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Development of a Certified Reference Material: Ethanol in Water - a Practical Case

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ABSTRACT

The present work describes the development of a certified reference material (CRM) composed of solutions of ethanol in water in five different concentrations, and all stages necessary for the development of an CRM, according to the guidelines of ISO GUIDES 30, 31, 32, 33, 34 and 35. The development of this CRM has been determined by Inmetro Rule n. 006 of 2002, which requires that all breath alcohol analyzers being used in Brazil shall have their respective models approved and that the subsequent verifications shall be performed based on tests with solutions of ethanol in water. As a result, the development of this CRM significantly contributes to the reliability of measurements performed with breath alcohol analyzers, with stated measurement uncertainty. Equally important, this development provides society with a CRM that may have multiple applications, not restricted to the verification of breath alcohol analyzers.

Key words: Certified reference material, reliability of measurements, and ethanol

INTRODUCTION

Inmetro has promulgated Rule n. 006 in 2002 (INMETRO/MDIC, 2002) based on the guidelines of the International Organization for Legal Metrology - OIML, determining that all breath alcohol analyzers being used in Brazil shall have their models approved and their respective initial, casual and periodic verifications performed on the basis of testing with solutions of ethanol in water, in five different concentrations. Breath alcohol analyzers are instruments for the determination of ethanol concentration in breath samples, internationally used to control drivers' conditions that could affect the safety of driving. In relation to the alcohol concentration that could impede a driver to legally conduct a vehicle, legislation varies nationally and, in some countries, from state to state. There is no international uniform parameter to indicate the maximum value allowed. However, as there is an international demand for reliability and traceability in measurements, these requirements are observed during testing with CRMs. The United States of America and Germany are countries where the tests performed on breath alcohol analyzers use a certified reference material (CRM) composed of a solution (or solutions) of ethanol in water.

Due to the urgency in adapting to OIML resolutions as directed by Inmetro Rule n. 006, the Directorate of Legal Metrology of Inmetro required the Directorate of Scientific and Industrial Metrology from the same institution to develop this CRM, in the ambit of the Chemical

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Metrology Division - DQUIM. After a training period (February, 2003) at the Federal Institute for Material Research and Testing - BAM in Germany, (an assigned metrology institute), Dquim initiated the process of development of this CRM. Concluded in August 2004, it resulted in five solutions of ethanol in water with certified values and respective stated measurement uncertainties. The training performed at BAM was focused only on the methodology used in that country, comprehending a procedure to individually prepare the solutions of the CRM. Inmetro has initially developed the procedure for individual preparation of the CRM in bottles of 0,5 L., but is presently considering the development of a batch procedure, i.e., the preparation of solutions of the CRM in bottles of 5,0 L and subsequent distribution in bottles of 0,5 L.

MATERIALS AND METHODS

Both in the individual preparation procedure (already developed and validated) and in the batch procedure (under development), the CRM solutions are prepared with Mili-Q water and HPLC (High Performance Liquid Chromatography) grade ethanol, 99,9% purity.

Procedure for individual preparation

The preparation procedure is entirely gravimetric, as gravimetry is a primary method (Milton and Quinn, 2001). No dilution is performed in the process. Ethanol and water are separately weighed and blended in 0,5L silica (boron silicate) glass bottles. After blending, the bottle containing the CRM hydro-alcoholic solution is manually shaken. Weighing is entirely performed using calibrated balances, with stated uncertainty values. These balances are appropriate for the concentration values sought for the CRM solutions to be prepared. Each CRM prepared is submitted to a gas chromatography analysis in order to validate the gravimetric preparation. The chromatographic techniques used include flame ionization detection and on-column injection as an injection technique. The column used is the DB-FFAP (60 m, 0,53 mm, 1μm). The conditions of the analysis are the following: Conditions of the Injector: 65 °C (1 min), 10 °C/min, 120 °C (1 min); Conditions of the column: 65 °C (1 min), 15 °C/min, 120 °C (3 min). Conditions of the detector: 220 °C. Internal standardization is used as quantification method, with HPLC grade n-propanol, 99,8% purity.

RESULTS

As the procedure for batch preparation of solutions is still under development, the results herein presented refer to the procedure for the individual preparation.

