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Efficacy and Toxicity of Hand Sanitizers: Detection of Impurities and Evaluation of Alcohol Content

By

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A thesis Submitted in partial fulfillment of the requirements

For the degree of Master of Science in Pharmaceutical Development and Management

School of Pharmacy

January 2022

LAU
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Lebanese American University

THESIS APPROVAL FORM

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Thesis Title: Efficacy and Toxicity of Hand Sanitizers: Detection of Impurities and Evaluation of Alcohol C

Program: Master of Science in Pharmaceutical Development and Management

Department: Pharmaceutical Sciences

School: School of Pharmacy

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To my loving parents, friends, and mentors

ACKNOWLEDGMENT

This project would not have been possible without the support of many people. Many thanks to my advisor, Dr. Yolande Saab, who read my numerous revisions and helped make some sense of the confusion. Also, thanks to my committee members, Dr. Rony Khonayzer, and Dr. Stephanie Mehanna, who offered guidance and support.

A special thanks to Dr. Cynthia El Hajjeh who was my research assistant and helped me throughout the process of the laboratory work.

A special thank you to Dr. Mroueh, without him this project wouldn't have seen the light.

I want to thank the Lebanese American University for trusting in me and this project.

And finally, thanks to my parents, and numerous friends who endured this long process with me, always offering support and love.

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ABSTRACT

Since the outbreak of Coronavirus Disease 2019 (CoVID-19), the World Health Organization has suggested that alcohol-based hand sanitizers should be used in the absence of soap and water to prevent the spread of SARS-CoV2.

This study aimed to assess the efficacy and toxicity of hand sanitizers produced and/or distributed on the Lebanese market. The study included 31 different brands of hand sanitizers that were analyzed using headspace gas chromatography- mass spectrometry to detect impurities and alcohol content. The impurities were identified based on a Mass Spectrometry database. Regression analysis was used to determine ethanol percentage. Accordingly, a toxicological review was conducted to assess the various risks associated with the usage of hand sanitizers.

Detected impurities mainly included but not limited to ethyl acetate, benzene, acetone, and acetal.

Among the ethanol-containing hand sanitizers, 71% contained less than 60% v/v alcohol not complying with the CDC and WHO recommendations which state that ethanol concentration should be at least 60%. Isobutanol and other non-recommended alcohols (methanol and 1-propanol) were also detected as contaminations.

Consumers should be aware that some hand sanitizers available on the Lebanese market are ineffective and include residues of harmful substances.

Keywords: Hand Sanitizer, COVID-19, Impurities, Headspace Gas Chromatography, Alcohol Content.

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List of Abbreviations and/or Symbols

COVID-19	Coronavirus disease 2019
SARS-CoV	Severe Acute Respiratory Syndrome
MERS-CoV	Middle East Respiratory Syndrome
CDC	Centers for Disease Control and Prevention
ABHS	Alcohol-based hand sanitizers
US FDA	United States Food and Drug Administration
WHO	World Health Organization
EPA	United States Environmental Protection Agency
QACs	Quaternary ammonium compounds
MS	Mass spectrometry
GC	Gas chromatography
NIST	National Institute of Standards and Technology
LC-MS	Liquid Chromatography – Mass spectrometry
GC/MS	Gas chromatography/Mass spectrometry
A_S	Area of the Sample
A_{IS}	Area of the Internal Standard

CEN	European Committee for Standardization
ASTM	American Society for Testing and Materials
FCC	The Food Chemicals Codex
USP	United States Pharmacopeia
IS	Internal Standard
TGE	Technical Grade Ethanol

Chapter 1
Introduction

Staring December 2019, cases of significant sickness inflicting the respiratory system and causing deaths were initially reported in Wuhan, China (Keni et al., 2020). Thereafter, the number of cases started to increase noticeably not only in China but worldwide (Muralidar et al., 2020). Novel coronavirus is the confirmed cause of the disease (Ahn et al., 2020). The total number of coronaviruses cases confirmed as of January 12 2022, is 314,405,420 confirmed human cases reported to the WHO with a death toll of 5,523,758 (fatality rate of 8.5%) (Ritchie et al., 2021). Patients with mild disease may experience cough, sore throat, high temperature, diarrhea, headache, muscle or joint pain, or loss of smell and taste; whereas patients with severe presentation of the disease may experience breathlessness, confusion, pneumonia, high temperature (above 38°C) (Struyf et al., 2020).

In Lebanon, the total number of coronavirus cases until August 19 2021, is 588,578 cases (*Lebanon COVID*, n.d.). According to Reuters COVID-19 tracker, Lebanon is reporting 1,458 new infections on average each day, 30% of the peak — the highest daily average reported on January 16. ("Lebanon," n.d.).

Diverse modes of transmission were reported for SARS-CoV-2, including aerosol, surface contamination, direct contact, and the fecal-oral route; yet their relative significance is still being under investigation. Respiratory droplets are predominantly the common route of coronavirus disease transmission (Harrison et al., 2020).

The manifestation of the novel virus, SARS-CoV-2, has created exceptional defies to public health around the world. Till date, there is no approved medication that has gone through controlled studies and showed an effect against COVID-19. The most successful method to manage the virus is the prevention of its spread. The approaches, presently, are mainly supportive and preventative; they are intended to reduce transmission. Prevention and control measures include early screening, early diagnosis, isolation, hand hygiene, personal protective equipment, social distancing, and vaccination (GÜNER et al., 2020). A simple and yet effective method to decrease transmission of infections in public or in healthcare settings is hand hygiene. During the current COVID-19 pandemic, there has been a significant emphasis on the importance of hand sanitization both in the health care sector and the public (Alzyood et al., 2020).

According to the study "Hand Hygiene Compliance Rate During the COVID-19 Pandemic" conducted at The University of Chicago Medical Center (UCMC), monthly hand hygiene compliance across all units was 54.5%. The compliance has augmented during the pandemic to reach a daily peak of 92.8% on March 29, 2020 in all hospital units. It was proposed that compliance had increased due to the increased awareness and prominent use of hand hygiene with the surge of the pandemic (Makhni et al., 2021).

If soap and water are not readily available, frequent and effective handwashing is one of the many ways applied to prevent the spread of this virus (Lotfi et al., 2020). The CDC recommends the use of hand sanitizers with at least 60% alcohol (often listed on the label as ethanol, ethyl alcohol, isopropanol, or 2-propanol). The use of hand sanitizers containing less than 60% alcohol is not advised. Moreover, the use of alcohol-free

sanitizers or alcohol-based hand sanitizers packaged in a container that resembles a food or beverage container is not advocated (CDC, 2020).

1.1 Formulation

Alcohol-based hand sanitizers have become a more popular substitute to conventional handwashing with soap and water. A variety of hand sanitizers are present with different combinations of ingredients and modes of delivery (Abuga & Nyamweya, 2021). Gels, foams, creams, sprays, and wipes are different forms of delivery systems for hand disinfectants (Jing et al., 2020).

The most widely used modes of delivery for hand sanitizers are low viscosity liquids and gels for the general people whereas in hospitals and other health facilities they are normally delivered in dispensers (Golin et al., 2020). Liquid hand sanitizers, are usually less viscous than the gels, they are portable and broadly available. Drawbacks of liquid formulations are the chance of spillage, dose metering, and the alcohol odor can be more obvious (Abuga & Nyamweya, 2021). Gel formulations have the same advantages as the liquid ones but with better dose metering and handling characteristics. The cons of gel delivery systems are spillage, the antimicrobial onset of action may not be as rapid as that of liquids, and a longer drying time than liquids (Abuga & Nyamweya, 2021). When in it comes to foam delivery systems, they are more expensive than liquids or gels but with diminished spillage. Spray delivery systems benefit from a controlled dose metering but the product may be lost to the atmosphere causing a potential risk for inhalation. In addition to that, sprays have a high flammability risk if incorrectly used (Abuga & Nyamweya, 2021). The wipes provide a convenient portable delivery mode with dose

metering advantage. However, they need to be designed to deliver adequate amount of alcohol in each wipe (Abuga & Nyamweya, 2021).

The foams and gels delivery system did not show a considerable difference in trials for the antimicrobial activity, however, the application volume and drying time had an intense effect on their efficacy. In another study, Singh and colleagues suggested that gel preparations may not replace alcohol-based liquid hand sanitizers due to their briefer application time (<30 seconds), which diminishes their effectiveness. Even though, gels decrease the skin irritation and dryness caused by the liquid preparations. (Singh et al., 2020).

1.2 Sanitizers types: Alcohol-based and Alcohol-free

Hand sanitizers can be commonly classified into two groups: alcohol-based or alcohol free. While the first type contains one or more type of alcohol, with or without excipients, the second type does not contain alcohol (Jing et al., 2020). The use of alcohol-free sanitizers against coronavirus is not recommended by the CDC.

