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OPTIMIZATION OF A FAST AND SIMPLE METHOD FOR THE DETERMINATION OF ETHANOL IN GEL BY HYDROGEN NUCLEAR MAGNETIC RESONANCE[1]

OTIMIZAÇÃO DE UM MÉTODO RÁPIDO E SIMPLES PARA DETERMINAÇÃO DE ETANOL EM GEL POR RESSONÂNCIA MAGNÉTICA NUCLEAR DE HIDROGÊNIO

Talal Suleiman Mahmoud[2], Alexandre Robison Meyer[3] e Pedro Toledo Netto[4]

ABSTRACT

Due to the COVID-19 pandemic, the use of alcohol-based sanitizers was intensified, resulting in the need for its monitoring. The most common techniques for determining the ethanol content are gas chromatography, spectrophotometry and alcoholometry, which are predominantly used for liquid solutions. NMR is one of the most important qualitative analysis technologies, however, it is rarely used for quantitative purposes such as the analysis of samples containing ethanol in gel. In this work, we proposed two methods for quantifying the ethanol content in gels using quantitative Hydrogen Nuclear Magnetic Resonance (^{1}H qNMR) spectroscopy. The quantification methods by external standardization and by standard addition showed selectivity, good linearity, good accuracy and precision with recoveries between 99 and 107% and coefficient of variation from 0.09 to 4.35% respectively, in accordance with the validation guidelines in chemical analysis. The ^{1}H qNMR optimized and validated in this work allowed the use of both methods in commercial samples of ethanol in gel. The external standardization method is the most recommended by the authors for being simpler and faster.

Keywords: alcohol; quantitative, validation, Covid.

RESUMO

Devido à pandemia do COVID-19, o uso de sanitizantes a base de álcool foi intensificada, surgindo a necessidade de seu monitoramento. As técnicas mais comuns para a determinação do teor de etanol são a cromatografia gasosa, a espectrofotometria e alcoometria, sendo aplicadas predominantemente para soluções líquidas. A RMN é uma das tecnologias de análise qualitativa mais importante e, ainda, é raramente usada para fins quantitativos para análise de amostras contendo etanol em gel. Neste trabalho, foram propostos dois métodos de quantificação do teor de etanol em gel por espectroscopia de Ressonância Magnética Nuclear quantitativa de Hidrogênio (RMNq ^{1}H). Os métodos de quantificação por padronização externa e por adição de padrão apresentaram seletividade, boa linearidade, boa exatidão e precisão com recuperações entre 99 e 107% e coeficiente de variação de 0,09 a 4,35% respectivamente, estando de acordo com os guias orientadores de validação em análise química. A RMNq ^{1}H otimizada e validada neste trabalho permitiu a aplicação dos

1 Chemical Laboratory of Natural Products and Organic Synthesis, Campus Pontal do Paraná - Center for Studies of the Sea - CPP-CEM/UFPR.

2 Professor Associado - Universidade Federal do Paraná. E-mail: talal@ufpr.br. ORCID: https://orcid.org/0000-0003-3198-7453

3 Químico - Universidade Federal do Paraná - UFPR. E-mail: alexandrerobisonmeyer@gmail.com. ORCID: https://orcid.org/0000-0002-3073-7973

4 Professor Adjunto do Centro de Estudos do Mar - Universidade Federal do Paraná -UFPR. E-mail: pedro.toledo@ufpr.br. ORCID: https://orcid.org/0000-0001-6614-4005

dois métodos em amostras comerciais de etanol em gel, sendo o método de padronização externo o mais indicado pelos autores por ser mais simples e rápido.

Palavras-chave: *álcool; quantitativo, validação, Covid.*

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the COVID-19 pandemic, which is why the World Health Organization (WHO) has advised the general people to develop the habit of often washing their hands to prevent infection. A greater emphasis has been placed on personal hygiene practices such hand washing with soap and water and the use of alcohol-based sanitizers, particularly when it comes to cleaning with gel alcohol (ANVISA, 2020a; FOOD AND DRUG ADMINISTRATION, 2020).

Due to the shortage caused by the rise in the usage of sanitizers with alcohol in their formulation, various agencies issued preparation guidelines for businesses that manufacture public health-related products in an effort to boost output. Among these agencies, the following stand out: ANVISA (National Health Surveillance Agency), USFDA (*US Food and Drug Administration*) and the WHO (World Health Organization) (ANVISA, 2020a; FOOD AND DRUG ADMINISTRATION, 2020).

The alcohol gel sold in Brazil has the following components, under the National Form of the Brazilian Pharmacopoeia: ethanol, distilled water or deionized water, triethanolamine, and carbomer 980. While Brazil has plenty of ethanol, triethanolamine, and the aforementioned types of water, Carbomer 980 was in insufficient supply to meet the enormous demand that had increased throughout the pandemic. This ingredient, which serves as a thickening, suspending, dispersing, and stabilizing agent, ensures consistency. According to Anvisa guidelines, triethanolamine is used as a neutralizing agent to bring the gel's pH level between 5 and 7, and ethanol is the sanitizing agent, which must contain 70° INPM or 70% (m/m) to eliminate some viruses and bacteria, including the new coronavirus that causes COVID-19. (ANVISA, 2010; BRAZILIAN PHARMACOPOEIA NATIONAL FORM, 2012).

