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HOMOGENEITY STUDY FOR CERTIFICATION OF A CANDIDATE REFERENCE MATERIAL FOR POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract – The objective of this work was to study the homogeneity of the lot of the candidate certified reference material (CRM) composed of 16 polycyclic aromatic hydrocarbons (PAHs) in toluene, with the purpose of evaluating the degree of homogeneity among its units and within a same unit, evaluating the uncertainty contribution of homogeneity to the certified value.

The method used complies with ISO Guide 30 series, being the ANOVA the statistical tool used to evaluate the variability among and within units. According to the statistical parameters, the homogeneity of the candidate PAHs CRM was confirmed for all substances with their respective property values.

Keywords: Certified reference material, homogeneity, PAHs, uncertainty.

1. INTRODUCTION

PAHs are ubiquitous environmental contaminants originated from anthropogenic, mainly pyrogenic and petrogenic, and natural sources [1]. Due to their persistency in the environment and their toxic potential (mutagenic and carcinogenic properties) several PAHs are on the list of priority pollutants of the U.S. According to the Environmental Protecting Agency (US/EPA) and regulatory organisms of many countries, PAHs are considered of interest for the public health [2]. The Fig. 1 shows the structure of the 16 PAHs considered as priority by Environmental Protection Agency (EPA).

Due to their relevant level of toxicity, analysis with a high degree of accuracy of these contaminants became fundamental, because they make possible the knowledge of the level of contamination with high reliability of the results.

This work aims to present the homogeneity study for certification of the candidate certified reference material (CRM) composed of 16 polycyclic aromatic hydrocarbons (PAHs) in toluene, whose main function is to ensure the measurement reliability, one of the main tools for ensuring traceability in chemical measurements.

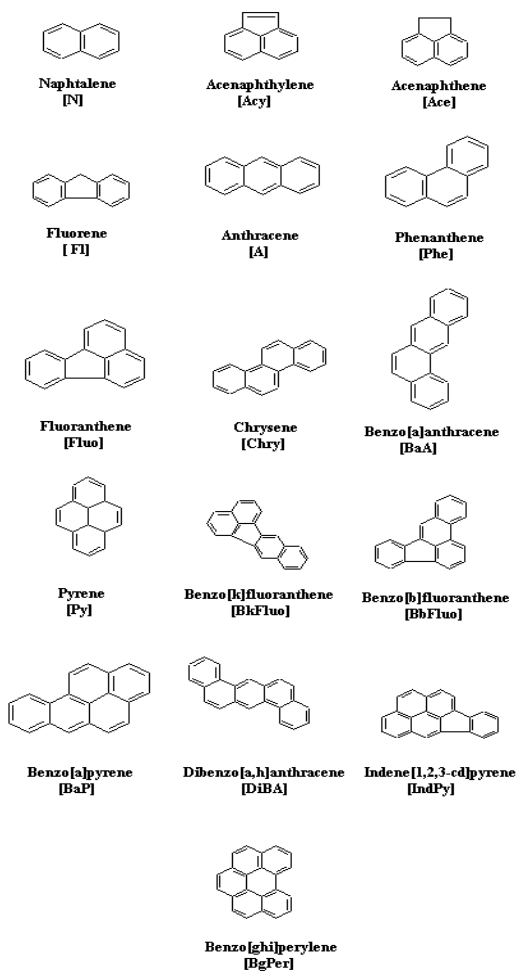

Fig. 1. Chemical structure of the PHAs considered as priority by US/EPA and their abbreviations.

The use of CRM on testing and calibration process is a requirement from ISO / IEC 17025, once that these materials are essential tools to the quality control of measurement methods [3]. The certification of a reference

material is carried out according to the requirements of ISO Guide 30 series [4-9]. The ISO Guide 35 states that the certification process of a CRM requires a careful study of all sources of uncertainty that can cause impact on the validity of the certified values. In general, these sources are relevant to characterization uncertainty, homogeneity uncertainty, uncertainty stability inherent to transport, uncertainty stability inherent to the storage, that are essential for the development and certification of a CRM [9].

The characterization is the process that determines the own values of a reference material, as a part of the certification process, and this procedure makes reference to the properties values of the lot [9].

