

Evaluation of Homogeneity in Certified Reference Materials (CRM)

Florbela A. Dias^{1*} and Carlos J. Costa¹

¹Instituto Português da Qualidade, Rua António Gião, 2, 2829-513 Caparica, Portugal

Abstract. The production of Certified Reference Materials (CRM), according to the international standard ISO 17034:2016, has been playing an increasingly role in measurements and accreditation activities of entities/laboratories, with special focus on the field of chemical, biological and clinical tests and physical properties. The IPQ - LGR is responsible for the production of various types of CRM, namely gas mixture standards of ethanol in nitrogen (C_2H_5OH/N_2), by gravimetric method according to the international standard ISO 6142-1:2015, and certification by non-dispersive infrared spectroscopy (NDIR) in accordance with ISO 6143:2001. According to ISO 17034:2016, these mixtures have to be evaluated regarding the homogeneity, which is one of the fundamental requirements for the production of CRM. In this communication, the results of the homogeneity study of standards gas mixtures of ethanol in nitrogen are presented, according to a validated measurement procedure, in order to guarantee the traceability to SI units and also to meet the requirements of ISO 17034:2016. The statistical treatment of the data is presented through analysis of variance ANOVA.

1 Introduction

Certified Reference Materials (CRM) are an important mean of disseminating SI units and are also a support in the credibility and reliability of a measurement or a laboratory. They can be used in the equipment calibration, validation of analytical methods and measurements quality control. They are also applied in interlaboratory comparisons allowing laboratories to evidence their best measurement and calibration capabilities (CMC). The use of CRM is also an important mean of method validation and accuracy and precision evaluation of the measurements, establishing the metrological traceability of the obtained results.

The Reference Gas Laboratory (LGR) of the Portuguese Institute for Quality (IPQ) carries out several activities, namely in the field of gas metrology, either by preparing primary gas mixtures, or certifying reference materials. Primary gas mixtures are prepared at the highest level of accuracy by gravimetric methods based on international standards. The composition of gas mixtures is certified by analytical reference methods. The LGR is responsible for the production of gas mixture standards of ethanol in nitrogen (C_2H_5OH/N_2) CRM, by gravimetric method according to the international standard ISO 6142-1:2015 [1], and certification by non-dispersive infrared spectroscopy (NDIR) in accordance with ISO 6143:2001 [2]. These gas mixtures of ethanol in nitrogen were prepared according to a validated measurement procedure, in order to guarantee the traceability to SI units and also

to meet the requirements of ISO 17034:2016 [3]. This standard establishes the necessary requirements for the planning and control of production of reference materials (RM) and certified reference materials (CRM) and the homogeneity study is one of the requirements. This study was carried out with twelve mixtures of C_2H_5OH/N_2 that were subjected to three rolling cycles and analysis. Later they were also analyzed over time, on different days, during six days. The statistical treatment of data is presented through analysis of variance ANOVA [4] and by the *t-student* statistical test [5], when the null hypothesis is rejected.

2 Preparation of Reference Gas Mixtures

The preparation of gas standards is carried out in accordance to an harmonised procedure, based on the international standard ISO 6142-1:2015 [1]. These primary mixtures are prepared at highest level of accuracy by gravimetric blending methods, as shown in figure 1. Their nominal composition (volume fractions) is estimated by the ideal gas equation, corrected by a compression factor.

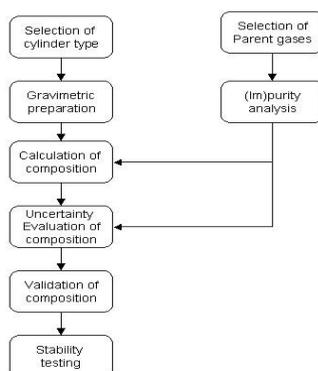


Fig. 1. Outline of the preparation process.

In an evacuated and passivated cylinder, each component is added through a filling station, where all equipment (pipes, valves etc.) is electro polished and free of oil (Fig. 2.). The mass of each gaseous component added to the cylinder can be determined through the mass comparator balance, in which masses are calibrated and traceable to the used national standard (Fig. 2.).