Anvisa published two Collegiate Board Resolutions (RDC): RDC 347, which allowed drug formulating pharmacies to produce alcohol gel and other sanitizers and market them freely; and RDC 350, which permitted companies with operating authorization and a valid health permit or license to produce alcohol-based antiseptic formulations without prior authorization from the federal agency itself (ANVISA, 2020a; ANVISA, 2020b). Consequently, in order to meet the population's increased demand due to the lack of Carbopol 980, various alternative components were used.

Due to the lack of alcohol-based sanitizing agents during the pandemic, Anvisa's authorization was a significant and crucial step. However, it resulted in a large number of erroneous goods being put on the market that did not account for the quantity of alcohol required to effectively eradicate the majority of bacteria and viruses, including the novel coronavirus. In order to stop the sale of

ineffective products, the competent agencies were to implement a monitoring program for the analysis of alcohol in sanitizers.

The most common techniques used to determine the ethanol content are gas chromatography, spectrophotometry and alcoholometry. Yet, they are predominantly applied to liquid ethanol solutions (FOOD AND DRUG ADMINISTRATION, 2020; RAMASAMI *et al*., 2005). Mid-infrared and near-infrared spectroscopy has been used to analyze alcohol gel samples, but it requires chemometric treatment to determine the ethanol content (FONSECA JR *et al*., 2020; NASCIMENTO *et al*., 2017).

The implementation of basic NMR theory is widely described in the literature, as well as its use in quantitative analysis: (BHARTI, S.K.; ROY, R., 2012) including analyzes in: coffee (OKARU, AO, *et al,* 2020), wine (SOLOVYEV, P.A., 2021), microplastics (PEEZ, N.; JANISKA, M.C.; IMHOF, W., 2019), agrochemicals (MANIARA, G., 1998), and drugs (HOLZGRABE, U., *et al* 2005). The relationship between the area of the ^{1}H signal is studied, being directly proportional to the number of ethanol methyl hydrogens that absorb energy at the specific radiofrequency.

For the relationship to be correct in pulsed NMR instruments, it is essential that the hydrogen spins are in thermal equilibrium and, necessarily, that the repetition of the pulse sequence is greater than five times, and then the NMR signal is acquired in the time interval.

In this work, we propose a fast and simple method for measuring the ethanol content in gels using the technique by spectroscopy of Hydrogen Quantitative Nuclear Magnetic Resonance (qNMR-^{1}H).

MATERIALS AND METHODS

REAGENTS AND OBTAINING SAMPLES

Absolute ethanol standard (HPLC-UV grade) was obtained from Dinâmica brand. Ultrapure water was obtained from an Integral 5 Milli-Q® purification system (Merck Millipore). Bottles of 70% ethanol gel (ºINPM) of three different brands were purchased in the commerce of Pontal do Paraná, Paraná, Brazil.

PREPARATION OF ANALYTICAL CURVES AND STANDARD ADDITION CURVES

Five standard solutions were prepared in quintuplicate from absolute ethanol at concentrations of 1%, 3%, 5%, 7%, and 9% (m/m), using ultrapure water as the solvent in order to design the analytical curve. The standard addition curves were made in quintuplicate by weighing 1g of the ethanol gel from each commercial sample at five different concentration levels, adding 0, 0.2, 0.4, 0.6, and 0.8 g of absolute ethanol, and then adding ultrapure water as a solvent. This produced mass percent concentrations of 0, 2.0, 4.0, 6.0, and 8.0%. Both the standard solutions of the analytical curves and the

standard addition curves were diluted with ultrapure water to a final mass of 10 g, using an analytical scale (ACZET, model CY 224C). All solutions were homogenized and 0.6 mL of it was transferred to standard 5 mm NMR tubes, followed by ^{1}H NMR analysis. Finally, the three commercial samples of different brands of ethanol gel were quantified (in quintuplicate) by the methods of external standardization and addition of standard to assess their performance.

ANALYSIS BY ^{1}H NMR

^{1}H NMR analyzes were carried out using a benchtop Oxford Nuclear Magnetic Resonance Spectrometer, model Pulsar, at a frequency of 60 MHz. The operational conditions were: Spectral frequency of 59.70 MHz; 5200 Hz spectral window; 8 scans; 32768 points per scan, recovery time between scans 12 seconds. For data acquisition, an aliquot of the ethanol gel and liquid ethanol samples was transferred to standard 5 mm NMR tubes and mounted in the NMR spectrometer at a temperature of 20°C. The samples were kept under these conditions for 5 minutes for temperature stabilization and, subsequently, 8 scans were acquired, totaling a time of 2 minutes and 30 seconds. The SoftLock option was used to ensure spectral stability, dispensing with the use of deuterated solvents. The SoftLock option guarantees absolute spectral stability without the need to use a deuterated solvent, allowing the superimposition of 2000 scans without the occurrence of baseline widening due to poor alignment.

