

# Final Report of the Interlaboratory Comparison in Anhydrous Bioethanol



Inmetro

Diretoria de Metrologia Científica e Industrial

**PEP-Dimci**

Programa de ensaios de proficiência em metrologia científica e industrial

# INTERLABORATORY COMPARISON IN ANHYDROUS BIOETHANOL

## FINAL REPORT – Nº 008/11

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## **1. Introduction**

Biofuel constitutes a viable alternative in relation to the fuel derived from petrol. It can be considered a significant model of energy source in the future. The participation of biofuel in the energetic matrix depends on a complex array of factors involving many production aspects (raw material and technology) and also the sustainability (social, economical and environmental) of this energy source.

The worldwide demand for renewable fuels is increasing, especially because of the reduction of noxious emissions and sulfur-containing gases which are blamed for global warming. More recently, the bioethanol quality played a decisive role on the need of ethanol conversion in commodities to the international market.

The ability to conquer overseas markets demands that the bioethanol meet the stringent quality requirements of these markets. Therefore, one of these requirements is the validation of the methods for bioethanol analysis. The validation is markedly dependent on three basic factors: normalized specifications; official methods of analysis and certified reference materials. The normalized specifications are a set of characteristics required to assure that the biofuel has the final destination as previously defined. The official methods are the necessary tools to construct the right evaluation for the normalized specification of the fuel. The certified reference materials are tools utilized to ensure reliability to analytical measurements. They can be used in method validation, calibration or as control samples.

Confidence in the analytical data requires thorough knowledge of the traceability chain which links results measured in the biofuel samples to the International System of Units (SI) or to an international agreed reference. Therefore, the metrology for biofuel focuses on providing traceable results by means of developing reference methods and suitable reference materials.

Inmetro, the Brazilian Metrology Institute, exerts a key role in the research of biofuels in general. Thus, Inmetro aims to contribute to fundamental research, development and production of reference materials to provide metrological traceability for national industrial laboratories by means of certified reference materials (CRMs), in accordance with international procedures which are necessary to make biofuels a commodity.

This report aims to present the results of performance evaluation of laboratories which participated in the interlaboratory comparison in anhydrous bioethanol for a project named Standards for the Ethanol of Africa and Latin America-PEAAL.

## **2. Objectives**

This Interlaboratory Comparison (IC) has the aim to assist the National Metrology Institutes (NMIs) and independent quality laboratories indicated and invited by the NMIs with technical qualification to participate in the Project “Standards for the Ethanol of Africa and Latin America” (PEAAL, in Portuguese), which are involved with the quality control of biofuels, mainly of anhydrous bioethanol.

Some parameters which were evaluated in this IC define the quality of ethanol used as a fuel with international acceptance. It is extremely important to carry out the determination of these parameters with confidence and traceability. Therefore, the IC was carried out with samples of ethanol fuel with certified values, allowing a more confident evaluation of the laboratories' performance regarding the determination of quality parameters for the ethanol.

## **3. Preparation of the Comparison Item**

The Chemical Metrology Division and the Mechanical Metrology Division from Inmetro were responsible for the preparation, bottling, characterization, homogeneity and stability tests of the comparison item (based on ISO GUIDE 35).

The comparison item was made up from samples of anhydrous bioethanol. The samples of bioethanol from sugar cane (approximately 99,6 % ethanol content) were provided by a Brazilian producer in a container of 200 L. The bioethanol was bottled in about 2500 amber glass ampoules of 20 mL which had been evacuated with argon and flame-sealed. The number of bioethanol samples needed for the determination of each parameter was sent by Inmetro to the participating laboratories.

## **4. Characterization, Homogeneity and Stability Tests of the Comparison Item**

### **4.1. Characterization**

As previously agreed, the material supplied was also measured for stability purposes.

In all parameters selected for the characterization of bioethanol, the results were available and they were consistent within their respective uncertainties.

#### **4.1.1. Acid number**

Five bottles were analyzed. Each bottle was prepared from eleven ampoules of 20 mL of bioethanol. It was needed 60 mL for each analysis. The acid number determination was carried out in triplicate by automatized volumetric titration with potentiometric end-point detection by means of a combined pH glass electrode with KCl internal filling solution previously checked by Inmetro CRM of pH 6,86 (MRC 03.2/10.0005) and pH 4,00 (MRC 03.1/09.0002). The bioethanol samples were titrated with NaOH 0,02 mol L<sup>-1</sup> solution standardized with HCl 0,01 mol L<sup>-1</sup> (MRC 08.2/10.0003). The result was expressed as quantity of acetic acid, in milligrams of acetic acid per liter of bioethanol.

#### **4.1.2. Copper content**

The result of characterization was based on the mean of results obtained in the homogeneity study. Each of the fifteen ampoules were analyzed in quintuplicate. The copper content was determined using an ICP OES coupled to ultrasonic nebulizer and membrane desolvator. The methodology was optimized and the analytical performance characteristics evaluated. Calibration curves were prepared in anhydrous ethanol using the SRM 3114 from NIST. The performance of the methodology was evaluated by recovery test using the CRM from INMETRO MRC 05.1/08.0001. The sample were diluted gravimetrically in anhydrous ethanol (1:10) and analyzed directly by the system.

#### **4.1.3. Electrolytic conductivity**

Each of the six bottles were analyzed in duplicate. Each bottle was prepared from five ampoules of 20 mL of bioethanol. It was needed 40 mL for each analysis. Electrolytic conductivity was determined by means of one conductivity meter coupled with a conductivity cell with constant value of  $0,090 \text{ cm}^{-1}$  (previously checked with Inmetro CRM of  $5 \mu\text{S cm}^{-1}$  (MRC 04.5/10.0002)), a magnetic stirrer and a resistance thermometer, Pt 100. All the measurements were carried out in duplicate at  $25 \text{ }^\circ\text{C}$ .

#### **4.1.4. Density**

Six ampoules of 20 mL of bioethanol were analyzed. The determination of density was carried out in two replicates by the use of a digital density meter, traceable to the hydrostatic weight system. All the measurements were done at  $20,0 \text{ }^\circ\text{C}$ . In each measurement, the internal cell of densimeter was washed with pure alcohol. The results are expressed as density, in  $\text{g mL}^{-1}$ .

#### **4.1.5. Sulphate content**

Each of the three ampoules were analyzed in quintuplicate. Sulphate content was determined using a bidimensional ion-exchange chromatography system with pre-concentration. All solutions were diluted gravimetrically using high purity water (type I) and anhydrous ethanol (9:1 w/w) at  $20 \text{ }^\circ\text{C}$ . In order to establish the traceability to the International System (SI) SRM 3181 from NIST was used.

#### **4.1.6. Water content**

The result of characterization was based on the mean of results obtained in the homogeneity study. Ten ampoules were analyzed. The water content was determined by coulometric Karl Fischer titration with Hydranal coulomat AG as the reagent, an electrode current generator of 400 mA and a platinum indicator electrode. The range of applied voltage was 50 to 70 mV. The titration was initiated after stabilization of equipment with a drift of  $20 \mu\text{g/min}$  at 120s and it finished with the relative value of  $\pm 5 \mu\text{g/min}$  compared with the initial drift. The titration curve was

performed with a measurement point every 2 s analysis. The samples were transferred to containers. Aliquots were injected into the equipment and the measurements were made in triplicate.

