Determination of PAHs: A Practical Example of Validation and Uncertainty Assessment

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The objective of this study was to present a reliable and practical example of method validation and uncertainty assessment with an analytical method for the determination of polycyclic aromatic hydrocarbons (PAHs) in urban dust. The method was gas chromatography–mass spectrometry in combination with isotope dilution mass spectrometry in the GC, can be minimized or eliminated, improving the quantification of losses during sampling clean-up and injection in the GC, can be minimized or eliminated, improving the performance of the analytical method (4). Deuterium-labeled (PAH-d) and 13C-labeled PAHs have been used as stable internal standards for the determination of PAHs by GC–IDMS.

In this study, a method based on GC–IDMS was implemented and validated for the analysis of five PAHs in urban dust, using PAH-d as internal standards. The CRM SRM 1649a (urban dust) of the U.S. National Institute of Standards and Technology (NIST) was used to validate the analytical method.

The International Organization for Standardization (ISO) standard ISO/IEC 17025, which defines general requirements for the competence of laboratories, reports the need for estimated measurement uncertainty and the validation of analytical methods (13). Method validation procedures have been described by Scientific and Industrial Metrology directorate, National Institute of Metrology, Quality and Technology (Inmetro) in a validation guidance document, which involves steps such as the evaluation of the estimated uncertainty and the repeatability and reproducibility. The concept of estimating measurement uncertainty was initially developed for physical processes, but it has recently been widely applied for chemical determinations (14). However, estimating the measurement uncertainty for the results of chemical analysis is much more...
complex than its application in physical processes, because a chemical measurement generally depends on a combination of physical data, chemical separation of the compound of interest and the appropriate selection portion of the material removed for analysis. Measurement uncertainty is useful to estimate the confidence of analytical results. Currently, a result without the uncertainty statement cannot be considered to be reliable, but the scientific literature still lacks examples of the estimate of measurement uncertainty. This paper presents a practical and reliable description of the measurement uncertainty estimation of the analytical determination of PAHs in urban dust.

The purpose of this paper is to provide a reliable and practical example for each step involved in method validation and uncertainty assessment. These steps were detailed to provide tools to analysts and to improve confidence in their analytical procedures.

**Experimental**

**Instrumentation and reagents**

Solid-phase cartridges of silica (SiO2, 500 mg, 3 mL) were purchased from Varian (Palo Alto, CA). Dichloromethane (HPLC/Spectro) and toluene (1 mL), which was used as a keeper for volatility. At the end of the procedure, the solution was concentrated to 2 mL. The concentrated extracts were cleaned up using SiO2 (SPE) cartridges previously activated with n-hexane. The PAHs were eluted with 3 mL of n-hexane–toluene (9:1) and analyzed.

**Analytical determination**

The GC was equipped with a VF-5MS 5%-phenyl-methyl-siloxane capillary column (30 m x 0.25 mm id and 0.25 μm film thickness) from Varian. Helium (99.999%) was used as carrier gas at a constant flow mode (1 mL/min). All injections were performed in split mode (1:50). All injections were performed in split mode (1:50). The transfer line was kept at 280°C. PAH quantification was obtained in the selected-ion monitoring mode (SIM) using molecular ions with 9 min of the dwell time. The oven temperature program was as follows: 60°C for 2 min; 40°C/min to 170°C; 6°C/min to 300°C, with a 10 min final hold. The retention times and m/z monitored for each PAH and PAH-d are presented in Table I.

**Results and Discussion**

The method was validated through parameter determination of the method performance for five PAHs (Phe, Flu, B[a]A, B[a]P and B[ghi]P). The evaluated performance parameters were: selectivity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), limit of repeatability (r) and uncertainty of measurement. The values determined for each performance parameter are shown in Table II. Each of the items in Table II are discussed as follows.

