Article

Certified Reference Material for Traceability in Environmental Analysis: PAHs in Toluene

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Material de referência certificado (CRM) desempenha um papel vital para a garantia de controle em análises ambientais, uma vez que a qualidade dos dados resultantes de medições ambientais tem importância crucial. Este artigo apresenta os resultados de estudos realizados para a certificação dos compostos planejados como candidato a material de referência, que são dezesseis hidrocarbonetos policíclicos aromáticos (PAHs) dissolvidos em tolueno (naftaleno, acenaftileno, acenafteno, fluoreno, antraceno, fenantreno, fluoranteno, criseno, benzo[*a*]antraceno, pireno, benzo[*k*]fluoranteno, benzo[*b*]fluoranteno, benzo[*a*]pireno, dibenzo[*a,h*]anthaceno, indeno[*1,2,3-cd*]pireno e benzo[*ghi*]perileno). Este é o primeiro CRM brasileiro de PAH desenvolvido pelo Inmetro. O processo de certificação inclui a validação do método GC/IDMS (espectrometria de massa de diluição isotópica acoplada a cromatografia gasosa), o estudo de homogeneidade, a estabilidade em condição de transporte e de armazenamento, além de cálculo de incertezas de medição.

Certified reference material (CRM) plays a vital role for quality control assurance in environmental analysis, because the quality of data arising from measurements has crucial importance. This article presents the results from studies carried out for certification of a candidate reference material composed by sixteen polycyclic aromatic hydrocarbons (PAHs) dissolved in toluene (naphthalene, acenaphthylene, acenaphtene, fluorene, anthracene, phenanthrene, fluoranthene, chrysene, benzo[*a*]anthracene, pyrene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, indeno[*123-cd*]pyrene and benzo[*ghi*]perylene). This is the first Brazilian PAH CRM developed by Inmetro. The certification procedure includes validation of the GC/IDMS method (isotopic dilution mass spectrometry coupled to gas chromatography), homogeneity study, stability studies under transport and storage conditions and estimation of measurement uncertainties.

Keywords: CRM, PAH, environmental analysis, GC/IDMS, uncertainty

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants from anthropogenic origin, mainly pyrogenic, petrogenic, and natural sources.[1] These compounds are contaminants in air, food, soil and water.[2-5] Due to its wide distribution and environmental lipophilic character, human contamination can occur by absorption, ingestion or inhalation, with rapid distribution throughout the body.[6]

Given the wide distribution of these compounds with inevitable environmental and human exposure, PAHs are measured in a variety of environmental matrices to ensure safety of people. PAHs are enzymatically activated to reactivate intermediate metabolites that cause mutations with the risk of developing tumors. Activated PAH metabolites can form adducts with DNA (PAH-DNA), which represent a DNA damaging event.[7,8] They also lead to error-prone repairs or failures to repair these adducts, leading to mutations.

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Due to their persistency in environment as pollution and potential toxicity, several PAHs are on the list of priority pollutants of the United States. According to the Environmental Protection Agency (US-EPA) and regulatory organisms of many countries, PAH are considered interesting for the public health.[9]

The growing concern about clean environment importance is an urgent demand and more accurate analysis methods are required. The use of certified reference material (CRM) provides quality assurance in environmental analysis and makes possible the reliability and traceability of the analytical results.[10]

Metrology science has advanced considerably with the development of improved primary reference measurement procedures and high-level reference materials, which are the base for accurate measurements. A rigorous approach has been established for making methods traceable to the highest-level reference measurement procedures and reference materials.

The National Institute of Metrology, Quality and Technology (Inmetro) has an important role in Brazil to provide traceability and reliability of measurements by activities of the Chemical Metrology Division (Dquim), which has the mission to develop reference procedures, primary methods and certified reference materials in chemistry.

According to ISO Guide 30 definition, a certified reference material (CRM) is a reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.[11]

The production and certification of reference materials is a key activity to the improvement and maintenance of a worldwide coherent system of measurements. As detailed in ISO Guide 32 and ISO Guide 33, CRMs are used for calibration, quality control and method validation purposes, as well as for the assignment of values to other materials, which in turn can also be CRMs.[12-14]

Inmetro is currently establishing a program for reference material certification in several areas, like health, food and environment. Related to this program, Inmetro produced and certified the reference material of PAHs in solution codified as CRM 8363.0001.

CRMs are capable of ensuring metrological traceability of measurement results to the International System of Units (SI), which corresponds to an "unbroken chain of calibrations, each contributing to the measurement uncertainty".[15]

Traceability is a requirement of ISO/IEC 17025.[16] Another requirement is the use of CRM on testing and calibration process. Therefore, accredited laboratories rely on the use of CRMs, once these materials are essential tools for the quality control of measurement methods.[16] Using CRMs, the traceability can be ensured and measurement results meaningfully compared "over time and place".[15]

The certification of a reference material is carried out according to the requirements of ISO Guides 31, 34 and 35, which are published by ISO REMCO (Reference Material Committee of the International Standardization Organization) and used worldwide by institutes engaged in producing and certifying reference material.[14,17,18]

The ISO Guide 35 states that certification process of a candidate CRM requires a careful study of all sources of uncertainty that may cause impact on the validity of certified values.[14] In general, the important uncertainty sources are characterization, homogeneity, transport and storage stability. These sources come from essential studies to the development and certification of CRM.[14] The characterization study determines the property values as part of the certification process.[11] Homogeneity study is necessary in certification projects to demonstrate that the batch of bottles (units) is sufficiently homogeneous. The determination of the remaining batch between-bottle variation is a necessary component to be included in the CRM uncertainty estimation.[11] A stability study aims to determine the remaining degree of instability of the candidate CRM after preparation, or to confirm the material stability. Such stability study is carried out by simulating transport and storage conditions, where temperature and time are varied.[11]

This article details all the studies performed by Inmetro regarding the first candidate batch of PAHs in solution CRM. The main work motivation was carried out in recent years for the development of CRM for environmental analysis of PAHs. Those studies started from planning activities, including the bottling of the material and certification steps up to the issuance of a CRM certificate. This CRM allows the improvement of measurement results since its can be reliably compared. Moreover, this improvement can be directly related to the quality assessment of these environmental contaminants.

