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RAPID GAS CHROMATOGRAPHIC ANALYSIS OF ACTIVE INGREDIENTS IN ALCOHOL BASED HAND SANITIZERS PRODUCTS

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ABSTRACT

Demand for hand sanitizer is surging around the globe as the new coronavirus (convid-19) spreads. Sales of hand sanitizers and similar products have swelled across several international markets since the COVID-19 outbreak began in January. The virus, which originated in China, has now spread to more than 190 countries. As of March, 2020 over 400,000 confirmed cases and more that 20,000 deaths worldwide. Alcohol based hand sanitizer companies are struggling to supply the world demand while assuring the public that the product meet requirements set by regulatory agencies and the World Health Organization regarding the content of active ingredients. The most common alcohols used in hand sanitizers are methanol, ethanol, iso-propyl alcohol, and n-propanol or a mixture of thereof. The firms must use the most accurate method of analysis available for verification of the alcohol content in samples of the finished product before each batch is released for distribution. Discussed here is rapid method to analyze for alcohols in finished hand sanitizer products using gas chromatography with flame ionization detection (GC-FID). The method is aimed at assuring that the product complies with the alcohol % specification in the product label.

KEYWORDS: Gas chromatography, flame ionization detector (FID), hand sanitizers, ethanol, 2-propanol, quantitative analysis.

INTRODUCTION

Hand sanitizer is a liquid or gel generally used to decrease infectious agents on the hands. Hand washing with soap and water, in most situations, is the most effective way to remove viruses and other infection agents from the hands. However there are situations where soap and water is neither feasible nor readily available thus making alcohol based hand sanitizer an effective way at killing microorganisms and viruses. In some cases hand sanitizers are better tolerated than soap and water.

Alcohol has been used as an antiseptic at least as early as 1363 with evidence to support its use becoming available in the late 1800s. Alcohol-based hand sanitizer has been commonly used in Europe since at least the 1980s. The alcohol-based version is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system.

Products with 60% to 95% alcohol by volume are effective antiseptics. Lower or higher concentrations are less effective; most products contain between 60% and 80% alcohol. In addition to alcohol (ethanol, isopropanol or n-propanol), hand sanitizers also contain the following: additional antiseptics such as chlorhexidine and quaternary ammonium derivatives; spermicides such as hydrogen peroxides that eliminate bacterial spores that may be present in ingredients; emollients and gelling agents to reduce skin dryness and irritation; small amount of sterile or distilled water; and sometimes foaming agents, colorants or fragrances. According to the Food and Drug Administration, ethyl alcohol is the most common of ingredients found in hand sanitizers and is the same ethanol found in beer and wine.

The United States Food and Drug Administration recommend manufacturing hand sanitizers using only the following United States Pharmacopeia (USP) grade ingredients in the preparation of the product (percentage in the final product preparation) and in accordance with World Health Organization (WHO) recommendations (see Table 1).[1,2]

Table 1: FDA recommended ingredients and concentrations to be used in hand sanitizer manufacturing.

Ingredient	% in final product
Ethanol (USP or Food Chemical Codex (FCC) grade); or Isopropyl Alcohol	80 %, volume/volume (v/v) in an aqueous solution^ 75 %, v/v in aqueous solution
Glycerol	1.45 v/v
Hydrogen peroxide	0.125 v/v
Sterile distilled water or boiled cold water	-

^- *denatured according to Alcohol and Tobacco Tax and Trade Bureau Regulations in 27 CFR part 20.*

Hand sanitizer's studies had focused on determining their efficiency as antimicrobial, antibacterial, or its antivirus potential. A study has shown the effectiveness of hand sanitizers in removing influenza A (H1N1) virus[3]. In another study the antimicrobial efficacy of different hand sanitizers against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis* was evaluated as well as assessing and comparing the antimicrobial effectiveness among the different hand sanitizers [4]. A different study analyzed the biofilm-forming potential of clinical isolates of Staphylococcus aureus and Pseudomonas aeruginosa, and to assess antimicrobial activity of commonly used hand sanitizers in hospital and laboratory settings.[5]