<table>
<thead>
<tr>
<th>PAHs</th>
<th>m/z</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe</td>
<td>178</td>
<td>10.241</td>
</tr>
<tr>
<td>Flu</td>
<td>202</td>
<td>13.880</td>
</tr>
<tr>
<td>B[a]A</td>
<td>228</td>
<td>19.084</td>
</tr>
<tr>
<td>B[a]P</td>
<td>252</td>
<td>24.004</td>
</tr>
<tr>
<td>B[ghi]P</td>
<td>276</td>
<td>28.396</td>
</tr>
<tr>
<td>Phe-d10</td>
<td>188</td>
<td>10.176</td>
</tr>
<tr>
<td>Flu-d10</td>
<td>212</td>
<td>13.810</td>
</tr>
<tr>
<td>B[a]A-d12</td>
<td>240</td>
<td>18.978</td>
</tr>
<tr>
<td>B[a]P-d12</td>
<td>264</td>
<td>23.930</td>
</tr>
</tbody>
</table>

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Selectivity

As a condition for the selectivity of the method, the absence of peaks in the region of the retention time for the investigated compounds was observed (18). Due to the non-availability of the matrix without the presence of the investigated analytes, it was not possible to verify the presence of interferents. However, due to the high selectivity of the detection technique (GC–MS) and the accuracy of the method in relation to the concentrations found for the CRMs, the selectivity of the method was accepted. The obtained chromatograms (Figure 1) verify an appropriate chromatographic separation. The peaks of the compounds with the same mass were separated with good resolution, allowing their quantification.

As selectivity criteria, the retention times of the five PAHs under investigation were determined. For this determination, six replicates of the standard solution were used.

The five PAHs were tested in the key comparison of CCQM-K50b to ensure proficiency in the measurements of the 16 priority PAHs. The selectivity proved the separation of all PAHs in GC (retention times) or MS (monitored m/z), proving that there was no interference in these PAHs.

Linearity

The linearity of the method was tested in the range of 1–7.5 µg/g. In the calibration curves, eight concentration levels were used, with a different concentration range for each PAH (levels of equidistant concentrations): Phe (2.5–6.0 µg/g), Flu (4.0–7.5 µg/g), B[a]A (1.0–4.5 µg/g), B[a]P (1.0–4.5 µg/g) and B[ghi]P (2.5–6.0 µg/g).

The calibration curves were constructed through the relation of the following ratios: area of compound peaks/area of internal standard with the mass of compounds/mass of internal standard. The least-squares estimation method was used to determine the calibration function. The applicability of this method demands that some conditions regarding the nature of errors associated with the measurements should be valid (19).

Some errors associated with measurements are caused by the design of the experimental study. For example, most errors are related to the preparation of the standard solutions (20). In this work, the steps were accomplished through a strict methodology using the gravimetrical method. These errors can be considered neglected compared to instrumental errors.

Initially, the presence of outliers among the data received for the construction of the calibration curve was verified using the Grubbs test (21). The variances of calibration curve residues, verified through the Cochran test, can be considered homogeneous (homoeddascidity) at the reliable level of 95%. Thus, the ordinary minimum squared method was used to construct the analytical curves (19).

In addition, the residual errors of calibration curves were also visually analyzed through the construction of graph residues. It was preliminarily evaluated if the linear model was adjusted, verifying the distribution of the residues at different levels in the analytical curve. The residual errors of calibration curves were randomly distributed around the zero line, thus indicating that the linear model is adequate, as shown in Figure 2. The coefficients of linear correlation were always greater than 0.99.

For the correct evaluation of a model and verification of whether the adjustment of the straight line is satisfactory, the F-test was used (19). For this, analysis of variance (ANOVA) was used at the reliability level of 95%. The model adjustment was considered satisfactory because the linear regression is accepted if the value of the significance, F, is smaller than the calculated value. Accordingly, the linear regression for all compounds of interest was confirmed and accepted.

Accuracy

The accuracy of an analytical method relates to the agreement of the results obtained by the method in the study with the value of a reference accepted conventionally as true (22, 23). This parameter is expressed in terms of random errors and systematic components (24). The most often used processes for the evaluation of the accuracy of a method are: CRMs, comparison of methods/interlaboratory assays; accomplishment of recovery assays and addition of standards (23).

With the availability of CRMs, the reference value accepted as true is the one described in the certificate. The method accuracy was determined using the SRM 1649a (urban dust), through the calculation of the relative error (RE), expressed in...
The recovery of B[ghi]P in the solvent used in the stage of clean-up, affect the recovery of B[ghi]P (28).

