A rapid and effective method for determination of ethanol content in hand sanitizers (alcohol gel)

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Abstract

The new Coronavirus-Disease (COVID-19) pandemic has increased public awareness about hand hygiene both in-house and in out-of-home situations. As a consequence, there was a shortage of alcohol gel in supermarkets and pharmacies which led to a rise of "alternative brands" and manufacturers for this product in the market. Ethanol is the main active ingredient in hand sanitizer products. For liquid formulas, the use of alcoholmeter is the standard method for quality-control of the ethanol concentration, but for gel products, with high viscosity, this method does not apply. In this paper, a rapid method for ethanol determination in hand sanitizers (alcohol gel) is presented, aiming at the quality-control of this product which is important to assure its efficacy in killing pathogenic microorganisms. The method was applied for seven samples of alcohol gel which showed ethanol mass fraction levels ranging from 53.9 % to 65.3 %, below the alleged content on their label and the recommended concentration for viruses and bacteria inactivation. The use of an ultrasonic bath was proved to be more suitable for sample homogenization rather than hand-shaking. Overall, the method has shown to be rapid and presented good specificity, repeatability, and reproducibility for the proposed application.

1 Introduction

The consumption of alcohol gel, the most common hand sanitizer, has sharply increased in Brazil after the outbreak of the new Coronavirus-Disease (COVID-19) pandemic (RIBEIRO, 2020; SANDES; PEREIRA, 2020). Since there was a shortage of this product in the market, several industries which have no previous experience in producing alcohol gel started to use their facilities to manufacture this product in order to help the government, but also to generate revenue with an alternative product (LEMOS, 2020; FUCUCHIMA, 2020; LABBATE, 2020).

Alcohol gel has been proved to be effective in eliminating viruses and bacteria from inanimate surfaces as well as from biological tissues (PARK et al., 2010; KAMPF, 2018; RABENAU et al., 2005), but the studies that have come to these conclusions also conclude that there is a range of concentration in which ethanol (the active component of most hand sanitizers) is effective in the elimination of hazardous microorganisms (HERNANDES et al., 2004; REYNOLDS et al., 2006). Products with ethanol mass fraction as low as 50 % do not kill viruses nor bacteria and therefore are recommended only for general cleaning of surfaces, such as tables, wardrobes, and countertops (ANVISA, 2002). Although efficient in killing microorganisms, alcohol content above 80 % (either by volume fraction or by mass fraction) is also not recommended because it evaporates quickly, leaving a short time range for the product to take effect. On the other hand, it increases the risk for domestic accidents, such as explosions, intoxication via ingestion and burning (CONSELHO FEDERAL DE QUÍMICA, 2020).

Most studies indicate a mass fraction range between 60 % and 80 % of ethanol for a good sanitizing activity (KAMPF et al., 2020). At these concentrations, ethanol molecules act in the same way as soaps, because of the nonpolar side of the molecular structure which binds to the lipid constituents of the cellular membrane of microorganisms, followed by interaction of the polar side with water molecules to facilitate the inactivation and the removal of the pathogenic agent from the skin (JANSEN, 2020).

In Brazil, the National Health Surveillance Agency (ANVISA) is the government department who regulates the production of hand sanitizers and analogous products and has established that the ethanol mass fraction in liquid hand sanitizer formulations must be between 60 % and 80 % but for alcohol gel formulations the minimum concentration must be 70 % (ANVISA, 2010; 2019). However, ANVISA does not specify a method for the determination of the final content of ethanol in these formulations. In a recently released guidance, the US-FDA recommends an ethanol volume fraction of at least 60 % for hand sanitizers (USFDA, 2020) and the World Health Organization (WHO) advises the use of a formula containing a volume fraction of 75 % or 80 % of ethanol (WHO, 2010).

Several standard methods are available for evaluating the efficacy of hand sanitizers in killing pathogenic microorganisms (AMERICAN SOCIETY FOR TESTING AND MATERIALS, 2013, 2015, 2016) but none of them focuses on the evaluation of the ethanol content. For liquid formulations, both WHO and ANVISA indicate the use of alcoholmeter in a graduate cylinder (WHO, 2010; ANVISA, 2020) but this method does not work for very viscous mixtures, such as gels. Therefore, it is necessary to have a specific and practical method for the quality control of the alcohol gel formulations.

