytical capabilities of NRLs to determine heavy metals in mushrooms. Two PTs were organised by IMEP on behalf of the EURL-HM using the same test item: IMEP-116 (for NRLs) and IMEP-39 (for official control laboratories, OCLs, and other laboratories).

This paper discusses and compares the outcome of both PTs.

TEST MATERIAL

A previous screening of Cd, Pb, As, Hg and iAs in several fresh mushrooms were performed by the University of Barcelona (UB). For this, fresh mushrooms were hand-cleaned for soil and moss. The end of the stalk that had been in contact with soil was then cut off using a stainless steel knife. Mushrooms were cut into pieces, air dried in a batch-type drying chamber at room temperature for 24 hours and dried in an oven at 40 °C for 24-48 hours. The dried mushrooms were minced using a commercial stainless steel mincer (Multiquick 5 Hand Processor, Braun), completely homogenized and analyzed. From the results, shiitake mushroom was selected as test

material. Then, 5 kg of the selected fresh shiitake mushrooms were sent to IRMM under refrigerated conditions.

Upon arrival, the material was stored at -20 °C until processing. At the time of processing the mushrooms were cut frozen in smaller pieces using an UMC-12 model cutter/mixer (Stephan Machinery GmbH, Hameln, Germany).

The material was freezed-dried in two cycles using a freeze-dryer Epsilon 2-10D (Martin Christ GmbH, Osterode, Germany). For each cycle five trays were filled with about 500 g each of pre-cut mushrooms. In total 5.27 kg were dried, giving 570 g of dried mushroom, corresponding to a mass loss of about 89 %.

Dried mushrooms were cryogenically milled using a Palla VM-KT vibrating mill (KDH, Humboldt-Wedag GmbH, Köln, Germany). All grinding elements in this system were made of high purity titanium to avoid contamination of the test material. After milling, this material was sieved over a 250 µm stainless steel sieve resulting in 522 g available for final mixing and homogenisation. Mixing was performed in a Dynamix CM-200 (WAB, Basel, Switzerland). Karl Fischer titration and laser diffraction analyses indicate that the material had a water content of 4 % (m/m) with a top particle size below 200 µm, respectively.

Finally, portions of 2.5 g were filled using an automatic filling machine (Allfill, Sandy, United Kingdom) into acid-washed 20 ml amber glass vials. The vials were closed with acid washed inserts and aluminium caps.

Each vial was uniquely identified with a number and the name of the PT exercise.

HOMOGENEITY AND STABILITY STUDIES

The measurements for homogeneity and stability studies were performed by *ALS Scandinavia AB* (Sweden) using inductively coupled plasma sector field mass spectrometry (ICP-SFMS) after sample digestion with a mixture of HNO_3/HF. Homogeneity was evaluated according to ISO 13528 [9]. The material proved to be adequately homogeneous for the total mass fraction of As, Cd, Pb and Hg.

The stability study was conducted following an isochronous experimental design [10, 11]. The material proved to be adequately stable for the eight weeks that elapsed between the dispatch of the samples and the deadline for submission of results and for all the four investigated total mass fractions (As, Cd, Pb and Hg).

The contributions to the uncertainty of the assigned value (u_{ref}), due to homogeneity (u_{bb}) and to stability (u_{st}), were calculated using statistical software SoftCRM [12]. On the basis of previous experience (IMEP-107), it was assumed that total As and iAs are similarly homogeneously distributed and stable in the test item investigated. Therefore, the same contributions were used for total As and for iAs.

INSTRUCTIONS TO PARTICIPANTS

Participants were asked to perform two or three independent measurements, correct their measurements for recovery and for the moisture content and report their calculated mean (expressed as mg kg^{-1} in dry mass) and its associated expanded measurement uncertainty (U_{lab}). The experimental protocol for the moisture content determination, described in the

accompanying letter, was optimised to yield the same result as the one obtained by Karl-Fisher titration which is specific for water in contrast to oven methods.

Participants received an individual code to access the online reporting interface, to report their measurement results and to complete the related questionnaire. The questionnaire was used to extract all relevant additional information related to laboratories and measurements.

Participants were informed that the procedure used for the analysis should resemble as closely as possible their respective routine procedures for these measurands (defined by specific matrix, analyte and concentration level).

ASSIGNED VALUES AND THEIR UNCERTAINTIES

Assigned values (X_{ref})

Five expert laboratories analysed the test item in order to determine the assigned values (Table 1): -Federal Institute for Materials Research and Testing, BAM, (Germany); -Laboratory of Public Health of Alicante, LSPA, (Spain); -Karl-Franzens-Universität Graz, KFUG, (Austria); -University of Barcelona, UB, (Spain); and -Instituto de Agroquímica y Tecnología de los Alimentos, Consejo Superior de Investigaciones Científicas, CSIC, (Spain). Not every laboratory analysed all measurands.

Experts were asked to use the method of their choice; no further requirements were imposed regarding methodology. Experts were also asked to report their measurement uncertainty with a

clear and detailed description on how the uncertainty budget was estimated. A detailed description of the methods reported by the expert laboratories is presented in Table 1.

The mean of the independent means provided by the expert laboratories was used to derive the assigned values (X_{ref}) for this PT according to ISO Guide 35 [13].

Associated standard uncertainties (u_{ref})

The standard uncertainties associated to the assigned values (u_{ref}) were calculated according to ISO/IEC Guide 98:2008 (GUM) [14] by combining the uncertainty of the characterisation (u_{char}) with a contribution for homogeneity (u_{bb}) and for stability (u_{st}) as follows:

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

Where:

u_{char} was calculated combining the standard uncertainties reported by the expert laboratories (u_i) [9]:

$$u_{char} = \frac{1.25}{p}\sqrt{\sum_1^p u_i^2} \qquad \text{Eq. 2}$$

Where *p* refers to the number of expert laboratories used to assign the reference value.

Table 2 presents the results reported by the expert laboratories, the standard uncertainty contributions, the reference values and the standard deviation for the PT assessment.

Standard deviation for proficiency assessment (σ_p)

The standard deviation for the proficiency assessment (σ_p) for total Pb and inorganic arsenic were calculated to be 20 and 19 %, respectively, using the Horwitz equation modified by Thompson [15]. For the rest of the measurands, σ_p was set by the advisory board of this PT to 15 % for total As and Hg and to 10 % for total Cd, on the basis of previous performance on similar measurands.

EVALUATION OF THE RESULTS REPORTED BY THE LABORATORIES TAKING PART IN IMEP-116 AND IMEP-39

In IMEP-116, 37 out of the 38 NRLs (from 25 countries) having registered reported results. In IMEP-39 results were received from 62 (from 36 countries) of the 71 registered laboratories. Laboratories reporting “less than X” were not evaluated. However, reported “less than X” values were compared with the corresponding "$X_{ref} - U_{ref}$". If the reported limit value “X” is lower than the corresponding "$X_{ref} - U_{ref}$", this statement is considered incorrect, since the laboratory should have been able to detect the respective element.

Scoring and evaluation criteria

Individual laboratory performance is expressed in terms of *z*- and *ζ-scores* in accordance with ISO 13528 [9]:

$$z = \frac{x_{lab} - X_{ref}}{\sigma_p} \qquad \text{Eq. 3}$$

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}} \qquad \text{Eq. 4}$$

where: x_{lab} is the measurement result reported by a participant

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

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

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

σ_p is the standard deviation for proficiency assessment

The interpretation of the *z*- and *ζ-score* is done as follows (according to ISO/IEC 17043 [16]):

Satisfactory performance, $|score| \leq 2$

Questionable performance, $2 < |score| < 3$

Unsatisfactory performance, $|score| \geq 3$

The z-score compares the participant's deviation from the reference value with the standard deviation for proficiency assessment (σ_p) used as common quality criterion. σ_p is defined by the PT organiser as the maximum acceptable standard uncertainty.

The ζ-score states if the laboratory result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value (u_{ref}) and the measurement uncertainty as stated by the laboratory (u_{lab}). The ζ-score is therefore the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value (assigned value), its uncertainty and the unit of the result as well as the uncertainty of the reported values. An unsatisfactory ζ-score can either be caused by an incorrect measurement result or an inappropriate estimation of its uncertainty, or both.

