

## New methodology for the analysis of volatile organic compounds (VOCs) in bioethanol by GC-MS

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**Resumo:** O presente estudo propõe o desenvolvimento de uma nova metodologia analítica para determinação de 11 compostos presentes no etanol, através da técnica de cromatografia gasosa acoplada à espectrometria de massa (CG-EM) e coluna cromatográfica de média polaridade, composta de 6% cianopropil-fenil e 94% dimetil polisiloxano. Os parâmetros de validação foram determinados de acordo com a NBR ISO 17025:2005. As taxas de recuperação dos compostos estudados foram de 100,4% a 114,7%. Os limites de quantificação calculados estão entre 2,4 mg.kg<sup>-1</sup> e 5,8 mg.kg<sup>-1</sup>. Para a estimativa da incerteza de medição foi adotado o guia para a expressão de incerteza de medição.

**Palavras-chave:** Bioetanol; Cromatografia Gasosa; Validação de Metodologia.

**Abstract:** The present study presents a new analytical methodology for the determination of 11 compounds present in ethanol samples through the gas chromatography coupled to mass spectrometry (GC-MS) technique using a medium polarity chromatography column composed of 6% cyanopropyl-phenyl And 94% dimethyl polysiloxane. The validation parameters were determined according to NBR ISO 17025:2005. The recovery rates of the studied compounds were 100.4% to 114.7%. The limits of quantification are between 2.4 mg.kg<sup>-1</sup> and 5.8 mg.kg<sup>-1</sup>. The uncertainty of the measurement was estimate in circa of 2%.

**Keywords:** Bioethanol; Gas chromatography; Validation of method

### 1. Introduction

Ethanol is a product of molecular formula CH<sub>3</sub>CH<sub>2</sub>OH, obtained by fermentation of simple sugar present in biomass [1,2]. It is colorless, volatile, flammable, water-soluble, with characteristic flavor and odor [4]. Ethanol is widely used in automotive industry various other industrial processes for the production cleaning

products, paints, perfums, cosmetics, varnishes, solvents among other products.

In Brazil, ethanol is used as biofuel produced from sugarcane, however, it can also be obtained by other renewable sources as the beetroot (Germany), corn (USA), sorghum saccharine (Africa) and wheat (Europe) can be used [1,2].

However, to be marketed in its various forms, it must comply several specifications regulated

by regulatory agencies, laws in force in each country or even by companies that are purchasing the product. As a consequence, daily, thousands analysis are performed in order to guarantee the quality of the product and its safe use. For this reason, it is necessary a continuous improvement of the used analytical, as well as the development of new ones which can improve all analytical process. Based on this premises, this work presents a new methodology for the simultaneous determinations of 11 VOCs in ethanol samples, based on gas chromatography coupled to mass spectrometry (GC-MS) technique.

## 2. Materials and Methods

### 2.1. Reagents and standards

All analytical reagents used in this study have purity greater than 99.0%. In the validation of the methodology, were used the reference materials: INMETRO – Cachaça Proficiency Testing – 4th round and NSI Lab Solutions – Lot 052316 – Expiration date: 05/31/2018.

### 2.2. Instrumentation

A chromatographic Shimadzu system composed of a gas chromatograph, model GC-17A, coupled to a quadrupole type mass spectrometer, model QP-5050 were used for the measurements. The sample introduction into the chromatograph was performed through an autosampler, model AOC-5000 Shimadzu.

The chromatography column used was a fused silica capillary DB-624 (6% cyanopropyl-phenyl 94% dimethyl polysiloxane), with dimensions of 60 m length x 0.25 mm inner diameter x 1.4um of film thickness, J Brand & W Scientific. Helium with 99.999% purity was used as carrier gas. The injection of sample was performed with a 10-uL Hamilton microsyringe. The analytical solutions were prepared gravimetrically, with automatic pipettes 10uL, 100uL, 1000uL, 10 mL volumetric flasks and analytical balance (accuracy of 0.0001 g). The samples were

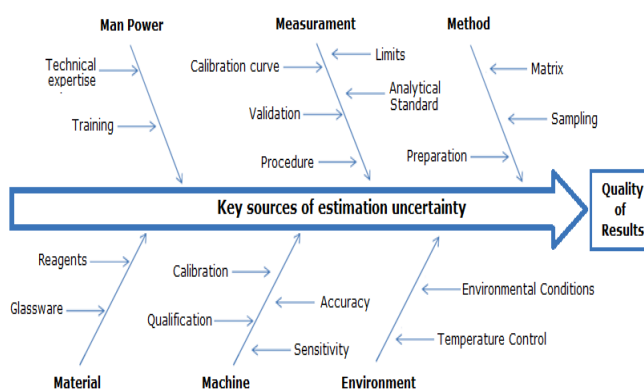
injected, without need for preparation, filtration or dilution.

### 2.3. Chromatographic conditions and validation parameters

The chromatographic conditions defined for the study were as follows: initial temperature of 35°C held for 10 minutes, followed by an increase of 10°C per minute until 80°C, remaining at this temperature for 5 minutes. Second heating ramp 5°C per minute until it reaches 120°C, remaining at this temperature for 7.5 minutes. Third heating ramp 15°C per minute until 150°C staying at this temperature for 3 minutes. With this temperature program the total chromatographic analysis will be of 40 minutes. The injection temperature was 230°C, and 2uL volume was injected in split mode with a split ratio of 1:25. Carrier gas was helium with 2.0 mL/min flow. The mass spectrometer was operated in electron impact (EI) mode, ion source temperature 230°C, MS Transfer Line 230°C, SIM mode; solvent delay 6.0 min

The validation parameters followed the requirements by NBR ISO 17025:2005: selectivity, linearity, limit of detection, limit of quantification, repeability, precision, accuracy, recovery and uncertainty measurement. In this last parameter, a cause and effect diagram (Ishikawa) was elaborated to identify the sources of uncertainty [8].