Due to the importance credited to those CRM not only National Metrology Institutes (NMI) are engaged to produce them. Other institutes or laboratories are formally recognized by accreditation bodies as CRM producers. Therefore, this article also intends to be a practical example for laboratories interested in producing and certifying CRM in order to establish traceability in environmental analysis.

Experimental

Reagents and chemicals

Toluene (HPLC/Spectro) was purchased from Tedia (Fairfield, USA). Solid PAH standards naphthalene (N, 99.7%), acenaphthylene (Acy, 99.0%), acenaphtene (Ace, 99.0%), fluorene (Fl, 98.0%), phenanthrene (Phe, 99.5%), anthracene (A, 99.0%), fluoranthene (Fluo, 99.0%), pyrene (Py, 98.0%), benzo[*a*]anthracene (B[*a*]A, 99.0%) and benzo[*ghi*]perylene (B[*ghi*]P, 99.0%) were purchased from Aldrich (St. Louis, USA). Solid PAH standards chrysene (Chry, 98.0%), benzo[*k*]fluoranthene (B[*k*]Fluo, 98.0%), benzo[*b*]fluoranthene (B[*b*]Fluo, 98.0%), benzo[*a*]pyrene (B[*a*]P, 90.0%), dibenzo[*a,h*]anthacene (DiBA, 98%), indeno[*123-cd*]pyrene (IndPy, 98%), were purchased from AccuStandard (New Haven, USA). The purity of solid PAH commercial standards was determined in-house.[19]

Deuterated PAH standards (IS) [^{2}H$_8$]naphthalene (N-d_{10}), [^{2}H$_{12}$]acenaphthylene (Acy-d_{10}), [^{2}H$_{10}$]acenaphtene (Ace-d_{10}), [^{2}H$_{10}$]fluorene (Fl-d_{10}), [^{2}H$_{10}$]anthracene (A-d_{10}), [^{2}H$_{10}$] phenanthrene (Phe-d_{10}), [^{2}H$_{10}$]fluoranthene (Flu-d_{10}), [^{2}H$_{12}$] chrysene (Chry-d_{12}), [^{2}H$_{12}$]benzo[*a*]anthracene (B[*a*]A-d_{12}), [^{2}H$_{12}$]pyrene (Py-d_{12}), [^{2}H$_{12}$]benzo[*k*]fluoranthene (B[*k*] Fluo-d_{12}), [^{2}H12]benzo[*b*]fluoranthene (B[*b*]Fluo-d_{12}), [^{2}H$_{12}$] benzo[*a*]pyrene (B[*a*]P-d_{12}), [^{2}H$_{14}$]dibenzo[*a,h*]anthracene (DiBA-d_{14}), [^{2}H$_{12}$]indeno[*123-cd*]pyrene (IndPy-d_{12}), [^{2}H$_{12}$] benzo [*ghi*]perylene (B[*ghi*]P-d_{12}) were obtained from Cambridge Isotope Laboratories (Andover, USA).

Certified Reference Material (SRM 2260a, NIST), supplied by the National Institute of Standards and Technology (NIST, USA) was used as quality control.[20]

Instruments and apparatus

All solutions were prepared gravimetrically using a 220 g semi-micro balance - XP205, readability of 0.00001 g (Mettler Toledo, Greifensee, Switzerland) and analytical balance of 1.2 kg - PR 1203, readability of 0.001 g (Mettler Toledo). These balances has been calibrated by Inmetro Mass Laboratory. All solutions were homogenized in a vortex (Phoenix, USA).

All analyses were carried out in temperature-controlled conditions of (20.1 ± 0.2) ºC and relative humidity of (65.9 ± 1.3)%; the thermo-hygrometer (Oregon Scientific, Portland, USA) used for these measurements was calibrated by Inmetro Hygrometry Laboratory.

A digital densimeter (Mettler Toledo) was used for conversion of the certified mass *per* mass (µg g^{-1}) in mass *per* volume (µg mL^{-1}). It was calibrated by Inmetro Fluids Laboratory.

A gas chromatograph with flame ionization detector (GC/FID) (CP-3800, Varian, Walnut Creek, CA, USA) was used to assess the purity of the 16 standards obtained from commercial sources. Two chromatographic columns of different polarities were used: DB-1ms (100% dimethylpolysiloxane) and VF-17ms (50% phenyl, 50% dimethylpolysiloxane).[19]

The analysis was performed by gas chromatography (CP-3800) coupled to ion trap mass spectrometer (GC/IT/MS, Saturn 2000, Varian), with electron ionization mode (70 eV) (Varian).