The WHO clarified that alcohols showed a significant activity against gram-positive bacteria, gram-negative bacteria, enveloped viruses, non-enveloped viruses, mycobacteria, and even fungi (Gold et al., 2021). The main components of alcohol-based hand sanitizers (ABHS) are an alcohol or an assortment of alcohols and water. Other ingredients may be present as well serving different functions.

The compliance rates at which the alcohol-based hand sanitizers successfully decrease or eliminate the bacterial/viral load varies between different formulations and preparations (Singh et al., 2020).

When alcohol-based hand sanitizers are used, a number of pathogens can be destroyed without the need of water or drying.

In the fight against SARS-CoV-2, ABHS have emerged as a crucial tool. The most widely used alcohols in ABHS are ethanol and isopropanol (2-propanol) (Dhama et al., 2021). ABHS are usually made up of water and a variety of additional substances like emollients, moisturizers, and perfumes (Leslie et al., 2021). Although the alcohol concentration has been the primary focus of ABHS performance, additional substances and auxiliary factors play a key role in their efficacy, safety, and long-term usability (Leslie et al., 2021).

The final product safety and efficacy are influenced by these different constituents and must be taken into consideration. Two formulations have been established by the WHO based on either ethanol (80% *v*/*v*) or isopropanol (75% *v*/*v*) with glycerol (1.45% *v*/*v*) and hydrogen peroxide (0.125% *v*/*v*). According to the WHO, these formulations cover a broad spectrum of microorganisms (Abuga & Nyamweya, 2021).

According to Kratzel et al., WHO-based formulations have a broad spectrum of antibacterial action, including efficacy against SARS-CoV-2. Firms that regularly produce commercial ABHS usually use proprietary formulas. According to their study, WHO-recommended formulations effectively inactivated SARS-CoV-2, supporting their usage in healthcare systems and viral outbreaks. (Kratzel, 2020).

Isopropanol is considered to have an improved activity towards bacteria whereas ethanol is more powerful against viruses (Gold et al., 2021). Nonetheless, the percentage concentrations of the alcohol and the physical properties of the object microorganism impact the efficacy of ABHS (Golin et al., 2020). Isopropanol is less effective against the hydrophilic viruses like polioviruses since it is more lipophilic than ethanol. SARS-CoV-

2 is more sensitive to isopropanol since it is a lipophilic enveloped virus (Singh et al., 2020).

The optimal solutions should contain 60 to 80% alcohol, noting that at higher concentrations the efficacy will be lower. This contradiction is clarified by the fact that proteins will not be straightforwardly denatured without a certain amount of water. According to the United States Food and Drug Administration (US FDA), CDC, and the WHO, concentrations of 60-95% (v/v) are suitable for eliminating microorganisms including SARS-CoV-2 (Villa & Russo, 2021).

Table 1. Alcohol- and alcohol-free hand sanitizers, and commonly used excipients (Jing et al., 2020).

Type of Hand Sanitizer	**Common chemicals present**	**Examples**
Alcohol based	Alcohol (60-95% v/v)	Ethanol, isopropanol, n-propanol
	Hydrogen peroxide	
Alcohol free	Antiseptics	Chlorhexidine, chloroxylenol, iodine/iodophors, quaternary ammonium compounds, triclosan
Common excipients to both types		Glycerin, fragrance, colorant

1.3 Ingredients in hand sanitizers

1.3.1. Glycerol

As previously mentioned, the alcohol-based hand sanitizers consist of an alcohol or a mixture of alcohols. Hydrogen peroxide could be also added. Most of the vegetative

forms of bacteria, fungi, and enveloped viruses are covered by the broad spectrum of the alcohols. Conversely, they are ineffectual against bacterial spores, that is why hydrogen peroxide (3%) may be added solve the problem (Jing et al., 2020).

Glycerol is incorporated in the formulation as a humectant to favorize the tolerability of the product. In addition, further humectants or emollients can be added to the mixture in the purpose of skin care given that they are inexpensive, available, miscible in water and alcohol, nontoxic, and hypoallergenic (WHO, 2009). In order to decrease the stickiness of the hand sanitizer, the percentage of glycerol may be lowered.

1.3.2 Chlorhexidine

Alcohol-free hand sanitizers consist of antiseptics such as chlorhexidine. Chlorhexidine is poorly water soluble but its gluconate form has good water solubility. It exerts its antimicrobial action by the disruption of the cytoplasmic membrane. consequently resulting in the precipitation of the cellular components (Golin et al., 2020). Chlorhexidine's onset of action is slower than that of alcohols (World Health Organization, 2009). Chlorhexidine is highly active against Gram-positive bacteria, slightly less active against Gram-negative bacteria and fungi, and minimal activity against mycobacteria (Bednarek et al., 2021). A notably better residual activity is created when low concentrations (0.5–1%) of chlorhexidine are added to alcohol-based mixtures (Bednarek et al., 2021). Chlorhexidine has a generally a good safety record when employed as indicated (World Health Organization, 2009).

1.3.3 Chloroxylenol

Chloroxylenol, another possible constituent of alcohol-free constituents, is an antimicrobial agent that exerts its action by inactivating the bacterial enzymes and

altering the cell walls (World Health Organization, 2009). It has been employed as a preservative in cosmetics and active antimicrobial substance in soaps (World Health Organization, 2009). It has shown a relatively good *in vitro* activity against Gram-positive bacteria and a partial activity against Gram-negative bacteria. Additionally, chloroxylenol has demonstrated as well an activity against Pseudomonas Aeruginosa (Berthelot et al., 2006).

1.3.4 Iodine

Iodine has been commonly as an antiseptic since 1800s, but it has been replaced widely by iodophors as active ingredients due to irritation and discoloration of skin caused by iodine-containing compounds (Bednarek et al., 2021). Iodine inactivates the microorganism's cells by creating complexes with amino acids and unsaturated fatty acids leading to an impaired protein synthesis and alteration of cell membrane (Eggers et al., 2015). Elemental iodine, iodide or triiodide, and a polymer carrier (complexing agent) of high molecular weight can be used as iodophors (Eggers et al., 2015).

1.3.5. Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) are the most widely present class of disinfectants on EPA's List N (when used according to labeled directions, EPA expects products on List N will kill all strains and variations of the coronavirus SARS-CoV-2 (US EPA, 2020). QACs consist of a nitrogen atom linked to four alkyl groups that differ in structure and complexity. Examples of QACs are alkyl benzalkonium chlorides, benzethonium chloride, cetrimide, and cetylpyridium chloride (Ogilvie et al., 2021). QACs exert their action by adsorption to the cytoplasmic membrane resulting in leakage

of low molecular weight cytoplasmic constituents. They exhibit an activity against lipophilic viruses (World Health Organization, 2009).

1.3.6 Triclosan

Triclosan is found in many products used to decrease or prevent bacterial spread. It can also be found in FDA-regulated products such as soaps, body washes and toothpastes. It can be found in non-FDA regulated products like clothing, kitchenware, toys and furniture (Commissioner, 2020b). The mode of action of triclosan is outlined by its effect on the cytoplasmic membrane and the synthesis of RNA, fatty acids, and proteins. Latest findings imply that the antibacterial activity of triclosan is attributed largely to binding to the active site of enoyl- acyl carrier protein reductase (World Health Organization, 2009).

1.4 Ingredients in Product Preparation: Additional Considerations

Alcohol (ethanol) generated utilizing fermentation and distillation procedures, that is commonly employed in the production of consumable products, and produced in a facility that manufactures consumable goods, could be employed for use in hand sanitizer if it meets interim impurity requirements in Table 2. Alcohol derived from synthetic processes may be used only if it meets USP or FCC guidelines (FDA, 2021).

Alcohol generated in facilities that ordinarily produce fuel or technical-grade alcohol (ethanol) may be considered for use in hand sanitizer if the following conditions are met:

- No further additives or chemicals have been added to the ethanol, which was created using fermentation and distillation procedures similar to those used in the production of consumable items.

- If the alcohol meets the USP or FCC grade criteria, or if the conditions in *Table 2.* are met.
- The alcohol has been checked for any other potentially dangerous contaminants that are not listed in the USP or FCC criteria but could be present due to the manufacturing environment.

1.5 Toxicology

Amid the widespread of Coronavirus disease, consumers are increasingly using hand sanitizers, which can cause an increased risk of accidental toxicity among users. The unavailability of hand sanitizers at times has led to an upsurge in the occurrence of falsified alcohol-based hand sanitizers. Falsification occurred at the level of the illegitimate addition of methanol or other impurities to hand sanitizers and the production of hand sanitizers with an alcohol concentration of less than 60 %. The presence of falsified alcohol-based sanitizers could pose a public health risk (Jairoun et al., 2021).