The standard uncertainty of the laboratory was estimated by dividing the reported expanded uncertainty by the reported coverage factor, *k*. When no uncertainty was reported, it was set to zero ($u_{lab} = 0$). When *k* was not specified, the reported expanded uncertainty was considered as the half-width of a rectangular distribution; u_{lab} was then calculated by dividing this half-width by √3, as recommended by Eurachem and CITAC [17].

Uncertainty estimation is not trivial; therefore an additional assessment was provided to each laboratory reporting uncertainty, indicating how reasonable their uncertainty estimate is. The standard uncertainty from the laboratory (u_{lab}) is most likely to fall in a range between a minimum uncertainty (u_{min}), and a maximum allowed (u_{max}, case "*a*"). u_{min} is set to the standard uncertainty of the reference value (u_{ref}). It is unlikely that a laboratory carrying out the analysis on a routine basis would measure the measurand with a smaller uncertainty than the expert

laboratories chosen to establish the assigned value. u_{max} is set to the standard deviation (σ_p) accepted for the PT assessment.

If u_{lab} is smaller than u_{min} (case "*b*") the laboratory may have underestimated its uncertainty. However, such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the uncertainty of the reference value also includes contributions of homogeneity and stability. If those are large, measurement uncertainties smaller than u_{min} (u_{ref}) are possible and plausible.

If u_{lab} is larger than u_{max}, (case "*c*") the laboratory may have overestimated the uncertainty. An evaluation of this statement can be made looking at the difference of the reported value and the assigned value: if the difference is small and the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty is properly assessed, but large. It should be pointed out that u_{max} is only a normative criterion if set down by legislation.

Laboratory results and scorings

Results as reported by the participants for total Cd, Pb, As, Hg and iAs mass fractions are summarised in Figures 1-5. These figures include the individual mean values and associated expanded uncertainties.

Figure 6 presents a general overview of z- and ζ-scores. In IMEP-116, 81 % (iAs) to 97 % (total Cd) of the NRLs performed satisfactorily ($z \leq 2$). The PT seems to have been more challenging for the laboratories taking part in IMEP-39 where 64 % (iAs) to 72 % (total Hg) of the reported

results were satisfactory. As shown in Figure 6, the percentage of laboratories obtaining satisfactory z-scores is higher for all measurands in IMEP-116 than in IMEP-39, the largest differences between the two populations occurring for total Pb, total As and iAs.

As indicated in *Scorings and evaluation criteria* "a", "b" and "c" scorings are just orientative assessments meant to help laboratories to evaluate the quality of the standard uncertainties associated to the results obtained with a certain method.

The assessment of reported uncertainties presented in Table 3 is based on the three uncertainty categories defined in the chapter on *Scorings and evaluation criteria:* "a" (realistic), "b" (underestimated) and "c" (overestimated/large). The first observation is that the percentage of laboratories reporting realistic uncertainties for all measurands is higher in IMEP-116 than in IMEP-39. The second observation is that while in IMEP-116 there is a clear tendency to overestimate the uncertainty, the opposite tendency took place in IMEP-39 where laboratories tended to underestimate the uncertainties associated to the reported results. Frequently underestimation of uncertainty occurs when repeatability is used as uncertainty. It also needs to be kept in mind that some laboratories did not report any uncertainties; in those cases IMEP considers the reported uncertainty to be zero and they are then counted as "b". This is done because Regulation (EC) No 333/2007 [18] indicates that in official control analysis results are to be reported as X ± U, being U the expanded associated uncertainty. A proper estimation of the standard uncertainties is of paramount importance for instance in cases of litigation. Along the years the EURL-HM organised several lectures providing NRLs with information about the different approaches which allow a sound estimation of the measurement uncertainties.

Additionally, every PT organised by the EURL-HM for the network of NRLs was an opportunity to review the quality of their uncertainty estimation.

It is clear that the values used for σ_p have an impact on the percentage of uncertainties being assessed as overestimated for a given PT. The lower σ_p the higher the chance that a laboratory would report an uncertainty assessed as overestimated. This could explain why most of the overestimated uncertainties were reported by the NRLs for total Cd and Pb. In the case of iAs, the proportion of overestimated uncertainties (31 %) could be explained by the fact that some NRLs have used an analytical method recently implemented, for which the laboratory is not fully confident thus resulting in larger standard uncertainties.

Mercury and arsenic speciation

In the preparatory phase of the PTs, it was decided to perform some preliminary studies to evaluate the content of the most toxic species of mercury and arsenic (methylmercury and iAs, respectively) in the test item.

The screening for methylmercury was performed by the Laboratory of Public Health of Alicante, using the analytical method validated by the EURL-HM in a collaborative trial (IMEP-115). The report of the collaborative trial [19] and the standard operational procedure (SOP) [20] can be downloaded from the EURL-HM web page [21].

For methylmercury an approximate concentration of 0.0042 mg kg^{-1} was found, which corresponds to about 5 % of the total content of mercury in the test item. This value can only be considered as approximate because the limit of quantification of the method used for the

screening is 0.010 mg kg^{-1}. The concentration found is in agreement with the information published in the literature [4], mentioning that methylmercury is normally present at a low percentage, rarely more than 16 %, of the total mercury mass fraction.

The screening of iAs performed by the University of Barcelona, indicates that around 50 % of the total As mass is present in the form of iAs. This was confirmed during the analysis conducted to establish the assigned value for that measurand (Table 2). Two of the expert laboratories having determined iAs using HPLC-ICP-MS, submitted chromatrograms showing the distribution of arsenic species in the test item (Figure 7). Both chromatograms show the same profile; iAs was identified by the two expert laboratories as the main arsenic species in the mushroom (*Lentinula edodes*) analysed. Dimethylarsinic acid (DMA) was also clearly detected. Traces of monomethylarsonic acid were also present. In the literature, it is indicated that the main arsenocompound detected in some mushroom species was arsenobetaine [4], although it depends on the type of mushroom, for instance DMA is the main arsenic species in *Laccaria Laccata* and *Volvariealla volvacea* [22]. In the test item used in the discussed PTs, arsenobetaine was not detected by any of the expert labs, although it has to be kept in mind that the chromatographic conditions used by the expert laboratories are those which best fit the determination of iAs, since that was the measurand in the discussed PTs.

Although in the present work the concentration of iAs in the dried test item is quite high, the corresponding iAs concentration in the natural material (with a moisture content of 90 %) could be ten times lower; nevertheless, mushrooms can also be consumed dried.

Additional information

When reporting their results participants were asked to answer a number of questions related to the analytical method used and to the quality assurance of their results. In order to allow the identification of all major potential sources of variability among the reported results we investigated (for each measurand) the relation between each reported value and the set of responses provided in the questionnaire. The statistical data treatment was performed using The Unscrambler X 10.1 (CAMO Software AS, Norway). Answers were first transformed into numerical variables, before applying partial least square regression modelling (*PLS-R*). Multivariate models succeed to "explain" a reasonable percentage of the total covariance relating the reported results and the set of answers. Furthermore, the model errors were generally lower than the observed variability for each corresponding set of reported values (expressed as the respective standard deviation). Therefore the multivariate models allowed reliable interpretations. Although no significant differences were observed among the participants, in general the better performing laboratories were characterized by: -having used microwave digestion with nitric acid and hydrogen peroxide for sample digestion, -some quality assurance issues (e.g. having a quality system in place, being accredited, use of CRMs for validation and/or calibration purposes and taking part regularly in PTs), and –having experience with this type of analysis/matrices.

When looking at the results reported in IMEP-39 two clear tendencies were observed:

1) *Tendency to underestimate the total As mass fraction*

At first glance this underestimation was directly related to the technique used, as illustrated in Figure 8. In general, participants using AAS-based techniques reported lower values than the

participants which used ICP-based techniques (ICP-MS and ICP-AES). The lower values reported by participants using AAS-based techniques resulted in a significantly lower percentage of satisfactory z-scores (35 %) when compared with those obtained by laboratories using ICP-based techniques (87 %). However, this clustering of results on the basis of the technique used could only be a secondary effect of a different primary cause, namely a non-quantitative digestion of the matrix. Some organic species of arsenic are difficult to digest and require digestion temperatures of around 280 °C when microwave digestion is used (most of the participants in IMEP-39 used microwave digestion). Most of the laboratories which clearly failed to quantify the total As mass fraction used temperatures in the range 190-200 °C with further hydride generation-AAS (HG-AAS).