The literature on the analysis performed on hand sanitizers to determine the concentration of their active ingredients is scarce. Gas chromatographic applications are used mainly for the analysis of blood ethanol or for the analysis of ethanol and other volatile compounds in alcoholic beverages. Applications to analyze alcohols, especially ethanol, involve the use of headspace gas chromatography with flame-ionization detection (HS-GC–FID). HS-GC-FID offers ease of automation, sensitivity, accuracy, and relative specificity [6]. To enhance specificity, many HS-GC–FID procedures use dual-column confirmation, which involves injecting a single sample and splitting to two chromatographic columns of sufficiently different polarity to change retention and elution order of ethanol and other volatiles of interest [7, 8]. However, several drawbacks are commonly associated with the use of headspace as a sample introduction procedure including the lack of sensitivity, need of an additional sample introduction equipment, reproducibility, and sample matrix effects.

Due to the high demand for the product a rapid method to determine the alcohol concentration is necessary. The aim of this study is to provide a fast gas chromatographic procedure using flame ionization detection to analyze for alcohols in hand sanitizer products. The method is fast, requires little sample preparation, and provides accurate and precise results that comply with regulatory agencies.

MATERIALS AND METHODS

A 1 mL of the hand sanitizer sample is dissolved in a 100 mL volumetric flask using deionized water. No further sample preparation is performed. 1 µL of the sample is injected into an Agilent 7890A gas chromatograph equipped with a flame ionization detector. Chromatographic conditions are shown in Table 2.

Table 2: Gas Chromatograph Operational Parameters.

Gas Chromatograph	Agilent
GC column	Restek Rxi-5HT 30 m fused silica capillary column; 0.32 mm ID x 0.25 µm thickness
Injector temperature	210^{o}C
Sample introduction	Split, 0.60
Oven temperature	32^{o}C, hold for 5 minutes, program at 10^{o}C/minute to 70^{o}C and hold.
Analysis time	8.8 minutes
Detector temperature	250^{o}C
Data management program	Chrom Perfect

To determine the concentration of the alcohol in the hand sanitizer product, the area of the alcohol, ethanol or 2-propanol, is compared to the area of a 10,000 ppm certified commercial standard ethanol solution (one point calibration). The 2-prpanol 10,000 ppm solution is prepared in the laboratory by diluting 1 mL of reagent grade 2-propanol into 100 mL with deionized water. The ratio of the area of the sample to the area of the standard multiplied by 100 gives the alcohol percentage in the hand sanitizer product. A reagent blank is analyzed before and after each batch of samples. To assess accuracy a sample duplicate is analyzed every 20 samples or per batch.

RESULTS AND DISCUSSION

A typical gas chromatogram is shown in Figure 1 for a 10,000 ppm ethanol standard solution and a hand sanitizer sample analyzed for ethanol (Figure 2). Figure 3 shows similar chromatograms for a 10,000 ppm 2-propanol standard solution and a hand sanitizer sample

analyzed for 2-propanol (Figure 4). The chromatograms show only one major peak corresponding to the alcohol active ingredient. To determine the capability to analyze for the alcohols in hand sanitizer samples four 500 ppm standard solutions were analyzed and their recoveries and standard deviation were calculated. Results for ethanol are shown in Table 3. In addition to determine the method detection limit (MDL) seven 100 ppm standard solutions were analyzed along with seven blanks. The MDL is calculated by multiplying the standard deviation by the t factor for six degrees of freedom (3.143). The results are shown in Table 4.