Each CRM prepared has a certified value and a particular expanded uncertainty, as each CRM is unique. Therefore, the results herein presented are based on one of the CRM preparation experiments. Table 1 shows the values for the ethanol concentration [ETOH], along with the respective expanded uncertainty.

Table 1 - Certified value and expanded uncertainty for the five concentrations of the prepared CRM:

CRM	Certified value and expanded uncertainty in % *
Concentration 1	[ETOH] = 0,050822 ± 0,000058 %
Concentration 2	[ETOH] = 0,08913 ± 0,00010 %
Concentration 3	[ETOH] = 0,12704 ± 0,00015 %
Concentration 4	[ETOH] = 0,38080 ± 0,00044 %
Concentration 5	[ETOH] = 0,49317 ± 0,00058 %

* % = percent per weight = g ethanol / 100 g solution

The ethanol concentration is calculated in Equation 1:

$$[ETOH] = \frac{mETOH}{(mETOH + mágua)} * p * eva \quad (1)$$

Where:

mETOH = mass of ethanol;
mágua = mass of water;
p = ethanol purity;
eva = evaporation factor of ethanol.
All uncertainty calculations are performed based on the Guide for the Expression of Measurement Uncertainty - ISO GUM (ABNT, INMETRO, 2003).
The expanded uncertainty, at a confidence level of 95 % for each concentration, is calculated in Equation 2.

$$U = k * u_c([ETOH]) \quad (2)$$

Where:

U = expanded uncertainty;
K = coverage factor;
u_c = combined standard uncertainty.

The combined standard uncertainty is calculated based on the uncertainty sources expressed on the cause-and-effect diagram shown in Exhibit 1.

Equation 3 shows the expression for the calculation of the combined standard uncertainty.

$$u_c([ETOH]) = \sqrt{(u(mETOH))^2 + (u(mágua))^2 + (u(p))^2 + (u(eva))^2} \quad (3)$$

Where:

$u_c([ETOH])$ = combined standard uncertainty of ethanol concentration;
u(mETOH) = uncertainty of the weighed mass of ethanol;
u(mágua) = uncertainty of the weighed mass of water;
u(p) = uncertainty of the ethanol purity;
u(eva) = uncertainty of the evaporation factor of ethanol;

Exhibit 2 shows the contribution of each uncertainty source (input quantity) in the calculation of the combined standard uncertainty in each concentration.
Table 2 expresses Exhibit 2 by presenting the contribution to uncertainty of each input quantity, as well as the coverage factor and the combined standard uncertainty for each concentration of the CRM.

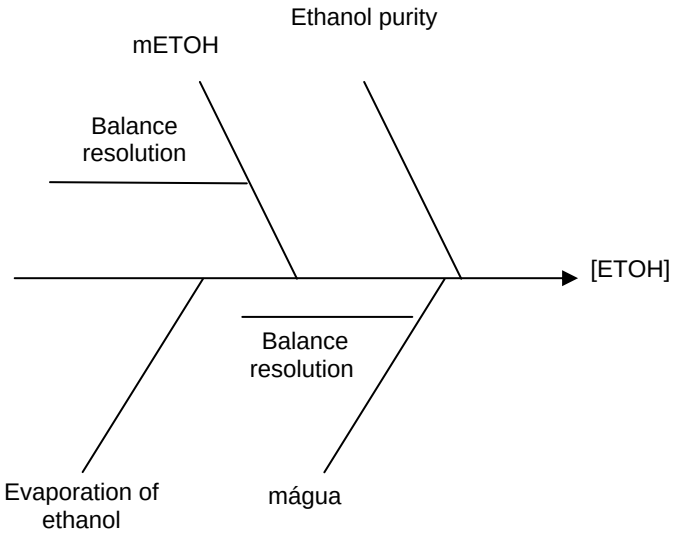

Exhibit 1 - Cause-and-effect diagram for the CRM.