The high temperatures reached in the plasma would eliminate that problem when ICP-based techniques are used. The same would apply to methods which involve a final determination of total As using electrothermal atomic absorption spectrometry (ET-AAS), since atomisation temperatures in the graphite furnace are also very high. The problem of non-quantitative digestion would mostly affect the results obtained with hydride generation because only inorganic arsenic species and, to a lesser extent, methylated arsenic species can generate the hydride. This would also explain the underestimation of the total As mass fraction in the result reported by L20, which used atomic fluorescence spectrometry (AFS); the technique also requires generation of the arsenic hydride before the final determination by AFS. Laboratories using HG-AAS must also keep in mind that after digestion of the matrix with a mixture HNO_3 and H_2O_2 (mixture used by most of the participants in IMEP-39), if the digestion is quantitative, most arsenic will be present in the form of As(V) and needs to be reduced to As(III) which is the arsenic species generating the hydride with a higher yield. This means that a reduction step must

be included and optimised prior to hydride generation to ensure quantitative reduction of As (V) to As(III).

For iAs determination, five out of the seven laboratories that obtained satisfactory z-scores, used AAS-based techniques. If proper method validation is carried out AAS-based methods can be used and they are cheap and easy-to-use methods which can provide correct results. Regarding the selective determination of iAs using HPLC-ICP-MS, it has been reported in the literature that a significant decrease in the relative sensitivity of arsenite as opposed to arsenate has been observed at the low flow rates used for that type of hyphenation [23]. Hence a significant bias can be introduced if the oxidation state of iAs in the analysed sample is different from that in the standard solution used for calibration purposes. Laboratories using HPLC-ICP-MS should keep this information in mind when validating their methods for determination of iAs.

The influence of the technique used was not so significant for the total Cd, Pb and Hg mass fractions. However, it should be noted that the four lowest values reported for total Cd (L38, L43, L48 and L50) used AAS or ET-AAS. A similar observation was made for the total Pb mass fraction for which the three laboratories obtaining an unsatisfactory z-score due to a serious underestimation of this measurand (L05, L38 and L53) used AAS and ET-AAS. The majority of these participants used microwave assisted digestion with a mixture HNO_3 and H_2O_2 with temperatures between 190-200 °C.

The observed underestimations are then not due to any effect directly related to AAS but to the use of low digestion temperatures. AAS-based techniques can be used if high temperatures are used for sample digestion (for instance dry ashing at 450 °C), as shown by L21.

2) *Tendency to overestimate the total Pb and Hg mass fractions*

A relatively high number of laboratories reported unsatisfactory results in terms of z-scores for total Pb and Hg due to overestimation regardless the technique used. Four of the laboratories which obtained an unsatisfactory z-score for total Pb due to overestimation also did for total Hg (L10, L20, L22 and L56). Overestimation of the total Pb mass fraction could be due to contamination problems. Laboratories must pay attention to the purity of the reagents used via blank control, must use clean laboratory material and must carry out analyses in clean environments. It was not possible to find a suitable explanation for the overestimation of total Hg. Contamination in this case is not as likely to occur as in total Pb analysis. Nevertheless, regular blank controls must be regularly included in the analytical sequence.

CONCLUSIONS

The performance of the network of NRLs for all the investigated measurands can be considered satisfactory. The overall rates of satisfactory performance obtained by the NRLs (expressed as z-scores) ranged from 10 % to 25 % higher than the same rates in IMEP-39.

Underestimation of the total arsenic mass fraction can occur if not high enough temperatures (higher than 280 °C) are used during the digestion of the sample. Laboratories using HG-AAS-based techniques for the final determination of arsenic should be particularly careful. The high temperatures reached in the plasma when using ICP-based techniques would eliminate this bias.

Particularly interesting is the case of inorganic arsenic. Sixteen NRLs reported values for this measurand (81 % of which obtained a satisfactory z-score) which is a considerably higher

number than in IMEP-107, the first PT organised by the EURL-HM in which inorganic arsenic was covered. In IMEP-39, five out of the seven laboratories which obtained a satisfactory z-score for iAs, have used AAS-based techniques, showing that sound determinations of iAs can be made without the use of expensive sophisticated instrumentation.

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Table 1: Analytical methods used by the expert laboratories

Certifier	Sample treatment / digestion / analytical method	Technique
BAM	Total As, Cd and Pb: 0.25 g of sample. Microwave-assisted digestion. 6 mL of HNO_3 (sub-boiling) in an Ultra Clave III. Power 1000 W, ramp 20 min. hold 30 min. Digestion temperature 250 °C at 100 bar. ICP equipped with a collision cell. Argon + helium as collision gas. Multi-point calibration from 0 - 10 µg L^{-1} (5 points) for total As and Pb, 0 – 25 µg L^{-1} for Cd.	ICP-MS
BAM	Total Hg: 0.25 g o f sample. Microwave-assisted digestion. 6 mL of HNO_3 (sub-boiling) in an Ultra Clave III. Power 1000 W, ramp 20 min. hold 30 min. Digestion temperature: 250 °C at 100 bar. CV-AFS, amalgamation mode (gold trap). Argon as gas. Multi-point calibration from 0-125 µg L^{-1} (5 points).	CV-AFS
BAM	Total Hg: 0.12 g of sample. Solid sampling cold-vapour AAS, combustion + amalgamation (gold trap). Advanced elementar mercury analyser (AMA-254) at the wavelength of 253.7 nm. Oxygen as gas mode. Multi-point calibration from 0.5 – 36 ng (9 points) and from 40 to 500 ng (9 points).	AMA-254
LSPA	Total As, Cd, Pb: The digestion of samples was carried out using a microwave digestion system, Ethos one (Milestone Inc., Shelton, USA), equipped with the Q-20 Quartz Rotor Ultratrace Analysis (20 mL quartz tubes, 250 ºC and 40 bars operating parameters). A unique sample digestion procedure was applied to all samples and analytes. 0.25 g of sample was weighted in quartz digestion vessels and 5 mL of HNO_3:H_2O 1:1 were added in a fume hood. The mixture was leaved to react over an hour approximately until finishing the gas generation process. Analysis were performed on an ELAN DRC II ICP-MS (PerkinElmer, Inc., Shelton, USA) equipped with a PFA standard nebulizer and a peltier cooled baffled glass cyclonic spray chamber (both from Elemental Scientific, Omaha, USA). Multi-element standard solutions were used for external calibration. Six standards in 2 % (w/w) HNO_3 matrix for As, Cd and Pb were prepared at levels ranging from 0.1 to 50 µg L^{-1}. The calibration curve was drawn from six points, including the calibration blank and there was applied a weighted linear regression approach with internal standardization.	ICP-MS
LSPA	Total Hg: 40 mg of sample was weighted directly in quartz samples boats and placed in the mercury analizer. To prevent explosions inside the catalizer, 500 µL of ultra-pure water were added in the quartz boats together with the samples. At least 2 quality control samples (CRM) were analysed in each sequence.	Elemental Hg analyser
KFUG	Total As: A portion of the powdered samples (about 250 mg weighed with a precision of 0.1 mg) was weighed directly into 12 mL quartz tubes, and concentrated nitric acid (2 mL) and H_2O (2 mL) were added. The tubes were transferred to a Teflon® rack of the Ultraclave microwave system (MLS GmbH, Leutkirch, Germany) and covered with Teflon® caps. After closing the system, an argon pressure of 4 x 106 Pa was applied and the mixture was heated to 250 °C for 30 minutes before being allowed to cool to room temperature. After mineralization, the samples were transferred to 15 mL polypropylene tubes (Greiner, Bio-one, Frickenhausen, Germany) and diluted with water to 9 mL (based on mass). Finally 1 mL of a solution containing 50 % methanol (to enhance the arsenic response) and 100 µg·L^{-1} each of Ge and In as internal standards were added to all digested samples giving a final concentration of 5 % methanol and 10 µg·L^{-1} of Ge and In. All standards for total arsenic determinations were prepared with 20% (v/v) of concentrated nitric acid and also 5% methanol for matrix matching with the digested samples. The arsenic concentrations in the digests were determined by ICP-MS using helium as collision cell gas.	ICP-MS
KFUG	Inorganic As: About 0.5 g of powder was weighed with a precision of 0.1 mg into 50 mL polypropylene tubes, and a solution (10 mL) of 20 mmol·L^{-1} trifluoracetic acid containing 50 µL of a 30 % H_2O_2 solution was added. Samples were extracted with a GFL-1083 shaking water bath (Gesellschaft für Labortechnik, Burkwedel, Germany) at 95 °C for 60 minutes. After cooling to room temperature the extracts were centrifuged for 15 min at 4700 g. An aliquot of 1 mL was transferred to Eppendorf vials and centrifuged for 15 min at 8900 g. The supernatant was used directly for HPLC-ICP-MS analysis.	HPLC-ICP-MS