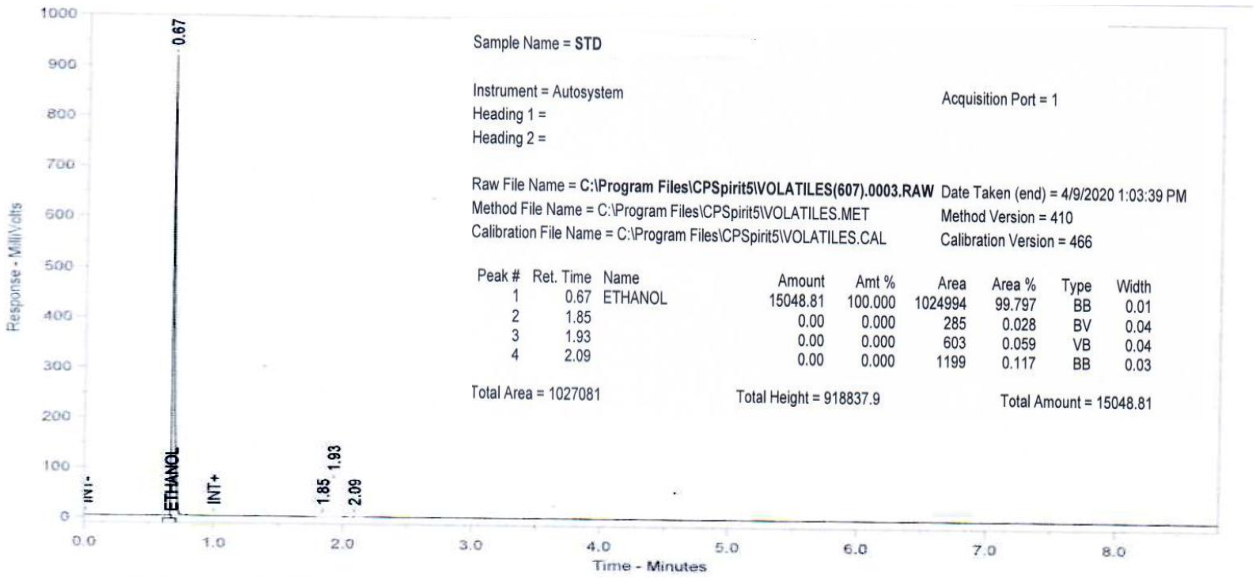

Fig. 1: Gas Chromatogram for a 10,000 ppm Ethanol Standard Solution.