#### **4.1.7. Ethanol content**

The result of characterization was based on the mean of results obtained in the homogeneity study. Each of the ten ampoules were analyzed in duplicate. The determination of ethanol content was performed by using gas chromatography with flame ionization detector (GC-FID) by the on-column injection technique and propanol was used as internal standard. Samples of bio ethanol were weighed in glass vials of 5 mL and transferred to containers containing 250 g of water. The solutions were homogenized for 12 h. An aliquot of each solution was diluted 1:1 with internal standard solution in a glass container. The ethanol content was determined by the equation of the calibration curve obtained by the method of least squares, applying the linear model.

### **4.2. Uncertainty of the Characterization**

The uncertainty budget was estimated according to ISO GUM.

#### **4.2.1. Acid number**

The uncertainty of the measurement for characterization ( $u_{\text{char}}$ ) of the acid number was estimated considering the following components: NaOH standard solution, repeatability, titrator, automatic burette (NaOH), automatic burette (sample), molar mass (acetic acid) and end-point detection.

The value of the characterization for acid number parameter is 45,2 mg L<sup>-1</sup>. The contribution of uncertainty due to characterization is 1,0 mg L<sup>-1</sup>.

#### **4.2.2. Copper content**

The value of the characterization of copper is 1,99 µg kg<sup>-1</sup> and its combined standard uncertainty was assumed as the uncertainty of the homogeneity study (0,165 µg kg<sup>-1</sup>).

#### **4.2.3. Density**

The uncertainty of the measurement for the characterization of the density was estimated considering the: repeatability and digital density meter certificate sources.

The value of the characterization for the density parameter is 0,79058 g mL<sup>-1</sup>. The contribution of uncertainty due to characterization is 0,00004 g mL<sup>-1</sup>.

#### **4.2.4. Electrolytic conductivity**

The uncertainty of the measurement for the characterization of the electrolytic conductivity was estimated considering the sources: CRM, repeatability, conductometer, temperature, cell constant and CO<sub>2</sub> concentration.

The value of the characterization for the electrolytic conductivity parameter is  $0,54 \mu\text{S cm}^{-1}$ . The contribution of uncertainty due to characterization is  $0,029 \mu\text{S cm}^{-1}$ .

#### **4.2.5. Sulphate content**

The following sources of uncertainty were considered: standard dilution, repeatability, sample dilution and calibration curve.

The value of the characterization for sulphate content is  $0,313 \text{ mg kg}^{-1}$ . The contribution of uncertainty due to characterization is  $0,006 \text{ mg kg}^{-1}$ .

#### **4.2.6. Water content**

The uncertainty of the measurement for characterization ( $u_{\text{char}}$ ) of the water content was estimated considering the balance resolution and the repeatability of analysis.

The value of the characterization for water content parameter is  $0,378 \%$  (in mass). The contribution of uncertainty due to characterization is  $0,0011 \%$  (in mass).

#### **4.2.7. Ethanol content**

The uncertainty of the measurement for characterization ( $u_{\text{char}}$ ) of the ethanol content was estimated considering the following components: calibration curve, repeatability of analysis, mass of internal standard solution, mass of sample solution, repeatability of area ratios and purity of ethanol used as calibrant.

The value of the characterization for ethanol content parameter is  $99,56 \%$  (in mass). The contribution of uncertainty due to characterization is  $0,20 \%$  (in mass).

### **4.3. Homogeneity**

After bottling, the homogeneity study of the batch of samples was carried out with the objective to determine the within and between-bottle variation. The samples used in this study were chosen randomly from the batch produced. The measurements were performed under repeatability conditions. The homogeneity study was performed for all parameters. The analyses of homogeneity study were performed at Inmetro.

The data evaluation of the between-bottle homogeneity study is based on the approach described in ISO Guide 35. First, the data was placed in order of measurement and visually inspected for irregularities. Then, by visual inspection, a check on possible drift was performed.

The data were then grouped by bottle (ampoule). ISO Guide 35 uses the concept "bottle" to represent one item of the batch. This concept is used here throughout the text. At the level of the bottles, the data were scrutinised for outliers by means of Grubbs' outlier test.



The homogeneity was assessed using a *one-way* analysis of variance. From mean squares, the between-bottle standard deviation can be computed as follows. The between-bottle standard deviation was computed using Equation 1.

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

where  $MS_{among}$  denotes the mean squares between bottles,  $MS_{within}$  within the bottles and  $n$  denotes the number of replicate measurements on a single bottle. In this evaluation, this approach is used if the repeatability of the measurement results is regarded as satisfactory.

In those cases where the repeatability of the test method is the limiting factor, the inhomogeneity 'hidden' by the repeatability can be converted into an estimate of the between-bottle standard deviation from Equation 2.

$$u_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt{\frac{2}{v_{MS_{within}}}} \quad (2)$$

where  $v_{MS_{within}}$  denotes the number of degrees-of-freedom of  $MS_{within}$ .

#### 4.4. Uncertainty of the Homogeneity

The between-bottle standard deviation ( $s_{bb}$ ) was considered as the uncertainty of the homogeneity ( $u_{bb}$ ) for each analyzed parameter. The uncertainty inherent to the homogeneity of the samples according to the parameter and its contribution to the reference value are presented in the Table 1.

According to the 8<sup>th</sup> edition of The International System of Units (SI) published by BIPM, it is permitted to use a point or a comma as a decimal marker. The chosen should be that which is customary in the context concerned. Therefore, in this report, a comma was used as a decimal maker since all laboratories have reported their results using a comma as a decimal maker.

Table 1. Results of the homogeneity study

Parameter	$u_{bb}$	$U_{bb, rel} (\%)$
Acid number	0,10 mg L <sup>-1</sup>	0,22
Copper	0,165 µg kg <sup>-1</sup>	8,29
Density	0,000008 g mL <sup>-1</sup>	0,001
Electrolytic conductivity	0,013 µS cm <sup>-1</sup>	2,4
Sulphate	0,0070 mg kg <sup>-1</sup>	2,2
Water content	0,0060 % (in mass)	1,6
Ethanol content	0,191 % (in mass)	0,19

For all parameters, the homogeneity uncertainty was judged to be acceptable for this intercomparison.

#### **4.5. Stability**

Stabilities studies are conducted to establish both dispatch conditions (short-term stability) as well as storage conditions (long-term stability). Short-term stability studies were performed during four weeks. Two ampoules were kept at 50 °C for 1, 2, 3 and 4 weeks. Another two samples were placed at the reference temperature of 4 °C. At the end, the samples were analyzed under repeatability conditions. From the results of this study, Inmetro ensures that the samples are stable for all measured parameters for 4 weeks at 50 °C and 4 °C.

A long-term stability study aims at determining the behavior of the material under storage conditions. All parameters of interest were assessed for their stability. This study was carried out using the classical design and evaluated by the protocol based on ISO Guide 35. In this design, the measurements were taken under within-laboratory reproducibility conditions. The long-term stability study of test material was evaluated using regression analysis. The material was found to be stable under the conditions of this long-term stability study for all parameters at the reference temperature of 20 °C. All measurements in the long-term stability study were performed at Inmetro.