CSIC	Inorganic As: 0.5-1 g of sample. Concentrated HCL is added and water. Reducing agent (2 mL of HBr and 1 mL of hydrazine sulphate) is added. 10 mL of $CHCl_3$. Agitate and separate the phases. Repeat the extraction 3 times. iAs is back-extracted with 10 mL of HCl. 2.5 mL of ashing aid suspension (20 % w/v $Mg(NO_3).6H_2O$ and 2 % w/v MgO) and 10 mL HNO_3 is added. Evaporated to dryness in a sand bath and place at a muffle at 150 °C. Increase the temperature to 425 ± 25 °C for 12 H. The white ash is dissolved in 6 mol L^{-1} HCl and reduced with pre-reducing solution (5 % w/v KI and 5 % w/v ascorbic acid). After 30 min, filter through Whatman N° 1 and dilute with 6 mol L^{-1} HCl. Samples are analysed by flow injection-hydride generation AAS.	FI-HG-AAS
UB	Inorganic As: A microwave digestion system, Ethos Touch Control (Milestone, Gomensoro, Barcelona, Spain), with a microwave power of 1000 W and temperature control, was used for extraction procedure. An Agilent 7500ce ICPMS was coupled to an Agilent 1200 LC quaternary pump to determine inorganic arsenic content. The analytical column Hamilton PRP-X100 (250x4.1 mm, 10 µm, Hamilton, USA) was protected by guard column filled with the corresponding stationary phase. The outlet of the LC column was connected via PEEK capillary tubing to the nebuliser (BURGENER Ari Mist HP type) of the ICP-MS system, which was the arsenic-selective detector. 0.25-g aliquots of the test material and three CRMs, for internal Quality Control, were weighed in PTFE vessels and then extracted by adding 10 mL of 0.2 % (w/v) HNO_3 and 1 % (w/v) H_2O_2 solution in a microwave digestion system. The temperature was raised first to 55 °C (and held for 10 min) then to 75 °C (and held for 10 min) and finally the digest was taken up to 95 °C and maintained for 30 min. Samples were cooled to room temperature and centrifuged at 3500 rpm for 12 min. The supernatant was filtered through PET filters (pore size 0.45 µm) and analyzed by HPLC-ICP-MS.	HPLC-ICP-MS

Table 2: Reported values by the expert laboratories (X_n), their uncertainty contributions (U_n), assigned value, standard and combined uncertainties u_{ref} (in mg kg^{-1})

	Total As	**Total Cd**	**Total Hg**	**Total Pb**	**iAs**
$X_n \pm U_n$	0.638 ± 0.026	4.42 ± 0.19	0.0782 ± 0.0032	0.274 ± 0.019	0.33 ± 0.014
	0.61 ± 0.06	3.99 ± 0.44	0.072 ± 0.007	0.26 ± 0.016	0.348 ± 0.026
(k=2)	0.69 ± 0.05		0.0781 ± 0.007		0.286 ± 0.037
X_{ref}	0.646	4.21	0.076	0.267	0.321
u_{char}	0.017	0.15	0.002	0.008	0.010
u_{bb}	0.007	0.04	0.002	0.009	0.004
u_{st}	0.015	0.06	0.002	0.010	0.007
u_{ref}	0.024	0.17	0.004	0.016	0.013
U_{ref} *(k=2)*	0.048	0.33	0.007	0.031	0.026
σ_p	0.10	0.42	0.01	0.05	0.06
σ_p(%)	15%	10%	15%	20%	19%

Note: Experts do not necessarily correspond to the order they were presented.

Table 3: Uncertainty assessment (in %)

Measurand	***Case "a"***		***Case "b"***		***Case "c"***	
	IMEP-116	*IMEP-39*	*IMEP-116*	*IMEP-39*	*IMEP-116*	*IMEP-39*
Total As	69	57	9	37	22	6
Total Cd	54	34	16	47	30	19
Total Hg	58	44	12	36	30	20
Total Pb	67	52	18	40	15	8
iAs	63	55	6	27	31	18

Figure Captions:

Figure 1: X_{lab} and U_{lab} as reported by the participants in IMEP-39 and IMEP-116 for the total mass fraction of Cd.

Figure 2: X_{lab} and U_{lab} as reported by the participants in IMEP-39 and IMEP-116 for the total mass fraction of Pb

Figure 3: X_{lab} and U_{lab} as reported by the participants in IMEP-39 and IMEP-116 for the total mass fraction of As

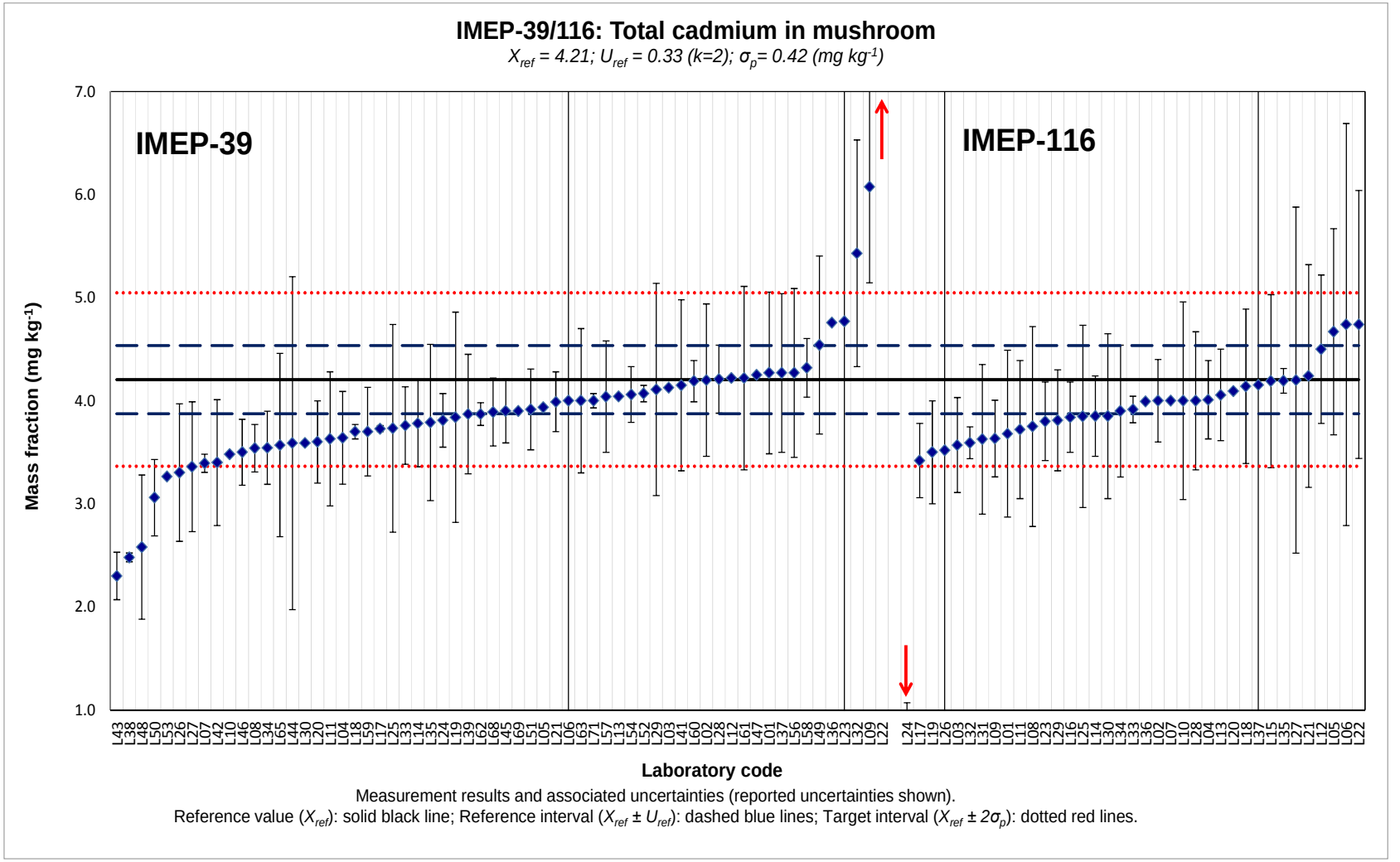
Figure 4: X_{lab} and U_{lab} as reported by the participants in IMEP-39 and IMEP-116 for the total mass fraction of Hg