Another way to evaluate the judgment of acceptance using CRM was published by the Institute for Reference Materials and Measurements (IRMM). This approach (29) takes into account the certified value, the measurement result and their respective uncertainties. These uncertainties are subsequently combined and the expanded uncertainty is compared to the difference. In general, after the measurement of a CRM, the absolute difference between the mean measured value and the certified value can be calculated by Equation 2, where $\Delta_m$ is the absolute difference between mean measured value and certified value, $X_{lab}$ is the mean measured value and $X_{CRM}$ is the value accepted as true (certified value of the CRM) (29).

$$\Delta_m = X_{lab} - X_{CRM}$$

The uncertainty of $\Delta_m$ is $u_\Delta$, which is calculated from the uncertainty of the certified value and the measurement result, according to Equation 3, where $u_m$ is the combined uncertainty of result and certified value (uncertainty of $\Delta_m$), $u_c$ is the uncertainty of the measurement result and $u_{CRM}$ is the uncertainty of the certified value.

$$u_\Delta = \sqrt{u_m^2 + u_{CRM}^2}$$

The expanded uncertainty $U_\Delta$ (Equation 4), corresponding to a confidence level of approximately 95%, is obtained by multiplication of $u_\Delta$ by a coverage factor ($k$), usually equal to 2.

$$U_\Delta = 2 \times u_\Delta$$

The method performance can be evaluated in this way; $\Delta_m$ is compared with $U_\Delta$, if $\Delta_m \leq U_\Delta$, then there is no significant difference between the measurement result and the certified value. The five PAHs were tested and there was no significant difference between the measurement result and the certified value.

**Precision**

The method precision was determined through repeatability studies, calculating the percent relative standard deviation (RSD), also known as coefficient of variation, of samples analyzed in replicate. Four samples were analyzed; from each one, three subsamples were taken. The average of the results of the samples was used to estimate the precision of the method.
RSD expresses the dispersion of the results among all accomplished replicates. With this intention, Equation 5 was applied, where \( s \) is the standard deviation (SD) of the readings at the studied concentration level and \( \bar{y} \) is the average of the experimental results.

\[
RSD(\%) = \frac{s}{\bar{y}} \times 100
\]  
(5)

As a criterion of evaluation, the RSD was considered as a function of the concentration range of the analytes. Trace analysis methods generally accept values up to 20% for the RSD (30). In accordance with AOAC recommendations, values of RSD up to 10% were accepted in the range of concentration used in this study (27, 31). Accordingly, the precision of the procedure was considered satisfactory because the RSD values were found to be lower than 7% for all investigated compounds (Table II). These values are in accordance with previously reported studies for the analysis of PAHs in particulate matter (9).

From the RSD of the assay results accomplished under the condition of repeatability, it is advisable to calculate \( r \), which is used as a decision criterion to verify whether the difference among the sample analyses in duplicate is significant (22). For a significance level of 5%, \( r \) can be determined by Equation 6, where \( Sr \) is the SD of repeatability associated with the results (22, 23). The values found for \( r \) and RSD are presented in Table I.

\[
r = 2.8Sr
\]  
(6)

**Limits of detection and quantification**

The LOD is the smallest detectable amount of an analyte present in a sample; however, it is not necessarily quantified under the established experimental conditions. LOQ is defined as the smallest concentration of the analyte that can be quantified in the sample with acceptable accuracy and precision, under the conditions of the analysis (32).

Several approaches for determining LOD and LOQ are possible; for example, based on visual evaluation, the SD of the response and slope or signal-to-noise ratio. The LOD and LOQ of the investigated compounds were determined by analysis of the signal-to-noise ratio (33).

Determination of the signal-to-noise ratio was performed by comparing measured signals from samples with known low concentrations of the analyte with those of blank samples, and by establishing the minimum concentration at which the analyte can be reliably quantified. This method assumes that the LOD and LOQ are noise–related, and because of the need to assess the noise, it can only be applied to methods that have baselines. This method allows a decrease of the signal (peak height) to be observed to the extent that the concentration is reduced through a series of dilutions (33).

The signal-to-noise ratios accepted as estimates of the LOD and LOQ were 3:1 and 10:1, respectively (33). The values found in this study are shown in Table II.