#### **4.6. Uncertainty of the Stability**

The uncertainty of the stability was estimated using regression analysis from the data of stability study (long-term stability). The observed slopes were tested for significance using a *t*-test ( $\alpha = 0,05$ , 95% confidence interval). It was considered one straight line ( $Y=b_1 X + b_0$ ) obtained by the measurements of the analyzed parameter (*y*-) axis versus the time (*x*-) axis. The uncertainty of the stability ( $u_{lts}$ ) was calculated by the Equation 3. This uncertainty was obtained from the product of the uncertainty of the slope of the straight line by the time of study that was shown in Table 2.

$$u_{lts} = s(b_1) \cdot t \quad (3)$$

Where:

$s(b_1)$  = uncertainty of the slope

$t$  = time of study.

The uncertainty due to the stability of the samples and the stability time for each parameter (time of study) are presented in Table 2.

Table 2. Results of the stability study

Parameter	$u_{\text{Its}}$	$t$ (days)
Acid number	0,37 mg L <sup>-1</sup>	105
Density	0,000009 g mL <sup>-1</sup>	190
Electrolytic conductivity	0,064 μS cm <sup>-1</sup>	189
Sulphate	0,0271 mg kg <sup>-1</sup>	189
Water content	0,0017 % (in mass)	714
Ethanol content	0,139 % (in mass)	623

The slope was not significant for all parameters at the 95 % level of confidence.

For copper content, due to the low concentration, it was observed that the reproducibility of measurements has affected the short and long-term stability studies. The final result obtained from these studies not reflected the instability of the material, but the influence of the method. Therefore, the contribution of uncertainty due to stability was not taken into account.

In conclusion, the material was suited to be used in the IC for all parameters.

#### 4.7. Estimate of the Uncertainty of the Comparison Item

The uncertainty estimated for the comparison item was obtained from the square root of the square sum of the uncertainties from characterization ( $u_{\text{char}}$ ), homogeneity ( $u_{\text{bb}}$ ) and long-term stability ( $u_{\text{Its}}$ ) studies according to the Equation 4.

$$u = \sqrt{u_{\text{char}}^2 + u_{\text{bb}}^2 + u_{\text{Its}}^2} \quad (4)$$

Where:

$u_{\text{char}}$  = standard uncertainty due to characterization

$u_{\text{bb}}$  = standard uncertainty due to between-bottle (in)homogeneity

$u_{\text{Its}}$  = standard uncertainty due to long-term stability

The combined standard uncertainty estimated for sulphate content includes also the uncertainty due to short-term stability study.

The expanded uncertainty (U) is given by the Equation 5, where  $k=2$  was chosen as coverage factor to provide a confidence level of approximately 95%.

$$U = k \cdot u \quad (5)$$

The references values assigned by Inmetro for this IC are summarized in Table 3.

Table 3. Reference values and expanded uncertainty.

Parameter	Reference Value	U (k=2)	Unit
Acid number	45,2	2,1	mg L <sup>-1</sup>
Copper	1,99	0,33	µg kg <sup>-1</sup>
Density	0,79058	0,00008	g mL <sup>-1</sup>
Electrolytic conductivity	0,54	0,15	µS cm <sup>-1</sup>
Sulphate	0,313	0,065	mg kg <sup>-1</sup>
Water content	0,378	0,013	% (in mass)
Ethanol content	99,56	0,62	% (in mass)

#### 4.8 Metrological Traceability

The laboratories responsible for the measurements have used only validated methods. Certified reference materials traceable to the SI were used to calibrate the equipments.

The realization of the above-mentioned conditions demonstrates that the reference values are traceable to the International System of Units (SI).

## 5. Statistical Analysis

### 5.1. z-score

The evaluation of the performance of the participant laboratories was done in accordance with ABNT ISO/IEC Guide 43-1: 1999. The z-score (zeta score) was used as the statistical test. It represents a measurement of the relative distance of the laboratory in relation to the reference value. The variability (s) was considered as the uncertainty of the CRM. The z-score for this interlaboratory comparison was calculated as the Equation 6:

$$z_i = \frac{y_i - y_{ref}}{s} \quad (6)$$

Where:

$y_{ref}$  is the reference value

$y_i$  is the average result of a specific laboratory i

s is the uncertainty of the CRM.

The value of zeta scores (for simplicity, "z" only) for each participant is reported and the performance of the laboratory is classified as satisfactory, unsatisfactory or questionable. The interpretation of zeta scores is presented as follows:

$|z| \leq 2$  - Satisfactory result;

$2 < |z| < 3$  - Questionable result;

$|z| \geq 3$  - Unsatisfactory result.

The z-score is shown in a graphical mode in order to present the performance of the number of the participating laboratories. The red line represents the unsatisfactory results ( $|z| \geq 3$ ); the range from the blue and red lines represents the questionable results ( $2 < |z| < 3$ ). The values between the two blue lines represent the satisfactory results ( $|z| \leq 2$ ).

## 6. Results and Evaluation of the Performance

### 6.1. Results of the Laboratories

The results presented in the Tables for each parameter are in accordance with the measured values that each laboratory have sent to Inmetro. The mean and standard deviation are presented with the number of significant figures in accordance with the reference value for each parameter. The results of z-score were reported only with one decimal number.

**In this Report each participant laboratory is only identified by its identification code in Tables and Figures.**

In Figures below, the reference value (Ref) is given as a black solid line, the limits denoted by the gray lines are the reference values  $\pm$  expanded uncertainty ( $\text{Ref} \pm U$ ), the limits denoted by the blue lines are  $\text{Ref} \pm 2U$  (reference value plus/minus 2 times the expanded uncertainty) and the limits denoted by the red lines are  $\text{Ref} \pm 3U$  (reference value plus/minus 3 times the expanded uncertainty). The uncertainty bars of these values denote the standard deviation from three measurements.

The results for Acid Number are given in Figure 1. Eight laboratories reported Acid Number results. Two laboratories reported results below 45,2 mg/L. The laboratories 39 and 67 were outside of the scale, because of that they can not be seen on the plot.

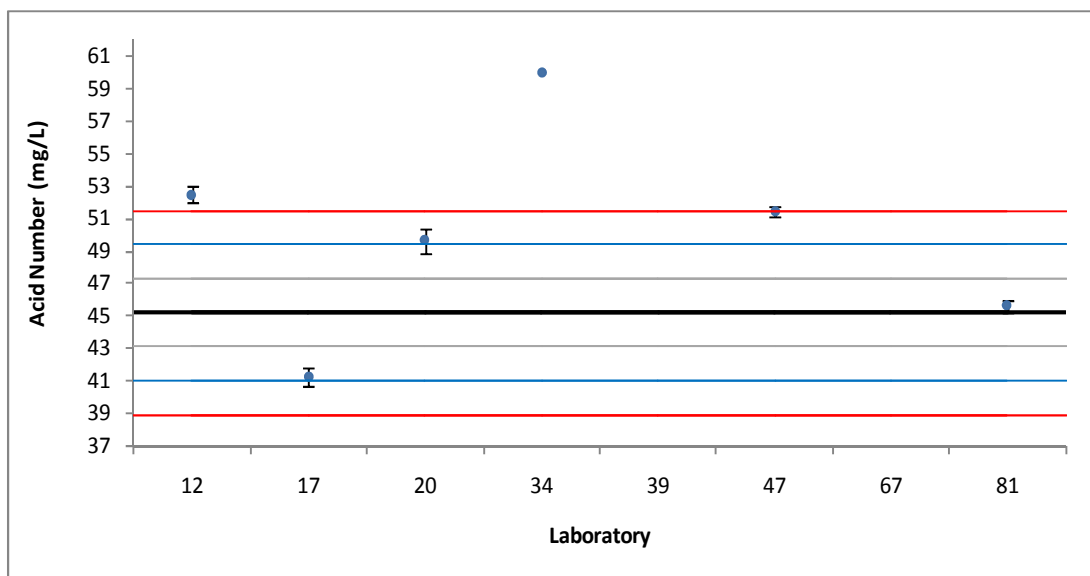


Figure 1. Results for acid number.