Figure 5: X_{lab} and U_{lab} as reported by the participants in IMEP-39 and IMEP-116 for the total mass fraction of iAs

Figure 6: Distribution of satisfactory, questionable and unsatisfactory a) z- and b) ζ-scores for IMEP-39 and IMEP-116.

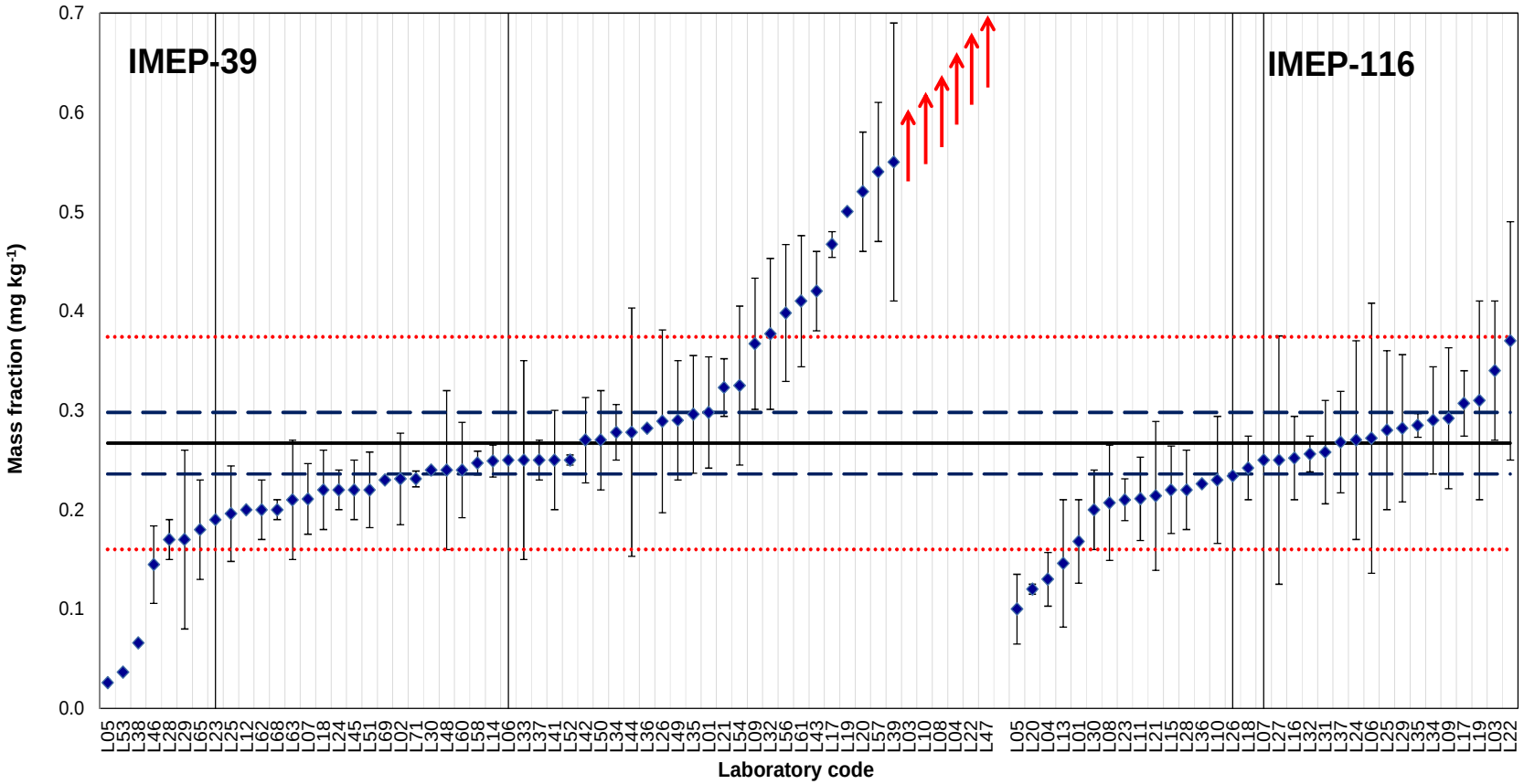
Figure 7: Chromatograms showing the distribution of arsenic species in the test item, as obtained by two expert laboratories using anion exchange-ICP-MS.

Figure 8: Distribution of results reported for the total mass fraction of arsenic on the basis of the technique used to perform the measurements.



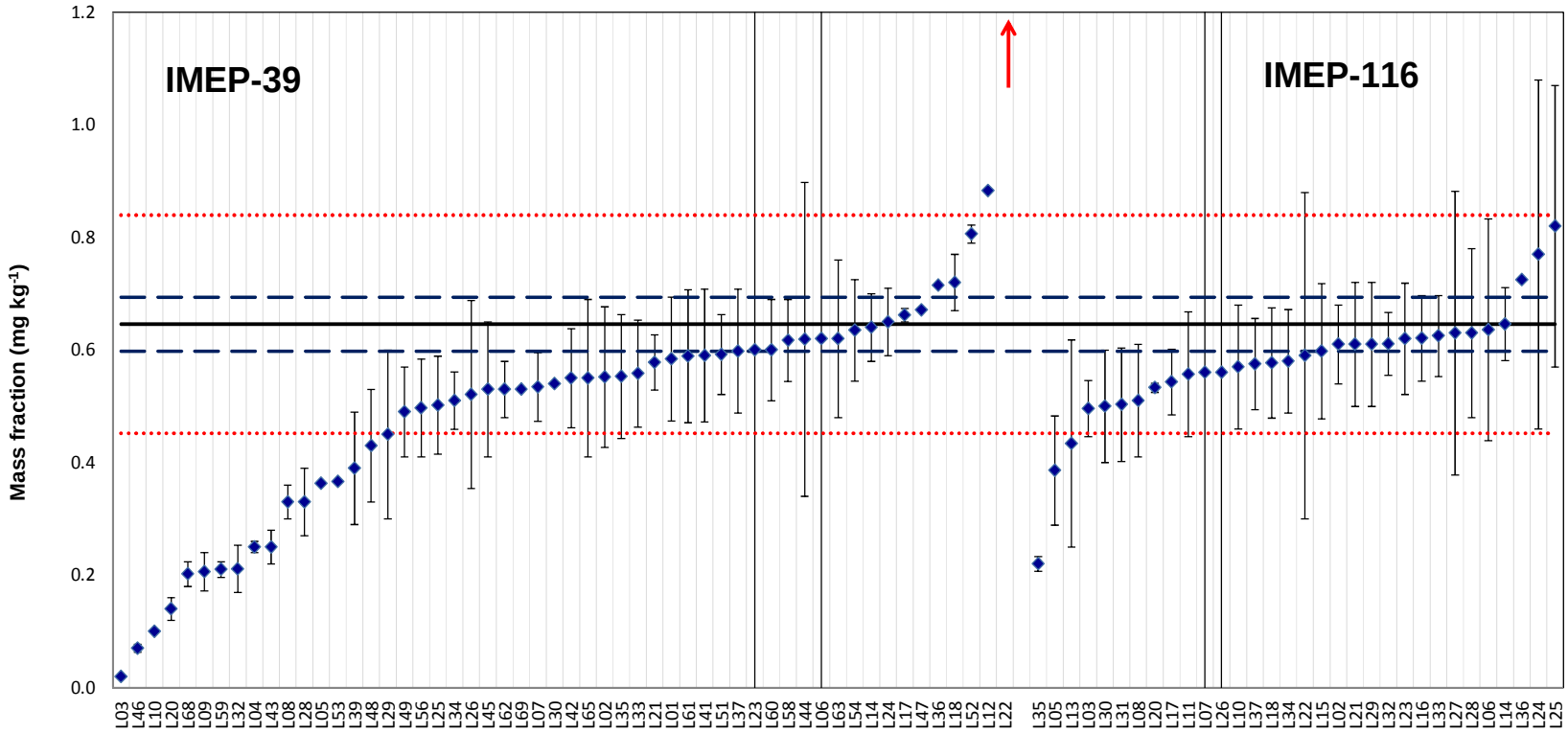
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Figure 1