Handwashing with water and soap for at least 20 seconds is a procedure as effective as the use of alcohol gel and the most recommended practice for the elimination of viruses and bacteria (WHO, 2009; RIDLEY, 2020). However, for "out-of-home" situations, such as when commuting in public transportation, the use of a hand sanitizer is strongly indicated to avoid contamination in pandemic times. Therefore, it is important to ensure that the ethanol concentration of a given hand sanitizer is in conformity with the suggestion by the sanitary authorities so the product can be safely used by the population.

To this end, we developed a rapid method for quality control of the ethanol content in hand sanitizers (alcohol gel) by gas chromatography with a flame ionization detector (GC-FID). We also applied this method for the quantification of ethanol in seven samples of alcohol gel and tested the effect of an ultrasonic bath in the sample preparation.

2 Materials and Methods

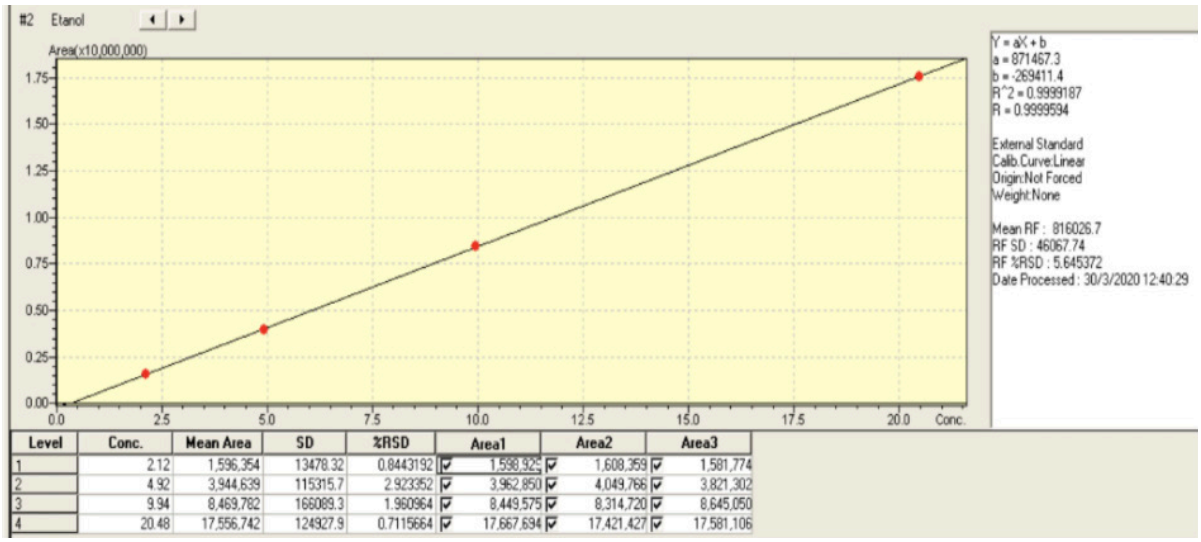
Seven batches of alcohol gel (ethanol mass fraction of 70 %, as declared on the label) were obtained from a local producer and identified with laboratory codes (A, B, C, D, E, F, and G). An aliquot of 1 g was weighed and diluted (1:10) with n-butanol, that is 1 gram of alcohol gel in 10 mL butanol (Vetec, Duque de Caxias-RJ, Brazil). During the preparation, it was noticed that the samples tend to stick to the narrow walls of volumetric flasks (due to its viscous characteristic), thus they were substituted for screw cap vials with a wide mouth for all preparations and the solvent was also weighed. The samples were prepared twice in triplicate (a total of six preparations for each sample). Half of the samples were hand-shaken for 2 min and the other half were put in an ultrasonic bath (UB) (Ultracleaner 1650A, Unique, Indaiatuba-SP, Brazil) for 2 min at 25 kHz. The short time was adopted because prolonged times in the UB would promote the heat of the solution, favoring the volatilization of ethanol. All samples were then filtered in a PTFE syringe filter (Chromafil Xtra 45/25, Macherey-Nagel, Duren, Germany) to a 1.5 ml vial and the GC-FID analysis was performed.