For the Copper, only two of the ten laboratories reported results, of which one reported non-numeric results ( $< 0,01$ ), of which the the value is below the limit of quantification/detection and the laboratory performance could not be evaluated.

The results for Electrolytic Conductivity are shown in Figure 2. Seven laboratories reported Electrolytic Conductivity results, of which only one laboratory reported results below  $0,54 \mu\text{S}/\text{cm}$ . The laboratory 67 reported the results higher than the reference value, therefore its result can not be seen on the plot.

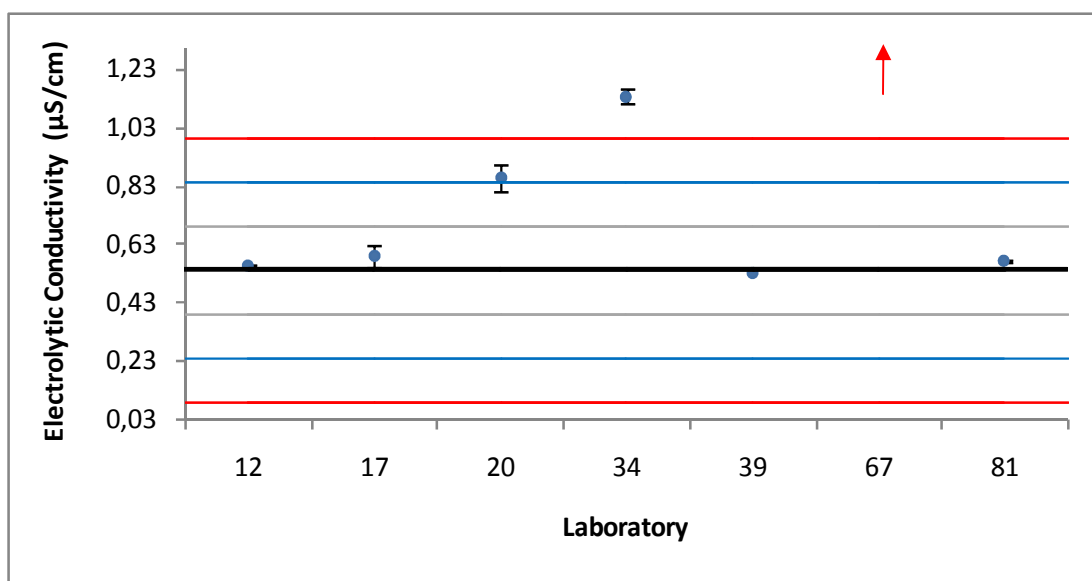


Figure 2. Results for Electrolytic Conductivity.

The results for Density are given in Figure 3. Nine laboratories reported Density results, of which two laboratories reported results below  $0,79058 \text{ g}/\text{cm}^3$ . The laboratory 34 reported a result that is quite high. This result presented the higher standard deviation.

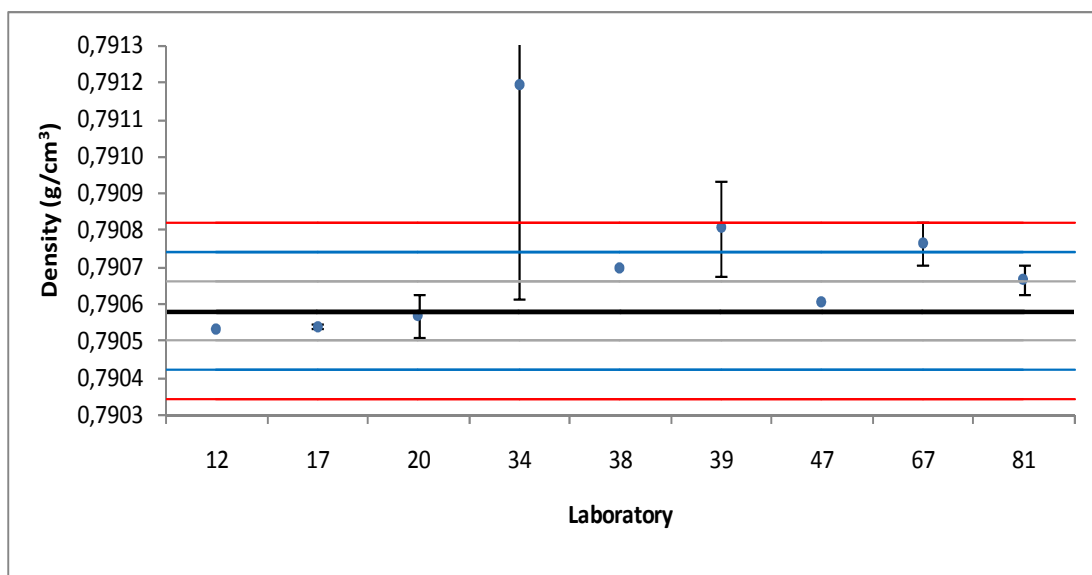


Figure 3. Results for Density.

For the Sulphate parameter, only one of the ten laboratories reported results. The comparability with the reference value (0,313 mg/kg) was good.

The results for Water Content are given in Figure 4. Seven laboratories reported Water content results. All of them reported results below 0,378 % (in mass). The laboratory 38 presented results that are quite different among the participants.

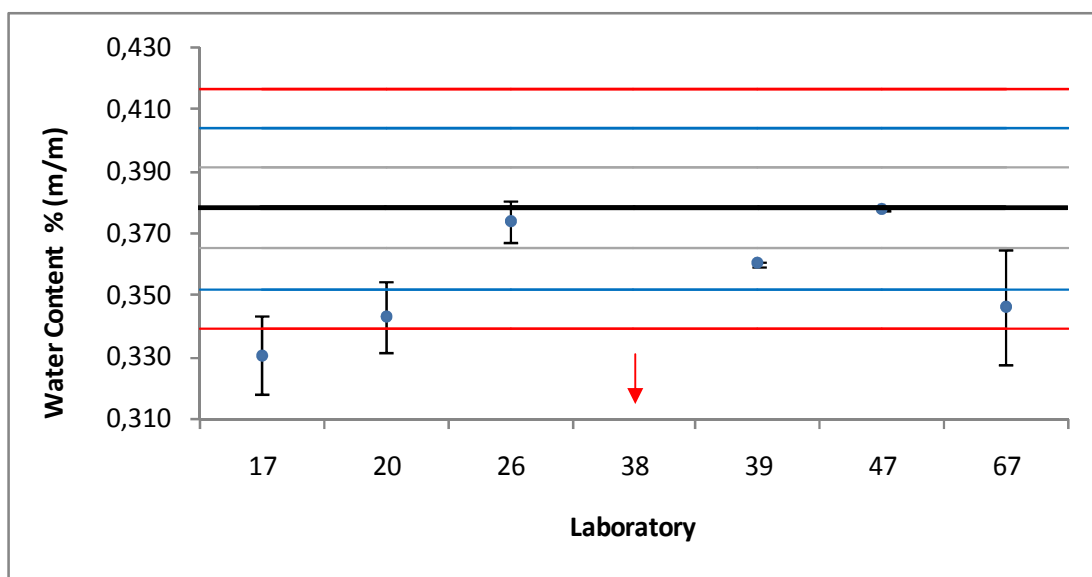


Figure 4. Results for Water Content.