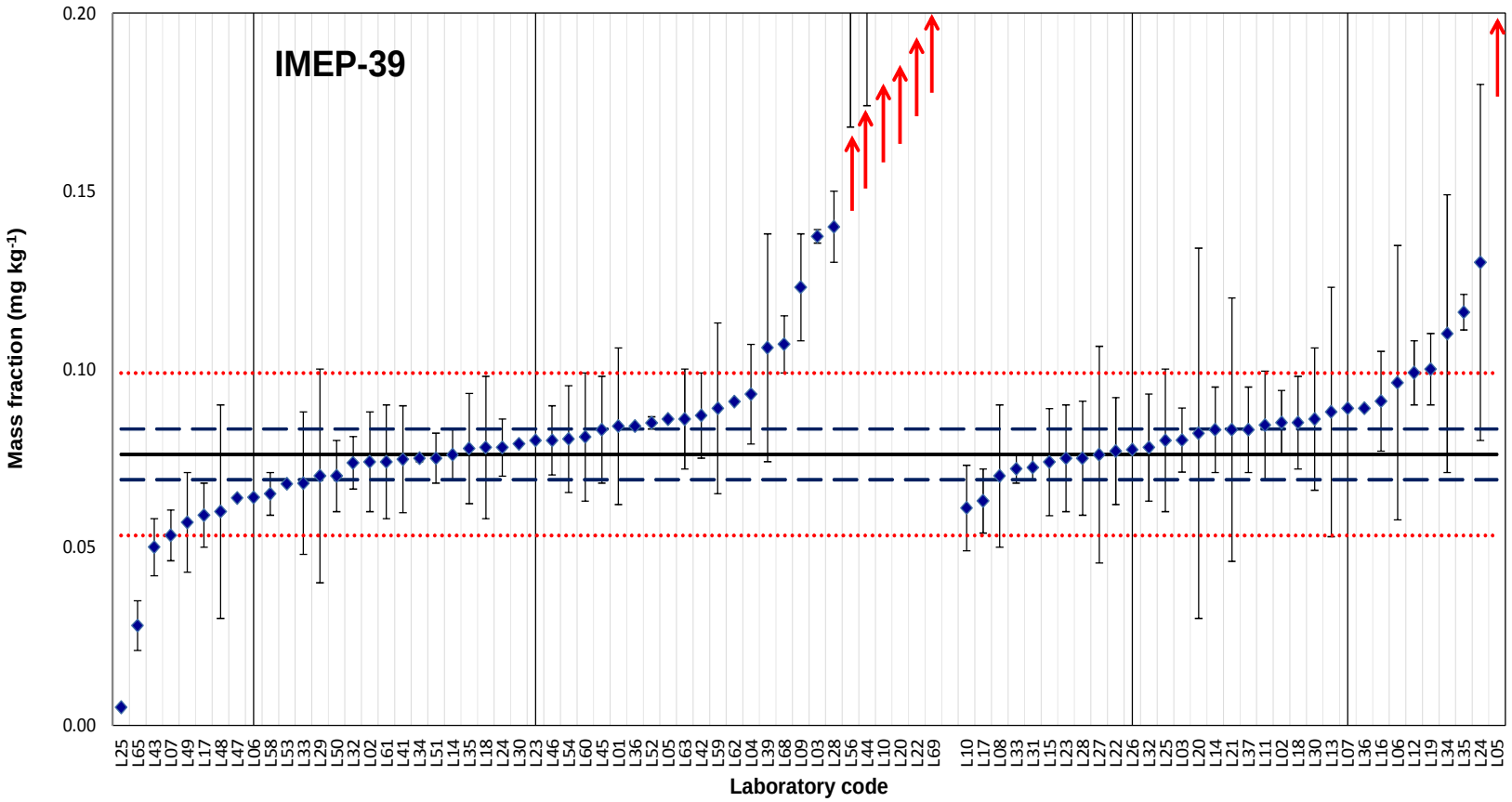
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Figure 2



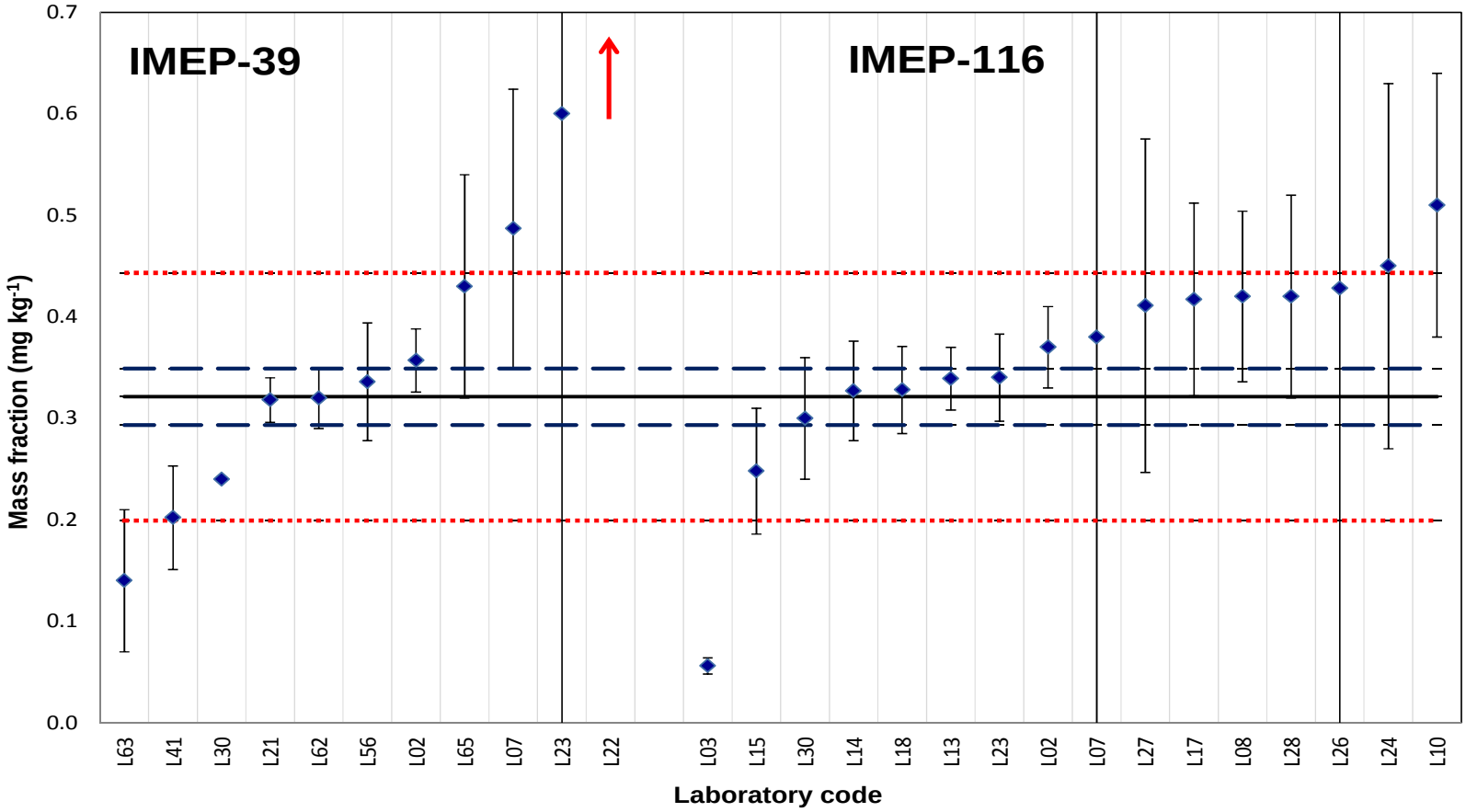
Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Figure 3



Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

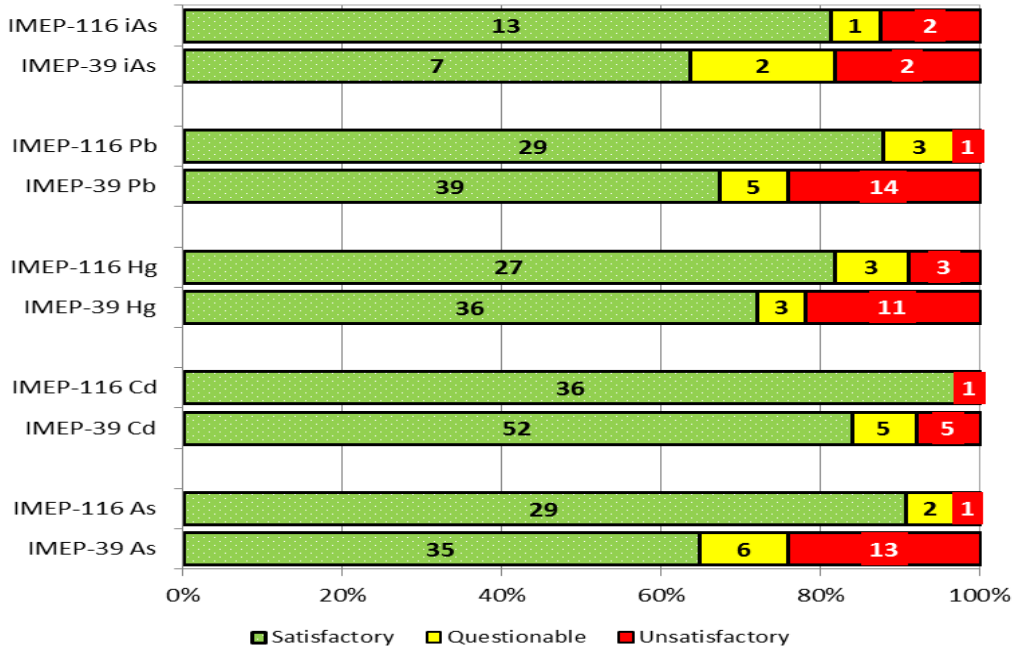
Figure 4



Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Figure 5

a)


IMEP-116 iAs
IMEP-39 iAs
IMEP-116 Pb
IMEP-39 Pb
IMEP-116 Hg
IMEP-39 Hg
IMEP-116 Cd
IMEP-39 Cd
IMEP-116 As
IMEP-39 As
13
1
2
7
2
2
29
3
1
39
5
14
27
3
3
36
3
11
36
1
52
5
5
29
2
1
35
6
13
0%
20%
40%
60%
80%
100%
Satisfactory
Questionable
Unsatisfactory

b)