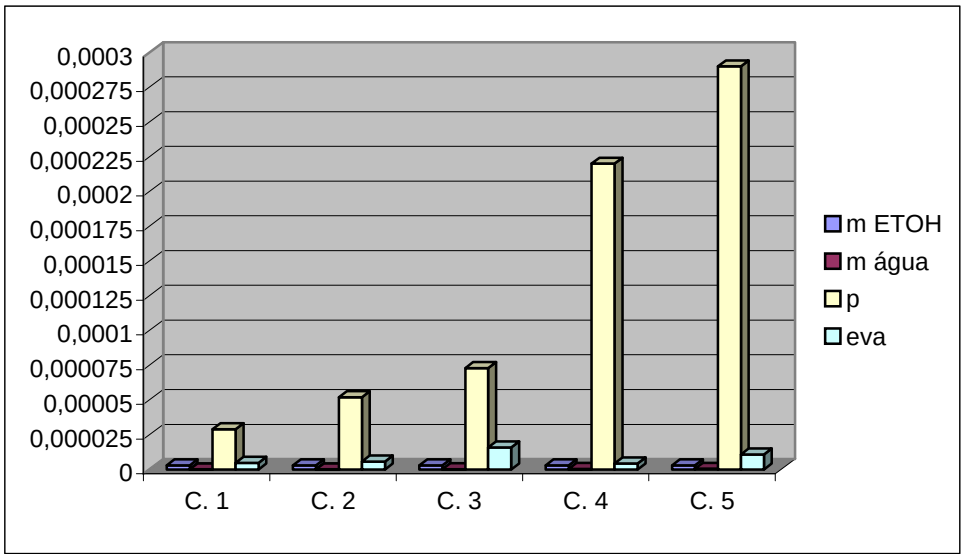

Exhibit 2 - Contribution of each uncertainty source in each concentration of the certified reference material ethanol in water.

Table 2 - Contribution to uncertainty of each input quantity, combined standard uncertainty and coverage factor for each concentration of the MRC.

Input quantity[1]	Type	C. 1[7] (%)	C. 2 (%)	C. 3 (%)	C. 4 (%)	C. 5 (%)
m ETOH[2]	B	$3.0*10^{-6}$	$3.0*10^{-6}$	$3.0*10^{-6}$	$3.0*10^{-6}$	$3.0*10^{-6}$
m water[3]	B	$5.1*10^{-8}$	$8.9*10^{-8}$	$1.3*10^{-7}$	$3.8*10^{-7}$	$4.9*10^{-7}$
p[4]	B	$2.9*10^{-5}$	$5.2*10^{-5}$	$7.3*10^{-5}$	$2.2*10^{-4}$	$2.9*10^{-4}$
eva[5]	B	$4.8*10^{-6}$	$5.7*10^{-6}$	$1.6*10^{-5}$	$4.4*10^{-6}$	$1.1*10^{-5}$
comb std uncert.[6]		$2.9*10^{-5}$	$5.2*10^{-5}$	$7.5*10^{-5}$	$2.2*10^{-4}$	$2.9*10^{-4}$
coverage factor		2	2	2	2	2

[1] Input quantity = Input quantity
[2] m ETOH = mass of ethanol;
[3] m water = mass of water;
[4] p = ethanol purity;
[5] eva = evaporation factor of ethanol;
[6] comb. std. uncert. = combined standard uncertainty;
[7] C. = concentration.

The validation of the procedure for the individual preparation was performed by means of a bilateral comparison with the Federal Institute for Material Research and Testing - BAM. The percent difference between the values determined by Inmetro for each concentration and those found by BAM, taking into account that each Institute has analyzed the same sample, is expressed in Table 3.
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Table 3 - Percent difference between values found by Inmetro and BAM for the same sample, considering the five concentrations of the MRC.

Concentration in %	Bottle 1	Bottle 2
0,0509	0,21 %	0,23 %
0,0890	0,15 %	0,15 %
0,1145	0,12 %	0,20 %
0,3800	0,17 %	xxxxx*
0,4900	0,25 %	0,27 %

*This bottle was broken during transportation.

DISCUSSION

The development and production of a certified reference material shall be based on the ISO GUIDES 30 to 35, which standardize everything, from the terms and definitions related to the reference materials to the statistical methods used (ABNT ISO GUIA 30, 2000), (ABNT ISO GUIA 31, 2000), (ABNT ISO GUIA 32, 2000), (ABNT ISO GUIA 33, 2000), (ABNT ISO GUIA 34, 2000) and (ABNT ISO GUIA 35, 1989). The development of the certified reference material presented in this work has been performed in compliance with the ISO GUIDES 30 to 35, as shown in Table 4.

Table 4 - ISO standards that are reference for the development and production of certified reference materials.