The traceability of the gas composition to the SI system is ensured by the use of calibrated instrumentation.



Fig. 2. Filling station. Mass comparator balance.

The mixture is prepared by gravimetric addition of each component. The mole fractions of the components in the final mixtures are calculated using the following equation (ISO 6142-1:2015) [1]:

$$x_i = \frac{\sum_{A=1}^P \left(\frac{x_{i,A} m_A}{\sum_{i=1}^n x_{i,A} M_i} \right)}{\sum_{A=1}^P \left(\frac{m_A}{\sum_{i=1}^n x_{i,A} M_i} \right)} \quad (1)$$

where

x_i is the mole fraction of the component i in the final mixture, $i = 1, \dots, n$

P is the total number of the parent gases

n is the total number of the components in the final mixture

m_A is the mass of parent gas A determined by weighing, $A = 1, \dots, P$

M_i is the molar mass of the component i

$x_{i,A}$ is the mole fraction of the component i .

3 Certification of Reference Gas Mixtures

The composition of a gas mixture is determined by separate analysis of the mole fraction of every individual analyte. The procedure for determining the mole fraction of only one individual analyte is described in ISO 6143:2001 [2]. The mixture composition is certified by comparison methods using a set of mixtures with pre-established assigned values. The traceability of these measurements is guaranteed by links to national and international primary gas mixtures standards. In order to establish the relationship between equipment response and the composition of the suite of calibration mixtures, the following aspects must be considered:

- Analytical method
- Calibration range
- Measuring conditions
- Number and sequence of replicate measurements

ISO 6143:2001 [2] and ISO 11095 [6] describe the bracketing and calibration curve methods for determining the composition of gas mixtures. The selection of method is dependent on

the available PRMs. Results are expressed together with their measurement uncertainty, according to GUM [7].

The composition of gas mixtures is certified by analytical reference methods such as Gas Chromatography (GC), Non Dispersive Infrared Spectroscopy (NDIR) and Ultra Violet (UV) (Fig. 3.).



Fig. 3. Analytical reference methods.

The CRM of gas mixture standards of ethanol in nitrogen (C_2H_5OH/N_2) are certified by non-dispersive infrared spectroscopy (NDIR).

4 Homogeneity evaluation

In the process of producing a certified reference material, the homogeneity evaluation is an important part of the planning, regarding the ISO 17034:2016 [3]. A material is perfectly homogeneous with respect to a given characteristic or composition if there is no difference between the obtained values of this characteristic from one part to another, according to ISO Guide 35 [8].

4.1. Homogenization System

Whenever a gas mixture is prepared it goes through a homogenization process. The cylinder containing the mixture is placed in a rolling system. See figure 4.



Fig. 4. Homogenization Systems.

In this study we give special attention to ethanol in nitrogen gas mixtures because the ethanol is a liquid at ambient temperature, which makes the mixing process more complex. In our experiment, we did the evaluation within-bottle homogeneity. This study is necessary to demonstrate that each mixture, prepared individually, is sufficiently homogeneous.

For homogeneity evaluation was chosen a highly repeatable method. This homogeneity study was carried out with twelve mixtures of C₂H₅OH/N₂ with molar fractions of 50×10^{-6} mol/mol, 212×10^{-6} mol/mol, 316×10^{-6} mol/mol and 500×10^{-6} mol/mol. Three mixtures of each molar fraction were prepared, then they were subjected to three rolling cycles and analysis, corresponding to different homogenization times.

4.2. Rolling time

After preparation, each mixture was subjected to an homogenization process on the rolling system and analyzes were carried out to each of the 12 mixtures at 0 minutes, 30 minutes, 60 minutes and 90 minutes of rolling. In Table 1 we can see all the results for the 12 molar fractions (line factor) for all 4 rolling times (column factor).