The spectra were processed and analyzed using the MestReNova Version 9.1.0 software. Manual phase adjustment was performed to obtain a precise fit. The chemical shift of the spectrum was calibrated using the methyl signal from ethanol (1.110 ppm) and the determination of ethanol content in both the gel and liquid ethanol was performed with the integration of the methyl signal. For accurate integration, this signal was integrated between chemical shifts from 0.8 to 1.4 ppm.

VALIDATION OF THE METHODOLOGY

The following performance parameters were evaluated for validating the ^{1}H NMR analysis methodology: matrix effect, selectivity, working range, linearity, trend (accuracy) and intermediate precision (inter-day). The limits of detection and quantification were not determined, as in the determination of major components (ethanol at 70% m/m) these parameters are unnecessary (NATIONAL INSTITUTE OF METROLOGY, QUALITY AND TECHNOLOGY, 2016).

The matrix effect and the selectivity were evaluated by comparing the external standardization analytical curves and the standard addition curves. If the slopes of these curves do not differ significantly, there is no matrix effect and, therefore, there is no interference from other chemical species present in the gel for the measurement of the methyl ethanol signal. The difference between the two curves mentioned can be graphically observed by the parallelism between the two straight

lines obtained by linear regression (NATIONAL INSTITUTE OF METROLOGY, QUALITY AND TECHNOLOGY, 2016).

The working range for the external standardization method was from 1 to 9% (m/m) and for the standard addition method from 0% to 8.0% (m/m). The homoscedasticity of the data along the analytical curves, that is, the variance of the residues, was verified using the Cochran Test (Equation 1). In this test, C_{Calc} is calculated and compared with C_{tab}; if the calculated value is less than the tabulated value, the hypothesis is accepted that the data present similar variances regardless of the concentration value and, therefore, the data are homoscedastics. Next, simple linear regression was applied using the least squares method, obtaining the straight line equations and the correlation coefficient between the methyl signals and the concentrations in mass percentage for each quantification method.

$$C_{calc} = \frac{s_{max}^2}{\sum_{i=1}^{p} s_i^2} \quad (1)$$

To obtain intra-assay precision (repeatability), the methyl ethanol signals at 11 concentration levels (1 to 21% m/m) were compared, in triplicate and on the same day of analysis. Intermediate precision was determined in this same concentration range, measurements of the methyl ethanol signal were carried out on two different days and with the same analyst.

Although the preparation of the gel involves only its dissolution in ultrapure water, causing no loss of the analyte, the tendency (accuracy) of the methodology was estimated using recovery tests through samples fortified with known amounts of ethanol. Both the external standardization method and the standard addition method were evaluated at three different ethanol concentrations (low, medium and high) using HPLC grade ethanol for fortification, with 1%, 5% and 9% (m /m) for the external standardization method and 2%, 4% and 8% (m/m) for the standard addition method.

RESULTS AND DISCUSSION

^{1}H NMR ANALYSIS OPTIMIZATION

^{1}H qNMR is an analytical technique that offers several advantages over conventional quantitative methods. Some of these are:

I) independent reference standard: unlike other techniques, ^{1}H qNMR does not require a reference standard structurally related to the substance of interest. This allows for more flexible and accurate analysis, as the structure of the reference standard does not influence the analysis.

II) Simultaneous identification and quantification: The technique allows the identification and quantification of several substances in a single spectrum. This is particularly useful in complex samples where multiple components are present.

III) Non-destructive method: ^{1}H qNMR analysis is non-destructive, which means that the sample is not altered during the analysis. This is especially important when the sample needs to be used later for other purposes.

These advantages have awakened the interest of many researchers. Currently, the technique is employed in several areas, such as:

I) Purity analysis: The ^{1}H qNMR is used to evaluate the purity of chemical substances, identifying impurities and determining the concentration of the components present.

II) Adulteration detection: In industries, the technique is used to identify adulterants in products, guaranteeing the quality and authenticity of the items manufactured.

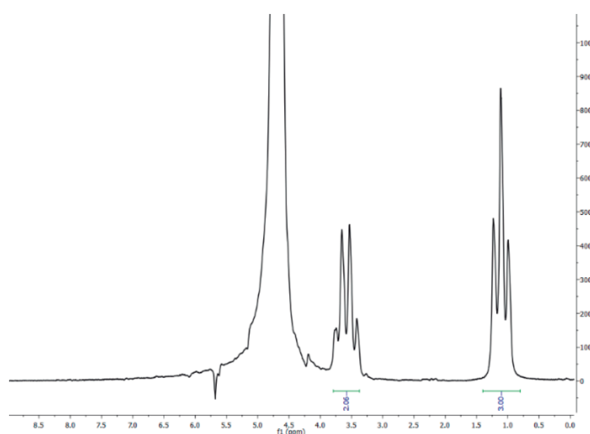
III) Studies of metabolic groups: In the Biology and Metabolomics fields, ^{1}HqNMR is used to study groups of metabolites, allowing the understanding of biochemical processes and their alterations.

The use of ^{1}H qNMR in scientific research has led to significant advances, mainly in the optimization of quantitative analyses. The ability to accurately and non-destructively identify, quantify and analyze substances makes this technique a valuable tool.