**Measurement uncertainty**

Measurement uncertainty is a parameter that is associated with the result of a measurement, which characterizes the dispersion of the values that can be attributed to a measurand. In this case, the uncertainty was determined based on the Guide to the Expression of Uncertainty in Measurement and the Eurachem/CITAC Guide (14, 34).

The values of measurement uncertainty were expressed in terms of expanded uncertainty (U), which was determined multiplying the coverage factor (k) by the combined standard uncertainty (\( u_c \ )) of the input quantity (Equation 7). The \( u_c \ ) is represented by Equation 8, where \( u_{A,\text{ratio}} \ ) is the contribution of uncertainty due to area ratio, \( u_{m_S} \ ) is the contribution of uncertainty due to calibration curve, \( u_{\text{ms}} \ ) is the contribution of uncertainty due to sample mass, \( u_{\text{pt}} \ ) is the contribution of uncertainty due to purity of analyte, and \( u_{\text{IS}} \ ) is the contribution of uncertainty due to repeatability of the process. The input quantities are those that contribute to the uncertainty of the analytical method.

\[
U = u_c \times k
\]  
(7)

\[
u_c = \sqrt{(u_{A,\text{ratio}})^2 + (u_{m_S})^2 + (u_{\text{ms}})^2 + (u_{\text{pt}})^2 + (u_{\text{IS}})^2}
\]  
(8)

The classical and relative methods were used to determine the uncertainties, according to the nature of the input quantity (Figure 3). The uncertainties referred to the area ratio (\( A_{\text{ratio}} \)), internal standard mass (\( m_S \)), sample mass (\( m_s \)) and purity of analytes (\( p \)), which were calculated by the classical method according to Equation 9, where \( u_{\text{IS}} \ ) is the input quantity uncertainty and \( (\Delta A_{\text{analyte}}/\Delta A_{\text{ratio}})^2 \ ) is the sensitivity coefficient related to the area ratio uncertainty. All other sensitivity coefficients were calculated by the same principle, because they are uncertainties originating from the equation that defines the measurand (Equation 10), using the derivative concept to standardize the units of the input quantity with relation to the unit of the statement (concentration). This equation is derived from the equation of the calibration curve (Equation 11) and from Equation 12 (35).

\[
u_{\text{IS}} = \sqrt{(\frac{\partial A_{\text{analyte}}}{\partial A_{\text{ratio}}} \times u_{A_{\text{ratio}}})^2 + (\frac{\partial A_{\text{analyte}}}{\partial m_S} \times u_{m_S})^2 + (\frac{\partial A_{\text{analyte}}}{\partial p} \times u_{p})^2}
\]  
(9)

\[
\frac{A_{\text{analyte}}}{A_{\text{IS}}} - b = \frac{D \times m_S}{a \times m_s}
\]  
(10)

\[
A_{\text{analyte}} = \frac{a \times m_{\text{analyte}}}{p \times m_s} + b
\]  
(11)

\[
m_{\text{analyte}} = [\text{analyte}] \times m_s
\]  
(12)

The cause and effect diagram (Figure 3) is constructed from the equation of the measurand. This diagram contains all input quantities derived from Equation 10 added to those derived from external sources and to those present in Equation 7.

The input quantities defined in the diagram of cause and effect are expressed in the equation of the combined standard uncertainty (\( u_c \ )) (Equation 8). The uncertainty due to purity
The purity is calculated as a function of three variables: chromatographic peak area of the analyte ($x_1$), total chromatographic peak area ($x_2$) (defined as the sum of all chromatographic peak areas, except the analyte) and chromatographic peak area of the solvent ($x_3$), which are used in the general formula for determination of the purity.

The partial purity ($p_i$) is a function of $x_1$, $x_2$ and $x_3$, defined as Equation 13:

$$p_i(x_1, x_2, x_3) = 100 \frac{x_i}{(x_2 - x_3)}$$ (13)

The purity uncertainty $u(P_i)$ is calculated by Equation 14:

$$u(P_i) = +\sqrt{u^2(P_i)}$$ (14)

Where $u^2(P_i)$ is expressed as Equation 15:

$$u^2(P_i) = \sum_{i=1}^{n} c_{xi}^2 u^2(x_i) + 2 \sum_{i=1}^{n} \sum_{j=i+1}^{n} c_{xi} c_{xj} u(x_i) u(x_j) r(x_i, x_j)$$ (15)

where $c_{xi}$ is the partial derivative of the function $P$ with respect to the variable $x_i$, (Equation 16).