Table 1. Results for the molar fractions 50 ppm, 212 ppm, 316 ppm and 500 ppm according to the rolling time. (1 ppm = 1×10^{-6} mol/mol)

Rolling time	Factor j (1... n)	1	2	3	4
Factor i (1... m)		0 min	30 min	60 min	90 min
1	50 ppm	51	50	50	50
		49	49	49	49
		50	49	49	49
2	212 ppm	203	207	208	206
		215	206	206	206
		211	206	205	205
3	316 ppm	315	313	312	312
		317	313	315	314
		325	318	317	317
4	500 ppm	522	528	527	526
		504	502	502	505
		507	507	502	501

4.3. Rest period

Each of the 12 mixtures were analysed in the Day 1, Day 2,..., until Day 6. In Table 2 we can see all the results for the 12 molar fractions (line factor) for all 6 rest periods (column factor).

Table 2. Results for the molar fractions 50 ppm, 212 ppm, 316 ppm and 500 ppm according to the rest period. (1 ppm = 1×10^{-6} mol/mol)

Rest period	Factor j (1... n)	1	2	3	4	5	6
Factor i (1... m)		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	50 ppm	50	51	48	47	51	52
		49	49	50	50	51	50
		49	50	50	51	52	50
2	212 ppm	206	207	207	207	208	209
		206	213	215	213	213	212
		205	213	210	211	213	212
3	316 ppm	312	315	320	319	319	319
		314	320	321	319	318	320
		317	324	321	319	318	320
4	500 ppm	526	537	516	525	523	522
		505	512	507	507	512	506
		501	511	506	507	506	504

5 Statistical analysis

The statistical analysis were applied not to absolute values but to the difference of each value and the sample mean.

5.1. Analysis of variance ANOVA

The Analysis of Variance (ANOVA) [4] which is a method of testing the equality of three or more population means, based on the analysis of the sample variances was applied.

Statistical model:

$$y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij} \quad (i = 1, 2, \dots, m \text{ e } j = 1, 2, \dots, n) \quad (2)$$

Where y_{ij} , represents the j^{th} observation taken at the factor level- i ; μ is a parameter common to all factors called the overall mean; τ_i is a random variable that corresponds to the i -level effect of the line factor; β_j is a random variable that corresponds to the j -level effect of the column factor and ε_{ij} the random component of the error associated with the j^{th} observation of the i -level.

The test is unilateral and the hypotheses are null:

$$H_0^{\text{line}} : \tau_1 = \tau_2 = \dots = \tau_m = 0 \quad (3)$$

and

$$H_0^{column} : \beta_1 = \beta_2 = \dots = \beta_n = 0 \quad (4)$$

the average of all populations are equal, it means that the factor has no effect.

We use the statistic F :

F is the *Fisher-Snedcor* [4] sampling distribution where:

$$F_0^{line} = \frac{MS_{line}}{MS_{Error}} \quad F_{\alpha, m-1, (m-1)(n-1)} \quad (5)$$

$$F_0^{column} = \frac{MS_{column}}{MS_{Error}} \quad F_{\alpha, n-1, (m-1)(n-1)} \quad (6)$$

where MS is the mean square between factors (line or column) and MS_{Error} is the mean square error.

If:

$$F_0^{line} > F_{\alpha, m-1, (m-1)(n-1)} \quad (7)$$

$$F_0^{column} > F_{\alpha, n-1, (m-1)(n-1)} \quad (8)$$

then the null hypothesis is rejected at a level of significance α , it means that not all population averages are equal, there is factor / treatment effect.

As we can see in Table 3, F_0 is higher than the critical value F_{critic} for the factor rolling time, indicating that there is at least one situation where the mixture is not homogeneous.

Table 3. ANOVA Table for the rolling time factor.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F_0	F_{critic}
Gas mixture	2,842x10 ⁻¹⁴	3	9,474x10 ⁻¹⁵	2,157x10 ⁻¹⁵	2,901
Rolling time	42,233	3	14,078	3,205	2,901
Error	140,538	32	4,392		
Total	218,5016	47			

In Table 4, we can observe that F_0 is higher than the critical value F_{critic} for the factor rest period, indicating that there is at least one situation where the mixture is not homogeneous.