Calibration curve preparation

The calibration curves with six equidistant levels (1, 3, 5, 7, 9 and 11 µg g^{-1}) were established from the gravimetric dilution of the PAH stock solutions in toluene.

Around 1.0 g of the internal standard (IS) solution was added gravimetrically to 1.0 g of each standard solution. The points of the calibration curve were injected in triplicate. The control sample used was SRM 2260a.[20] This methodology was used to perform all analysis by isotopic dilution mass spectrometry coupled to gas chromatography (GC/IDMS) and the compound identification was confirmed by the obtained mass spectra.

Production of PAH candidate CRM

The amount of high-purity materials used for the batch preparation of the candidate certified reference material was approximately 5 mg. The analytical balance XP205 was used to weigh high-purity materials.

A 1 L amber glass bottle with Teflon-lined caps was used for preparation of the PAH solution candidate CRM. After the weighing of the bottle containing the high purity materials in toluene, the bottle was placed on a rotating mixer for 12 h to achieve complete homogenization. Subsequently, this solution was bottled in 373 ampoules, each one containing 1.5 mL of the candidate CRM 8363.0001. The material was stored at the so-called reference temperature of (4 ± 2) ºC. Preparation of the candidate CRM and the studies for its certification were performed according to the ISO Guides 31, 34 and 35.[14,17,18]

GC/IDMS method

Previous validation study of the analytical methodology using GC/IDMS technique for the determination of sixteen PAHs in toluene has been carried out.[21] Chromatographic conditions are: splitless injection with ratio of 1:10 after 1 min of run time; injection volume of 1 µL, the injector,

trap, transfer line and manifold temperatures were set at 290, 230, 280 and 80 °C, respectively; detection in SCAN mode and SIM mode, chromatographic column DB-5ms (60 m × 250 mm × 0.25 μm); the GC oven operating conditions were 60 °C (2 min), 120 °C (20 °C min^{-1}; 2 min), 180 °C (6.0 °C min^{-1}; 2 min), 290 °C (3.0 °C min^{-1}; 29.33 min).[21] Helium 6.0 was used as carrier gas with constant flow of 1.0 mL min^{-1}. The retention times and the monitored ions for each PAH and PAH-*d* are presented in Table 1.

Homogeneity study

The homogeneity study was performed with representative samples of the whole lot. The between-bottle variation of the CRM was evaluated using eleven CRM ampoules selected randomly from 373 ampoules. All measurements were performed on the same day under repeatability conditions. The ampoules were sub-fractioned into A and B, as shown in Figure 1, and after being weighed, each sample was diluted with deuterated solution (1:1; m/m), homogenized in vortex and subjected to triplicate analyses by GC/IDMS (Figure 1). The analysis of variance (ANOVA) statistical test was applied.

Table 1. Gas chromatography retention time and monitored ion for each PAH and PAH-*d*

PAH/PAH-*d*	Ion[a] / (*m/z*)	Retention time / min
N / N-d_{10}	128 / 136	15.11 / 15.05
Acy / Acy-d_{10}	152 / 160	21.52 / 21.48
Ace / Ace-d_{10}	154 / 164	22.42 / 22.25
Fl / Fl-d_{10}	166 / 176	25.26 / 25.08
Phe / Phe-d_{10}	178 / 188.3	31.38 / 31.19
A / A-d_{10}	178 / 188.3	31.73 / 31.57
Flu / Flu-d_{10}	202 / 212.3	39.89 / 39.76
Py / Py-d_{12}	202 / 212.3	41.56 / 41.42
B[*a*]A / B[*a*]A-d_{12}	228.2 / 240.4	50.77 / 50.59
Chry / Chry-d_{12}	228.2 / 240.4	51.03 / 50.82
B[*b*]Fluo / B[*b*]Fluo-d_{12}	252.4 / 264.4	58.78 / 58.59
B[*k*]Fluo / B[*k*]Fluo-d_{12}	252.4 / 264.4	59.01 / 58.84
B[*a*]P / B[*a*]P-d_{12}	252.4 / 264.4	61.47 / 61.25
IndPy / IndPy-d_{12}	276.4 / 288.4	73.34 / 73.04
DiBa / DiBa-d_{14}	278.4 / 292.4	73.86 / 73.44
B[*ghi*]P / B[*ghi*]P-d_{12}	276.4 / 288.4	76.75 / 73.36

[a]mass-to-charge ratio.

Characterization study

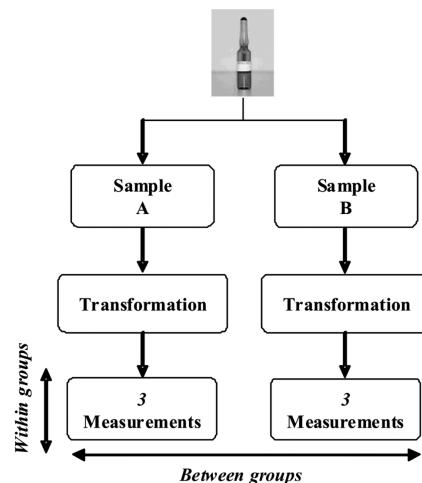The characterization study approach involved one primary method for measuring each PAH under certification

Figure 1. Layout homogeneity study between-ampoules.

in the batch.[14] The mean result of the homogeneity study of eleven ampoules was used for the characterization study. The GC/IDMS presents metrological traceability and the sources for uncertainty estimation are calibration curve, area ratio (*AR*), sample mass (m_s), internal standard mass (m_{IS}), purity of analyte *i* (p_i) and repeatability.