The homogeneity study is necessary in the certification process of a lot of a RM to demonstrate that the units of this lot are sufficiently homogeneous amongst themselves. The homogeneity should be evaluated between different units of the candidate CRM lot (ampoules at this case) and also within the same unit. This parameter is an element of uncertainty to be included in the uncertainty estimate of the property value of the CRM [9].

2. PURPOSE

The objective of this work was to study the homogeneity of the lot of the candidate certified reference material (CRM) composed of 16 polycyclic aromatic hydrocarbons (PAHs) in toluene, with the purpose of evaluating the degree of homogeneity among its units and within a same unit, determining the uncertainty contribution of homogeneity to the certified value.

3. METHODS

The methodologies were validated through the determination of the performance parameters of the method, to the 16 US/EPA priority PAHs, with basis on the guidance document of the National Institute of Metrology, Standardization and Industrial Quality - INMETRO, the AOAC (Association of Analytical Communities) and ISO/IEC 17025 [10, 11, 3].

The procedure for the production of the reference material and the homogeneity study has been carefully designed and optimized step by step.

Firstly it was determined the purity of the 16 standards obtained from commercial sources and used in the preparation of this CRM. It was used a gas chromatograph with a flame ionization detector (GC/FID) (CP-3800, Varian) for the purity determination of 16 natives PAHs. Two chromatographic columns of different polarity were used: DB-1ms (100% dimethylpolysiloxane) and VF-17ms (50% phenyl, 50% dimetilolisiloxano) [12]. The perdeuterated PAHs were obtained from Cambridge Isotope Laboratories, Andover, MA.

Soon after a series of solutions were prepared, being them: solution with the 16 deuterated PAHs; preparation of the stock solution of the 16 native PAHs; preparation of calibration solutions (native / deuterated) and preparation of reference material. All the solutions were obtained under gravimetric preparation, since gravimetry is a primary method [8,9]. Gravimetric preparation of all solutions is an essential part of the production of calibration solution CRMs.

Optimization of equipment, procedure, and method of preparation contribute to reduction of uncertainties related to the variation in preparation. High-purity materials are generally expensive and available in limited amounts. In addition, replicate preparations of solutions are required for estimation of preparation variation and stability assessment as described below.

Therefore, the amount of high-purity materials amenable to a single preparation lot of the candidate certified reference material is limited to about 5 mg. An analytical balance of 220 g (Model XP205, Mettler Toledo, resolution of 0,01 mg) was used to weigh high-purity materials, since the precision and accuracy of weighing directly affect those of the certified values.

The use of glass boats for weighing and transferring high-purity materials is intended for good repeatability of operation and good visibility of materials which may contribute to reduction of preparation variation. For each one of the two solutions prepared (native and deuterated) this procedure was repeated for the 16 PAHs analyzed. The outline of the scheme is shown in Fig. 2.

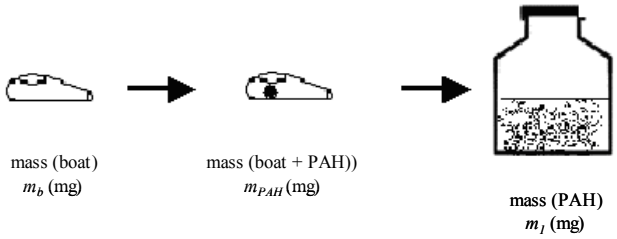

Fig. 2. Scheme of gravimetric preparation of candidate PAH CRM – First step.

A clear amber glass bottle of about 1 L capacity with an specific covers in which a Teflon packing was placed as the seal insert was used for the preparation. A large balance of 1,2 Kg (Model PR1203, Mettler Toledo, resolution of 1 mg) with about 5 kg capacity was used for the weighing. After the weighing of the bottle containing the high purity materials in solvent, the bottle was placed on a rotating mixer for 12 hours to achieve complete homogenization (Fig. 3).

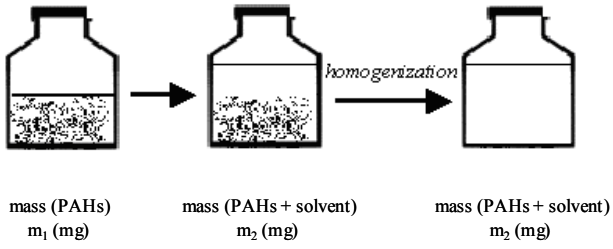

Fig. 3. Scheme of gravimetric preparation of candidate PAH CRM – Second step.