The results for Ethanol Content are given in Figure 5. Eight laboratories reported Ethanol Content results. Three laboratories reported results below 99,56 % (in mass). There was a good comparability for them.

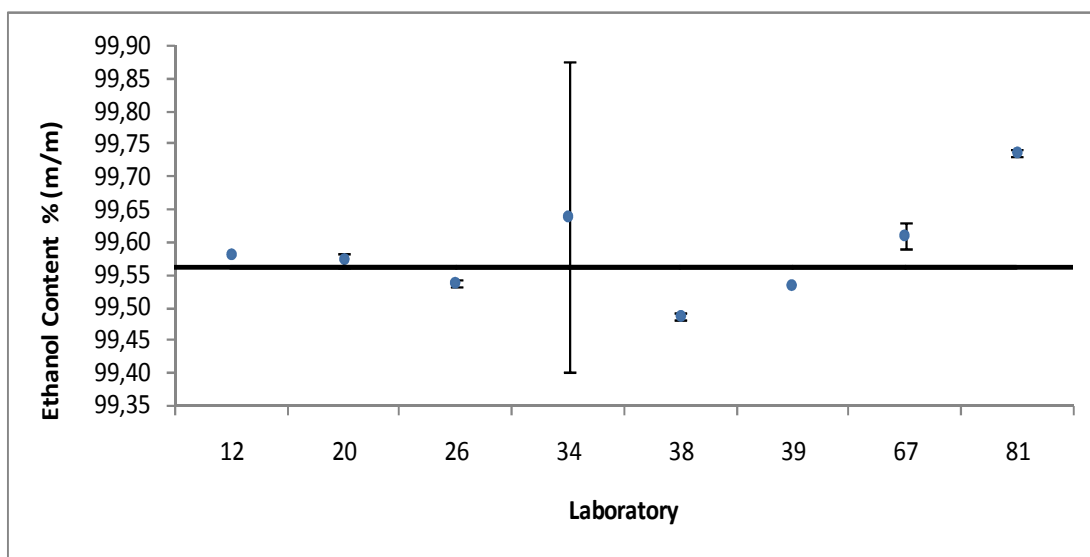


Figure 5. Results for Ethanol Content.

### 6.1.1. Acid Number

The reported results from each participant laboratory, as well as the mean and standard deviation are presented in Table 4.

Table 4. Results of the laboratories for the acid number parameter.

Laboratory	Measurement	Result (mg L <sup>-1</sup> )	Mean	Standard Deviation
12	1	52,2	52,5	0,5
	2	52,2		
	3	53,1		
17	1	40,5379	41,2	0,6
	2	41,5267		
	3	41,5267		
20	1	49,2	49,6	0,8
	2	50,5		
	3	49,2		
34	1	60	60,0	0,0
	2	60		
	3	60		
39	1	0,9068	0,9	0,005
	2	0,9017		
	3	0,9108		
47	1	51,06	51,5	0,4
	2	51,67		
	3	51,67		
67	1	117,9	117,4	3,9
	2	121		
	3	113,2		
81	1	45,513	45,6	0,4
	2	45,31		
	3	46,00		



Table 5 presents the results of z-score for acid number calculated from the results reported by the laboratories.

Table 5. Values of z-score for the acid number parameter

Laboratory	z-score
12	3,5 <sup>a</sup>
17	-1,9
20	2,1 <sup>b</sup>
34	7,0 <sup>a</sup>
39	-21,1 <sup>a</sup>
47	3,0 <sup>a</sup>
67	34,4 <sup>a</sup>
81	0,2

<sup>a</sup> Unsatisfactory result

<sup>b</sup> Questionable result

In Figure 6, the result of z-score for acid number calculated for each laboratory is presented graphically.

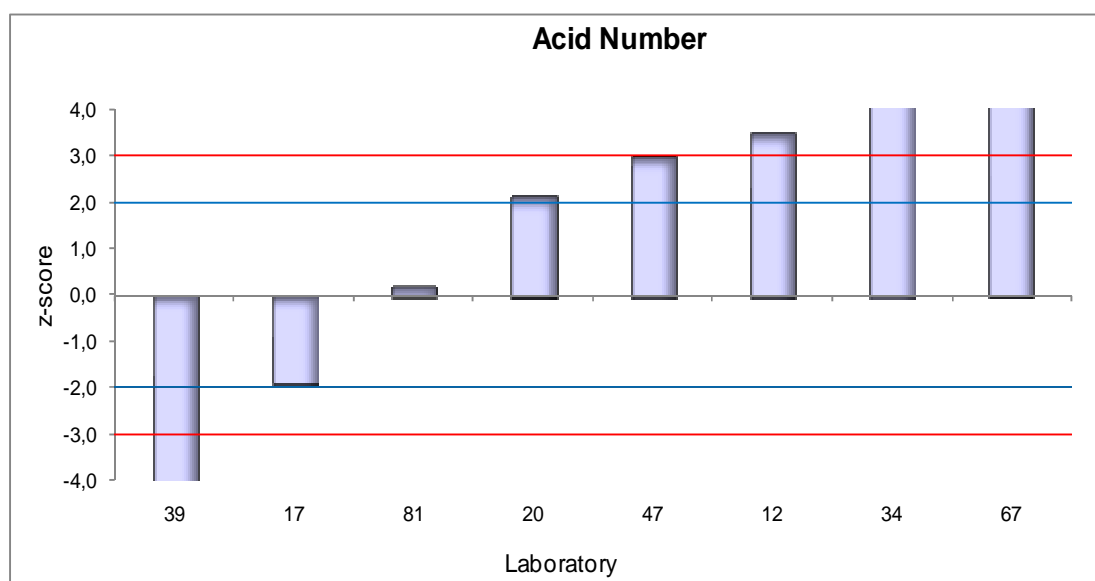


Figure 6. Graph of z-score on the measurement of acid number.

### 6.1.2. Copper

The reported results from each participant laboratory, as well as the mean and standard deviation are presented in Table 6.

Table 6. Results of the laboratories for the copper parameter.

Laboratory	Measurement	Result ( $\mu\text{g kg}^{-1}$ )	Mean	Standard Deviation
17	1	< 0,01	-	-
	2	< 0,01		
	3	< 0,01		
20	1	7	5,33	2,08
	2	6		
	3	3		

In Table 7 are presented the results of z-score for copper calculated from the results reported by the laboratories. The Participant 17 reported its results as lower than 0,01, therefore was not possible to assess its performance.

Table 7. Values of z-score for the copper parameter

Laboratory	z-score
20	10,1 <sup>a</sup>

<sup>a</sup> Unsatisfactory result

### 6.1.3 Electrolytic Conductivity

The reported results of each participant laboratory, as well as the mean and standard deviation are presented in the Table 8.