$$c_{xi} = \frac{\partial p}{\partial x_i}$$ (16)

The $c_{xi}$s are commonly denominated by the sensibility coefficient in metrological terms. The partial derivatives follow, defined as Equations 17, 18 and 19:

$$c_{x1} = \frac{\partial p}{\partial x_1} = \frac{100}{(x_2 - x_3)}$$ (17)

$$c_{x2} = \frac{\partial p}{\partial x_2} = \frac{-100 x_1}{(x_2 - x_3)^2}$$ (18)

$$c_{x3} = \frac{\partial p}{\partial x_3} = \frac{100 x_1}{(x_2 - x_3)^2}$$ (19)

The uncertainty of $x_i$ $u(x_i)$ is its sample mean SD (Equation 20):

$$u(x_i) = +\sqrt{s^2_i / n}$$ (20)

The final purity ($P_F$) is defined as the average of different partial purities ($P_{ci}$), relative to a specific chromatographic column, where the $P_{ci}$ are determined by GC–flame ionization detection (FID) on two chromatographic columns with different polarities (Equation 22).

$$P_F = \frac{1}{n} \sum_{i=1}^{n} P_{ci}$$ (22)

The $P_F$ uncertainty $[u(P_F)]$ is expressed as Equation 23:

$$u(P_F) = +\sqrt{u^2(P_F)}$$ (23)

Here, $u^2(P_F)$ can be determined by the application of Equation 23. In this particular case, the partial purities are uncorrelated and all sensibility coefficients are equal to Equation 13, so Equation 23 simplifies to Equation 24.

$$u^2(P_F) = \sum_{i=1}^{n} u^2(P_{ci})$$ (24)

The $uA_{rs}$ was calculated through the product of the concentration derivative of the analyte with relation to the ratio of the area (sensibility coefficient) for the uncertainty of the ratio in its own unit ($u^r$) (Equation 25). The sensibility coefficient was calculated by deriving the analyte concentration as a function of the area ratio $A_{rs}$ (Equation 26), where $m_s$ is the internal standard mass added to the sample, $a$ is the angular coefficient of the straight line equation and $n$ is the sample mass (Equation 26). The uncertainty of the $A_{rs}$ which is related to the experimental measurement, is given by Equation 27, where $s$ is the SD of the area ratio measurements and $n$ is the number of replicates.

$$uA_{rs} = \frac{d[analyte]}{d(A_{analyte}/A_{rs})} \times u'(A_{analyte}/A_{rs})$$ (25)

$$\frac{d[analyte]}{d(A_{analyte}/A_{rs})} = \frac{p \times m_s}{a \times m_a}$$ (26)

$$u'(A_{analyte}) = \frac{s}{\sqrt{n}}$$ (27)

All other uncertainties originating from the Equation 10 were calculated in the same way as $uA_{rs}$, presenting the uncertainties and the sensitivity coefficients for each other. Thus, the multiplication of the sensitivity coefficient, expressed as a function of the area ratio, by the uncertainty of the area ratio in its unit, supplies a result in the same unit as the measurand.

The other input quantities (repeatability and calibration curve) had their uncertainty calculated using the relative method. In this case, the input quantity uncertainty was divided by the value of the quantity. As an example, the
Table III
Uncertainty Components in Relative Form (Equation 30) of the Investigated PAHs*

<table>
<thead>
<tr>
<th>Uncertainty components in relative form (Equation 30)</th>
<th>PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.482 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>5.091 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>7.279 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>5.201 × 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>1.541 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>4.593 × 10⁻²</td>
</tr>
<tr>
<td></td>
<td>7.328 × 10⁻³</td>
</tr>
</tbody>
</table>

*Note: Equation 30 = $u_c = \sqrt{\left(\sum u_{IQ}^2\right)} + \left(\frac{u_{acc}}{m_{analyte}/m_{IS}}\right)^2 + \left(\frac{u_{up}}{m_{analyte}}\right)^2$ where $u_{IQ}^2 = u_{ratio}^2$ uncertainty components in relative form due to area ratio; $u_{acc}$ uncertainty components in relative form due to internal standard mass; $u_{up}$ uncertainty components in relative form due to purity of analyte; $u_{ms}$ uncertainty components in relative form due to sample mass; $u_{analyte}$ uncertainty components in relative form due to accuracy.