Stability study

The short-term stability study has been carried out to simulate the conditions of transport. It was conducted with the isochronous design method, at repeatability condition.[22] It was performed using seventeen ampoules randomly selected. A group of eight ampoules was stored at temperature of T_{s1} (18 ± 2) °C, and a group of eight ampoules was stored at temperature of T_{s2} (40 ± 2) °C. In each pre-determined time (1, 4, 7, 11, 16, 21, 25 and 30 days), one ampoule was removed from the study temperature and kept at the reference temperature (T_r) of (4 ± 2) °C (Figure 2). One ampoule was stored all the time at the reference temperature.

After 30 days, when all ampoules had been submitted to the reference temperature (T_r), the group was exposed to the laboratory room temperature (21 ± 1) °C. The ampoules were sub-fractioned into A and B; after being weighed, each sample was diluted with a deuterated solution (1:1), homogenized in vortex and subjected to triplicate analyses by GC/IDMS. SRM 2260a was used as a control sample, prepared as ampoules.[20]

The long-term stability study has been carried out to simulate storage conditions. This study was conducted with the classical design method, at reproducibility conditions.[22] The study was performed using 18 randomly selected ampoules. A group of six ampoules was stored at temperature (30 ± 2) °C, and a group of six ampoules
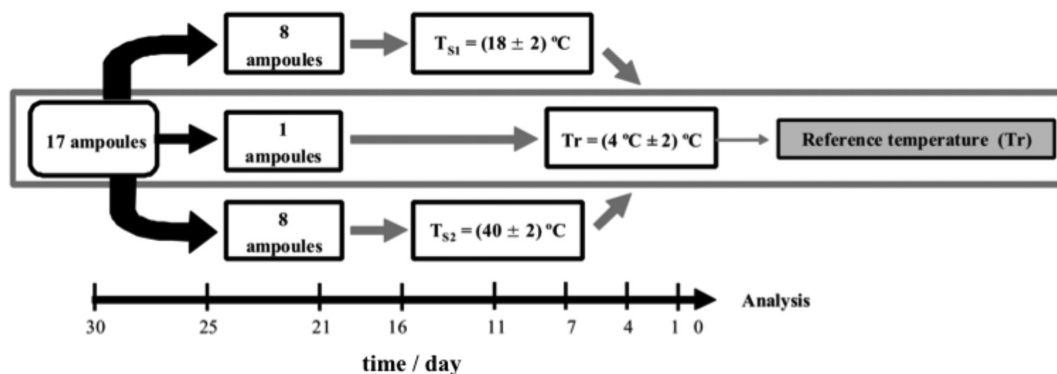
Figure 2. Scheme for short-term stability study of PAH certified reference material.

were stored at the temperature of (0 ± 2) °C. A group of six ampoules was stored at the reference temperature (T_r) of (4 ± 2) °C. CRM samples stored at the three temperatures were analyzed six times throughout the 240 days of the certification study. At each time, three ampoules were sub-fractioned into A and B; after being weighed, each sample was diluted with a deuterated solution (1:1), homogenized in vortex and subjected to triplicate analyses by GC/IT/MS. SRM 2260a was used as a control sample.[20] Linear regression was the statistical test used to calculate the HPA sample stability for each temperature.[14]

Measurement uncertainty

The combined standard uncertainty (u_{CRM}) for each CRM component is calculated by combining the contributions of uncertainties of characterization (u_{char}), homogeneity, which is related to possible between-bottle variation (u_{bb}), instability upon long-term storage (u_{lts}) and instability during transport to the customer (u_{sts}).[14,23] As provided in ISO Guide 35 the short-term stability uncertainty was not included in u_{CRM}, because it was studied only to define the transport conditions and it is negligible in relation to long-term stability uncertainty.[14] The combined standard uncertainty (u_{CRM}) was calculated using equation 1.

$$u_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2} \tag{1}$$

The characterization uncertainty was calculated based on the cause and effect diagram (Figure 3), that took into account the sources presented in the equation of the measurand according to equation 2, where w_i is the mass fraction of the analyte i, A_i is the area of the analyte GC peak, A_{IS} is the area of the internal standard GC peak, b is intercept of calibration curve, a is the slope of calibration curve, p_i is the purity of the analyte, m_{IS} is the mass of the internal standard and m_s is the mass of the sample.
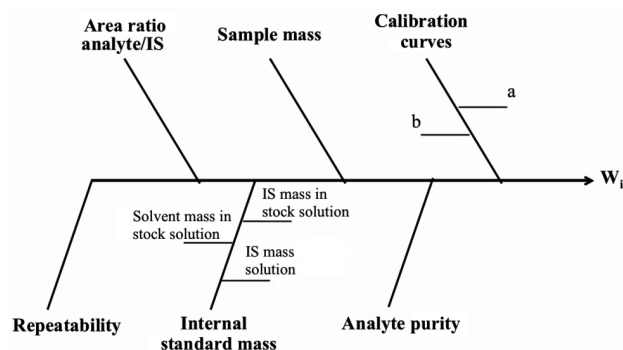

Figure 3. Cause-and-effect diagram of characterization uncertainty sources.

$$w_i = \left(\frac{A_i}{A_{IS}} - b \right) \times \frac{p_i \times m_{IS}}{a \times m_s} \tag{2}$$