Table 4. ANOVA Table for the rest period factor.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F_0	F_{critic}
Gas mixture	$1,137 \times 10^{-13}$	3	$3,790 \times 10^{-14}$	$8,987 \times 10^{-15}$	2,798
Rest period	169,998	5	34,000	8,063	2,409
Error	202,408	48	4,217		
Total	580,882	71			

5.2. Statistical t-student test

With the ANOVA calculations, we have identified the existence of a mixture or mixtures where the value of molar fractions may be considered outliers.

When the null hypothesis is rejected, we can use the *t-student* statistical test [5] for a significance value of 5 %, that is a criteria to deal with outlying measurements. With these criteria, we can use a sample statistic to be compared with a critical value in order to determine the measurements to reject.

The statistical test to detect the outliers was the test of the mean of a normal distribution with unknown variance for a significance value of 5 %.

The statistical test used is:

$$t_0 = \frac{\bar{x} - \mu_0}{\frac{s}{\sqrt{n}}} \quad (9)$$

and the null hypothesis will be rejected if $|t_0| > t_{\alpha/2, n-1}$ where $t_{\alpha/2, n-1}$ is a bilateral test with $n-1$ degrees of freedom.

The average of the 48 measurements related with the rolling time factor, will be considered the mean μ_0 . The 4 rolling time averages will be used to calculate the $t_{0,025, 3} = 4,176$. By the observation of Table 5 we can conclude that the measurements from the 0 minutes rolling time should be rejected.

Table 5. Statistical t-student test for the rolling time factor.

Rolling time	0 min	30 min	60 min	90 min
$ t_0 $	5,033	0,501	1,995	2,537
$t(0,025 ; 3)$	4,176			

Also applying the *t-student* statistical test, we have the average of the 72 measurements related with the rest period factor that will be considered the mean μ_0 . The 6 days rest period averages will be used to calculate the $t_{0,025, 5} = 3,163$. By the observation of Table 6 we can conclude that the measurements from the day 1 and day 2 should be rejected.

Table 6. Statistical *t-student* test for the rest period factor.

Rest period	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
$ t_0 $	5,839	4,818	0,557	0,003	1,545	0,029
$t(0,025 ; 5)$	3,163					

6 Conclusions

Tests for homogeneity of 12 gas mixture standards of ethanol in nitrogen for candidate reference material were carried out using the ANOVA statistical tool and the *t-student* statistical test when the null hypothesis is rejected.

With the ANOVA evaluation, we conclude that for the rolling time and for the rest period, $F_0 > F_c$ or at least one mean is statistically different, which means that there is at least one situation where the mixture is not homogeneous.

With the *t-student* test we conclude that:

- $t_0 > t_c$, for the 0 min rolling time, which means that the cylinder has to roll for at least 30 minutes.
- $t_0 > t_c$, for the rest period of 1 and 2 days, which means that the cylinder must be on rest period for at least two days before carrying out the analysis.

References

1. ISO 6142-1:2015 – Gas analysis — Preparation of calibration gas mixtures — Part 1: Gravimetric method for Class I mixtures
2. ISO 6143:2001 - Gas analysis — Comparison methods for determining and checking the composition of calibration gas mixtures
3. ISO 17034:2016 - General requirements for the competence of reference material producers
4. D. C. Montgomery, *Introduction to Statistical Quality Control*, Singapore: John Wiley & Sons, 3rd ed., ISBN 0-471-51988-X, 485-524 (1996).
5. E. Filipe, AMCTM VI, editors P. Ciarlini *et al* , World Scientific Publishing Co., *Validation of Calibration Methods – A Practical Approach*, Singapore, ISBN 981-238-904-0, **66**, 285-292 (2004)
6. ISO 11095:1996, Linear calibration using reference materials. Switzerland: ISO. 29 p.
7. Guide to the expression of uncertainty in measurement. ISO, Geneva (1993). (Reprinted 1995: Reissued as ISO Guide 98-3 (2008), JCGM 100:2008
8. ISO Guide 35:2006 – Reference materials – General and statistical principles for certification