To calculate the combined standard uncertainty ($u_c$), it is necessary that all considered entrance input quantities be expressed in the same unit or dimensionless. The relative uncertainties for all greatnesses were calculated through the ratio of the uncertainties by the value of the property. In this way, Equation 8 was rewritten in accordance with Equation 30, where $u_{IQ}$ is the uncertainty of the equation (Equation 31).

$$u_c = \sqrt{\left(\sum u_{IQ}^2\right)} + \left(\frac{u_{acc}}{m_{analyte}/m_{IS}}\right)^2 + \left(\frac{u_{up}}{m_{analyte}}\right)^2$$

$$\sum u_{IQ}^2 = (u_{analyte})^2 + (u_{ms})^2 + (u_{ms})^2 + (u_p)^2$$

This stage is of utmost importance, because to combine uncertainties from all sources to calculate the combined standard uncertainty expressed in Equation 8, it is necessary that all are in the same unit or that they are dimensionless. Thus, the relative uncertainties for all sources are calculated, dividing the respective uncertainties by the property value. For each PAH, Table III presents the values of the input quantities in their relative forms and normalized by the measurand.

Figure 4 presents the contribution of the input quantity considered for the calculation of the measurement uncertainty, expressed in function of the measurand (concentration).

Table IV shows the results of the uncertainties for all sources.
It was determined that the major uncertainty contributions come from the accuracy of each analyzed PAH and the repeatability of the process. Among the uncertainties of the entrance input quantity, those provided from the gravimetric primary method presented the lowest values among all uncertainties. This result is in accordance with the requirements for this technique, because its only source of uncertainty comes from the balance. The uncertainty values of the area ratio, which can be considered to be low, indicate the repeatability of the chromatographic system, because this uncertainty is provided from the shunting line standard of the analyses. The values found for the measurement uncertainty of the investigated compounds are presented in Table II.

The internal standard purity, unlike the standard purity, is not a source of uncertainty in this analysis, because, independently from its value, the internal standard is added to the points of the calibration curve and to the sample at the same concentration, in which both are divided by the same factor.

Application to real sample
This method was used in the key comparison CCQM-K50 of the Consultative Committee for Amount of Substance (CCQM), which is the committee for chemical metrology in the International Bureau of Weights and Measures (BIPM) (16).

The participation of National Metrology Institutes (NMIs) in key comparisons (KCs) is essential to demonstrate their technical competence and measurement capability. KCs may be performed in two ways: (i) samples of known composition are sent to the participant NMIs to check their capacity to measure one or more property values with accuracy; or (ii) samples of unknown composition are sent to the NMIs, the results of which will be used to estimate a consensus property value and its corresponding expanded uncertainty. A good performance of NMIs in KCs is the technical basis for the international recognition of their calibration and measurement capabilities (CMCs) by BIPM (36).

In the KC CCQM-K50b, "PAHs in Particulate Matter," five target PAHs, Phe, Flu, B(ghi)P, B(ghi)A, were selected as representatives for the measurement of individual compounds. They are of toxicological relevance and span the volatility and concentration ranges of the PAHs commonly quantified in environmental samples. B(ghi)P is often used as a marker for total PAH exposure. The target compounds also include some potentially problematic GC separations (Phe/anthracene, B(ghi)A/triphenylene/chrysene) (16).
Each participant of the CCQM received two bottles of K50b and one bottle of SRM 1649a (control sample). The exercise instructions requested the analysis of three aliquots from each of the four bottles (for a total of six determinations per sample) (16).

Graphical versions of the results submitted by the national metrology institutes that took part in CCQM-K50b are shown in Figures 5 and 6. The graphics summarize the results and their expanded uncertainties (95% confidence interval), as reported by the participants. Mass fractions of the target analytes in K50b particulate are the means of six independent determinations (three per bottle). Mass fractions in the SRM 1649a control sample are the means of one to three determinations.