Since the start of the public health emergency, FDA has seen an increase in reports of significant adverse events (including some associated or reported deaths) linked to alcohol-based hand sanitizer intake (both unintentional and purposeful). If the following conditions are met, there have also been multiple complaints of cutaneous toxicity linked to these products, according to the FDA. FDA reported a number of hand sanitizer items that were advertised to contain ethanol but tested positive for methanol contamination in the spring of 2020; usage of some of these hand sanitizer products resulted in serious adverse outcomes consistent with methanol poisoning (FDA, 2021).

When mishandled these formulations might shift to a toxic hazard to human health and the environment. At the beginning of 2020, the American Association of Poison Control

Center reported 9504 alcoholic hand sanitizer exposure cases in children under the age of 12 years and it was noted that even a minimal quantity of alcohol is capable of causing alcohol poisoning in children resulting in confusion, vomiting and drowsiness and sometimes more serious cases such as respiratory arrest and death (Hakimi & Armstrong, 2020).

The FDA issued in the FDA guidance for hand sanitizer products a list of impurities classified as Level 1 or Level 2, relying on the toxicity of the impurities in the sanitizer, where Level 1 impurities are considered to be more toxic. Table 2, displays the Level 1 and Level 2 impurities and their limit values. (Analysis of Impurities in Alcohol-Based Hand Sanitizers by GC-MS | SHIMADZU (Shimadzu Corporation), n.d.).

To be sold as USP-grade ethanol, solutions must contain less than 300 μg/mL as total sum of level 1 impurities (Table 2). When the sum of all level 1impurities in a sample exceeds the interim limit of 300 ppm in fuel or technical grade ethanol, all individual impurities are identified and meet the interim limits in level 2 (Tse, Nelson, et al., 2021). In reaction to this pandemic, regulatory organizations including the FDA and Health Canada eased standards, allowed alcohols with higher levels of particular impurities than pharmaceutical-grade ethanol to be used as hand sanitizers (*Worrying Levels of Some Impurities in Hand Sanitizers, Chemists Find*, n.d.).

Table 2. FDA Listed Impurities and Detectable Concentration Ranges

		Compound Name	Interim Limit Listed in FDA Guidances (ppm)	Concentration Ranges for this Method (µg/mL)
Impurity	Level 1	Methanol	NMT 630	15.82 - 791
		Benzene	NMT 2	0.044 - 2.2
		Acetaldehyde	NMT 50	1.178 - 58.9
		1,1-diethoxyethane (acetal)	NMT 50	1.245 - 62.25
	Level 2	Acetone	NMT 4400	15.8 - 790
		1-Propanol	NMT 1000	16.08 - 804
		Ethyl Acetate	NMT 2200	18.04 - 902
		2-Butanol	NMT 6200	16.16 - 808
		Isobutanol	NMT 21700	16.06 - 803
		1-Butanol	NMT 1000	16.2 - 810
		3-Metyl-1-Butanol	NMT 4100	16.18 - 809
		Amyl Alcohol	NMT 4100	16.22 - 811

The FDA is warning consumers and health care professionals that hand sanitizer products branded as containing ethanol (also known as ethyl alcohol) but have tested positive for methanol or 1-propanol contamination have increased dramatically (FDA, 2021).

1.5.1 Methanol toxicology

Methanol, often known as wood alcohol, is a poisonous chemical that can be life-threatening if consumed or absorbed through the skin (FDA, 2021). At least 77 hand sanitizers have been discovered by the US Food and Drug Administration (FDA) as containing unsafe quantities of methanol, a toxic chemical that can induce nausea, nerve damage, and blindness when absorbed through the skin and causes death if consumed orally (Lanese, 2020).

1.5.2 Benzene toxicology

Moreover, according to data given by fuel ethanol manufacturers who produce ethanol by fermentation and distillation, at least some of their products contained dangerous substances such as gasoline and benzene, which is a proven human carcinogen (cancer-causing agent) (McCue, n.d.).

Furthermore, FDA has received information indicating that fuel ethanol products contain high quantities of acetaldehyde, which appears to be genotoxic when in direct contact with tissues.(McCue, n.d.).

1.5.3 Inactive substances toxicology

Beyond the active ingredients, hand sanitizers contain inactive substances, which may be specified separately on the label. These inactive components may pose a health concern as well (Center for Drug Evaluation and Research, 2020). Artificial dyes are made from petroleum and can be found in meals, cosmetics, and personal care goods like hand sanitizers. Dyes are one of the few terms on the ingredient list that can be identified and pronounced on the packaging of conventional items. Blue 1, Red 40, and Yellow 5 are some examples. Side effects such as accelerated dermal absorption in damaged skin,

allergic responses, and hyperactivity. Unfortunately, there are still significant data gaps on artificial dyes, therefore, using the cautious approach (i.e., avoiding substances for which there is insufficient data), may be the best approach.

PEGs are thickening, softening, and penetration-enhancing polyethylene glycol compounds. Hand sanitizers are only one example of where they might be found. Ethoxylation produces PEG compounds, which indicates they're probably polluted with carcinogens like ethylene oxide and/or 1,4 dioxane. These pollutants will not be indicated on the ingredient label because they are not purposely added substances, but rather by-products of the production process (Cann, 2021).

1.6 Recommendations

The standards issued by the American Society for Testing and Materials, the European Committee for Standardization (CEN) and FDA are the two most extensively used recommendations for testing and regulation of hand disinfectants (ASTM).

Thus, during the public health emergency posed by COVID-19, FDA published guidelines to communicate its policies for the temporary manufacture or compounding of alcohol-based hand sanitizer products and ethanol for use in alcohol-based hand sanitizer by some enterprises and pharmacies (temporary policies) (FDA, 2021). In addition to that, the Food and Drug Administration (FDA) announced a rule to guarantee that over-the-counter (OTC) hand sanitizers are safe and effective for consumers who use them. Certain active chemicals are prohibited from being used in OTC hand sanitizers, also known as topical consumer antiseptic rub products, that are designed for use without water and are marketed under the FDA's OTC Drug Review (FDA, 2019).

Moreover, the infection control guidelines recommend using alcohol-based hand rub solutions to inactivate coronavirus that causes severe acute respiratory syndrome (Kratzel, 2020). (Kratzel, 2020). The CDC recommends using alcohol-based sanitizers that contain at least 60% ethanol or 70% isopropanol (Kratzel, 2020).

In addition to that, according to the FDA, the hand sanitizer can be manufactured using only the following ingredients in the preparation of the product:

- Alcohol (ethanol) that is not less than 94.9% ethanol by volume or United States Pharmacopeia (USP grade) Isopropyl Alcohol (IPA)
- Glycerin (glycerol) USP or Food Chemical Codex (FCC) (also known as “food grade”)
- Hydrogen peroxide
- Sterile water (McCue, n.d.)

Chapter 2

Objectives and Aims

The demand and consumption of hand sanitizers has increased thus leading to a shortage in these products at all levels. The purpose of this study is the evaluation of finished hand sanitizer products present on the Lebanese market and the determination of alcohol percentage. Detection of the targeted impurities enumerated in the FDA was carried out in the study using headspace gas chromatography coupled to mass spectrometry detector.

Objective 1. Determination of the impurities present in the hand sanitizers.

- Identification of the impurities based on mass spectrometry database.
- Semi-quantification of the impurities by comparing peak area of an impurity in a sample chromatogram to the peak area of an internal standard.

Objective 2. Determination of percentage alcohol preset in the hand sanitizers.

- Quantitation of the alcohol present in the samples is performed by using external calibration curve.

Objective 3. Performance of a toxicological evaluation on the detected impurities

Chapter 3

Materials and Methods

3.1 Evaluation Method

The need for methods to determine the quality and safety of the hand sanitizers has recently became of great significance. As a result, the FDA has set a "Direct Injection Gas Chromatography Mass Spectrometry (GC-MS) Method for the Detection of Listed Impurities in Hand Sanitizers" for quality assessment of hand sanitizer (*Direct Injection Gas Chromatography Mass Spectrometry (GC-MS) Method for the Detection of Listed Impurities in Hand Sanitizers*, n.d.).

The National Institute of Standards and Technology (NIST), has as well developed four quantitative methods for the evaluation of finished hand sanitizer products. These methods include gas chromatography with flame ionization detection (GC-FID), liquid chromatography with ultraviolet absorbance detection (LC-UV), quantitative nuclear magnetic resonance spectroscopy (qNMR), and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) (Bedner et al., 2021).

In this study, the qualitative and quantitative analysis of impurities in alcohol-based sanitizers was realized using headspace GC/MS, in compliance with the FDA hand sanitizer analysis method. The analysis parameters designated in this article can be employed to detect impurities in alcohol-based sanitizers. Additionally, the listed impurities by to the FDA, can be identified over a wide concentration range. Other impurities can also be detected using the Scan and the SIM mode (*Analysis of Impurities in Alcohol-Based Hand Sanitizers by GC-MS | SHIMADZU (Shimadzu Corporation)*, n.d.).