The feasibility of using ^{1}H qNMR in the determination of ethanol in commercial gels began with the search for a technique parameter that could respond selectively and linearly by varying the ethanol concentration. The ^{1}H NMR spectrum (Figure 1) obtained in this work shows that the ethanol methyl peak (triplet at 1.11 ppm) appears with good resolution and far from interference from other signals, and can be selectively integrated. Thus, the variation of the absolute area of the methyl signal can be monitored with the variation of the ethanol concentration.

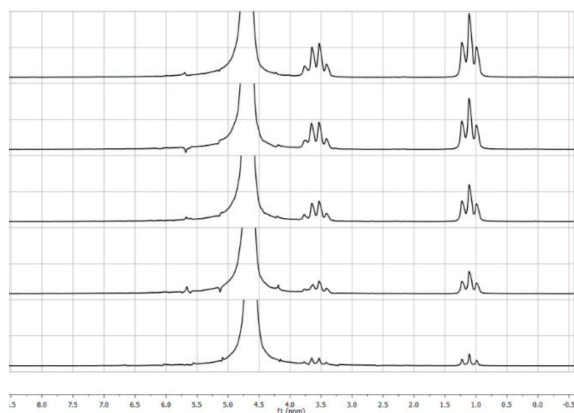
It was necessary to work with diluted concentrations of ethanol to allow the solubilization of the gels sold at 70º INPM. Thus, the range from 1 to 9% (w/w) was chosen, as in this range the gel became solubilized. The spectra of Figure 2 show the variation of the intensity of the ^{1}H NMR signals with the increase of the ethanol concentration from 1 to 9% (m/m) and qualitatively demonstrate that the intensity of the signals increases with the ethanol concentration.

Figure 1 - ^{1}H NMR spectrum of HPLC grade ethanol at 0.7% m/m concentration in ultrapure water.



Source: The author.

Figure 2 - Intensity variation of ^{1}H NMR signals with increasing ethanol concentration (m/m), from bottom to top: 1% ethanol, 3% ethanol, 5% ethanol, 7% ethanol and 9% of ethanol (concentration m/m).



Source: The author.

In the concentration range studied (1 to 9% m/m), the methyl area of ethanol proved to be very susceptible to phase adjustment. This phase adjustment was achieved by expanding the spectrum so that the signals of interest could be observed in detail. This fixed the distorted signals. Correction is done manually by dragging up and down to correct phases 0 and 1 of the spectrum into the intended signal (FOREZI & CASTELO-BRANCO, 2017).

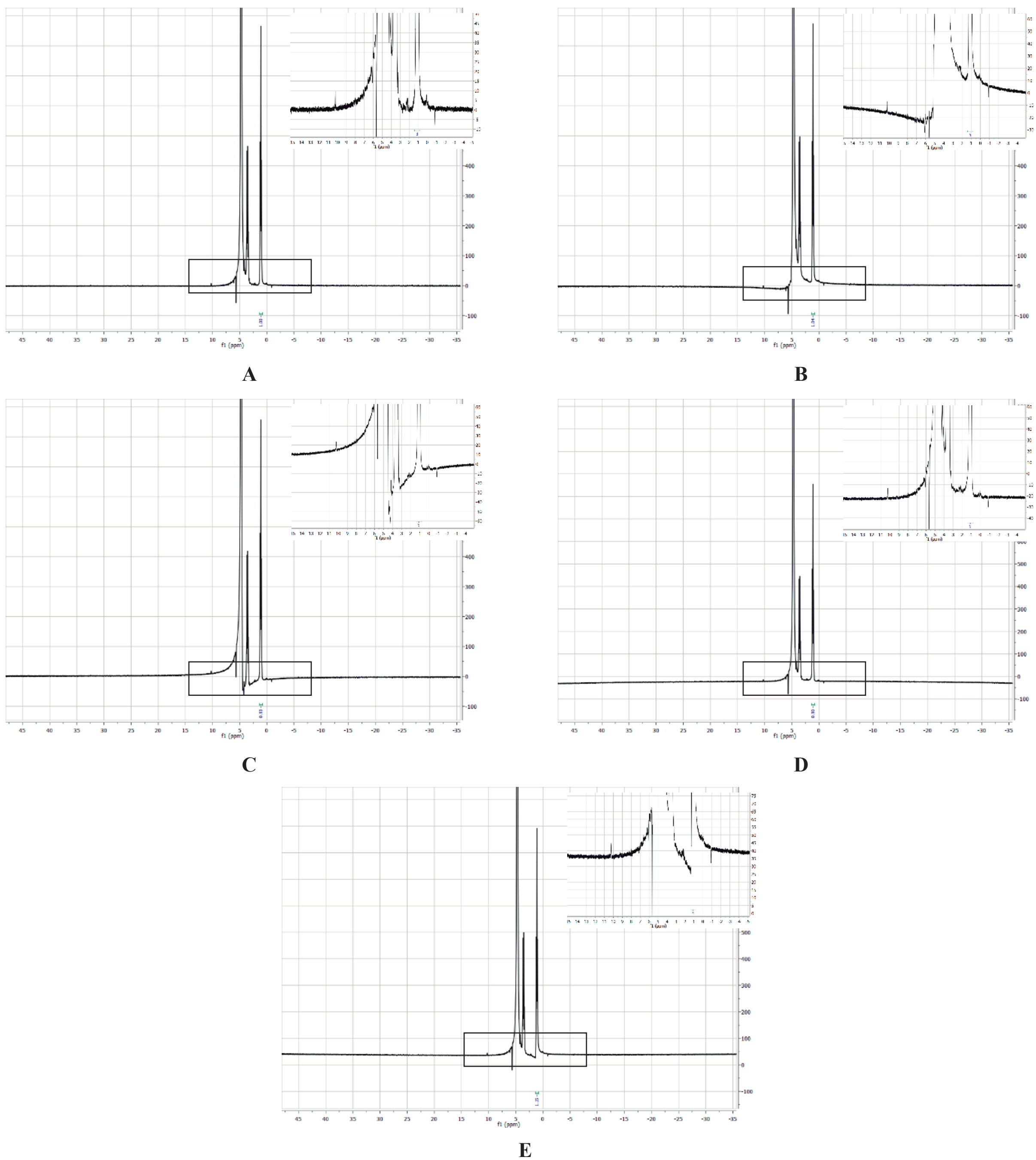
Meticulous manual adjustment of the spectrum line is important to ensure that the origin is correctly aligned, which is crucial for obtaining accurate results in methodology validation and sample quantification. This means that the spectrum settings are being precisely and carefully adjusted in order to minimize measurement errors and uncertainties.