Figure 5 displays the analytical results in K50b with KC reference values (KCRVs) calculated by the arithmetic mean of the participants, with results represented by the black solid line and the standard and expanded uncertainty at 95% confidence level represented by the dashed lines.

Figure 6 displays the analytical results in the SRM 1649a control sample with the certified value, which is represented by the black solid line, and uncertainty of the certified CRM, represented by the dashed lines.

Although all laboratories used GC–MS for separation and detection, the layout of the extraction and cleanup steps (if applicable) was different. Extractions were done by Soxhlet or pressurized liquid extraction (PLE) (16). Regarding the air particulate sample, one might recognize a certain impact of the extraction solvent distinguishing a group using dichloromethane from another group using toluene (16).

The analysis capability of a laboratory is considered satisfactory when its results are within the range represented by the dotted lines in the graphics presented in Figures 5 and 6. The results produced by Inmetro in the CCQM-K50 KC for the control sample (SRM 1649a) agreed well with those from other participating national metrology institutes and with the value of the CRM, as Figure 6 shows, therefore validating the isotope-dilution mass spectrometric procedures used at Inmetro for a wide range of reference measurement applications.

In the CCQM-K50b particulate, the results were satisfactory for all PAHs, except for B[ghi]P. The quantification procedures were not appropriate for this PAH, because its deuterated form was not available, as explained previously, thus interfering with the quantification of this analyte. Beyond that, the acceptable range (uncertainty of reference value) for this PAH in SRM 1649a was much larger than the sample of the CCQM-K50b, as shown in Figure 6 (SRM 1649a) where the range is 3 to 5 μg/g. In Figure 5 (CCQM-K50b), the acceptable range is 3.8 to 4.4 μg/g.

The acceptable range for the CCQM-K50b sample corresponds to the SD of the participating laboratories, whereas the control sample (SRM 1649a) corresponds to the uncertainty of the CRM.

Conclusions
A method based on GC–IDMS was validated for the determination of PAHs in urban dust. PAH-nds were satisfactorily used as internal standards. All evaluated parameters of the performance (selectivity, linearity, accuracy, precision, LOD, LOQ, limit of repeatability and uncertainty of measurement) were adjusted to the target of the study. The developed methodology presented good accuracy and precision, indicating the efficiency of the quantification method. The method also showed adequate sensitivity. The CRM is presented as an important tool in the determination of the accuracy and precision of the analytical method. Results for repeatability showed the precision of the method, with the values of RSD always lower than 7%. The relative error values (accuracy) varied between −5.5 and 2.4%, thus indicating the accuracy of the method.

The results found for B[ghi]P in the analysis of sample CCQM-K50b highlights the importance of using isotopically labeled internal standard of each PAH to obtain results with
great accuracy. The use of CCQM-K50b was successful for the other PAHs (Phe, Flu, B[a]A and B(a)P), obtaining good results and low uncertainty, providing very accurate and precise results for analysis and indicating the importance of the method validated by GC–IDMS.

Importantly, the quality of a measurement result is dependent on the uncertainty of the reference value.

The participation in CCQM KCs allows Inmetro to demonstrate competence in measurements through a comparison with other NMI, in this case, in measuring PAH in urban dust.

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References
1. Mastral, A.M., Callén, M.S.; A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation; Environmental Science and Technology, (2000); 34: 3051–3057.
8. Karthikeyan, S., Balasubramanian, R., Sen, S.W.; Optimization and validation of a low temperature microwave-assisted extraction method for analysis of polycyclic aromatic hydrocarbons in airborne particulate matter; Talanta, (2006); 69: 79–86.
12. Quinn, T.J.; Primary methods of measurement and primary standards; Metrologia, (1997); 34: 61–65.
17. Christensen, A., Ostman, C., Westerholm, R.; Ultrasound-assisted extraction and on-line LC-GC-MS for determination of polycyclic aromatic hydrocarbons (PAH) in urban dust and diesel particulate matter; Analytical and Bioanalytical Chemistry, (2005); 391: 1206–1216.

35. de la Cruz, M.H.C., Rodrigues, J.M., Couto, P.R.G., Cunha, V.S., Bremser, W.; Estimativa da incerteza de medição em análise cromatográfica: abordagem sobre a quantificação de carbamato de etila em cachaça; *Química Nova*, (2010); 33: 1578–1584.