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EVALUATION OF STABILITY OF ETHANOL IN WATER CERTIFIED REFERENCE MATERIAL: MEASUREMENTUNCERTAINTY UNDER TRANSPORT AND STORAGE CONDITIONS

Vanderléa de Souza[1], *Janaína Marques R. Caixeiro* [1], *Raquel D. C. Cunha* [1] , *Laura A. N. Valente* [1], *Renata M. H. Borges* [2], *Rafael A. L. da Silva* [1]

[1] INMETRO, Directorate of Scientific and Industrial Metrology, Chemical Metrology Division, Duque de Caxias, Brazil
[2] INMETRO, General Coordination of Accreditation, Rio de Janeiro, Brazil
jmrodrigues@inmetro.gov.br

Abstract:

This study simulated the transport and storage conditions of ethanol in water certified reference material (CRM) produced by the Chemical Metrology Division of Inmetro – DQUIM with the purpose to estimate the measurement uncertainty related to stability. The short-term stability study was performed on five different concentrations in terms of g ethanol/100g solution (%) of the ethanol in water CRM: 0.0509%, 0.0890%, 0.1145%, 0.3820% and 0.4960%, at the temperatures of 4 ºC and 60 ºC. On the other hand, the long-term stability study was developed on four different concentrations: 0.0509%, 0.0890%, 0.1145% and 0.4960%. In this paper will be shown data from the long- term stability study concerning 20 weeks.

The method used complies with ISO GUIDE 35, BCR-Guideline for Feasibility Studies and ISO GUIDE 34.

According to the statistical parameters used in both studies the stability of the ethanol in water CRM was confirmed for all concentrations studied.

Keywords: certified reference material, stability, ethanol in water, uncertainty.

1. INTRODUCTION

Stability and homogeneity are key characteristics in the certification of reference materials, as they impact the validity of the certified values and their uncertainties. Hence, in the certification process, the reference materials shall be submitted to stability and homogeneity tests [1].

ISO GUIDE 35 [2] establishes that the certification of a reference material requires a detailed study of all sources of uncertainty that affect the certified value. Among these sources are the uncertainties of characterization, homogeneity, transport and storage.

The Ethanol in Water certified reference material (CRM) is produced by the Chemical Metrology Division of Inmetro - DQUIM to test breath alcohol analyzers used to determine the content of alcohol (ethanol) present in the expired air of vehicle drivers.

The uncertainty concerning the storage and transport of a CRM is estimated based on a stability study of long-term and short-term, respectively [2]. In the case of ethanol in water solutions the estimation of the uncertainty related to stability is extremely important as ethanol is volatile and the impact of its volatility in regarding to storage and transport conditions is crucial for quality assurance of the material.

The short-term stability study was performed in five different concentrations in g ethanol/100g solution (%) of the ethanol in water CRM: 0,0509%, 0,0890%, 0,1145%, 0,3820% and 0,4960%. The follow-up of the short-term stability was performed at the temperatures of 4ºC and 60ºC. The long-term stability study was developed in four different concentrations: 0,0509%, 0,0890%, 0,1145% and 0,4960%. In this paper it is shown data from the long-term stability study concerning 20 weeks. However, this studies continue under development at DQUIM/ DIMCI/INMETRO.

All the procedures used in this study are complied with ISO GUIDE 35, ISO GUIDE 34 and the BCR document-Guideline for Feasibility Studies [2-5].

2. PURPOSE

The aim of this work is to demonstrate the estimation of uncertainty related to the transport (short-term stabillity study) and storage (long-term stability study) of the ethanol in water certified reference material in five different concentrations.

The short-term stability study was to assess the influence of temperature on the stability of the five different concentrations of the ethanol in water CRM, by submitting them to temperatures above and below room temperature, simulating extreme conditions. The long-term stability study consisted of following up quantitatively, for a period of 20 weeks to estimate the impact of the storage on the concentration of ethanol at room temperature. To the room temperature is been considered the range from (20 ± 0,3) °C to (25± 0,3) °C.

3. METHODS

During the stability studies the ethanol in water solutions were considered as reference materials (RM's) under a certification process.

For both stability studies each RM was prepared in a 5 L bottle subsequently subdivided into nine 500 mL bottles.