By doing so, the reliability of the results obtained in the validation step of the methodology and in the determination of the amounts of substances present in the samples increases. This is especially important in fields such as analytical chemistry and materials analysis, where measurement accuracy is essential to ensure the quality of the data and the conclusions drawn from it.

Since manual adjustment requires an in-depth knowledge of the principles of spectroscopy and the features of the instruments utilized, the effectiveness of this operation may also depend on the operator's technical expertise and experience.

In order to validate the methods and quantify the samples, we were able to achieve high precision through meticulous manual modification with the spectrum line passing through the origin. Figure 3 shows different phase adjustment errors and their impact on the value obtained for the methyl area.

Figure 3 - Phase adjustment errors for the 7% (w/w) ethanol spectrum. A) perfect fit; B) positive PH0 adjustment error; C) negative PH0 fit error; D) negative PH1 fit error; E) positive PH1 adjustment error. Absolute areas respectively: A) 105536.72; B) 98055.53; C) 110364.70; D) 95198.21; E) 121986.35



Source: The author

STUDY OF PERFORMANCE PARAMETERS

The data in Table 1 were used to determine the intermediate precision (inter-day), linearity, working range and homoscedasticity, working with 11 levels of ethanol concentration (%, m/m) HPLC grade in ultrapure water and in three different days.

Table 1 - Areas, area mean and relative standard deviation (RSD) of ethanol methyl signals at 11 concentration levels and on three different days.

Days	Ethanol concentration in % m/m	Ethanol methyl signal áreas	Area mean	*RSD (%)
1	1.09	17651.1		
2	1.02	17633.4	17480.8	1.6
3	1.07	17157.8		
1	3.00	47949.7		
2	3.03	46379.5	47165.3	1.7
3	3.04	47166.7		
1	5.11	78454.3		
2	5.00	76100.4	77652.4	1.7
3	5.01	78402.5		
1	7.04	107469.9		
2	7.06	107470.7	107534.0	0.1
3	7.04	107661.3		
1	9.01	137987.8		
2	9.07	138534.3	138723.5	0.6
3	9.05	139648.4		
1	10.79	165283.1		
2	11.07	167200.4	167142.2	1.1
3	11.06	168943.3		
1	13.04	197541.1		
2	13.11	197575.9	199144.8	1.4
3	12.98	202317.4		
1	15.04	231111.2		
2	15.03	228816.7	228933.8	0.9
3	15.06	226873.6		
1	17.05	255866.9		
2	17.01	254662.4	256235.9	0.7
3	16.84	258178.5		
1	19.11	287377.8		
2	18.97	286948.5	287024.0	0.1
3	19.03	286745.8		
1	21.03	317658.4		
2	21.03	316553.2	316402.1	0.4
3	20.95	314994.7		

*Intermediate Precision RSD

Source: The author

The relative standard deviation (RSD) of the intermediate precision for the methyl areas ranged 0.1 to 1.7%, showing excellent precision for the preparation of standard solutions on three different days, being in accordance with the values established by the National Agency of Sanitary Surveillance (ANVISA) for bioanalytical assays, which admits values of less than 20% for the lowest concentration and 15% for other concentration levels (ANVISA, 2017).