The ampoules were potted using a procedure based on the certificate of the Certified Reference Material SRM 2260a of NIST, in which a solution must be cooled to - 5 °C and a volume of approximately 1,5 mL waived for an amber vial of 2 mL (Fig. 4).

Fig. 4. Candidate PAH CRM.

The calibration curves solutions were prepared by diluting the stock solutions with toluene. The dilutions had been accomplished by gravimetric techniques through the preparation of solution-standard of compounds of interest in six levels of equidistant concentrations in the range of 1 to 11 (μg g^{-1}). The method of internal standardization was applied (isotope dilution) by adding known amounts of the deuterated analytes of the respective analytes under investigation, for subsequent quantification**.**

The points of the calibration curve, as so as the ampoules of the candidates CRM, were injected in triplicate. As a control, it was used the SRM 2260a obtained from NIST, National Institute of Standards and Technology. The analysis were performed in a gas chromatography coupled to a ion trap mass spectrometer (GC/IT/MS) Varian model CP-3800 (CG) Saturn 2000 (IT/MS) under the following chromatographic conditions: splitless ratio of 1:10 after 1 minute; injection volume of 1 μL, the injector temperature, the trap, the line of transfer and the mainfold were set at 290°C, 230 °C, 280 °C and 80 °C, respectively; detection in scan mode and sis mode, chromatographic column DB – 5ms (60m x 250mm x 0,25μm); the oven program: 60 °C (2 min), 120 °C (20 °C/2 min), 180 °C (6,0 °C/2 min), 290 °C (3 °C/29,33 min), totalling 85 minutes. It was used as carrier gas helium 6,0 with constant flow of 1,0 mL/min.

The respective diagnoses ions monitored are presented in Table 1.

To the homogeneity study it was analyzed representative samples of the whole lot, eleven ampoules of the candidate CRM were randomly selected for this study and two aliquots of each one was taken for analysis on each aliquot was added the solution of the deuterated internal standards on the same grounds; the samples were injected in triplicate in GC/MS. The uncertainty of homogeneity was based on the analysis of variance (ANOVA). The property value of the lot for each PAH was achieved by the average of data from the homogeneity study.

Table 1. 16 PAHs (native / deuterated) and monitored ions.

PAHs native / deuterated	Monitored ions
N / perdeuterated naphthalene[a]	128 / 136
Acy / perdeuterated acenaphthylene[a]	152 / 160
Ace / perdeuterated acenaphthene[a]	154 / 164
Fl / perdeuterated fluorene[a]	166 / 176
Phe / perdeuterated phenanthrene[a]	178 / 188,3
A / perdeuterated anthracene[a]	178 / 188,3
Fluo / perdeuterated fluoranthene[a]	202 / 212,3
Py / perdeuterated pyrene[a]	202 / 212,3
BaA / perdeuterated benz[*a*]anthracene[a]	228,2 / 240,4
Chry / perdeuterated chrysene[a]	228,2 / 240,4
BbFluo / perdeuterated benzo(b)fluoranthene[a]	252,4 / 264,4
BkFluo / perdeuterated benzo(k)fluoranthene[a]	252,4 / 264,4
BaP / perdeuterated benzo(a)pyrene[a]	252,4 / 264,4
IndPy / perdeuterated dibenzo[b,c]fluoranthene[a]	276,4 / 288,4
DiBa / perdeuterated dibenz[*a,h*]anthracene[a]	278,4 / 292,4
BgPer / perdeuterated benzo[*ghi*] perylene[a]	276,4 / 288,4

(a) Internal standards

4. RESULTS

It was produced 373 ampoules of the candidate reference material of 16 PAHs, named as HPA_001 to HPA_373, in order of production, and maintained at 4 °C, except the ampoules randomly selected for certification studies. Soon after the sampling, the material was submitted to the characterization, homogeneity and stability studies.

The between-ampoule variance of the CRM was evaluated by analyzing eleven ampoules selected from 373 ampoules. The ampoules were sub-fractioned in A and B, subjected to triplicate analyses by GC/IT/MS (fig. 5).