In short-term stability study were prepared two different groups as follows: three bottles were considered as reference samples and, after being weighed, were stored at 4 ºC (reference temperature). The other six bottles were weighed and stored in an stove at 60 ºC. Each two days, two bottles were removed from the stove and left in the laboratory at room temperature for two hours. Immediately after, they were weighed and stored at the reference temperature (4 ºC). At the end of eight days, when all bottles had already been submitted to the reference temperature, the group was exposed to the laboratory room temperature (from (20 ± 0,3) °C to (25± 0,3) °C), and weighed and analyzed by gas chromatography.

Comentário: O símbolo da unidade deve ser sempre separado do valor por um espaço! Por exemplo: 5 m; 2 cm; 1 g.

In long-term stability study, the monitoring of each analyte concentration in the RM was performed in two different cycles in time. The first cycle began one week after the preparation of the calibration curve and the samples. The analyses were performed once a week, during a period of four weeks. In the same week the preparation of calibration curves and samples was performed, the respective "time-zero" analyses (the first analyses before initiating the cycles) were also performed. The internal standard solution was added to the calibration solutions and new calibration solutions and a new internal standard solution were prepared. In the second cycle the analyses were performed once a month during a period of five months (20 weeks). All material was stored and protected against light at the temperature range from (20 ± 0,3) °C to (25± 0,3) °C.

The analysis was performed by gas chromatography with flame ionization detector (GC/FID), *on-column* injector (65°C, rate: 16°C/min, 125ºC (3min)), and the quantification was performed by the addition of 1-propanol as internal standard. The column used was a DB-FFAP of 60m (1,00µm of phase thickness 0,53mm of external diameter). The temperature program was: 65°C (10min), rate of 15°C/min up to 120°C (6min), column flow rate 5,65mL/min. The injection volume was 1µL. The detector was kept at 220°C. The calibration curves were made with gravimetrically prepared standards by using two curves, with 8 points each, in the concentration range of 0,035% to 0,105% and in the concentration range of 0,085% to 0,58%, respectively.

The method also included checking the homogeneity within each bottle, as two aliquots were analyzed for each bottle, each one of them in duplicate.

The statistical criteria adopted for assessing the stability and uncertainty in both studies were based on linear regression and residue analysis in conjunction with analysis of variance (ANOVA). Two criteria shall be simultaneously met in order to determine the stability of the material: a p-level greater than 0,05 and an angular coefficient (B) near to zero [2].

To the short-term study two more criteria were evaluated: the maximum percent differences acceptable for sample mass of each bottle before and after the study temperature (60ºC) and the ethanol concentration of each bottle submitted to study temperature (60ºC) against ethanol concentration of bottles submitted to reference temperature (4ºC). These two parameters are presented at Table 1.

Table 1: Parameters and maximum percent differences for the short-term stability study

Parameter	Maximum Percent difference (%)
Sample mass before and after the study temperature (60 °C)	0.05
Analyte concentration after study temperature against analyte concentration at reference temperature	1.0

RESULTS

According to the number of days in the stove the ethanol concentrations obtained for each bottle in short-term stability study after them being submitted to the reference temperature are shown in the Table 2.

Table 2: Ethanol concentration (g ethanol/100 g solution) after bottles were submitted to the temperature of 4ºC

	Number of days in stove			
Concentration	0	2	4	7
[1]	0.0507	0.0507	0.0508	0.0507
[1]	0.0507	0.0508	0.0506	0.0507
[2]	0.0891	0.0888	0.0892	0.0889
[2]	0.0891	0.0890	0.0893	0.0892
[3]	0.1155	0.1158	0.1155	0.1157
[3]	0.1155	0.1155	0.1154	0.1153
[4]	0.3887	0.3893	0.3898	0.3885
[4]	0.3887	0.3909	0.3905	0.3909
[5]	0.4576	0.4587	0.4591	0.4588
[5]	0.4576	0.4577	0.4583	0.4567

Figure 1 shows, for each RM concentration, the ratio between the ethanol concentration found in each bottle submitted to the temperature of 60°C and the bottles considered as reference samples, submitted only to the temperature of 4°C.