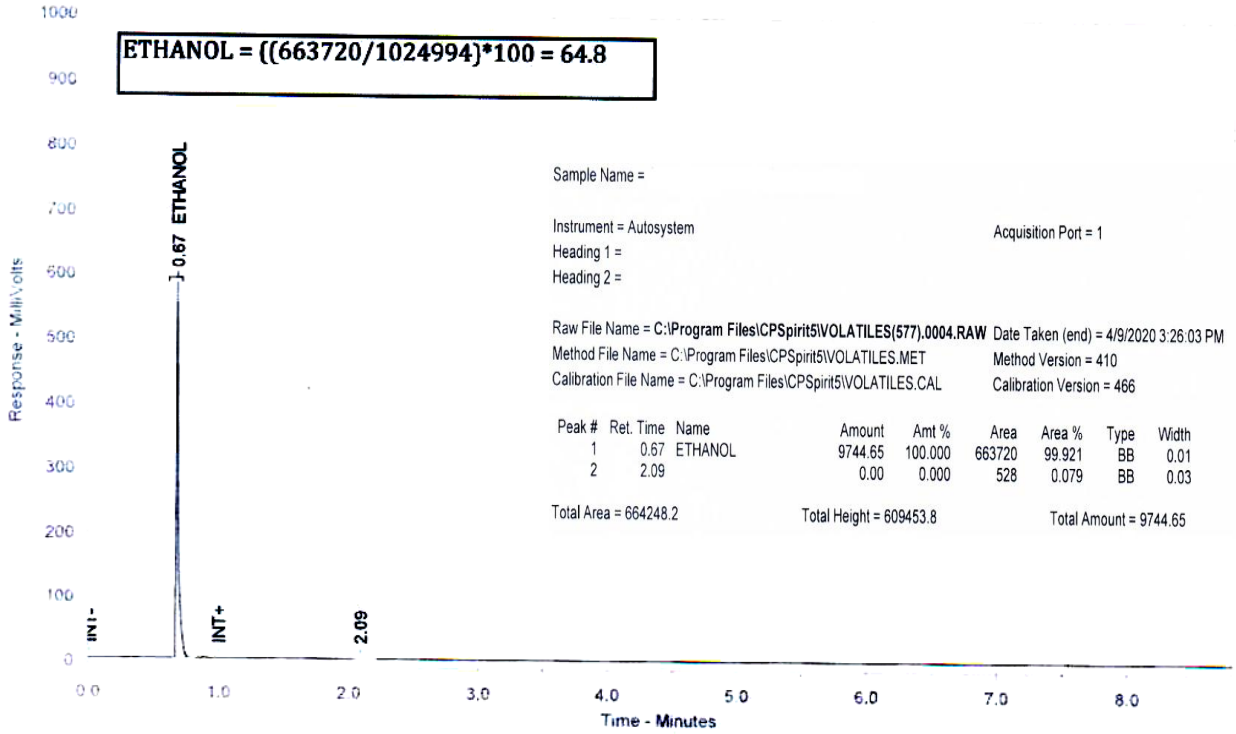

Fig. 2: Gas Chromatogram for a Hand Sanitizer Sample Analyzed for Ethanol.

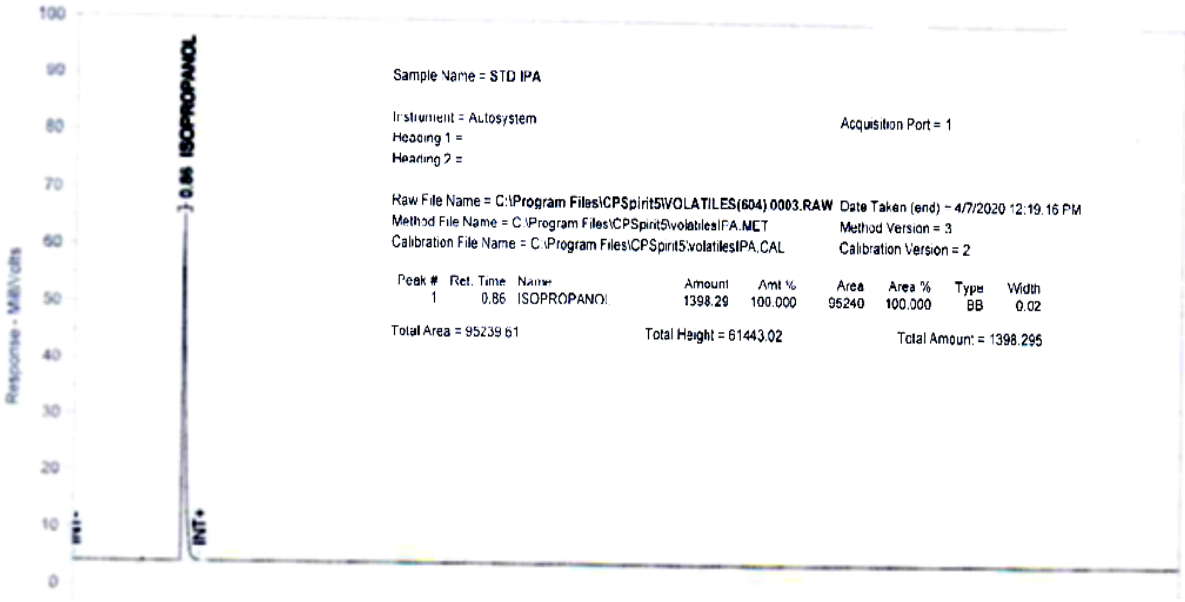

Fig. 3: Gas Chromatogram for a 10,000 ppm 2-Propanol Standard Solution.