The calibration was done in a gas chromatograph with a flame ionization detector (GC 2010, Shimadzu Corporation, Kyoto, Japan) with four solutions of ethanol (Honeywell, Charlotte, USA) ranging from 2.0 % to 20.0 % of mass fraction in n-butanol, prepared in a laboratory. The GC programming was as follows: inlet temperature: 250 °C; injection mode: split (1:50); carrier gas: helium (3.00 ml/min); column: Poraplot Q (10 m x 0,32 mm x 0,10 µm, Agilent, Santa Clara, USA); oven temperature: initial 85 °C for 2 min, ramp of 35 °C/min up to 260 °C, maintained for 5 min (total run time: 15 min); detector temperature: 280 °C; injected volume: 0.5 µL. The software for data acquisition and processing was the GC Solutions (version 2.40.00; Shimadzu Corporation, Kyoto, Japan). The identification of the ethanol peak was done by comparison with the retention time (RT) of the standard solutions (expected RT for this configuration: 3.8 min). All calibration points were analyzed in triplicate.

3 Results and Discussion

*The calibration curve obtained is presented in **Figure 1**. The regression coefficient (r2) for this curve was 0,9999 with a relative standard deviation (RSD) of 5.64 % which denotes good linearity for the proposed working range. The results for ethanol content in the seven samples are presented in Table 1. An aliquot of sample G was also injected in triplicate on the GC system to assess the method variability, the results of this replicate are also presented in **Table 1**.*

Figure 1- Calibration curve.



Level	Conc.	Mean Area	SD	%RSD	Area1	Area2	Area3
1	2.12	1,596,354	13478.32	0.8443192	1,598,925	1,608,359	1,581,774
2	4.92	3,944,639	115315.7	2.923352	3,962,850	4,049,766	3,821,302
3	9.94	8,469,782	166089.3	1.960964	8,449,575	8,314,720	8,645,050
4	20.48	17,556,742	124927.9	0.7115664	17,667,694	17,421,427	17,581,106

Source: prepared by the authors

Table 1- Quantitative results for ethanol in alcohol gel samples (mass fraction in %)

Sample	Replicates (preparations)									
	Handshake			Mean	CV %	Handshake			Mean	CV %
	1	2	3			1	2	3		
A	61.9	52.7	47.2	53.9	13.8	63.5	62.3	61.0	62.3	2.0
B	50.6	59.3	59.5	56.4	9.0	60.6	60.5	61.1	60.7	0.6
C	64.8	64.2	64.9	64.7	0.6	63.9	63.9	64.9	64.2	0.9
D	63.0	62.1	61.2	62.1	1.4	64.0	63.5	64.0	63.8	0.4
E	65.3	65.2	59.7	63.4	5.1	65.6	65.6	64.7	65.3	0.8
F	65.3	56.7	63.5	61.8	7.4	63.5	63.1	64.2	63.6	0.9
G	64.8	62.6	61.8	62.8	2.9	63.3	65.0	64.4	64.2	1.3

Repeatability test	Injection 1	Injection 2	Injection 3	Mean	CV (%)
G (Handshake 1)	64.8	63.5	64.6	64.3	1.1