Table 8. Results of the laboratories for the electrolytic conductivity parameter.

Laboratory	Measurement	Result ( $\mu\text{S cm}^{-1}$ )	Mean	Standard Deviation
12	1	0,552	0,55	0,002
	2	0,555		
	3	0,555		
17	1	0,556	0,59	0,04
	2	0,580		
	3	0,629		
20	1	0,91	0,86	0,05
	2	0,85		
	3	0,82		
34	1	1,160	1,14	0,03
	2	1,110		
	3	1,140		
39	1	0,530	0,53	0,00
	2	0,530		
	3	-		
67	1	111	87,10	20,84
	2	72,7		
	3	77,6		
81	1	0,57	0,57	0,01
	2	0,57		
	3	0,58		

In Table 9 are presented the results of z-score calculated from electrolytic conductivity results reported by the laboratories.

Table 9. Values of z-score for the electrolytic conductivity parameter

Laboratory	z-score
12	0,1
17	0,3
20	2,1 <sup>b</sup>
34	4,0 <sup>a</sup>
39	-0,1
67	577,1 <sup>a</sup>
81	0,2

<sup>a</sup> Unsatisfactory result

<sup>b</sup> Questionable result

In Figure 7, the result of z-score for electrolytic conductivity calculated for each laboratory is presented graphically.

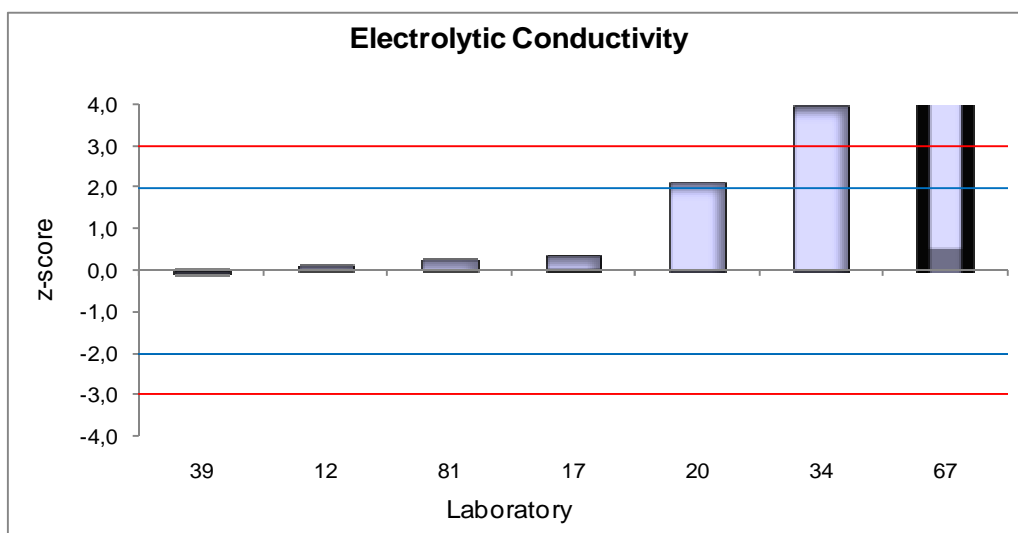


Figure 7. Graph of z-score on the measurement of electrolytic conductivity.

#### 6.1.4 Density

The reported results of each participant laboratory, as well as the mean and standard deviation are presented in Table 10.

Table 10. Results of the laboratories for the density parameter.

Laboratory	Measurement	Result (g mL <sup>-1</sup> )	Mean	Standard Deviation	Uncertainty (k=2)
12	1	0,79053	0,79053	0,00000	0,00002
	2	0,79053			
	3	0,79053			
17	1	0,790548	0,790540	0,00007	-
	2	0,790537			
	3	0,790536			
20	1	0,7906	0,7906	0,0001	-
	2	0,7905-			
	3	0,7906			
34	1	0,79101	0,79120	0,00058	-
	2	0,79185			
	3	0,79073			
38	1	0,7907	0,7907	0,0000	0,001
	2	0,7907			
	3	0,7907			
39	1	0,7907709	0,7908054	0,0001285	0,000451
	2	0,7909476			
	3	0,7906976			
47	1	0,790604	0,790603	0,000001	0,000017
	2	0,790602			
	3	-			
67	1	0,7908	0,7908	0,0001	-
	2	0,7908			
	3	0,7907			
81	1	0,79070	0,79067	0,00004	0,00007572
	2	0,79068			
	3	0,79062			

In Table 11 are presented the results of z-score for density calculated from the results reported by the laboratories.

Table 11. Values of z-score for the density parameter

Laboratory	z-score
12	0,6
17	0,5
20	0,2
34	7,7 <sup>a</sup>
38	1,5
39	2,8 <sup>b</sup>
47	0,3
67	2,3 <sup>b</sup>
81	1,1

<sup>a</sup> Unsatisfactory result

<sup>b</sup> Questionable result

In Figure 8, the result of z-score for density calculated for each laboratory is presented graphically.

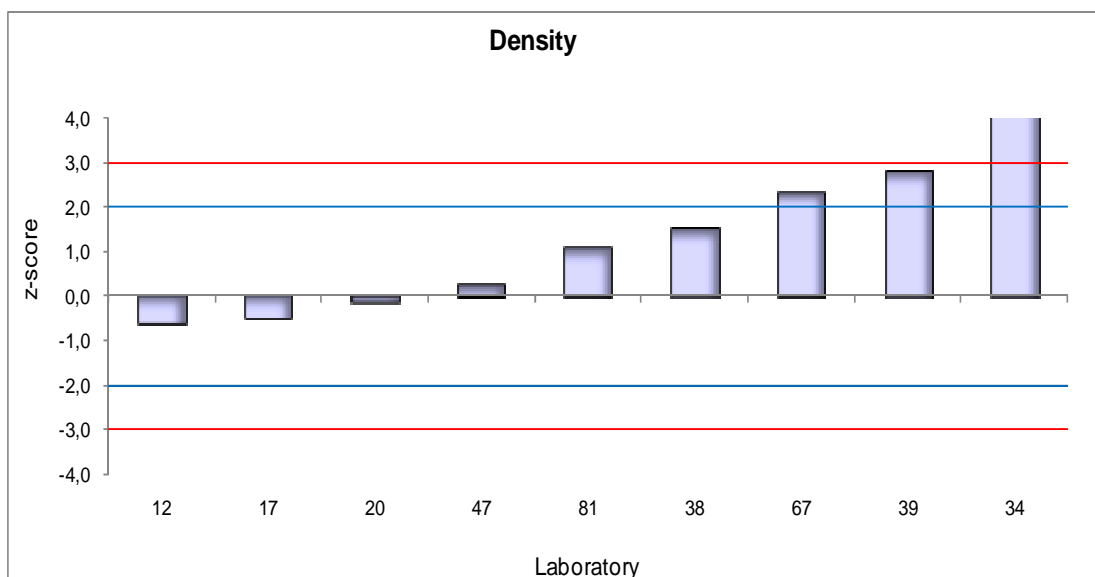


Figure 8. Graph of z-score on the measurement of density.

### 6.1.5 Sulphate

The reported results of each participant laboratory, as well as the mean and standard deviation are presented in Table 12.