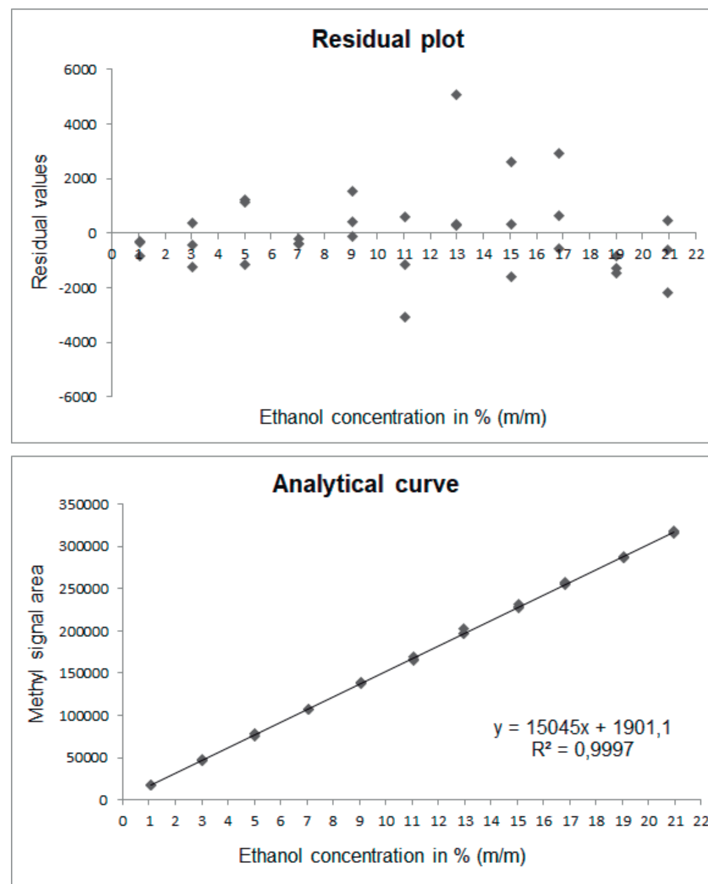
The Cochran Test showed that the data are homocedastic, as the C_{calc} (0.318) is smaller than the C_{tabel} (0.417), with 11 replicates and a 5% significance level (Table 2). The residuals graph in Figure 4 shows that the difference between the expected value and the value calculated by the equation of the regression line for each point of the analytical curve varies randomly (free of tendency), reinforcing that the data are homoscedastic allowing, therefore, the application of the method of least squares. Figure 4 also presents the analytical curve, showing that there is linearity throughout the investigated range (from 1 to 21% m/m), since the correlation coefficient is greater than 0.99 (ANVISA, 2017; NATIONAL INSTITUTE OF METROLOGY, QUALITY AND TECHNOLOGY, 2016).

Table 2 - Evaluation of the homoscedasticity of the data in Table 1.

Cochran test (Evaluation of homoscedasticity)	
Hypothesis	If Ccal < Ctab , the data is homoscedastic
Higher variance value between the variances of the methyl areas (data from Table 1)	7549346
Sum of variances of methyl areas (data from Table 1)	23719009
C_{calc}	0.318
C_{tab} (11 concentrations, n = 3, 5% significance)	0.417
Result	Ccalc < Ctab , data is homoscedastic

Source: The author.

Figure 4 - Residue plot and analytical curve with linear regression parameters.



Source: The author.

Since the ethanol gel had to be diluted ten times in ultrapure water for its solubilization, the working range for the external standardization method and the standard addition has been limited to 1 to 9% (m/m) and 0 to 8.0% (m/m), respectively. As a result, the expected concentration in the commercial samples (70º INPM) decreases by this same dilution factor.