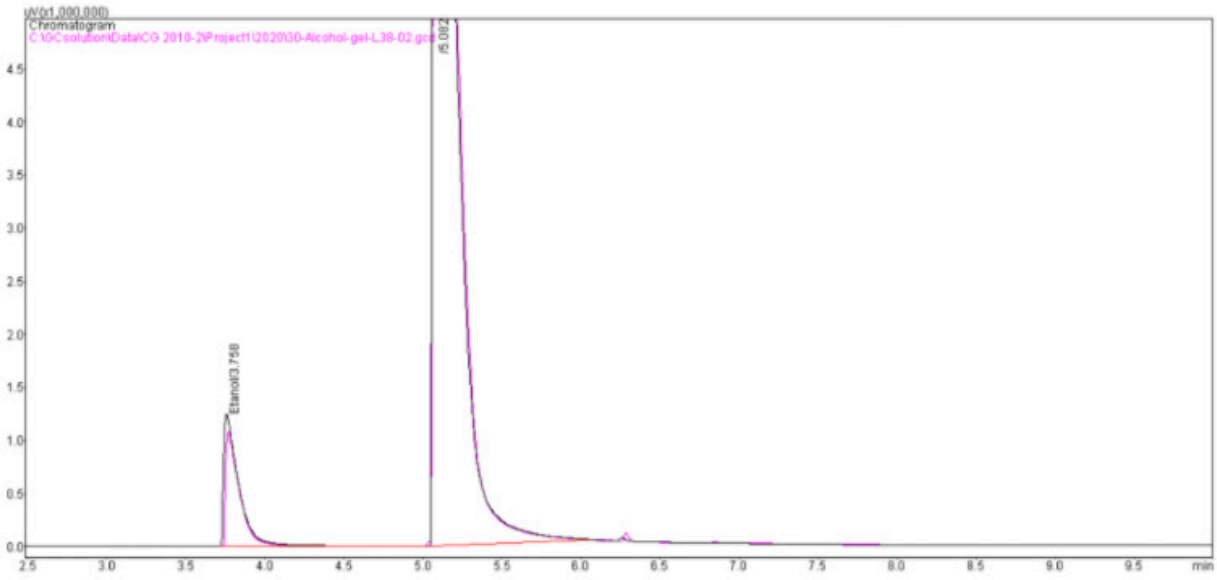
* CV = coefficient of variation

Source: prepared by the authors

All samples showed ethanol mass fractions ranging from 53.9 % to 65.3 % which is below the declared label concentration and the ANVISA's specifications for alcohol gel formulations (ANVISA, 2002). This difference may be attributed to the manufacturing process which requires long times of the homogenization step in order to incorporate the ethanol into the gel matrix. As ethanol is a quite volatile substance, if the formulation does not account for some losses due to volatilization along its production process, the final concentration can be rather lower than the theoretical value.

Figure 2 *shows the chromatographic profile of Sample D, where no interfering compounds can be seen, indicating a good specificity of the method. The coefficient of variation (CV) for the replicate analysis of Sample G (preparation handshake 1) shows also good repeatability of the GC system. As for the comparison between hand-shaking and the ultrasonic-bath (UB) shaking in sample preparation, the CV indicates that UB is the best recommended method as it increases the reproducibility of the results. A statistical analysis also indicates a difference in results obtained by the two preparation techniques (F test; p = 0.033). The difference can be explained by the viscous nature of the sample matrix which adheres to the inner surfaces of the glassware and makes it difficult to completely dissolve just by hand shaking. An ultrasonic bath has been proved an efficient tool for a sample preparation in analytical chemistry, because it promotes the homogenization and agitation of solutions in the molecular level, producing more reproductive results and improving recoveries in extraction applications (ASHLEY et al., 2001; Zuo et al., 2004).*

Figure 2- Chromatographic profile of Sample D (in red) compared to the calibration point 3 (in black).



Source: prepared by the authors

4 Conclusions

The herein developed method has shown good repeatability, reproducibility, and specificity within the application range which are important validation parameters to demonstrate the applicability of a given analytical method. From sample preparation to obtained results, the total time of analysis was 20 minutes, which is quite fast for chromatographic methods.

An ultrasonic bath seems to be a more reliable tool for sample homogenization than hand-shaking, which can be seen by the lower CV of samples prepared by the former technique. Moreover, it was also noticed that screw cap vials are better suited for sample weighing than volumetric flasks, as gel samples would stick to the inner surfaces of the flasks, making the addition of solvent difficult.

All samples showed ethanol concentrations below the minimum content specified by ANVISA for alcohol gel formulations. Regardless of the process, or formulation issues, it is important to implement a systematic monitoring strategy to assure that products being commercialized during the present pandemic event are safe to be used by the population and do not pose another threat for people's health.

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