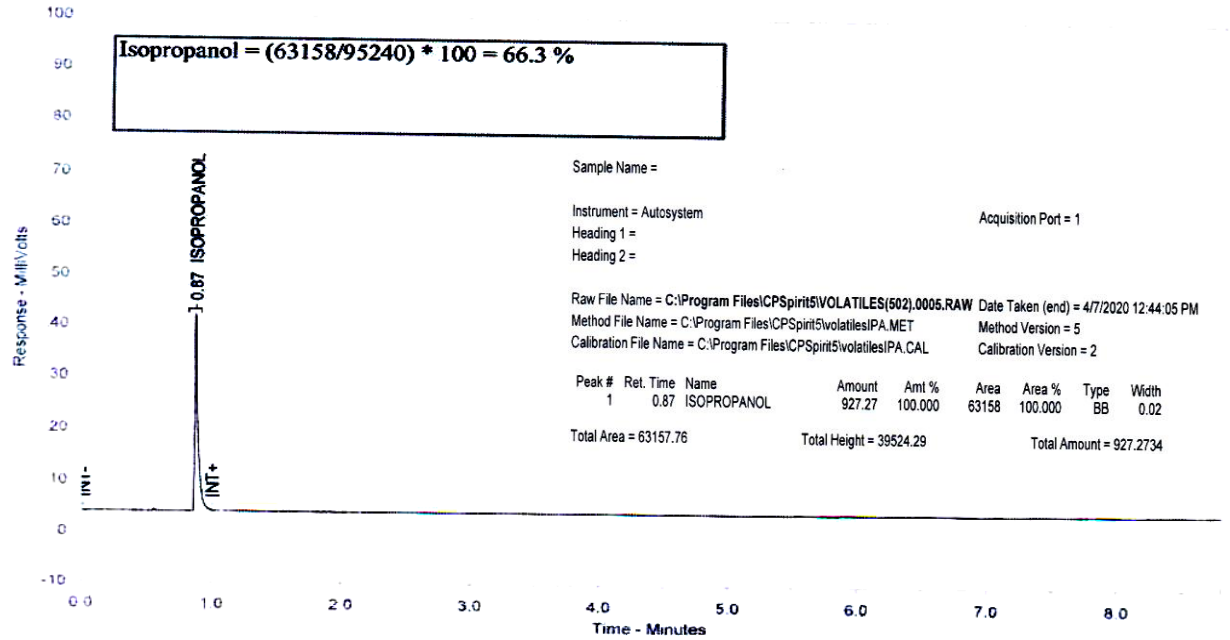

Fig. 4: Gas Chromatogram for a Hand Sanitizer Sample Analyzed for 2-Propanol.

Table 3: Initial Demonstration of Capability for Ethanol.

SPIKE LEVEL	IDC1	IDC2	IDC3	IDC4	RECOVERY				AVG. % REC.	STD. DEV.
					%	%	%	%		
500.0	492.9	447.8	449.2	478.4	98.6	89.6	89.8	95.7	93.4	4.46

All concentration values are in ppm.

To determine the method precision and uncertainty the peak area for nine injections of a 10,000 ppm certified standard solution was obtained. The percentages of the individual area to the average area were calculated. The average and standard deviation were also calculated. The expanded uncertainty of the method at the 95 % confidence level was determined as 2 times the standard deviation. Results for ethanol are shown in Table 5.

Table 4: Determination of the MDL.

MDL 1	MDL 2	MDL 3	MDL 4	MDL 5	MDL 6	MDL 7	MEAN	STD. DEV.
92.2	**93.8**	**110**	**108**	**85.2**	**84.3**	**89.8**	**95.4**	**10.3**
t-VALUE		MDL		MEAN/MDL		MEAN/MDL THEORETICAL		
3.143		32.3		2.95		3.10		

All concentration values are in ppm.