The input quantities defined in the cause and effect diagram are expressed in the equation of characterization uncertainty (u_{char}) expressed in equation 3, where u_{AR} is the contribution of uncertainty due to area ratio, u_{cc} is the contribution of uncertainty due to calibration curve, u_{ms} is the contribution of uncertainty due to mass sample, u_{mIS} is the contribution of uncertainty due to internal standard mass, u_p is the contribution of uncertainty due to analyte purity and u_{rpt} is the contribution of uncertainty due to the process repeatability. The input quantities are those that contribute for the uncertainty of the analytical method.[24]

$$u_{char} = \sqrt{(u_{AR})^2 + (u_{cc})^2 + (u_{m_s})^2 + (u_{m_{IS}})^2 + (u_p)^2 + (u_{rpt})^2} \tag{3}$$

The homogeneity uncertainty (u_{bb}) was calculated according to ISO Guide 35, based on analysis of variance (ANOVA).[14] The mean squares within each group (MS_{within}) and among the groups (MS_{among}) were calculated. Standard deviations between ampoules (u_{bb}) were calculated with equation 4, where n represents the number of measurements by ampoule.[14]

$$u_{bb} = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \qquad (4)$$

The contribution of the heterogeneity (C (%)) for the studied material was calculated by equation 5, where $\bar{X}$ is the mean of all measurements from the homogeneity study.

$$C\ (\%) = \frac{u_{bb}}{\bar{X}}\ 100 \qquad (5)$$

The long-term stability uncertainties (u_{lts}) was calculated by multiplying the uncertainty of the slope (s_b), equivalent to standard error in b at the regression analysis, and the time of study (t) as shown in equation 6, according to the ISO Guide 35.[14]

$$u_{lts} = s_b \times t \qquad (6)$$

The uncertainty of the certified value for each PAH was expressed in terms of the expanded uncertainty (U), which was calculated by multiplying the combined uncertainty (u_{CRM}) by the coverage factor k for approximately 95% confidence level, according to equation 7.[25]

$$U = u_{CRM} \times k \qquad (7)$$

Results and Discussion

Certification of reference material for PAHs is an important process because this type of CRM assists the laboratories on quality control assurance of environmental analysis.

Beyond the declaration of traceability, the uncertainty associated to the property value (certified value) is what distinguishes a CRM from a common reference material, which usually is not certified. Therefore, for each CRM 8363.0001 certification step, a detailed uncertainty estimation, considering all relevant sources, is described in this work.

Purity study

To provide results traceable to an SI unit (mol), the purity of the PAH standards was assessed with its uncertainty.[19] The purity of PAH standards were determined in-house by gas chromatography with flame ionization detection (GC/FID) using columns of different polarities: DB-1ms (100% dimethylpolysiloxane) and VF-17ms (50% phenyl, 50% dimethylpolysiloxane). The final purity is the average of both columns and the uncertainties were combined.[24] Table 2 shows the values for the purity of each analyte followed by their associated uncertainties. The

purity was used to correct the gravimetric preparation of the standard solutions, ensuring metrological traceability. The uncertainty due to the purity was included as an uncertainty source in the characterization study of the certified reference material.

Table 2. In-house purity for all PAH by GC/FID

PAH	Purity / ($\mu g\ g^{-1}$)	Expanded uncertainty (U) / ($\mu g\ g^{-1}$)
N	0.9994	6.22×10^{-3}
Acy	0.9951	1.16×10^{-1}
Ace	0.9996	2.40×10^{-1}
Fl	0.9905	2.28×10^{-2}
Phe	0.9937	4.78×10^{-2}
A	0.9951	1.71×10^{-1}
Fluo	0.9937	2.18×10^{-2}
Py	0.9874	4.14×10^{-2}
B[a]A	0.9866	2.16×10^{-2}
Chry	0.9990	1.99×10^{-1}
B[b]Fluo	0.9978	2.50×10^{-2}
B[k]Fluo	0.9931	3.20×10^{-2}
B[a]P	0.9912	2.02×10^{-2}
IndPy	0.9952	5.02×10^{-2}
DiBa	0.9884	1.91×10^{-2}
B[ghi]P	0.9842	2.06×10^{-2}

Characterization study

The property values for each PAH were assigned using the primary method GC/IDMS that is one of the four approaches settled by ISO Guide 34.[18] The accuracy of the determination was evaluated by using SRM 2260a as a control sample.[24] The standard uncertainty due to characterization was calculated according to the ISO GUM approach.[25]

The classical and relative methods were used for determination of characterization uncertainty, including all sources according to the nature of the input quantity. The uncertainties referred to the area ratio (AR), internal standard mass (m_{IS}), sample mass (m_s) and purity of the analyte (p_i), were calculated by the classical method according to equation 8, where u_{IQ} is the input quantity uncertainty and $(\sigma w_i / \sigma AR)^2$ is the sensitivity coefficients related to the area ratio uncertainty. All other sensitivity coefficients were calculated by the same principle, since they are uncertainties originated from the equation that defines the measurand as expressed in equation 2, using the derivative concept to standardize the units of the input quantity in relation to the unit of the statement (mass fraction). This equation is derived from the calibration curve equation as described in equation 9 and equation 10.[24]

$$u_{IQ} = \sqrt{\left(\frac{\partial w_i}{\partial AR} \times u'_{Aratio}\right)^2 + \left(\frac{\partial w_i}{\partial m_{IS}} \times u'_{mIS}\right)^2 + \left(\frac{\partial w_i}{\partial m_S} \times u'_{ms}\right)^2 + \left(\frac{\partial w_i}{\partial p_i} \times u'_{p_i}\right)^2} \quad (8)$$