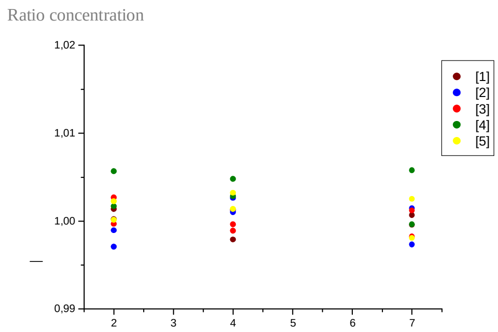

Figure 1: Ratio between the ethanol concentration for each bottle submitted to 60 ºC and the ethanol concentration for the bottles submitted only to the temperature of 4 ºC (reference), for each RM concentration.

Figure 2 shows, for each bottle in each RM concentration, the mass percent difference of each bottle before and after the study temperature (60 ºC).

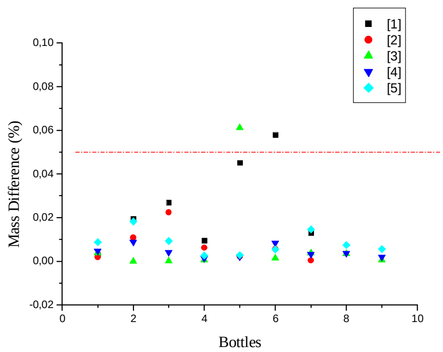

Figure 2: Mass Percent Difference after reference temperature for each bottle in each RM concentration .

In long-term stability study, the results obtained for the [A], [B], [C] and [D] ethanol concentrations during the 20 weeks are displayed in Table 3.

Table 3. Ethanol concentrations (g ethanol/100 g solution) obtained during the period of study.

	Period of Study (weeks)					
Ethanol	0	2	3	4	16	20
[A]	0.05090	0.05098	0.05081	0.05076	0.05097	0.05088
[B]	0.08940	0.08966	0.08876	0.08888	0.09043	0.08993
[C]	0.11499	0.11508	0.11418	0.11461	0.11603	0.11531
[D]	0.45841	0.46276	0.45918	0.45820	0.46556	0.46100

Figures 4 and 5 show the variation of the RM concentrations [A] and [B], [C] and [D] during the period of study.

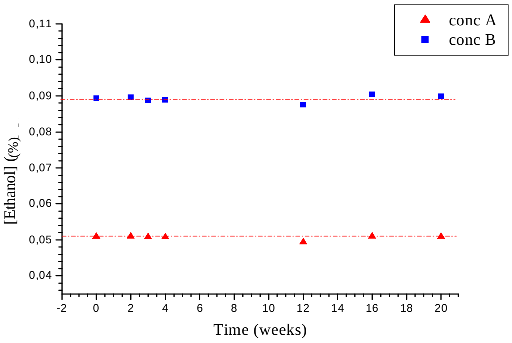

Figure 4: RM concentrations [A] and [B], obtained during the period of study.

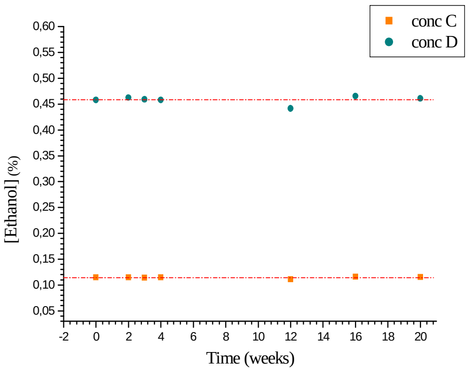

Figure 5: RM concentrations [C] and [D], obtained during the period of study.

ISO GUIDE 34 [3] establishes that the uncertainty inherent to a CRM shall be calculated on the basis of the combination of all sources involved in the certification process, i.e., the combination of the uncertainties taken from the characterization, the homogeneity, the storage and the transport, being the two latter the object under estimation in this study.

The uncertainties related to storage and transport, long-term and short-term studies, respectively, were calculated through an spreadsheet, that provide the following parameters: the standard deviation of ethanol concentration in relation to the time of study, the angular coefficient of the curve related to the variation of ethanol concentration with the time and p-level. Table 4 and Table 5 show respectively to the short and long-term stability study, the uncertainty related to the transport and storage to each CRM concentration, the angular coefficient of the curve, the p-level and standard deviation (STD) in relation to time.