According to Dai and collegues, headspace sampling is more reliable than liquid injections of solutions. The headspace GC is preferably used because of its ability to quantify individual solvents (Dai et al., 2010).

Headspace technique has been used since the late 1950s, it is a sample preparation method to identify volatile compounds in solid and liquid samples. It is a simple and very clean method of introducing volatile analytes into the GC machine and require little maintenance (Penton, 2002). The headspace sampling technique is basically a separation technique based on the concept in which volatile material may be extracted from a bulkier sample matrix and injected into a gas chromatograph for evaluation (Tipler, 2014). Separation will depend on the compound volatility; the more volatile compounds will lean to move towards the gas phase or headspace. On the contrary, components that stay in the liquid phase are less volatile (Tipler, 2014.).

Headspace gas chromatography is a technique that can be potentially used for the analyses of volatile substances; it has practical applications in food sciences, forensic sciences, clinical chemistry, toxicology, and environmental sciences (Seto, 1994). Headspace GC is thought to be a better method for the determination of residual solvents in nonvolatile materials such as drug compounds and drug products, because only volatile components are injected into the GC system, and hencethe risk of contamination is reduced, and the system's robustness is improved (*A LEAN Approach for the Determination of Residual Solvents Using Headspace Gas Chromatography with Relative Response Factors*, n.d.). The headspace GC/MS method is more advantageous over the conventional headspace GC method that uses the flame ionization detection (Xiao et al., 2014). Viewing that laboratories are currently turning more towards GC/MS

currently, the method employed in this article would be of good value for clinical and health purposes (Xiao et al., 2014).

3.2 Analytical system and conditions

HS-20 headspace – GCMS-QP2020 NX (Shimadzu Corporation, Japan) were employed in this work. The chromatograph is equipped with split/spitless injector (SPL) and mass spectrometry detector (MS). Helium was used as the carrier gas at constant linear velocity mode of 43.4 cm/s. PoraBOND Q (25 m x 0.25 mm I.D., d.f. = 3 μm) is the column employed. The initial gas chromatograph oven temperature was 50°C (held for one minute) and increased to 250°C (held for nine minutes) at rate of 20°C per minute. The headspace sample is connected to the GC via fused silica capillary transfer line through the split/splitless injector. The headspace oven temperature was set at 85°C. The headspace sample line and transfer line temperature were set at 150 and 160°C, respectively. The mass spectrometry detector was operated in scan/SIM mode and the analysis was done over a mass range of m/z from 24 to 200. The analytical conditions details are illustrated in *Table 3*.

Table 3. HS-GC-MS analytical conditions for analysis of impurities in hand sanitizers

Analytical System	
GC-MS	GCMS-QP2020 NX
Headspace Autosampler	HS-20
Column	PoraBOND Q (25 m x 0.25 mm I.D., d.f. = 3 μm)
HS Parameters	
Oven Temperature	85°C
Sample Line Temperature	150°C
Transfer Line Temperature	160°C
Injection Time	1 min
Pressurizing Gas Pressure	90 kPa
Equilibrating Time	10 mins
Shaking Level	2
GC Cycle Time	30 mins
GC Parameters	
Column temperature	50°C
Injection Mode	Split mode Split ratio 50
Carrier Gas	Helium
Gas Flow Condition	Constant linear velocity mode Linear velocity 43.4 cm/s
Oven Temperature Programming	50°C (1 min) → 20°C/min to 250°C (9 mins)
Column Flow	1.19 mL/min
Injection Volume	1.0μL
MS Parameters	
Ion source temperature	230°C
Interface temperature	300°C
Measurement mode	Scan/SIM
Scan range	*m/z* 24 to 200
MSD Solvent Delay	No solvent delay
MSD m/z Settings	Start time (0 min) End Time (20 min) Acquisition mode (SIM/Scan)

Table 4. MSD m/z settings

Time (min)	Acquisition mode	Channel	SIM ions (target or identity) m/z	Compound Name
0-20	SIM	1	78	Benzene
		2	74	Isopropanol
		3	51	Benzene
		4	43	Glycerin
		5	61	Glycerin
		6	31	Methanol
		7	29	Methanol
		8	44	Glycerin
		9	32	Methanol
		10	30	Methanol
		11	46	Ethanol
		12	45	Ethanol
		13	31	Ethanol
		14	27	Ethanol
0-20	Scan		24-200	-

3.3 Equipment/Instrument

Among the equipment and instruments employed was the HS-20 headspace – GCMS-QP2020 NX machine to perform the experiments. The column used in the study was PoraBOND Q (25 m x 0.25 mm I.D., d.f. = 3 μm). Solutions were measured and transferred using eppendorf Pipets and pipet tips. Conical tubes served as containers for the final diluted solutions of hand sanitizers. A 100 mL beaker was utilized to measure solvents and solutions. Finally, a 20 mL Headspace vial w/ 20mm AL Crimp Cap & Silicone/PTFE Septa was utilized for the assessment of samples using HS-GCMS.

3.4 Chemicals

LC-MS grade water (Lot No. 1714121, Code: X/0112/17) was kindly provided by Fisher SCIENTIFIC, UK. Acetonitrile >= 99.9% (Lot. No. 1872272, Code: A/0638/17). Absolute ethanol HPLC grade (Lot. No. 1852958, Code: E/0665DF/17) was purchased from Fisher SCIENTIFIC UK. 1-Butanol 99%, extra pure (Lot. No. A0324323, Code: 107690025) was purchased from Acros Organics.

3.5 Hand sanitizer samples

The study was performed on hand sanitizers found and manufactured in Lebanon. The hand sanitizers were provided by the School of Pharmacy at the Lebanese American University. The samples were collected from different pharmacies and supermarkets on the Lebanese market. The study included 31 different brands of hand sanitizers manufactured and marketed in Lebanon. The hand sanitizers' formulation varied between gels and sprays. 38.7% of the samples in the study were sprays and 61.2% of the samples were gels (Figure1). The samples used in this research were all ethanol-based.

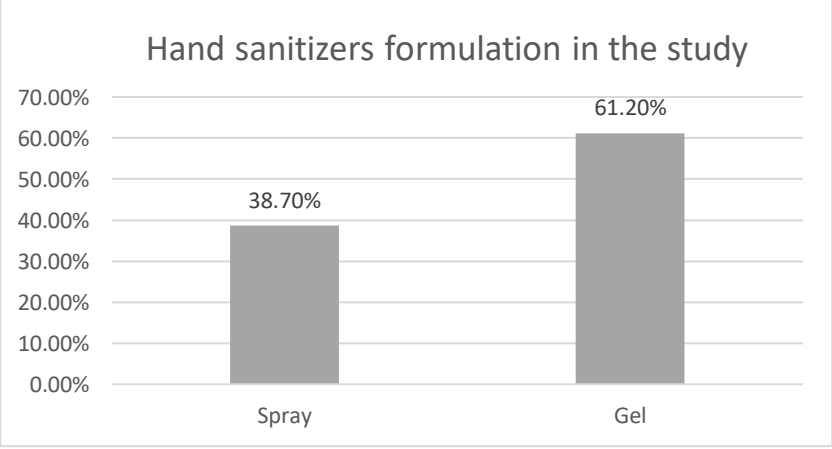

Figure 1. Sample formulations in the study

3.6 Development and validation of headspace GC/MS method for the determination and semi-quantification of impurities

A developed and validated method was used to determine and relatively quantify the impurities present in the samples. The standards and samples were prepared in accordance with the FDA hand sanitizer analysis method. In order to prepare the diluted hand sanitizer solution, 1 mL of hand sanitizer was measured using a micropipette and topped up to 44 mL with LC-MS grade water. 90 µL of acetonitrile (surrogate standard) and 90 µL of n-butanol (internal standard) at 2% (1620 µg/mL) were added to the dilution to reach a final volume of 45.18 mL. From this prepared mixture, 5 mL were transferred to a 20 mL headspace vial for the analysis. This method of preparation was performed for all 31 samples of hand sanitizers. The experiment was carried out in triplicate and repeated three times on consecutive days. A GCMS-QP2020 NX gas chromatograph mass spectrometer and GCMS Real Time Analysis software were used for the detection (Schimadzu, Japan). The results are measured as mean +/- standard deviation. Impurities were identified and semi-quantified based based on internal calibration using data obtained from the mass spectrometer. The relative concentrations of the impurities were therefore determined based the internal standard concentration (1620 µg/mL). The estimation was based on the concentration and area under the curve of the internal standard in relation to the area under the curve of the desired impurity.

A blank solution was injected (Water- LC-MS Grade) at least once at the beginning of a sequence and between samples. The hand sanitizer sample solutions were injected for three replicates. The system suitability was verified regularly.