$$\frac{A_i}{A_{IS}} = a \times \frac{m_i}{p_i \times m_{IS}} + b \quad (9)$$

$$m_i = w_i \times m_s \quad (10)$$

The u_{AR} was calculated through the product of the mass fraction derivative of the analyte in relation to the ratio area for the uncertainty of the ratio in its own unit (u') as expressed in equation 11. The sensitivity coefficient was calculated deriving the mass fraction of the PAH in function of its area ratio, where m_{IS} is the internal standard mass added to the sample, a is the angular coefficient of the straight-line equation and m_s is the sample mass as described in equation 12. The uncertainty of the ratio area, which is related to the experimental measurement, is given by equation 13, where s is the standard deviation of the area ratio measurements and n is the number of replicates.[24,25]

$$u_{A_{ratio}} = \frac{dw_i}{d(A_i / A_{IS})} \times u'(A_i / A_{IS}) \quad (11)$$

$$\frac{dw_i}{d(A_i / A_{IS})} = \frac{p \times m_{IS}}{a \times m_s} \quad (12)$$

$$u'(A_i / A_{IS}) = \frac{s}{\sqrt{n}} \quad (13)$$

All other uncertainties originated from equation 2 were calculated and presented in the same way as for u_{AR}. Therefore, the multiplication of the sensitivity coefficients, expressed as function of the area ratio, by the respective uncertainty in its unit, supplies the result in the same unit of the measurand.[24]

The other input quantities (repeatability and calibration curve) have their uncertainties calculated using the relative method. In this case the input uncertainty was divided by the value of the quantity. As an example, it is shown the determination of uncertainty regarding the repeatability of the process. Dealing with an experimental measurement, its uncertainty is calculated through equation 14, where s is the standard deviation of the results and n is the number of replicates. However, since this uncertainty is calculated as a relative form, the result of this equation should be divided by the mean mass fraction of analyte, as shown in equation 15, where u_{rpt} is the uncertainty of the repeatability, w_i is the mean mass fraction of the PAH and u'_{rpt} is the relative uncertainty of the repeatability.[24]

$$u''_{rpt} = \frac{s}{\sqrt{n}} \quad (14)$$

$$u'_{rpt} = \frac{u''_{rpt}}{w_i} \quad (15)$$

To estimate the combined standard uncertainty (u_c) it is necessary that all the considered entrance input quantities be expressed in the same unit or as dimensionless. The relative uncertainties for all the sources are calculated dividing the respective uncertainties by the property value. In this way, equation 3 was rewritten in accordance with equation 16, where u_{IQ} is the uncertainty of the equation expressed in equation 17.[24]

$$u_c = u_{char} = \sqrt{\left(\frac{\sum u_{IQ}}{w_i}\right)^2 + \left(\frac{u_{cc}}{(m_s / m_{IS})}\right)^2 + \left(\frac{u_{acc}}{w_i}\right)^2 + \left(\frac{u''_{rpt}}{w_i}\right)^2} \quad (16)$$

$$\sum u_{IQ} = (u_{A_{ratio}})^2 + (u_{m_{IS}})^2 + (u_{m_S})^2 + (u_p)^2 \quad (17)$$

Table 3 shows the results of the uncertainties ($\mu g\ g^{-1}$) for all sources and the uncertainties obtained from the study of characterization for each PAH.

Among the uncertainties of the entrance input quantity, the ones provided from the gravimetric primary method present the lowest values. This result is in accordance with the requirements for the used technique, where its only uncertainty source comes from the balance.

The internal standard purity is not a source of uncertainty in this analysis, as, independently from its value, this material is added to the points of the calibration curve and to the PAH sample at the same mass fraction, where both are divided by the same factor. It does not contribute for the uncertainty in the analysis.

Homogeneity study

Homogeneity is an important property of a CRM. It is, nevertheless, a relative concept, closely related to the distribution of components in the material, the sample size, and the number of samples that has been selected to measure homogeneity.[14]

A material is perfectly homogeneous related to a given parameter (property value) if there is no difference among all the aliquots taken from the sample with a determined mass. However, in practice, a material is accepted to be homogeneous if this difference is negligible when compared to the uncertainty component from, for example, characterization.[14]

The uncertainty component due to batch heterogeneity is denoted as u_{bb}. Care should be taken in order not

Table 3. Uncertainty components obtained from the characterization study for 16 PAHs