Table 5: Determination of Precision and Uncertainty for Ethanol in Hand Sanitizers.

Area of Ethanol Standard Solution (10,000 ppm)		(Area of Ethanol Standard 10,000ppm / Mean Area) x 100	
502783		99.6	
596594		118	
474265		94.0	
453677		89.9	
449413		89.0	
456445		90.4	
476548		94.4	
553186		110	
580270		115	
Mean:	504798	100	
Standard Deviation	10.35	Uncertainty (95 % confidence level)	22.7 %

Figure 5 shows a graph for the results obtained for the % ethanol in final product for several lots of a particular brand of hand sanitizer. The recommended product specifications are between 60 and 70 % ethanol. Results are compared to the control limits determined as the average $\pm$ 2 times the standard deviation for the % ethanol in the samples analyzed. Only one sample felt below the control limit and the method specification. The lot not meeting specifications needed to be reprocessed.

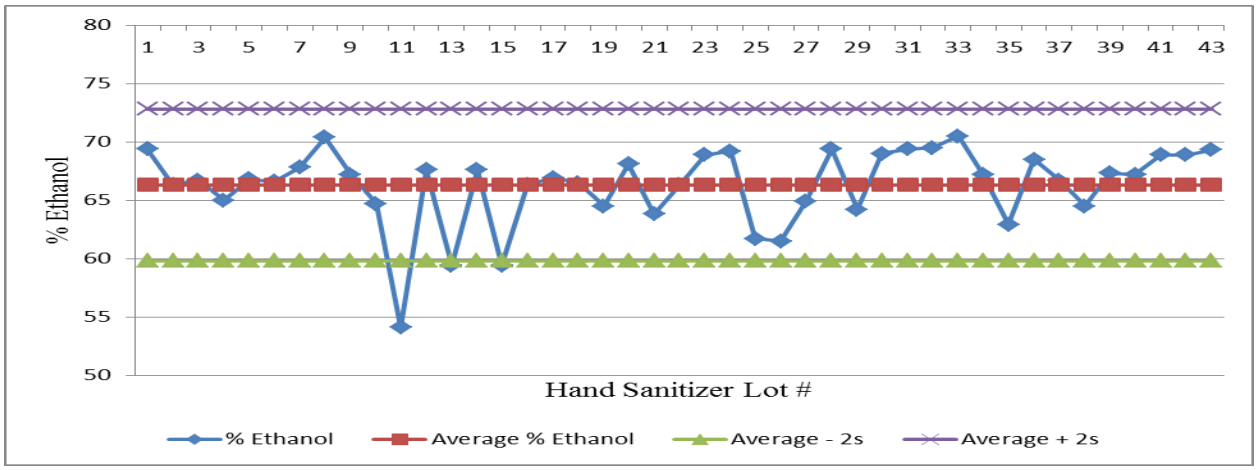

Fig. 5: % Ethanol in Finished Hand Sanitizer Product.

Table 6 shows the results from a different hand sanitizer product that utilizes 2- propanol as the active ingredient. On a limited number of samples, the average and standard deviation obtained for the percentage of 2-propanol in the hand sanitizer are similar to the percentage and standard deviation of ethanol in a larger data set.

Table 6: % of 2-Propanol in Hand Sanitizer Product.

Lot #	% 2-Propanol	Lot #	% 2-Propanol
3-21-2020	60.3	4-6-2020	66.3
4-4-2020	69.1	4-3-2020	67.4
Average	65.8		
Standard Deviation	3.83		

CONCLUSIONS

A rapid test has been developed to analyze for the active alcohol ingredient in hand sanitizer products. The test is aimed at determining if the product complies with the active ingredient specification on the product's label of 60 – 70 % alcohol. The test is based in gas chromatography with flame ionization detection. No sample preparation is required. Quantitation is based on the ratio between the area response of a 10,000 ppm standard alcohol solution and the area response of the hand sanitizer sample. Excellent precision is achieved.

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