3.7 Development and validation of headspace GC/MS assay method for the determination of alcohol % in hand sanitizer samples

The same developed and validated method that was used to determine and quantify the impurities present in the samples was employed to determine the alcohol % in samples. For the determination of ethanol content in hand sanitizers, quantitative dilutions were performed from the stock solution to prepare standard solutions at 90%, 80%, 70%, 60%, 50% of ethanol respectively. To obtain the ethanol percentages of 90%, 80%, 70%, 60%, 50%, a pure ethanol solution (99.9% w/w) was mixed with LC-MS grade water. From each dilution solution sample (Vf = 5 mL) 1 mL was poured into a 45 mL conical tube. Each dilution solution was then spiked with 90 µL of acetonitrile (surrogate standard) and 90 µL of n-butanol (internal standard) at 2%. The final volume of 45.18 mL was obtained by adding LC-MS grade water. 5 mL of each final preparation was transferred to the headspace vial for measurement. All concentrations were measured in triplicates.

A regression line was drawn between the GC peak area under curve (A) ratio of ethanol to n-butanol (y-axis) (A_S /A_{IS}) and the ethanol concentration ratio (x-axis) (C_S).

A blank solution was injected (Water- LC-MS Grade) at least once at the beginning of a sequence and between samples. The hand sanitizer sample solutions were injected for three replicates. The system suitability was verified regularly.

Chapter 4

Results

4.1 Determination and semi-quantification of impurities

The GC settings used in this study, which are detailed in the materials and methods, were chosen after many runs to get the best resolution. The retention time of ethanol was 5.78 minutes and n- butanol which used as internal standard solution was 9.81 minutes.

Thirty-one hand sanitizers samples manufactured in Lebanon were tested for the presence of impurities. 32% of the samples contained impurities while the rest 68% were free of impurities. Analysis was conducted using HS/GC-MS (Figure 2).

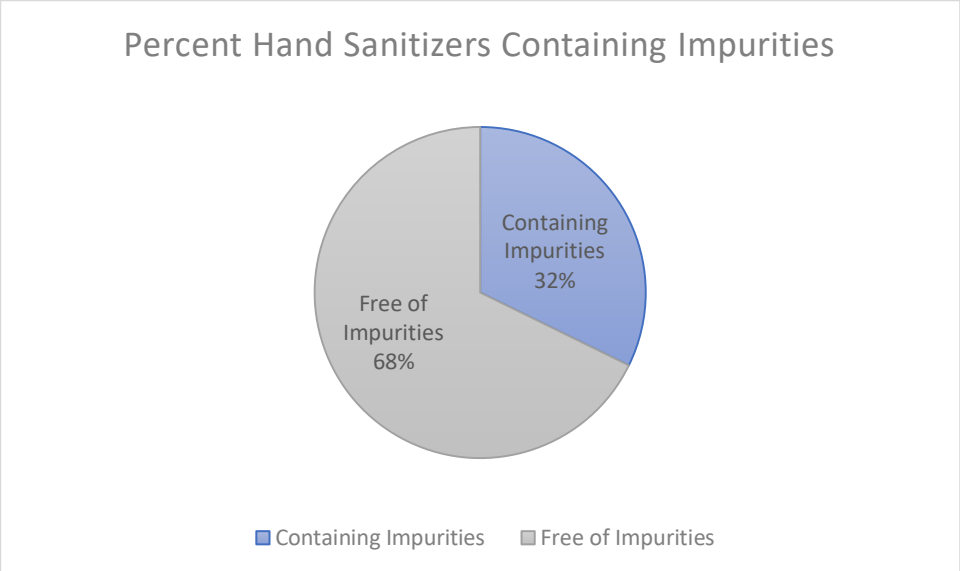

Figure 2. Percentage of hand sanitizers containing impurities

According to Table 5, seven different impurities were detected in the hand sanitizer samples: 2-methyl-1-propanol, ethyl acetate, 1-Propanol, benzene, acetone, methanol, 1,1-diethoxyethane (Acetal). The sample IDs containing the impurities were: 6, 9, 10, 12, 16, 18, 19, 20, 21, 30.

In sample 6, 2-methyl-1-propanol was detected with an average area percentage of 11.03% and a concentration ranging between ~ 665–690 ug/ml, exceeding the FDA concentration limit. 2-methyl-1-propanol is a level 2 impurity.

Sample 9, included the highest number of impurities with four impurities detected: ethyl acetate, 1-Propanol, benzene, and acetone with an average area percentage of 11.47%, 1.18%, 0.86%, and 0.28% respectively. The concentrations of impurities detected were below the FDA concentration limit except for benzene: ~ 16–19 ug/ml, exceeding the recommended limit. The impurities identified in this sample belonged to both level 1 and level 2 (Figure 3).

Table 5. Impurities detected in hand sanitizers samples with chromatographic results and concentration levels

Sample ID	Impurity	Ret. Time	Average Area of Runs	Average area %	Average SI	Concentration (ug/ml)
6	2-methyl-1-propanol	8.68	143841350	11.03	97	~ 665 - 690*
9	Ethyl Acetate	7.21	45494639	11.47	99	~ 215 – 218
	1-Propanol	7.47	4673404	1.18	98	~ 21 – 24
	Benzene	11.71	3403431	0.86	97	~ 16 – 19*
	Acetone	12.54	1103226	0.28	97	~ 5 – 6
10	Methanol	3.87	14745922	1.97	98	~ 69 – 73
	1,1-diethoxyethane	11.84	1819445	0.23	96	~ 8 – 10
12	1,1-diethoxyethane	11.85	870397	0.12	96	~ 4 - 5
16	1,1-diethoxyethane	11.85	540876	0.06	94	~ 2 - 3
18	1,1-diethoxyethane	11.85	2854351	0.26	96	~ 11 - 14
19	1,1-diethoxyethane	11.19	253495	0.02	96	~ 1 - 2
20	1-Propanol	7.47	31523599	3.74	99	~ 150 - 170
21	1,1-diethoxyethane	11.85	242509	0.03	93	~ 1 - 2
30	1-Propanol	7.37	353794471	30.8	95	~ 1660 - 1720*

*exceeds FDA limit

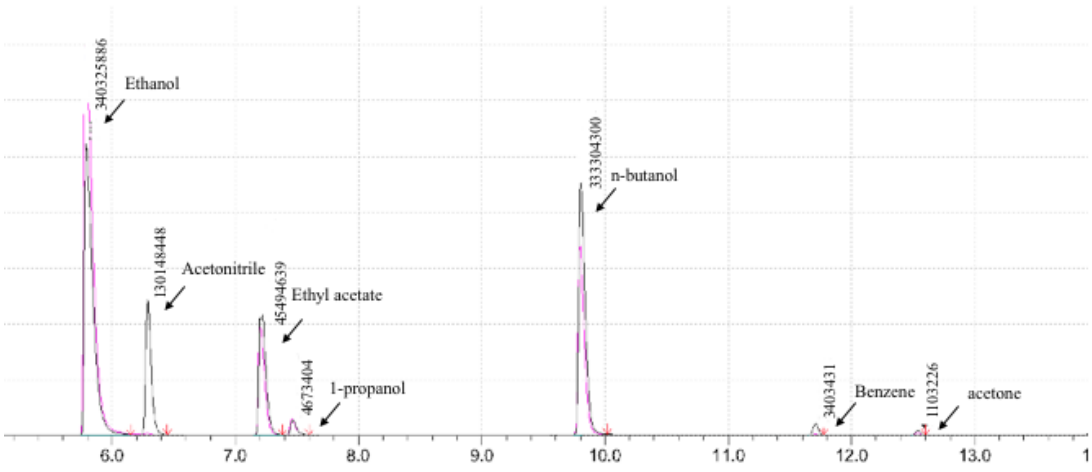

Figure 3. Chromatograph of Sample 9 showing peaks detected

In sample 10, two impurities were detected classified as level 1: methanol and 1,1-diethoxyethane (acetal) with an area percentage of 1.97% and 0.23% respectively. The concentration of these impurities did not exceed the concentration limit set by the FDA (Figure 4).