PAH	Uncertainty components / ($\mu g\ g^{-1}$)						u_{char} / ($\mu g\ g^{-1}$)
	$(u_{AR})^2$	$(u_{cc})^2$	$(um_S)^2$	$(u_{mIS})^2$	$(u_p)^2$	$(u_{rpt})^2$	
N	3.88×10^{-2}	2.72×10^{-2}	1.80×10^{-4}	4.05×10^{-2}	3.11×10^{-3}	2.52×10^{-2}	0.07
Acy	4.75×10^{-2}	3.38×10^{-2}	1.94×10^{-4}	5.29×10^{-2}	5.80×10^{-2}	3.08×10^{-2}	0.10
Ace	3.38×10^{-2}	5.28×10^{-2}	1.88×10^{-4}	5.21×10^{-2}	1.20×10^{-1}	2.23×10^{-2}	0.15
Fl	4.90×10^{-2}	6.73×10^{-2}	2.00×10^{-4}	5.02×10^{-2}	1.14×10^{-2}	1.90×10^{-2}	0.11
Phe	4.46×10^{-2}	4.19×10^{-2}	1.82×10^{-4}	5.01×10^{-2}	2.39×10^{-2}	6.48×10^{-4}	0.09
A	5.43×10^{-2}	6.16×10^{-2}	2.00×10^{-4}	5.06×10^{-2}	8.57×10^{-2}	4.90×10^{-2}	0.15
Fluo	9.99×10^{-2}	3.62×10^{-2}	1.91×10^{-4}	4.92×10^{-2}	1.09×10^{-2}	3.37×10^{-2}	0.12
Py	4.29×10^{-2}	4.29×10^{-2}	1.86×10^{-4}	4.28×10^{-2}	2.07×10^{-2}	4.89×10^{-2}	0.12
B[*a*]A	7.92×10^{-2}	1.74×10^{-2}	1.87×10^{-4}	4.85×10^{-2}	1.08×10^{-2}	7.18×10^{-2}	0.21
Chry	1.37×10^{-1}	9.11×10^{-2}	2.09×10^{-4}	4.49×10^{-2}	9.95×10^{-2}	3.21×10^{-2}	0.20
B[*b*]Fluo	1.42×10^{-1}	7.30×10^{-2}	1.78×10^{-4}	5.04×10^{-2}	1.25×10^{-2}	5.38×10^{-2}	0.17
B[*k*]Fluo	9.87×10^{-2}	5.42×10^{-2}	1.99×10^{-4}	4.48×10^{-2}	1.60×10^{-2}	4.23×10^{-2}	0.13
B[*a*]P	1.41×10^{-1}	1.24×10^{-1}	1.83×10^{-4}	4.43×10^{-2}	1.01×10^{-2}	7.21×10^{-2}	0.21
IndPy	1.03×10^{-1}	8.64×10^{-2}	1.97×10^{-4}	5.59×10^{-2}	2.51×10^{-2}	4.95×10^{-2}	0.16
DiBa	1.34×10^{-1}	4.70×10^{-2}	1.79×10^{-4}	2.82×10^{-2}	9.54×10^{-3}	4.74×10^{-2}	0.15
B[*ghi*]P	9.18×10^{-2}	7.88×10^{-2}	1.83×10^{-4}	3.46×10^{-2}	1.03×10^{-2}	2.72×10^{-2}	0.13

$u_{char} = \sqrt{(u_{AR})^2 + (u_{cc})^2 + (u_{m_s})^2 + (u_{mIS})^2 + (u_p)^2 + (u_{rpt})^2}$; u_{AR} = contribution of uncertainty due to area ratio; u_{cc} = contribution of uncertainty due to calibration curve; u_{mS} = contribution of uncertainty due to sample mass; u_{mIS} = contribution of uncertainty due to internal standard mass; u_p = contribution of uncertainty due to purity of the analyte; u_{rpt} = contribution of uncertainty due to repeatability of the process.

to underestimate this uncertainty component due to limitations arising from the measurement method. When the only available methods display poor repeatability, these underestimation risks should be taken into account. Furthermore, if the subsamples taken for measurement are sufficiently large this type of subsampling does not contribute significantly to uncertainty due to measurement repeatability.[14]

For all certified PAHs, the u_{bb} was calculated through equation 4 because the variance analysis of the samples showed high variation between the samples ($MS_{among} > MS_{within}$). If the lot reference material homogeneity is not proven, its certification is not possible, since this homogeneity is one of the most important requirements demanded by the ISO Guides 34 and 35.[14,18] Table 4 shows the mean data in $\mu g\ g^{-1}$ from homogeneity study and standard uncertainty due to between-bottle heterogeneity (u_{bb}) obtained for each of the 16 PAHs.

Heterogeneity less than 3% was the criterion established for the certification of all 16 PAHs for this reference material. The range of heterogeneity varied from 0.63% (Chry) to 2.66% (B[*b*]Fluo) as presented in Table 4.

Stability study

The short-term stability study set the conditions of transport of CRM 8363.0001 as a maximum period of

Table 4. Mean data and standard uncertainty due to between-bottle heterogeneity (u_{bb}) obtained from the homogeneity study for each of the 16 PAHs

PAH	Mean data / ($\mu g\ g^{-1}$)	u_{bb} / ($\mu g\ g^{-1}$)	PAH	Mean data / ($\mu g\ g^{-1}$)	u_{bb} / ($\mu g\ g^{-1}$)
N	4.58	0.04	B[*a*]A	4.73	0.04
Acy	4.92	0.08	Chry	5.29	0.03
Ace	4.76	0.03	B[*b*]Fluo	4.51	0.12
Fl	5.07	0.04	B[*k*]Fluo	5.06	0.05
Phe	4.61	0.03	B[*a*]P	4.65	0.08
A	5.09	0.05	IndPy	5.00	0.04
Fluo	4.85	0.10	DiBa	4.54	0.08
Py	4.73	0.12	B[*ghi*]P	4.65	0.07

60 days at (30 ± 2) °C. With these conditions it is not expected changes in the property values of the CRM. The short-term stability uncertainty (u_{sts}) was not considered relevant as an uncertainty component and it was not included in the u_{CRM} uncertainty calculation.[14]

The long-term stability study was based on linear regression and no trend was observed in the property values during the period of study (240 days). The *p*-value indicates that the regression was insignificant (it becomes significant for a level of confidence of, for example, 95% for 0.05). Therefore, the batch of PAH CRM was confirmed to be stable and able for certification. The standard uncertainty

due to long-term stability (u_{lts}) was estimated according to equation 6. The results are described in Table 5 for all PAHs.