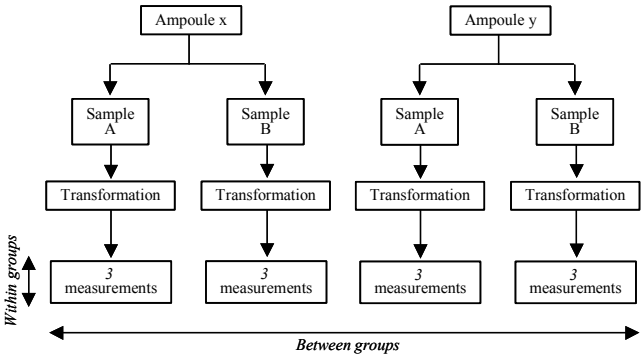

Fig. 5. Layout of a between-ampoule homogeneity study (from Reference [9]).

Homogeneity is an important property of CRMs. 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 have been selected to measure the homogeneity.

The uncertainty inherent to the homogeneity was calculated according to ISO Guide 35. The mass fractions (μg g^{-1}) and uncertainties obtained from the study of homogeneity are described in Table 2 for each one of 16 PAHs.

Table 2. Mass fractions of 16 PAHs (μg g^{-1}) with the uncertainties of homogeneity study and repeatability of the method.

PAHs (abbreviations)	[μg g^{-1}]	$u_{bb.}$ ($C_{\%}$)	$u_{rep.}$ (RSD)
N	5,34	0,90	0,74
Acy	5,03	2,07	0,81
Ace	4,86	0,84	0,60
Fl	5,60	1,09	0,84
Phe	4,72	1,25	0,83
A	5,67	1,11	0,95
Fluo	5,00	2,25	1,73
Py	4,85	2,51	1,56
BaA	4,76	1,19	0,89
Chry	5,45	0,95	2,18
BbFluo	4,65	2,55	2,67
BkFluo	5,17	1,05	1,74
BaP	4,76	1,59	2,64
IndPy	5,21	0,88	1,75
DiBa	4,65	1,88	2,59
BgPer	4,79	1,23	1,78

($C_{\%}$) Contribution of the inhomogeneity
(RSD) Relative Standard Deviation

5. DISCUSSION

Chemical Metrology Division of Inmetro – Dquim aims through these certified reference material, provides to testing laboratories a tool for them achieve reliability and accuracy on PAHs measurements. To get this goal, Dquim certificates it CRM through an analytical technique that offers greater accuracy and less uncertainty, such as GC-MS/IDMS.

The methods adopted for gravimetric preparation and ampouling of solutions were qualified and optimized to reduce the uncertainties of certified values due to these factors.

The homogeneity uncertainty was calculated based on ISO Guide 35, the ANOVA of the raw peak areas of the main component was performed and mean squares within each group (MS_{within}) and among the groups (MS_{among}) were calculated. Standard deviations between ampoules ($u_{bb(1)}$) were then calculated by use of (1), where n represents the number of measurements per ampoule:

$$u_{bb(1)} = u_A = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \quad (1)$$

Equation 1 calculated the uncertainty inherent to the heterogeneity of the samples, since the analysis of variance of the samples showed that the variation between the samples is higher than the variation within the same sample ($MS_{among} > MS_{within}$).

In the case of insufficient repeatability of the measurement method, the influence of analytical variation on the standard deviation between units ($u_{bb(1)}$) was calculated and used as the estimate of inhomogeneity. The $u_{bb(2)}$ was calculated by using (2).

$$u_{bb(2)} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MS_{within}}}} \quad (2)$$

The contribution of the inhomogeneity (C) for the material studied was calculated using (3).

$$C_{(\%)} = \frac{u_{(bb)} \cdot 100}{\overline{X}} \quad (3)$$

Were:

$\overline{X}$ is the average of all measurements of homogeneity study.

Through this contribution is possible to verify the impact of the homogeneity uncertainty in the value of the parameter studied. A value of inhomogeneity less than 3%, for all 16 PAHs, was the criteria established for the development of this reference material. The range of inhomogeneity varied from 0,84 % (Ace) to 2,55 % (BbFluo) what is inside on the acceptable limit. So, the material is homogeneous according to the statistical tests applied.