Standard	Definition
ISO GUIDE 30	Terms and definitions used in connection with reference materials
ISO GUIDE 31	Reference Materials - Contents of certificates and labels
ISO GUIDE 32	Calibration in analytical chemistry and use of certified reference materials
ISO GUIDE 33	Uses of certified reference materials
ISO GUIDE 34	General requirements for the competence of reference materials producers
ISO GUIDE 35	Certification of reference materials– General and statistical principles

The ISO GUIDE 17025 in the general requirements for the competence of calibration and testing laboratories shall also be a reference for the activities of development and production of a certified reference material (NBR ISO/IEC 17025, 2001).

The International Vocabulary of Basic and General Terms in Metrology (VIM) (INMETRO, 2003) and ISO GUIDE 30 (ABNT ISO GUIA 30, 2000) , define a certified reference material as a reference material, accompanied by a certificate, in which one or more of its property values are certified by a procedure that establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Based on the definition of certified reference material and on ISO GUIDES 30 to 35, the outcomes of the present work are presented in the following paragraphs.

In regard to the development of a certified reference material composed of solutions of ethanol in water in five different concentrations, the preparation procedure is entirely performed by means of gravimetry, as it is a primary method (Milton and Quinn, 2001). General chemical analysis laboratories frequently use volumetry in the preparation of solutions. However, as the uncertainty related to the volumetric method is greater than that related to the gravimetric method,

the latter is recommended as the method to be used in the preparation of solutions and in the general handling of samples (Ávila et al., 2004). A 0,00001 g resolution balance has been used to weigh ethanol and a 0,001 g resolution balance has been used to weigh water, during the preparation of the solutions that comprise the certified reference material. Both balances have been calibrated by the Mass Laboratory of the Mechanic Metrology Division of the Directorate of Scientific and Industrial Metrology of Inmetro, assuring the traceability of the measurements to the International System of Units (SI).

When weighing ethanol, two crucial aspects are its volatility and hygroscopic potential. These require a fast handling of this substance in order to prevent its evaporation or the absorption of moisture from the environment, assuring that the weighed values are as accurate as possible. It is worth mentioning that no dilution is performed, minimizing the effects of evaporation.

Based on Table 2 and Exhibit 2, it may be noticed that ethanol purity is the predominant uncertainty source during the individual preparation of the solutions of ethanol in water, constituents of the certified reference material. Presently, the value attributed to ethanol purity is that stated by the manufacturer of ethanol, 99,9 %. Thus, the predominant uncertainty in the process is of type B, and the coverage factor (K) = 2. The procedure used to consider ethanol purity is being improved, with the purpose of being experimentally determined, so the uncertainty of this source might be considered of type A. Using the ethanol purity value stated by the manufacturer does not imply to rely on an error, as it simply means to take into account data from a certificate. However, in order to improve the reliability of the process, the Chemical Metrology Division of Inmetro is preparing the determination of ethanol purity by means of the Differential Scanning Calorimetry - DSC technique. This technique is a primary measurement method, i.e., it is not traceable to any other technique or standard, and is presently the most reliable method available in the metrological hierarchy for the determination of the purity of substances.

The evaluation of Table 2 and Exhibit 2 also indicate the robustness of the procedure of weighing ethanol, as the uncertainty related to this process does not vary when the weighed mass increases, within the range of the concentration of the CRM prepared.

Each reference material prepared by gravimetry is also analyzed by gas chromatography to validate the procedure of gravimetric preparation. The criterion of comparison between these techniques is the Analysis of Variance (ANOVA).

The uncertainty inherent to the evaporation of ethanol was determined experimentally, by observing the loss of ethanol mass during the period of time when the flask containing ethanol was being weighed, opened and exposed to the environment, and consequently exposed to the effects of evaporation. This experiment was carried out separately, and its value will be considered a constant in the process. As a result, this uncertainty source is classified as type B, as its determination is not carried out during the same routine of the preparation of the certified reference material.

Besides the measurement uncertainty inherent to the development process, each certified reference material, as its designation denotes, shall be accompanied by a certificate. The ISO GUIDE 31 describes the requirements of a reference material certificate. The requirements described as essential by this Guide and also included in the reference material certificate developed in the Chemical Metrology Division of Inmetro - DQUIM, are:

a) name of the reference material;
b) producer of the reference material;
c) code of the reference material;
d) purpose;
e) instructions for use;
f) instructions for storage;
g) certified values with the respective measurement uncertainties;
h) methods used;
i) period of validity.