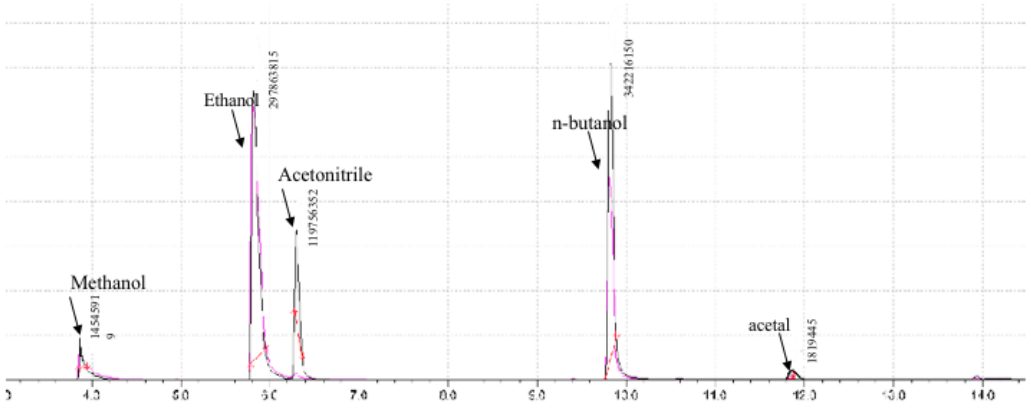

Figure 4. Chromatograph of Sample 10 showing peaks detected

Samples 12,16, 18, 19, and 21 contained 1,1-diethoxyethane (acetal) as the sole impurity. Acetal belongs to level 1 impurities with the respective area percentages of: 0.12%,

0.06%, 0.26%, 0.02%, and 0.03%. None of the impurities present in the above-mentioned samples exceeded the limits set by the FDA.

1-propanol was the detected impurity in samples 20 and 30 with an area percentage of 3.74% and 30.8% respectively. 1-propanol is a level-2 impurity. In sample 30, the concentration of 1-propanol, ~ 1660-1720 ug/ml exceeded the limits set by the FDA (Figure 5).

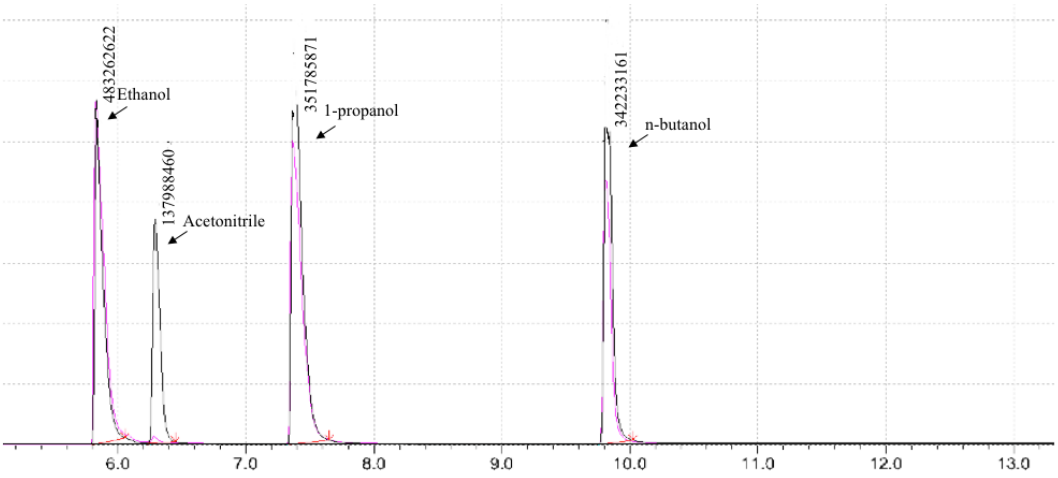

Figure 5. Chromatograph of Sample 30 showing peaks detected

Table 6. Impurities detected in hand sanitizer samples and interim concentrations limit in FDA guidance

Impurity	**Concentrations Limit in FDA Guidance (µg/mL)**
2-methyl-1-propanol	16.06 – 642
Ethyl Acetate	18.04 - 902
1-Propanol	16.08 - 804
Benzene	0.044 - 2.2
Acetone	15.8 - 790
Methanol	15.82 - 791
1,1-diethoxyethane (Acetal)	1.245 - 62.25

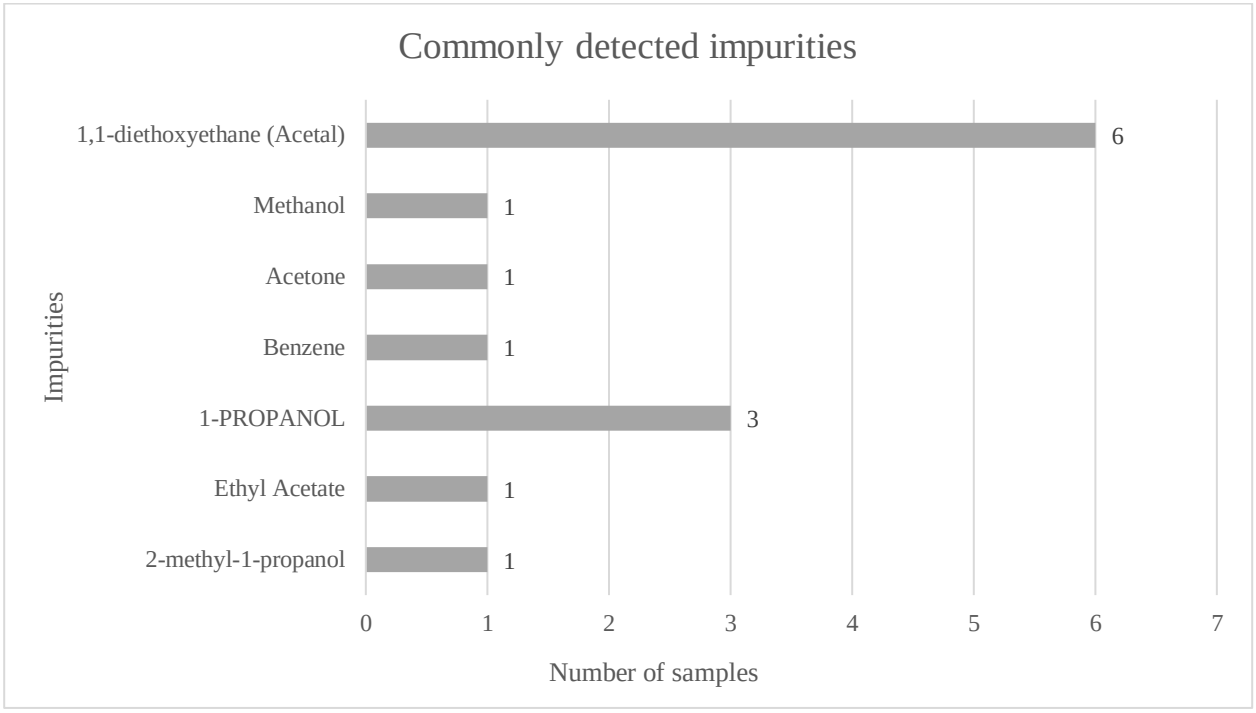

Figure 6. Recurrence of impurities in hand sanitizer samples

1,1-diethoxyethane (Acetal) was the most detected impurity, it was identified in six different samples (Figure 6). In all samples, the concentration of acetal detected did not exceed the limit set by FDA (Table 6). The second most detected impurity in this study was 1-propanol, it was found in three samples (Figure 6). Only in one sample the concentration of 1-Propanol was exceeding the limit set by FDA (Table 6). The rest of the detected impurities were recognized in one sample only (Figure 6).

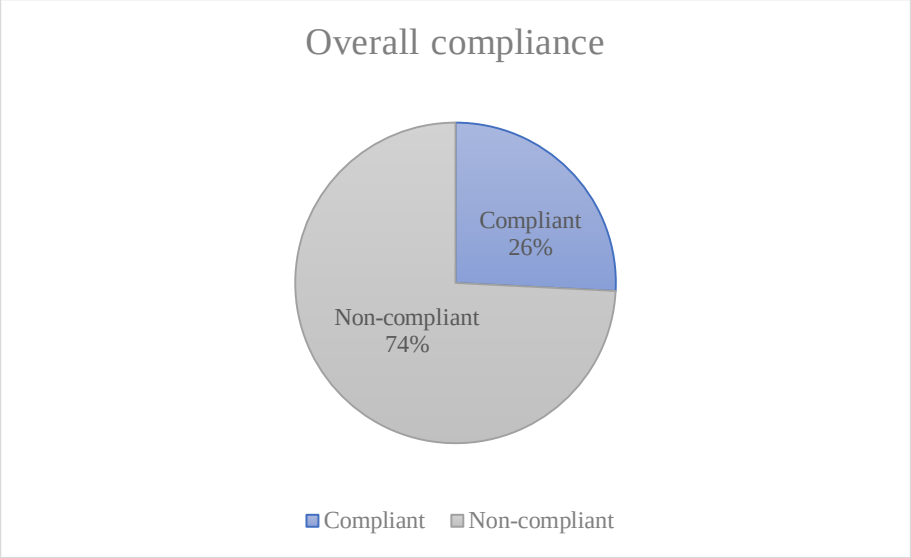

Figure 7. Overall compliance of hand sanitizer samples

In the conducted study, 26% of the tested hand sanitizers were compliant (figure 7.) to both the alcohol percentage recommended by the CDC guidelines and the absence of impurities listed in the FDA guidance. Whereas 74% of the tested samples were not compliant to the recommendations (Figure 7).