If the homogeneity of the lot of a candidate reference material were not proven, its certification would not be possible, since the homogeneity of the lot of a reference material is one of the requirements demanded by the ISO Guide 35. Fortunately, this lot under certification showed a good agreement among its units (ampoules) for each one of the 16 PAHs.

The repeatability of the test method for each PAH was the square root of MS_{within} as described in (4) [14].

$$u_{rep} = \sqrt{MS_{within}} \quad (4)$$

The results obtained for the repeatability of the method were expressed as Relative Standard Deviation (RSD). A value of RSD less than 3%, for all 16 PAHs, was the criteria established for the development of the method. The range varied from 0,60 % (Ace) to 2,67 % (BbFluo) what is inside in the acceptable limit.

6. CONCLUSION

Inmetro has recently begun the development of CRMs for organic calibration solutions. Our goal as a CRM producer is to establish highly reliable certified values which are traceable to SI units as far as possible, since the dissemination of CRMs traceable to the SI units contributes to establishment of international comparability of measurements in chemistry. Among the requirements for reliability of CRMs are the technical improvements, especially in determination of the purity of starting materials, and assessment of the homogeneity and stability of solutions.

The certification of the PAHs RM by Inmetro will provide the country with a national reference standard, constituting in 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.

The candidate CRM under study is homogeneous for each one of the 16 PAHs according to the statistical tests used. This result makes possible to continue the certification study to the step of stability tests that will be conducted in order to estimate the transport and storage conditions associated with their uncertainties.

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REFERENCES

[1] M. Mastral, M. Callén, "A Review on Polycyclic Aromatic Hydrocarbon (PAH) Emissions from Energy Generation", *Environmental Science & Technology*, vol. 34, pp. 3051, 2000.

[2] IARC, "Mixtures and Exposures Evaluated and Their Classification", *International Agency for Research on Cancer*, Lyon , France, 2007.

[3] ISO / IEC 17025:2005 "General requirements for the competence of testing and calibration laboratories", 2ª Edition, 2005.

[4] ABNT ISO GUIA 30:2000, "Terms and definitions used in connection with reference materials", 1st Edition, 2000.

[5] ABNT ISO GUIA 31:2000, "Reference materials – Contents of certificates and labels", 1st Edition, 2000.

[6] ABNT ISO GUIA 32:2000, "Calibration in analytical chemistry and use of certified reference materials", 1st Edition, 2000.

[7] ABNT ISO GUIA 33:2000, "Uses of certified reference materials", 1st Edition, 2000.

[8] ISO GUIDE 34:2000 (E), "General requirements for the competence of reference material producers", 2nd Edition, 2000.

[9] ISO GUIDE 35:2006 (E), "Reference materials – General and statistical principles for certification", 3rd Edition, 2006.

[10] DOQ-CGCRE-008. "Guidance on validation of methods for chemical testing - Orientação sobre validação de métodos de ensaios químicos", *INMETRO*, rev.02, Brazil, 2007.

[11] AOAC, "Guidelines for Single Laboratory Validation of Analytical Methods for Trace-level Concentrations of Organic Chemicals", *AOAC*, Washington, 1999.

[12] E. F. Guimarães, J. M. R. Caixeiro, M. H. C. de la Cruz, A. V. Sartori, A. B. Silva, V. Souza, " Measure uncertainty on purity determination of polycyclic aromatic hydrocarbons (PAHs) by gas chromatography with flame ionization detector - Incertidumbre de medida en la determinación de la pureza de hidrocarduros policíclicos aromáticos (HPAs) por cromatografía a gas con detector por ionización de llama", *Simposio de Metrología 2008*, Santiago de Querétaro, Qro, México, Oct 2008.

[13] SRM 2260a. "Certificate of Analysis - Standard Reference Material - Aromatic Hydrocarbons in toluene", *National Institute of Standards and Technology*, Gaithersburg, USA, 2004.

[14] A. M. H. Van der Veen, T. Linsinger, J. Pauwels, "Uncertainty calculations in the certification of reference materials - 2 part: Homogeneity study", *Accreditation and Quality Assurance*, vol. 6, pp. 26-30, 2001.