Table 4:- Uncertainty for each concentration of CRM, obtained by statistical treatment (linear regression)

Concentratior	Angular Coefficient (B)	p-level (p)	Uncertainty	STD
[1]	-1.589E-06	0.8127	0.000041	5.89184E-06
[2]	5.293E-06	0.8875	0.00023	3.30646E-05
[3]	1.822E-05	0.4244	0.00013	1.83044E-05
[4]	1.216E-04	0.4713	0.00097	0.000138037
[5]	2.359E-05	0.8506	0.00077	0.00011042

The uncertainty is expressed in g ethanol/100g solution.

Table 5: Statistical results obtained by linear regression for the respective ethanol concentrations.

Concentration	Angular Coefficient (B)	p-level (p)	Uncertainty	STD
[A]	2.370E-06	0.6641	0.00010	5.0635E-06
[B]	5.353E-05	0.1187	0.00054	2.7021E-05
[C]	4.978E-05	0.1536	0.00057	2.83232E-05
[D]	1.913E-04	0.2550	0.0029	0.000144097

* The uncertainty is expressed in g ethanol/100g solution.

The uncertainty related to the transport and storage (short and long term stability study) for each CRM ethanol concentration is calculated by the product of standard deviation and the time of study as show in Equation 1. To the short-term stability study t = 7 days and to the long-term stability study t=20weeks.

$$U_{Stability} = STD * t \quad (1)$$

where:

$U_{Stability}$ = Uncertainty Stability
STD= Standard Deviation
t= time

4. DISCUSSION

In short-term stability study, the ratio between the ethanol concentrations found for each bottle at 60 ºC and the bottles submitted only to the temperature of 4 ºC (reference samples) was approximately 1, showing that no significant variation of concentration occurred when submitting the CRM to this range of temperature.

In relation to the study to evaluate the mass difference of the bottles before and after they had been submitted to the study temperature (60 ^{o}C) it can be seen that there was no lost of mass evidencing the stability of ethanol solutions for all concentrations.

As the study evidenced the CRM stability in the temperature range of 4 °C to 60 °C for 7 days, the uncertainty obtained is deemed to be the uncertainty inherent to the transport of CRM, since during the transport a material may be submitted to variable conditions and the range of temperature studied simulates extreme conditions.

In relation to long-term stability study, the Figures 4 and 5 show that for all ethanol concentrations no significant difference was detected during the period of study (20 weeks). The calculations performed demonstrated that the variation of each concentration, in each week, compared to time zero (t=0) is not greater than 1.0%, indicating a satisfactory concordance of data and evidencing the stability of the material.

The results of this study made possible the estimative of the impact of the CRM storage on the ethanol concentration, and also the subsequent determination of the uncertainty taken from the CRM storage during the period of study. This uncertainty can be seen at the Table 5.

The statistical parameters used in both stability studies are those obtained from linear regression and residues analysis. These parameters, expressed by the angular coefficient (B) and the p-level (p), indicate if a material is or not stable. A material is deemed to be stable when the following criteria are simultaneously met: $B \cong 0$ and $p > 0,05$. At the Tables 4 and 5 can be seen that these two criteria were met, confirming the stability of the ethanol in water CRM for all concentrations studied.

These results meet the criteria established in ISO GUIDE 35, ISO GUIDE 34 and BCR Guidelines for Feasibility Studies, evidencing the certification of the RM for the period of study.

6. CONCLUSION

The short-term stability study characterized for all concentrations studied the stability of the ethanol in water CRM in the range of temperature from 4 ºC to 60 ºC at the period of 7 days, providing an estimation, for each concentration, of the uncertainty inherent to the transport of this material.

The long-term stability study of the ethanol in water RM demonstrated the stability of the CRM along the 20 weeks period of study (5 months). Hence, this period corresponds to the period of storage shown in the CRM certificate. This study also facilitated the estimation of the uncertainty of the ethanol in water CRM storage, in regard to the certified values of ethanol concentration.

It is important to mention that the long-term stability study of ethanol in water CRM is under development at DQUIM/ DIMCI/ INMETRO with the objective to reach the total time of 13 months.

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