4.2 Determination of alcohol % in hand sanitizer samples

4.2.1 Calibration curve for ethanol

Knowing that the hand sanitizers samples in the study were ethanol based, the calibration curve was prepared using ethanol. To improve accuracy, these tests frequently include an internal standard (IS). Ethanol elutes away from n-butanol, and the latter is not known to induce GC system deterioration. It is often used as an IS for ethanol in blood alcohol

content measurements. As a result, the IS in this investigation was n-butanol (Paulo Amorim De Lacerda et al., 2020).

Acetonitrile used is also listed as an IS for ethanol in the USP method but it elutes closer to ethanol and can lead to column/liner degradation with repeated injections n-butanol was selected as the IS.

The calibration curve (Figure 8*)* was fitted to linear regression without pushing through zero using internal standard quantification methods.

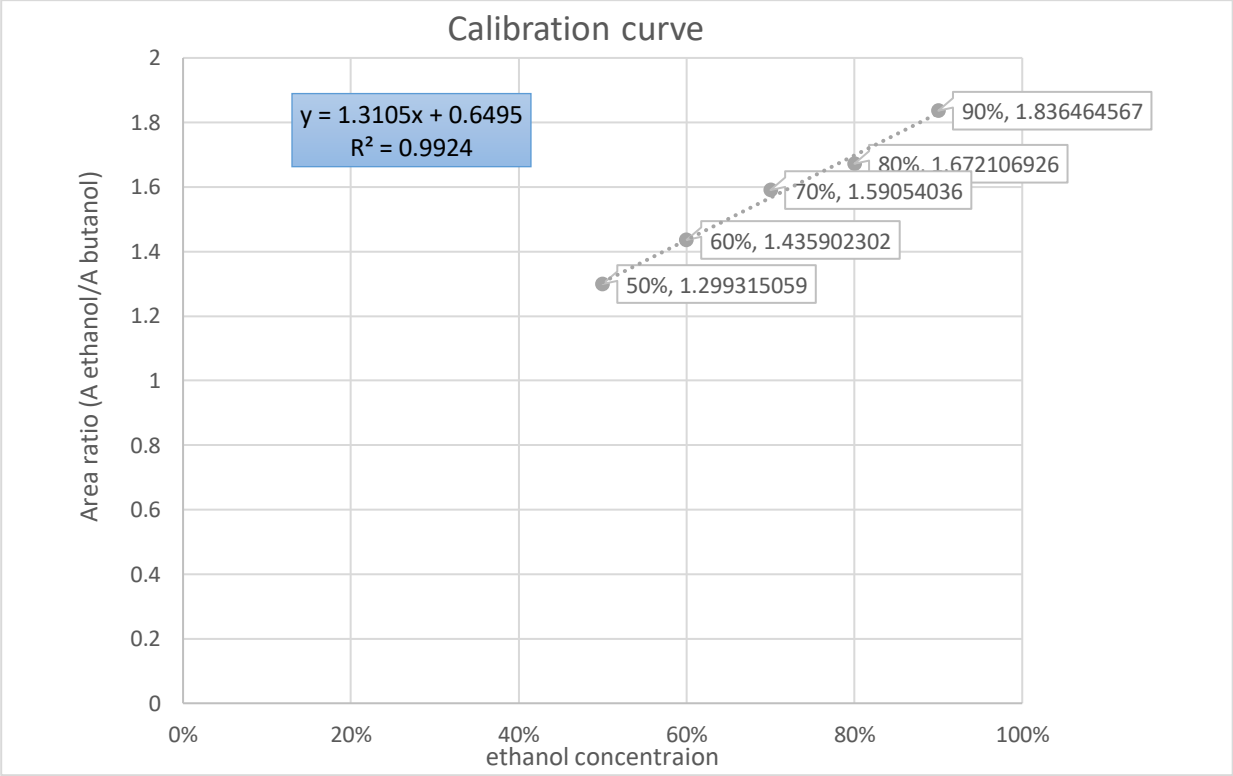

Figure 8. Linear Regression of Calibration curve of ethanol concentration with (AS/AIS)

A five-point calibration curve was derived for all concentrations listed in the table below (Table 7) with an $R^2 > 0.99$ and an RSD $\leq 5\%$.

A regression line was generated as peak area ratios of ethanol standard solutions (A_S) to n-butanol (internal standard, AI_S). (A_S/AI_S) against the concentration of ethanol (C_S).

Table 7. Ethanol content using gas chromatography by headspace gas chromatography method with RSD

Ethanol Content (%)	**A_S[1] /A_{IS}[2]**
50	1.30
60	1.43
70	1.58
80	1.67
90	1.84

[1]A_S: area of ethanol
[2]A_{IS}: are of internal standard

Linearity was determined by visually inspecting the calibration curve all across the defined range. The following equations were used to calculate the detection and quantitation limits using regression analysis.

$$\text{Quantitation Limit} = \frac{10 \times \sigma}{S}$$

$$\text{Detection Limit} = \frac{3.3 \times \sigma}{S}$$

where: σ = standard deviation of y-intercept of the regression line

S = slope of the calibration curve

4.2.2 Alcohol percentage determination

Ethanol content was calculated using the following equation:

$$\text{Cs (\%) (ethanol percentage)} = \frac{A\ ratio - 0.6495\ (intercept)}{1.3105\ (slope)}$$

31 samples were analyzed for their alcohol content. Of the 31 samples analyzed, only 29% of the samples were compliant with the CDC recommendations (ethanol ≥ 60%) (Table 8). Whereas 71% samples (Figure 9) were not compliant with the CDC recommendations and contained a percentage of alcohol less than 60%.

Table 8. Ethanol percentage present in hand sanitizer samples and compliance to CDC recommendations

Sample	Area ratio (A ethanol/A butanol)	Ethanol % based on linear equation	Compliance to CDC recommendations
1	1.63	75.15 +/- 0.95	**Yes**
2	1.45	61.15 +/-1.14	**Yes**
3	1.41	57.67 +/- 0.80	No
4	1.41	57.82 +/- 0.65	No
5	1.50	64.62 +/- 0.82	**Yes**
6	1.48	63.69 +/- 1.54	**Yes**
7	1.44	60.04 +/- 1.15	**Yes**
8	1.36	54.20 +/- 0.85	No
9	0.99	26.30 +/- 0.76	No
10	0.88	17.70 +/- 1.11	No
11	1.16	39.03 +/- 1.23	No
12	1.13	36.56 +/- 0.72	No
13	1.08	33.16 +/- 1.39	No
14	1.36	54.41 +/- 0.50	No
15	1.16	39.26 +/- 1.07	No
16	1.19	41.40 +/-1.29	No
17	1.41	57.77 +/- 0.99	No
18	1.45	60.78 +/- 0.92	**Yes**

19	1.45	61.35 +/- 0.46	**Yes**
20	1.37	55.19 +/- 1.20	No
21	1.00	26.74 +/- 0.40	No
22	1.15	37.93 +/- 1.12	No
23	0.98	24.96 +/- 0.69	No
24	1.53	66.83 +/- 0.90	**Yes**
25	1.10	34.18 +/- 0.68	No
26	1.61	73.42 +/- 0.89	**Yes**
27	1.00	26.62 +/- 0.93	No
28	1.30	49.98 +/- 0.56	No
29	1.21	42.41 +/- 0.90	No
30	1.40	57.32 +/- 1.10	No
31	1.14	37.59 +/- 0.56	No