**Figure 01.** Ishikawa diagram



### 3. Results and Discussion

In order to demonstrate capability of the methodology, 6 constituents were selected, 5 of them polar: methanol, 1-propanol, Isobutanol, 1-butanol, 2-butanol and 1 apolar: cyclohexane.

#### 3.1. Limit of detection and quantification

Limit of detection is the lowest concentration of analyte in the sample that can be detected but not necessarily quantified under the conditions established for the assay. Limit of quantification is the smallest amount of analyte in the sample that can be quantitatively determined with acceptable accuracy and accuracy [3].

**Table 1.** Limits of detection and limit of quantification in mg.kg<sup>-1</sup> (ppm).

Limits	LOD	LOQ
Methanol	1,8	5,8
1-propanol	0,8	2,4
Isobutanol	1,6	5,6
1-Butanol	1,1	3,9
2-Butanol	1,0	3,3
Cyclohexane	0,03	0,11

In this study, the LOD and LOQ were calculated by the method based on analytical curve parameters, corresponding to 3 times and 10 times standard deviation of the linear coefficient of the equation by the angular coefficient of the analytical curve, respectively.

#### 3.2. Selectivity

Is the degree to which the method can quantify the analyte in the presence of other analytes, matrices or other potentially interfering material [3].

#### 3.3. Linearity

Linearity refers to the ability of the method to generate results linearly proportional to the analyte concentration in the sample [5]. In this study, linearity ( $R^2 > 0.9999$ ) was obtained in the range from 2.0 mg.kg<sup>-1</sup> to 300 mg.kg<sup>-1</sup>.

#### 3.4. Precision

Precision is the general term for evaluating the dispersion of results between independent assays, repeated in the same sample, similar samples or standards under defined conditions [3].

#### 3.5. Repeatability

It is the measurement condition in a set of conditions, which includes the same measurement procedure, the same operators, the same measuring system, the same operating conditions and the same place, as well as repeated measurements on the same object or similar objects for a short time [3].

**Table 2.** Comparison of relative standard deviations at different concentration levels.

Compound	Level	Average Intensity	Standard Deviation	%RSD
Methanol	Low	2,9	6980	<b>9,87</b>
	Medium	29,4	17126	<b>2,60</b>
	High	147,7	31107	<b>0,96</b>
1-propanol	Low	3,1	4774	<b>3,64</b>
	Medium	31,0	42904	<b>2,94</b>
	High	155,6	101018	<b>1,33</b>
Isobutanol	Low	3,1	2099	<b>3,94</b>
	Medium	31,2	13838	<b>2,27</b>
	High	156,7	40063	<b>1,25</b>
1-Butanol	Low	3,6	2798	<b>5,28</b>
	Medium	36,1	14743	<b>2,43</b>
	High	181,1	44661	<b>1,41</b>
2-Butanol	Low	3,5	2854	<b>2,22</b>
	Medium	14,3	7702	<b>1,37</b>
	High	71,2	33091	<b>1,15</b>
Cyclohexane	Low	0,09	130	<b>2,39</b>
	Medium	1,99	903	<b>1,41</b>
	High	10,7	7709	<b>1,23</b>

The values shown in Table 02 show that the lower the concentration of the analyte greater the value of the relative default deviation.

#### 3.6. Recovery

Recovery is the proportion of the amount of the substance of interest, present or added in the

analytical portion of the analyzed material, which is extracted and quantifiable. It can be estimated as the percentage of the analyte after processing the sample compared to that of a solution containing the analyte in a concentration corresponding to 100%. The reference materials used in recovery, were: INMETRO – Cachaça Proficiency Testing – 4th round and NSI Lab Solutions – Lot 052316 – Expiration date: 05/31/2018. The expected values are shown in table 3.

**Table 3.** Recovery rate of the studied compounds.

Parameters	Reference Value	Concentration	Recovery (%)
Methanol	82,4	87,9	106,7
1-Propanol	268,0	307,4	114,7
Isobutanol	209,6	210,5	100,4
1-Butanol	35,8	37,9	106,0
2-Butanol	62,1	64,3	103,6
Cyclohexane	3,1	3,2	103,2

### 3.7. Measurement uncertainty

According to Eurachem/CITAC, the uncertainty is defined as “a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand” [7]. So, the uncertainty of an analytical methodology is a parameter characterizing the dispersion of the values attributed to a measured quantity considering all steps of the analytical procedure.

**Table 4.** Combined and expanded uncertainty in the studied method.

Parameters	Concentration (mg.kg <sup>-1</sup> )	u <sup>a</sup>	U <sup>b</sup>	U(%)
Methanol	88,2	0,6	1,3	1,5
1-Propanol	306,3	2,3	4,5	1,5
Isobutanol	223,4	1,8	3,6	1,6
1-Butanol	37,0	0,4	0,8	2,2
2-Butanol	63,8	0,5	1,1	1,7
Cyclohexane	3,0	0,03	0,06	2,0

u<sup>a</sup> - Combined uncertainty

U<sup>b</sup> - Expanded uncertainty, coverage factor k = 2

U(%) - Relative expanded uncertainty

## 4. Conclusions

As described previously, the method presented good results of selectivity, linearity, repeatability, recovery and the expanded uncertainties and analytical quality similar to the commonly used methods for this same purpose. In addition, once it uses only one chromatography column for the analysis of compounds with different polarity, it allows a cost savings and enhancement in the laboratory throughput.

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