Another important component of a certified reference material is the receptacle label, which shall have the following major features, according to ISO GUIDE 34:

a) name of the provider;
b) address of the provider;
c) name of the reference material;
d) code of the reference material.

Exhibit 3 shows the label used for the CRM composed of solutions of ethanol in water, in five different concentrations.

Exhibit 3 - Label of the certified reference material developed in the Chemical Metrology Division of Inmetro.

The stability study of this reference material is presently under development and was initiated three months ago. This is an on-going process and its purpose is to study the stability for a 13 months period of time, as the breath alcohol analyzers in Brazil are mandatorily verified every 12 months.

The procedure for batch preparation of the solutions is being finalized. A control chart is being established to evaluate the homogeneity of the bottles derived from the same gallon and the bottles prepared by the different technicians involved in the preparation. The statistical technique being used is the Analysis of Variance, known as ANOVA. The ANOVA is the statistical tool recommended by ISO GUIDE 35 (ABNT ISO GUIA 35, 1989) for the assessment of variances between experiment results.

CONCLUSION

The certified reference material composed of solutions of ethanol in water in five different concentrations, developed by the Chemical Metrology Division of Inmetro, already has immediate application in the society, especially in the forensic field, as it is determined by law that all breath alcohol analyzers in the country shall have their respective models approved and submitted to verifications consisting of tests performed with the mentioned solutions. Consequently, Brazil now performs the measurement of ethanol in water achieving more reliability, following the example of countries like the United Kingdom, Germany and the United States, that perform breath alcohol analyzers testing based on certified reference materials developed and produced by their respective national institutes of metrology. It is worth mentioning that the development of these solutions and their respective applications are not restricted to the forensic field, as they may be developed for any other purposes that may require hydro-alcoholic solutions with traceability.

The development of this certified reference material might be considered an on-going process, as new improvements are about to be incorporated, like the use of the DSC technique for the determination of ethanol purity and the finalization of the development of the batch procedure. Both represent important steps in the process of speeding up the preparation of these solutions.

By making this certified reference material available, Inmetro is playing one of its statutory roles, i.e., providing reliability and traceability to measurements, while improving health and safety, which represent two of the three foundational elements of its mission: Health, Safety and Environment.

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RESUMO

O presente trabalho descreve o desenvolvimento de um material de referência certificado (MRC) referente a soluções de etanol em água em cinco concentrações diferentes, abordando todas as etapas necessárias para o desenvolvimento de um MRC, conforme prescreve as ISO GUIDE 30, 31,

32, 33, 34 e 35. O desenvolvimento deste MRC originou-se nas determinações da Portaria Inmetro n° 006 de 2002, que assegura que todos os etilômetros a serem utilizados no Brasil deverão ter os seus modelos aprovados, e as verificações realizadas a partir de ensaios com soluções de etanol em água. Após um treinamento, em fevereiro de 2003, no The Federal Institute for Material Research and Testing - BAM, que é um instituto metrológico alemão designado, a Dquim iniciou o processo de desenvolvimento deste MRC, o qual foi concluído em agosto de 2004. No Inmetro desenvolveu-se inicialmente o procedimento de preparo individual, onde as soluções do MRC são preparadas em garrafas de 0,5 L. Atualmente está se estudando, no Inmetro, o desenvolvimento do procedimento em batelada, o que corresponde ao preparo das soluções do MRC em garrafas de 5,0 L, com seqüente distribuição em garrafas de 0,5 L. Pode-se considerar que o processo de desenvolvimento deste material de referência certificado está num período contínuo, onde melhorias estão sendo viabilizadas, como a utilização da técnica de DSC para a determinação da pureza do etanol, e a finalização do desenvolvimento do procedimento em batelada, que será um importante passo para a agilização no processo de preparo destas soluções. Desta forma, o desenvolvimento deste MRC contribui de forma significativa, para confiabilidade nas medições realizadas com etilômetros, tendo incerteza de medição declarada. Outro ponto importante é que a partir deste desenvolvimento, possibilita-se a sociedade um MRC que pode ter aplicações variadas, não se restringindo somente a ensaios com etilômetros.

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