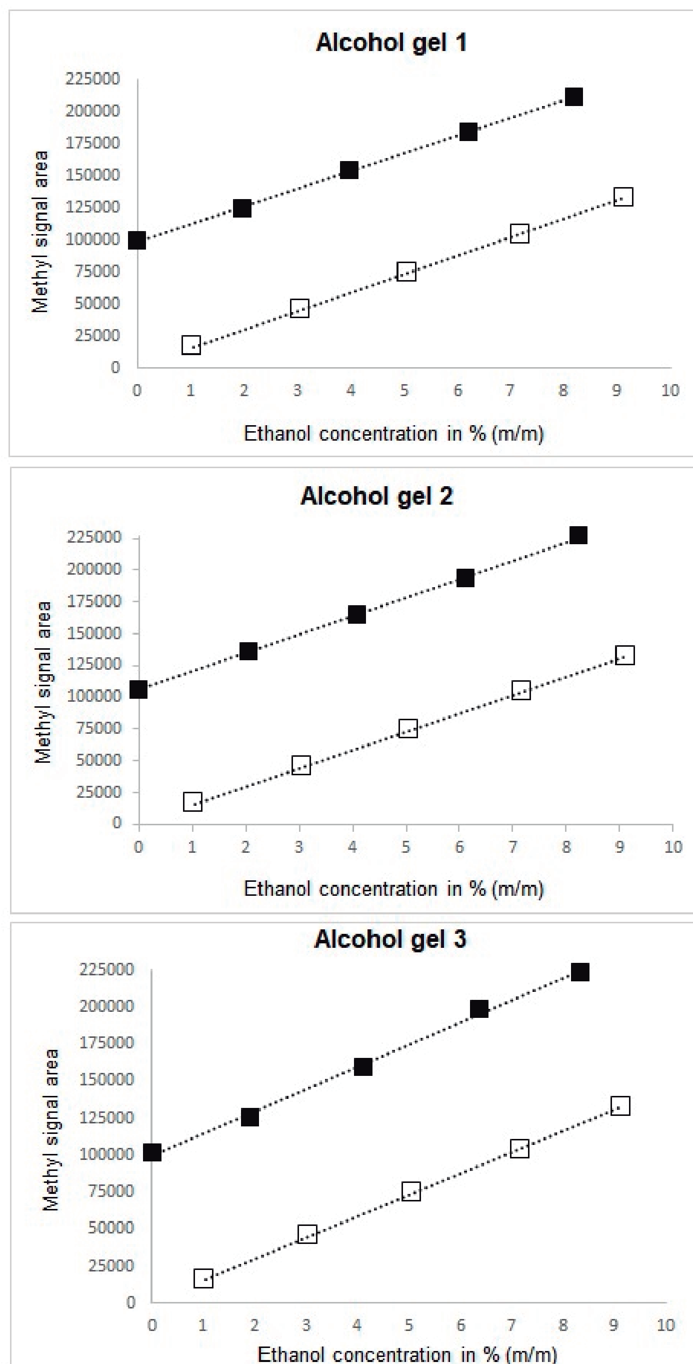
The standard addition method and the external calibration method both use a set of analyte standard solutions to determine the concentration. Standard is added to the sample in the standard addition method, however, to account for "matrix effects," which are changes in the analytical signal brought on by any component of the sample other than the analyte. In this work, treating a gel sample that is analogous to the material being studied but devoid of ethanol and adding this alcohol in known concentrations to create standard solutions would be one way to address this issue. The calibration curve would then be built using a gel matrix similar to the sample; however, due to the fact that matrix effects vary from one gel to another, this treatment would not entirely eradicate them. Furthermore, finding a commercial gel without ethanol would be difficult and unlikely.

As a result, the standard addition method - which involved adding ethanol standard in escalating volumes to commercial samples before preparing them for ^{1}H qNMR analysis - became practical. To obtain the spectra, commercial gel samples with added 100% ethanol were employed. Analytical curves were built to correlate the areas obtained with the amounts of standard ethanol added to the gels. The area of the substance being determined corresponds to the point where the straight line crosses the ordinate axis without the inclusion of the ethanol standard. On the abscissa axis, the straight line extrapolation defines the ethanol concentration in the examined gels.

Despite the fact that the standard addition approach lessens the matrix effect problem, it has significant drawbacks, including difficulty automating and the need to work with more samples than the external standardization method. Another drawback is that it uses an extrapolation method, which is less precise than interpolation-based quantification techniques like the external standardization.

Figure 5's graphs show the parallelism between the lines obtained using the standard addition and external standardization methods. Thus, it can be said that neither the gel nor the liquid alcohol contain any chemical species that would interfere with the measurement of the methyl ethanol signal, demonstrating the selectivity of both quantification techniques. (NATIONAL INSTITUTE OF METROLOGY, QUALITY AND TECHNOLOGY , 2016).

Figure 5 - Parallelism between the standard addition curves of three brands of commercial alcohol gel (filled squares) and the external standardization curves (empty squares)



Source: The author

Table 3 shows that the external standardization and standard addition methods showed good accuracy and precision at the three concentration levels evaluated, with recoveries between 99 and 107% and coefficient of variation from 0.09 to 4.35%, respectively. These values are in accordance with ANVISA, which recommends recoveries between 70 and 120% with coefficients of variation lower than 20% (ANVISA, 2017).

Table 3 - Results of accuracy and precision of quantification methods (n = 3).

RECOVERY STUDY - EXTERNAL STANDARDIZATION				
replicas	**Ethanol concentration level (%, m/m)**	**Recovery (%)**	**Mean (%)**	**Coefficient of variation (%)**
1		109.36		
2	1	105.99	106.53	2.44
3		104.25		
1		103.84		
2	5	104.64	104.19	0.39
3		104.08		
1		104.56		
2	9	99.72	102.36	2.39
3		102.81		
RECOVERY STUDY - STANDARD ADDITION				
replicas	**Ethanol concentration level (%, m/m)**	**Recovery (%)**	**Mean (%)**	**Coefficient of variation (%)**
1		103.61		
2	2	95.07	99.82	4.36
3		100.79		
1		99.68		
2	4	99.52	99.58	0.09
3		99.52		
1		96.61		
2	8	98.78	100.22	4.48
3		105.25		

Source: The author.

QUANTIFICATION OF COMMERCIAL ALCOHOL GEL SAMPLES

The percentage of ethanol in the three commercial samples evaluated in this work was quantified both by the external standardization method and by the standard addition method, as both showed adequate precision and accuracy. The results of these determinations are presented in Table 4.

Table 4 - Ethanol concentration in % (m/m) in the analyzed gels.