Table 12. Results of the laboratories for the sulphate parameter.

Laboratory	Measurement	Result (mg kg <sup>-1</sup> )	Mean	Standard Deviation
17	1	0,262	0,265	0,007
	2	0,273		
	3	0,260		

In Table 13 are presented the results of z-score for sulphate calculated from the results reported by the laboratories.

Table 13. Values of z-score for the sulphate parameter

Laboratory	z-score
17	-0,7

### 6.1.6 Water Content

The reported results of each participant laboratory, as well as the mean and standard deviation are presented in Table 14.

Table 14. Results of the laboratories for the water content parameter.

Laboratory	Measurement	Result % (in mass)	Mean	Standard Deviation
17	1	0,325	0,331	0,013
	2	0,322		
	3	0,345		
20	1	0,33	0,343	0,012
	2	0,35		
	3	0,35		
26	1	0,3806	0,374	0,007
	2	0,3666		
	3	0,3748		
38	1	0,07321	0,073	0,0004
	2	0,07381		
	3	0,07303		
39	1	0,36100	0,360	0,001
	2	0,35954		
	3	0,36025		
47	1	0,3783	0,378	0,0003
	2	0,3777		
	3	0,3781		
67	1	0,347	0,346	0,019
	2	0,364		
	3	0,327		
81	1	0,375	0,377	0,002
	2	0,378		
	3	0,377		

In Table 15 are presented the results of z-score for water content calculated from the results reported by the laboratories.

Table 15. Values of z-score for the water content parameter

Laboratory	z-score
17	-3,6 <sup>a</sup>
20	-2,7 <sup>b</sup>
26	-0,3
38	-23,4 <sup>a</sup>
39	-1,4
47	0,0
67	-2,5 <sup>b</sup>
81	-0,1

<sup>a</sup> Unsatisfactory result

<sup>b</sup> Questionable result

In Figure 9, the result of z-score for water content calculated for each laboratory is presented graphically.

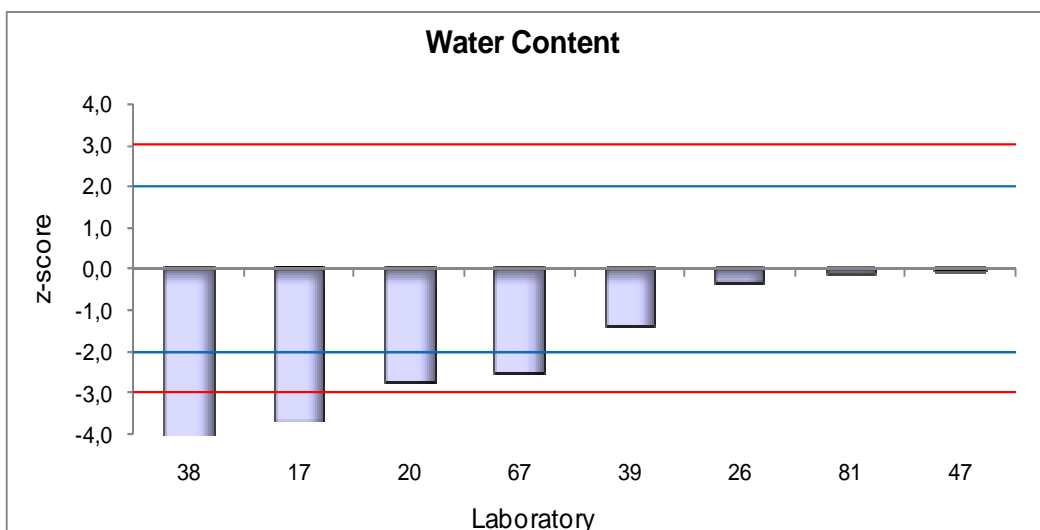


Figure 9. Graph of z-score on the measurement of water content.

### 6.1.7 Ethanol Content

The reported results of each participant laboratory, as well as the mean and standard deviation are presented in Table 16.

Table 16. Results of the laboratories for the ethanol content parameter.

Laboratory	Measurement	Result % (in mass)	Mean	Standard Deviation
12	1	99,58	99,58	0,00
	2	99,58		
	3	99,58		
20	1	99,58	99,57	0,01
	2	99,58		
	3	99,56		
26	1	99,53	99,54	0,01
	2	99,54		
	3	99,54		
34	1	99,46	99,64	0,24
	2	99,91		
	3	99,55		
38	1	99,49	99,49	0,01
	2	99,48		
	3	99,49		
39	1	99,534	99,53	0,001
	2	99,533		
	3	99,533		
67	1	99,61	99,61	0,02
	2	99,59		
	3	99,63		
81	1	99,73	99,74	0,01
	2	99,74		
	3	99,74		

In Table 17 are presented the results of z-score for ethanol content calculated from the reported results by the laboratories.

Table 17. Values of z-score for the ethanol content parameter.

Laboratory	z-score
12	0,0
20	0,0
26	0,0
34	0,1
38	-0,1
39	0,0
67	0,1
81	0,3

In Figure 10, the result of z-score for ethanol content calculated for each laboratory is presented graphically.

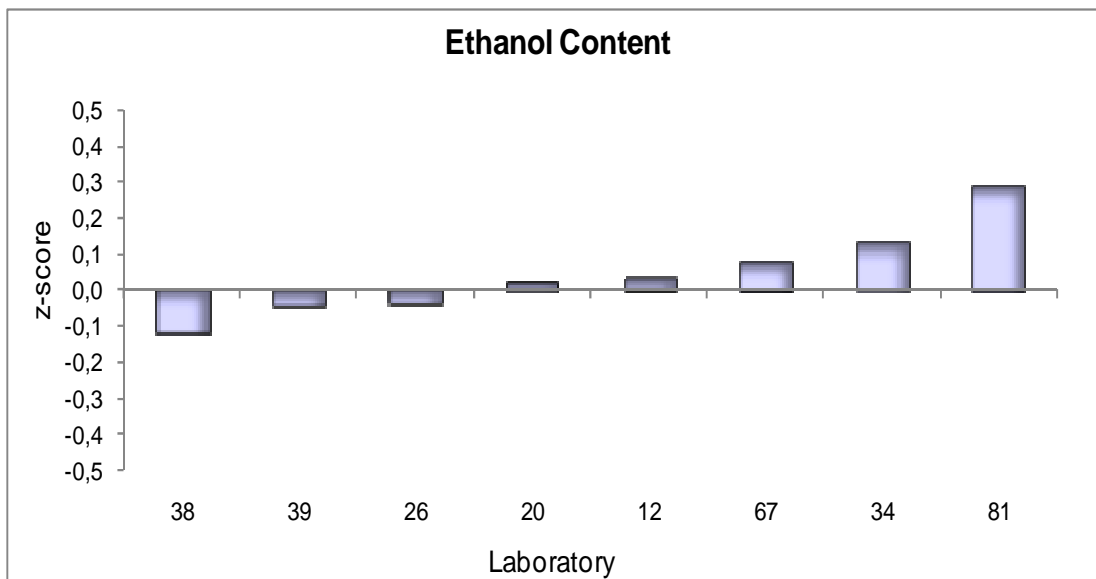


Figure 10. Graph of z-score on the measurement of ethanol content.