Table 5. Standard uncertainty due to long-term stability (u_{lts}) in μg g^{-1} for each of the 16 PAHs

PAH	u_{lts} / (μg g^{-1})	PAH	u_{lts} / (μg g^{-1})
N	0.06	B[*a*]A	0.08
Acy	0.05	Chry	0.14
Ace	0.11	B[*b*]Fluo	0.11
Fl	0.11	B[*k*]Fluo	0.12
Phe	0.13	B[*a*]P	0.35
A	0.04	IndPy	0.36
Fluo	0.05	DiBa	0.09
Py	0.12	B[*ghi*]P	0.14

CRM 8363.0001 should be stored in the dark at temperatures below (4 ± 2) °C, but it cannot be frozen.

Certified values

The certified property value for each PAH was achieved by the mean data from the homogeneity study (Table 4). It is important to point out that the primary method, GC/IDMS, was used in the analysis in accordance to one of the four possible methodologies settled by ISO Guide 34 to characterize a CRM.[18]

Table 6 presents the results for certification of all PAHs. These results include the combined standard uncertainty (u_{MRC}) in μg g^{-1}, expanded uncertainty (U) and certified values in μg g^{-1}, as well as certified values in mass per volume (μg mL^{-1}).[14]

The certified value in mass *per* volume (μg mL^{-1}) was calculated by converting the mass *per* mass value (μg g^{-1}) considering the density of solution (0.8668689 ± 0.0000011) g cm^3 at 20 °C (k = 2.95%), determined by a digital densimeter (Mettler Toledo).

After the CRM begins to be used commercially, its stability is monitored continuously as long as there are CRMs of the PAH batches on the shelf.

Conclusions

The Chemical Metrology Division of Inmetro - Dquim aims through this CRM development to provide CRM producers with a feasible case of CRM certification, as well as providing testing laboratories with a tool to guarantee reliability and accuracy on PAH measurements. To achieve this goal, Inmetro followed all requirements from ISO Guides 31, 34 and 35, and certificated this CRM using isotope labeled standards as internal standards,[14,17,18] and

Table 6. Combined standard uncertainty (u_{MRC}), expanded uncertainty (U) and certified values in two units for all PAHs

PAH	u_{MRC} / (μg g^{-1})	U^a / (μg g^{-1})	Certified value / (μg g^{-1})	Certified value / (μg mL^{-1})
N	0.10	0.20	4.58 ± 0.20	3.97 ± 0.18
Acy	0.14	0.28	4.92 ± 0.28	4.27 ± 0.24
Ace	0.19	0.37	4.76 ± 0.37	4.13 ± 0.32
Fl	0.16	0.32	5.07 ± 0.32	4.40 ± 0.28
Phe	0.16	0.31	4.61 ± 0.31	4.00 ± 0.27
A	0.16	0.32	5.09 ± 0.32	4.41 ± 0.27
Fluo	0.17	0.33	4.85 ± 0.33	4.20 ± 0.29
Py	0.20	0.40	4.73 ± 0.40	4.10 ± 0.35
B[*a*]A	0.23	0.46	4.73 ± 0.46	4.10 ± 0.40
Chry	0.25	0.49	5.29 ± 0.49	4.59 ± 0.43
B[*b*]Fluo	0.24	0.47	4.51 ± 0.47	3.91 ± 0.41
B[*k*]Fluo	0.18	0.36	5.06 ± 0.36	4.39 ± 0.31
B[*a*]P	0.42	0.83	4.65 ± 0.83	4.03 ± 0.72
IndPy	0.39	0.79	5.00 ± 0.79	4.34 ± 0.68
DiBa	0.19	0.38	4.54 ± 0.38	3.94 ± 0.33
B[*ghi*]P	0.21	0.41	4.65 ± 0.41	4.03 ± 0.36

^{a}k = 2.95%.

assigned its actual code as CRM 8363.0001. This CRM is homogeneous and stable at (4 ± 2) °C for all 16 PAHs according to the used statistical tests. The range of certified values varies from 4.51 to 5.29 μg g^{-1}.

In 2007, Inmetro participated in the key comparison CCQM-K50 to demonstrate its measurement capability to provide the traceability and acceptability of this CRM that was certified in 2011.[24,26] The CRM 8363.0001 was audited in a peer review process, being approved and available in the Key Comparison Database (KCDB) of the International Bureau of Weights and Measures (BIPM) through the publication of Calibration and Measurement Capability of Inmetro for measurement and production of PAH CRM in toluene.[24,27]

The certification of the CRM 8363.0001 by Inmetro will provide Brazilian laboratories with a national reference standard, which is one of the main tools for the assurance of the traceability, analytical measurement reliability and greater access of the Brazilian laboratories to this kind of CRM. It is intended primarily for use in the calibration of chromatographic instrumentation.

PAHs exposition can increase the occurrence of cancer and pulmonary problems, as well as other types of intoxications in living organisms, with emphasis on mammal species, especially on human beings. The development of this work is especially important for the analysis and control of PAHs in the atmosphere, a fundamental process for environmental monitoring.

Supplementary Information

Supplementary information is available free of change at http://jbcs.sbq.org.br, as PDF file.

Acknowledgement

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