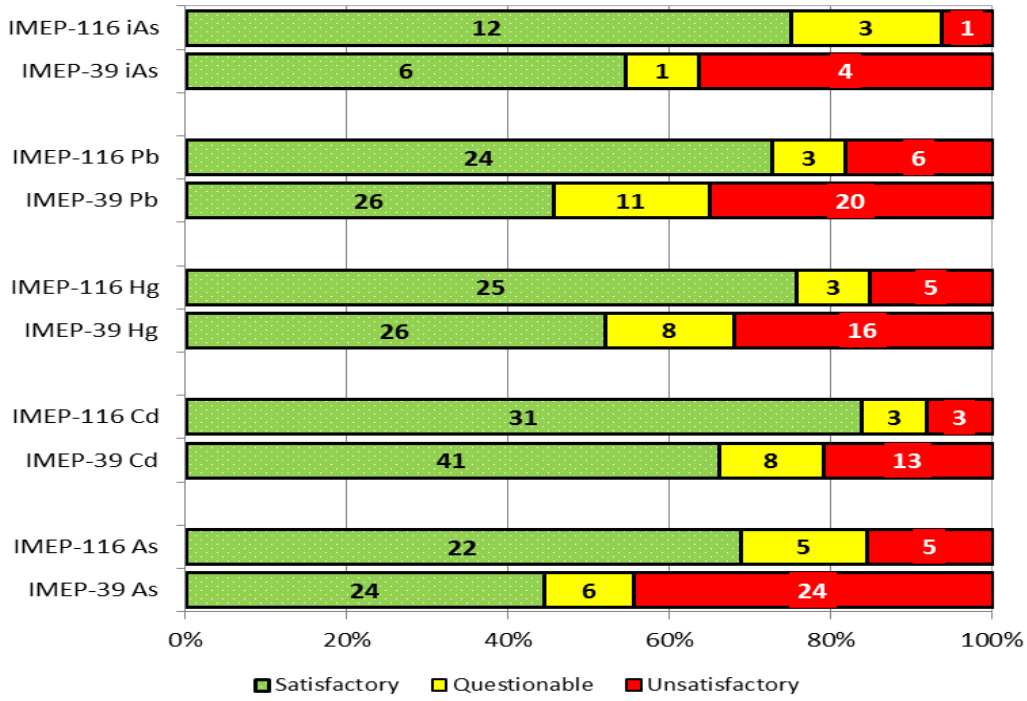
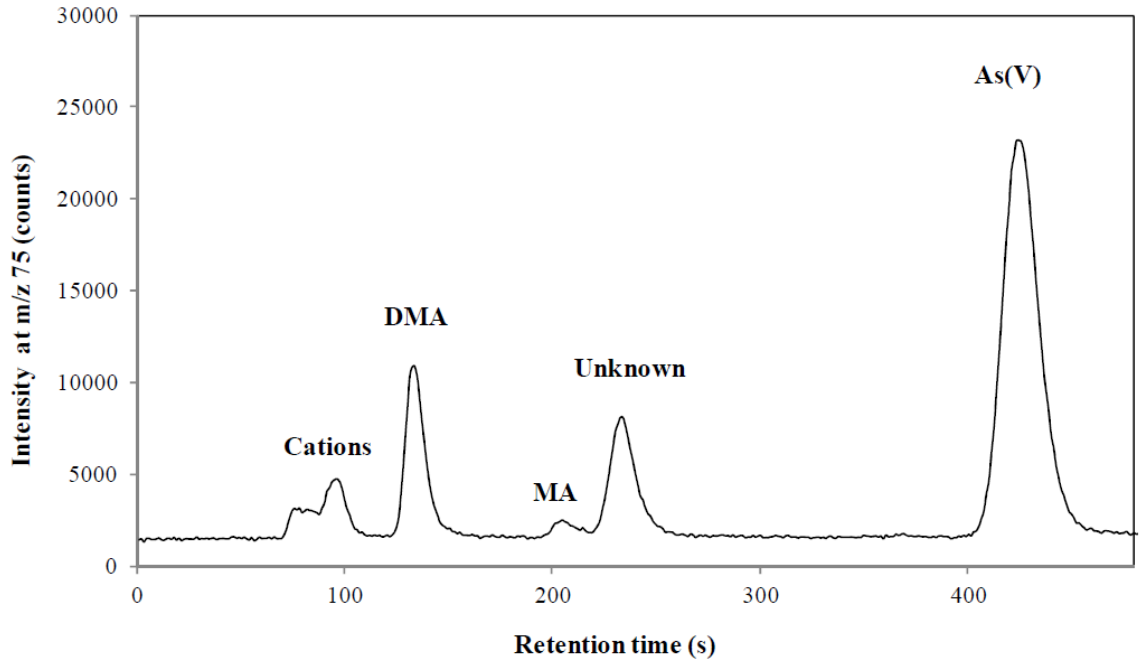
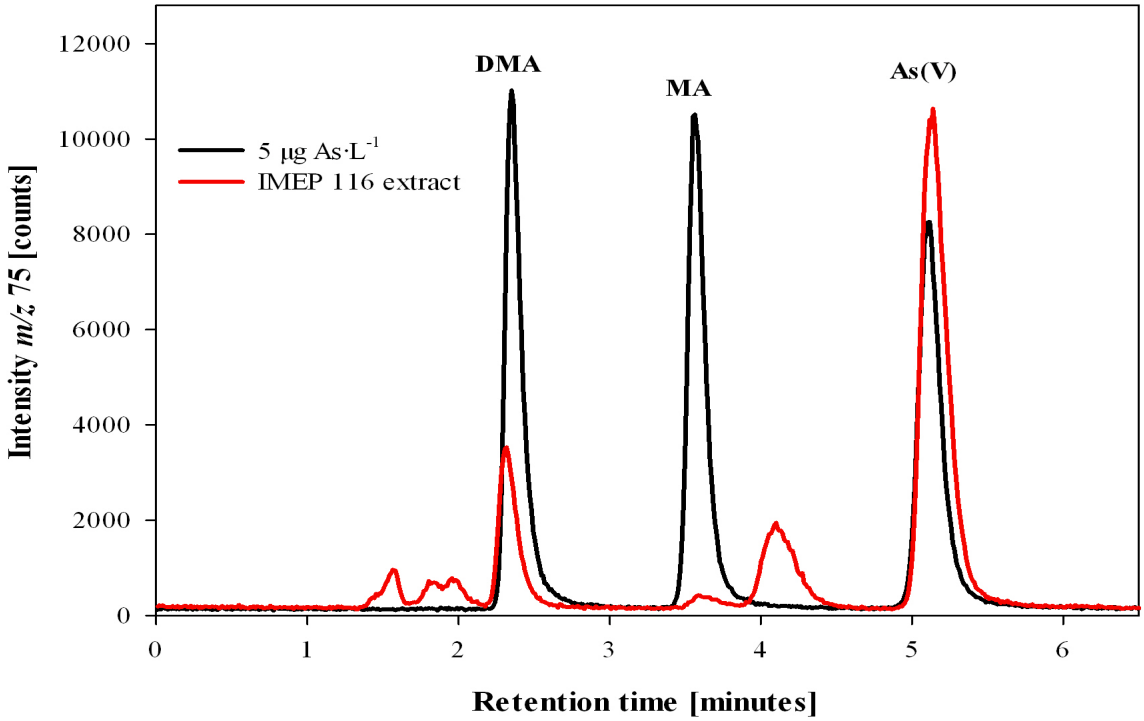
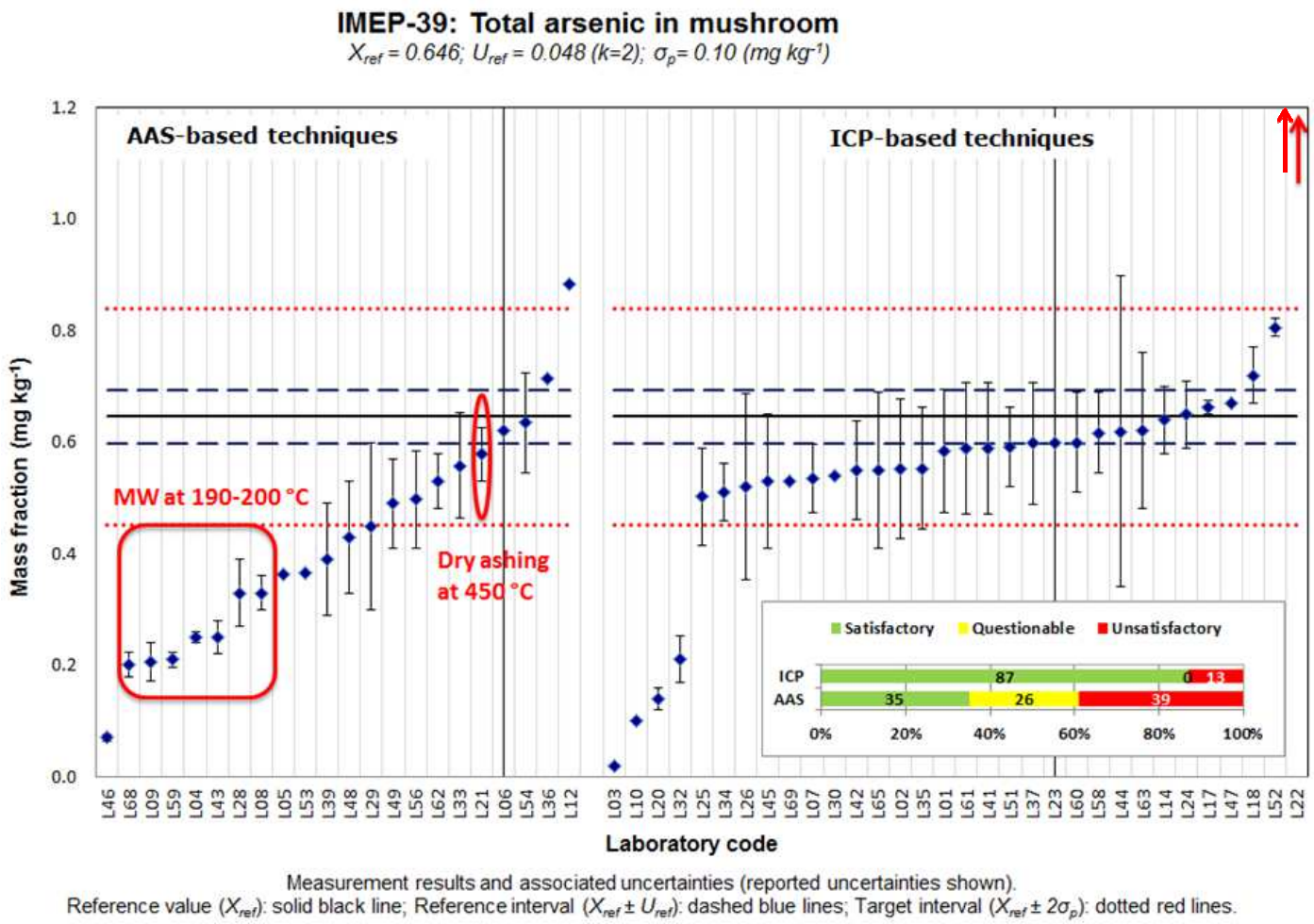

IMEP-116 iAs
IMEP-39 iAs
IMEP-116 Pb
IMEP-39 Pb
IMEP-116 Hg
IMEP-39 Hg
IMEP-116 Cd
IMEP-39 Cd
IMEP-116 As
IMEP-39 As
12
3
1
6
1
4
24
3
6
26
11
20
25
3
5
26
8
16
31
3
3
41
8
13
22
5
5
24
6
24
0%
20%
40%
60%
80%
100%
Satisfactory
Questionable
Unsatisfactory

Figure 6

Figure 7


30000
25000
20000
15000
10000
5000
0
Intensity at m/z 75 (counts)
0
100
200
300
400
Retention time (s)
Cations
DMA
MA
Unknown
As(V)


12000
10000
8000
6000
4000
2000
0
Intensity m/z 75 [counts]
0
1
2
3
4
5
6
Retention time [minutes]
5 µg As·L-1
IMEP 116 extract
DMA
MA
As(V)



Measurement results and associated uncertainties (reported uncertainties shown).
Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\sigma_p$): dotted red lines.

Figure 8