Quantification by external standardization			
Replicas	**% Ethanol (m/m) - Commercial Gel 1**	**% Ethanol (m/m) - Commercial Gel 2**	**% Ethanol (m/m) - Commercial Gel 3**
1	67.98	72.95	68.83
2	68.06	72.19	68.79
3	67.68	71.48	68.07
4	67.88	72.70	68.78
5	67.88	72.47	68.35
Mean ± SD	67.90 ± 0.14	72.36 ± 0.57	68.56 ± 0.34
Variation in relation to the alcohol concentration sated in the product (70% m/m or 70º INPM)	-3.01%	+3.37%	-2.05%

Quantification by standard addition			
Replicas	**% Ethanol (m/m) - Commercial Gel 1**	**% Ethanol (m/m) - Commercial Gel 2**	**% Ethanol (m/m) - Commercial Gel 3**
1	71.57	72.94	66.16
2	69.28	75.36	67.10
3	69.24	75.63	67.87
4	70.53	73.45	65.09
5	69.47	73.45	67.18
Mean ± SD	70.02 ± 1.02	74.17 ± 1.23	66.68 ± 1.08
Variation in relation to the alcohol concentration stated in the product (70% m/m or 70º INPM)	-0.03%	+5.95%	-4.74%
Variation in relation to the two quantification methods	-3.03%	-2.43%	+2.83%

Source: The author.

As shown in Table 4, the variation between the two quantification methods was small, being -3.03%, -2.43% and 2.83% for commercial gels 1, 2 and 3 respectively, so that they can both be used for the determination of ethanol in commercial samples of ethanol gel. The standard addition method sample preparation procedure is more time consuming than the external standardization method because it is necessary to add known amounts of HPLC grade ethanol to commercial ethanol gel samples for each concentration level. Due to its volatility, the ethanol concentration can undergo random variations in the standard addition method and showed a higher standard deviation (from 1.02 to 1.23%) in relation to quantification by external standardization (from 0.14 to 0. 57%). External standardization is the suggested technique for ethanol quantification by Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) in samples of ethanol gel. This is due to the method's ease of use and higher precision, which leads to a smaller relative standard deviation between determinations and suggests more dependable and consistent results. External standardization is a process that involves creating a number of standards with known ethanol concentrations and measuring them with ^{1}H NMR. The NMR signal strength is then correlated with the known concentrations using a calibration curve. By comparing the signal intensities of the samples' ethanol gel samples with the calibration curve by interpolation, this curve can then be used to measure the amount of ethanol present in the samples.

When we compared the ethanol concentrations obtained in this work using the standard addition method and the external standardization method with the concentrations listed on the labels of the three brands of alcohol gel we analyzed, we found that the first method had a lower variation range, ranging from 2.05 to 3.37% (standard addition) and from 0.03 to 5.95% (external standardization). ANVISA states in its Collegiate Board Resolution - RDC No. 422, of September 16, 2020, that the alcohol concentration in cosmetic products cannot vary by more than 10% in relation to the concentration of ethanol stated on the product in °INPM (or %m/m) (ANVISA, 2020c). This is done in order to guide the production and preparation of alcohol antiseptics. As a result, the gels assessed in this study comply to these ANVISA recommendations.

According to a study carried out by Kampf *et al*. (2020), the inactivation of the coronavirus on surfaces such as metals, glass and various plastics is effective with the use of sanitizers containing an ethanol content between 62 and 71% (the article does not specify whether the content is in m/m or v/v) if the contact time is one minute. Collegiate Board Resolution - RDC No. 422, of September 16, 2020, says that cosmetics used in health services, except in liquid form, must respect the minimum content of 68.25% (m/m) (ANVISA, 2020c). Other studies point out that concentrations of a minimum of 60% and a maximum of 80% (unspecified content if m/m or v/v) are also effective, although they may not have the same effectiveness as a concentration of 70% (m/m) (FEDERAL CHEMISTRY COUNCIL, 2023). Since the concentrations of ethanol (m/m) obtained in this work were between 66.68% (gel 3) and 74.17% (gel 2), it can be inferred that all of them are capable of inactivating the coronavirus.

CONCLUSIONS

The ^{1}H qNMR technique presents itself as a valuable alternative for the unequivocal (selective) determination of ethanol in commercial samples of ethanol gel because it is simple and fast, as the sample does not undergo any treatment, only dissolution. Although mid-infrared and near-infrared spectroscopy can be used for ethanol gel samples, it requires chemometric treatment, making the method more complex and time-consuming. Other analytical techniques such as gas chromatography, spectrophotometry and alcoholometry have also been used, but are predominantly limited to liquid solutions.

External standardization and standard addition methods were used as quantification methods in this work. In the standard addition method, a known amount of standard ethanol was added to the gel sample and ^{1}H qNMR signals were measured as a result. A calibration curve was created by the external standardization method using various known concentrations of standard ethanol and their respective ^{1}H qNMR signals. After that, the ethanol concentration in the gel sample was determined using this calibration curve. Our study showed that the external standardization method is the most appropriate due to its ease of execution and the better presented accuracy, offering more reliable results when compared to the standard addition method.

Finally, we strongly recommend the quantitative ^{1}H NMR for ethanol content quality control in commercial gels as well as those made in universities, institutes, and other institutions.

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