## **7. Conclusion**

Ten laboratories from different countries participated in this exercise of Interlaboratory Comparison in order to measure quality parameters in anhydrous bioethanol. However, not all laboratories measured the seven parameters.

Eight laboratories measured the acid number parameter: five laboratories presented unsatisfactory results; one laboratory presented a questionable result, and two laboratories presented satisfactory results. The participant laboratories presented different performance due to the use of different kinds of indicators, such as phenolphthalein (laboratories 20, 39, 47 and 81), bromothymol blue (laboratory 34), alpha-naphtholphthalein (laboratories 17 and 67) and also different standards such as ASTM D1613 or NBR 9866. Moreover, the laboratory 34 did not mention which standard was followed.

Two laboratories measured the copper content: one laboratory presented unsatisfactory result and the other presented the result as below of the limit of detection. The laboratory 17 followed the standard NBR 11331, while the laboratory 20 the NBR 10893. Therefore, the comparability of the results could not be evaluated.

Nine laboratories participated for the density quantity measurement in this intercomparison. However, four of these did not evaluate the uncertainty of measurement. From the five laboratories that evaluated the uncertainty of measurement, two expressed the uncertainty using more than 2 significant figures. This means that some of these laboratories need to acquire the knowledge about evaluation and expression of uncertainty of measurement according to ISO GUM.

The laboratories' performance for density measurement, according to the z-score, shows that six laboratories (12, 17, 20, 38, 47 and 81) reached satisfactory results, and the laboratories 39 and 67 obtained questionable results. The laboratory 34, which had an unsatisfactory result, and the two laboratories with questionable results (39 and 67) must investigate the reasons behind this performance. It may be originated from the measurement process or even from the calibration of the instruments. A recommended practice according to ISO GUIDE 34 is to use a certified reference material to check the accuracy of the instrument.

From the seven participant laboratories in the measurement of electrolytic conductivity parameter, four laboratories presented satisfactory results; two laboratories presented unsatisfactory results and only one laboratory presented questionable result. Two laboratories (34 and 39) did not mention which standard was followed in the measurements, and three laboratories (12, 20 and 81) carried out the measurements by using the ASTM D1125 standard. The laboratories 17 and 67 followed the requirements of the NBR 10547 standard. Most of the participating laboratories used 3 ampoules for each measurement with a total of 9 measurements.

It is worth to mention that the electrolytic conductivity measurement is strongly influenced by the temperature of the measurement. Thus, the use of calibrated thermometer inside the sample is needed to obtain good results, as well as the use of CRM which has its metrological traceability guaranteed for calibrating the conductivity cell.

For the sulphate parameter, only one participant presented result and its performance was satisfactory. The participant did not specify the method or the standard which was followed.

Eight laboratories measured the water content parameter. According to the z-score, four laboratories presented satisfactory results (26, 39, 47 and 81), two laboratories presented unsatisfactory results (17 and 38) and two laboratories presented questionable results (20 and 67). The laboratories which had unsatisfactory and questionable results must investigate the reasons behind this performance. It may be originated from the measurement process. Since these laboratories have experience in measuring lower values of water content, probably there were losses of mass of bioethanol during the weighing processes and injection.

Eight laboratories measured the ethanol content parameter. According to the z-score, all laboratories presented satisfactory results. However, it is important to note that only laboratories 26, 39 and 67 used gas chromatography with flame ionization detector that is a selective technique. The other laboratories used density meter and laboratory 34 used pycnometer. Although the use of alcoholimetric tables is a common practice to determine ethanol content, it is not a selective method to determine this parameter.

## 8. Participant Laboratories

Ten laboratories have registered in the interlaboratory comparison in anhydrous bioethanol quality measurement parameters.

The list of laboratories which submitted the form of results' records to the coordination of this comparison is presented in Table 18. The numeration of the laboratories in the table is only indicates the number of participants in the comparison, it is not associated with the identification of the laboratories in the presentation of the results in this report.

**Table 18 – Participant Laboratories**

Institution	
1.	Agência Nacional do Petróleo, Gás Natural e Biocombustíveis – ANP
2.	Administración Nacional de Combustibles, Alcoholes y Portland – ANCAP
3.	Centro de Pesquisas da Petrobras – CENPES
4.	Centro Nacional de Metrología, México
5.	Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual – INDECOPI
6.	Instituto Boliviano de Metrologia
7.	Kenya Bureau of Standards
8.	Laboratorio Costarricense de Metrología – LACOMET
9.	National Metrology Institute of South Africa – NMISA
10.	The Bureau os Standards Jamaica - BSJ

## 9. References

- [1] Spitzer, P., Fisicaro, P., Seitz S., Champion, R., “pH and conductivity as parameters to characterize bioethanol”, *Accred Qual Assur*, 2009.
- [2] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION – ISO/IEC 17043 - Conformity assessment – General requirement for proficiency testing. Geneva, 2010.
- [3] ISO 5725 (E), “Accuracy (trueness and precision) of measurement methods and results”, 1994.
- [4] NRB ISO GUIDE 43, “Proficiency Testing by Interlaboratory Comparisons – Part 1: Development and Operation of Programs of Proficiency Testing”, 1999.
- [5] Vocabulário Internacional de Metrologia – Conceitos Fundamentais e Termos Associados (VIM 2008), Inmetro, 2009.
- [6] Cortez, L.A.B., Sugarcane Bioethanol R & D for Productivity and Sustainability, São Paulo, Blücher, Fapesp, 2010.
- [7] BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML (2008) “Evaluation of measurement data — Guide to the expression of uncertainty in measurement”, first edition, GUM:1995 with minor corrections.
- [8] Eurachem, CITAC, “Quantifying uncertainty in analytical measurement”, 2<sup>nd</sup> edition, 2000, <http://www.eurachem.ul.pt/>.
- [9] International Organization for Standardization, “ISO Guide 35 – Reference Materials – General and statistical principles for certification”, 3<sup>rd</sup> edition, ISO Geneva, 2006.
- [10] International Organization for Standardization, “ISO 5725-2:1994 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method”, *Statistical methods for quality control*, Vol. 2 (1994), pp 30-74.
- [11] Van der Veen A.M.H., Pauwels J., “Uncertainty calculations in the certification of reference materials. 1. Principles of analysis of variance”, *Accreditation and Quality Assurance* **5** (2000), pp. 464-469.
- [12] Van der Veen A.M.H., Linsinger T.P.J, Pauwels J., “Uncertainty calculations in the certification of reference materials. 2. Homogeneity study”, *Accreditation and Quality Assurance* **6** (2001), pp. 26-30.
- [13] Linsinger T.P.J., Pauwels J., Van der Veen A.M.H., Schimmel H., Lamberty A., “Homogeneity and Stability of Reference Materials”, *Accreditation and Quality Assurance* **6** (2001), pp. 20-25
- [14] ABNT NBR 10547, “Álcool etílico – Determinação da condutividade elétrica”, 2006, <http://www.abnt.org.br>.
- [15] ASTM D 1125, “Standard test methods for electrical conductivity and resistivity of water”, 2009.

- [16] NBR 9866, "Álcool Etílico – Verificação da Alcalinidade e Determinação da Acidez Total", Rio de Janeiro, ABNT, 1987.
- [17] ASTM D 1613, "Standard test method for acidity in volatile solvents and chemical intermediates used in paint, varnish, lacquer, and related products", 2006.



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