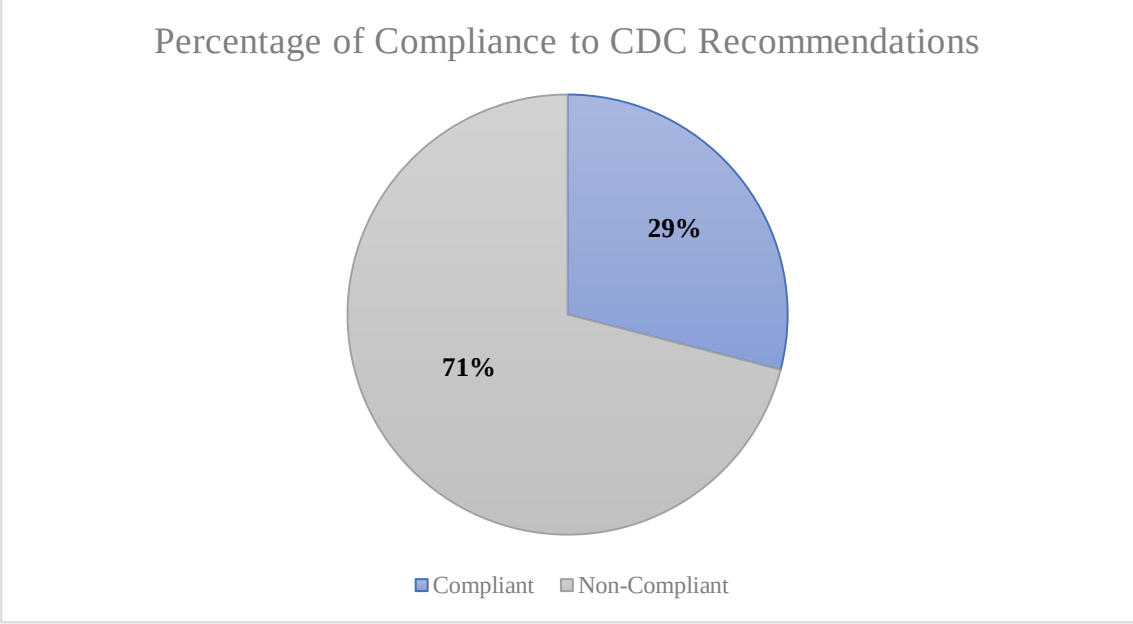

Figure 9. Percentage of hand sanitizers compliant to CDC recommendations

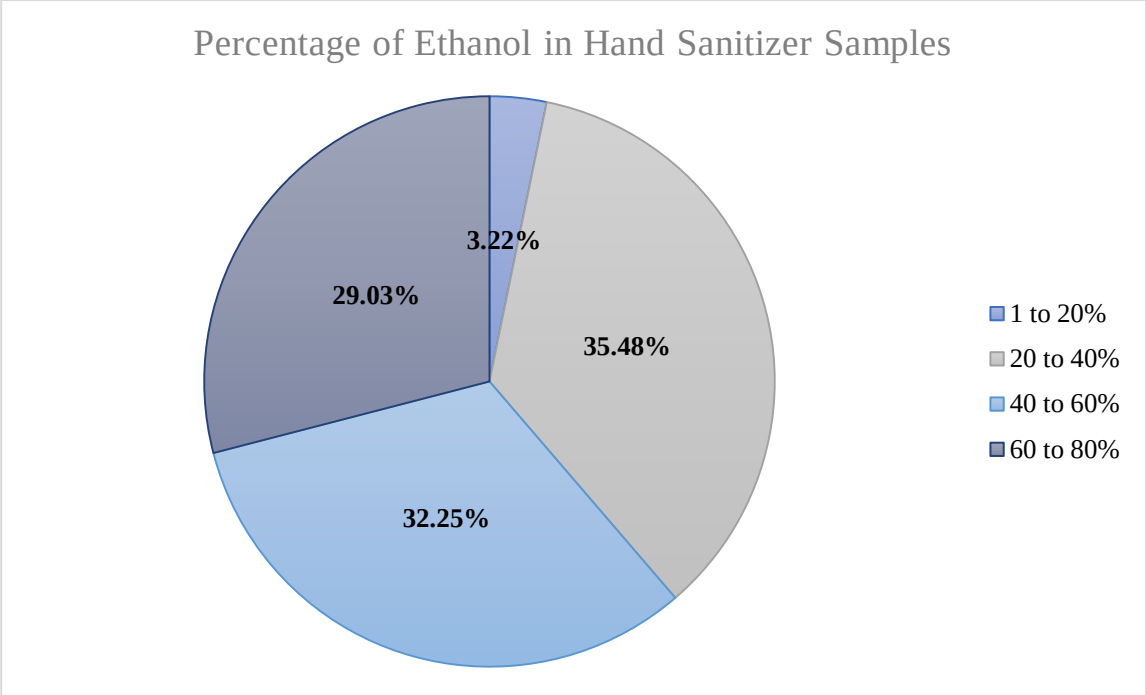

Figure 10. Percentage of ethanol in hand sanitizer samples

As shown in Figure 10, only 29% of the samples contained ethanol with a percentage higher than 60% while the other samples comprised ethanol in a lower percentage. Most of the samples (35.48%) had an ethanol percentage between 20-40%. While 32.25% of the samples contained ethanol in a percentage of 40-60%. 3.22% of the samples demonstrated an ethanol percentage range between 1-20%.

Chapter 5
Discussion

5.1 Impurities detected in hand sanitizers

The COVID-19 pandemic's extraordinary impact has triggered a global catastrophe, resulting in widespread panic buying of vital products, such as hand sanitizer and surface disinfectants. Due to the high demand, commercial vital products were in short supply, prompting government regulatory bodies to temporarily allow the use of lower-quality raw materials and substitutes in ABHRs (Tse, Nelson, et al., 2021).

Seven impurities were detected in the samples analyzed in this study: 2-methyl-1-propanol, ethyl acetate, 1-Propanol, benzene, acetone, methanol, 1,1-diethoxyethane (Acetal). Among these samples only three compounds exceeded the limit set by the FDA guidance: 2-methyl-1-propanol (sample 6), benzene (sample 9), and 1-Propanol (sample 30).

Within all the 31 samples studied none of them had a sum of Level 1 impurities that exceeded 300 μg/mL which suggest that they can be sold in the market according to the latest FDA guidance in February 2021.

In sample 6, 2-methyl-1-propanol (isobutanol) was detected with a concentration of 677.68 μg/mL, exceeding the FDA limit by approximately 6%. Isobutyl alcohol is a colorless liquid with a sweet, musty smell. It is utilized as a solvent and in the production of other compounds (Brownstein, 2015). According to the United State Environmental Protection Agency, the inhalation reference concentration of isobutanol is not available (*Provisional Peer Reviewed Toxicity Values for Isobutanol (CASRN 78-83-1)*, n.d.).

If inhaled, the LD50/LC50 of isobutanol in rats is 19,200 ppm/4 h. The LD50/LC50 for dermal exposure in rabbits is 3400 mg/ kg and the oral LD50/LC50 is rats is 2743 mg/ kg (*Provisional Peer Reviewed Toxicity Values for Isobutanol (CASRN 78-83-1)*, n.d.).

Isobutanol's mutagenic or carcinogenic effects have only been studied in a few cases. However, isobutanol has been shown to have tumorigenic and teratogenic effects in animal studies. These compounds have also been shown to have reproductive impacts in animal models. Due to the bioaccumulation of these compounds in breastmilk, this could pose a risk to children or those who are breastfeeding. (Tse, Purdy, et al., 2021).

In sample 30, 1-Propanol was identified with a concentration of 1674µg/mL, exceeding the limit set by FDA. The major use of 1-Propanol is as a solvent and it is considered one of the most important industrial alcohols (EPA, 2021).

According to the article "Poisoning with 1-propanol and 2-propanol", the oral lethal dose of 1-Propanol is not known yet (Vujasinović et al., 2007). Exposure to n-propanol via skin contact, inhalation, or oral consumption has been shown to be harmless to animals and humans (Tse, Purdy, et al., 2021). However, at doses exceeding occupational exposure, developmental and reproductive problems have been found (Tse, Purdy, et al., 2021). In terms of pharmacokinetics, isopropanol is identical to ethanol, but it has a higher narcotic/intoxicating impact due to central nervous system depression (CNS) (Tse, Purdy, et al., 2021). Isopropanol-related deaths are uncommon, yet increased exposure can cause altered sensorium, hypotension, hypothermia, and cardiac collapse (Tse, Purdy, et al., 2021). Mild erythema and cutaneous absorption into the circulatory system can occur after repeated use (Tse, Purdy, et al., 2021). Furthermore, isopropanol was converted to

acetone during exposure, and acetone accumulation over time could contribute to extended activity and toxicity. (Tse, Purdy, et al., 2021).

Swallowing a hand sanitizer containing 1-propanol can cause significant symptoms such as decreased breathing and heart rate, as well as death. Hand sanitizer contaminated with 1-propanol might irritate the skin (or eyes, if exposed) (FDA, 2020).

These contaminants are not expected in a regular fermentation and distillation process, although they could be present due to the industrial environment (e.g., equipment, containers) (McCue, n.d.).

In sample 9, benzene was identified with a concentration of 16.10 μg/mL, exceeding the limit set by FDA by approximately 8 times approximately. Benzene is found in the air as a result of coal and oil combustion, gasoline service stations, and automobile exhaust. Acute (short-term) inhalation exposure to benzene in humans can produce drowsiness, dizziness, headaches, eye, skin, and respiratory tract irritation, as well as unconsciousness at high doses (EPA, n.d.). Benzene can be used to produce absolute ethanol. The water can subsequently be removed from absolute ethanol by fractional distillation. Furthermore, unless cross contamination occurs, ethanol produced from grain fermentations is normally benzene-free (Tse, Purdy, et al., 2021). Benzene is a recognized human carcinogen and mutagen that can enter the body through inhalation, cutaneous absorption, and oral absorption (Tse, Purdy, et al., 2021). Drowsiness, tremors, headaches, vomiting, irritability, convulsions, irregular heartbeat, and mortality are all common symptoms (Snyder et al., 1993). Occupational exposure limits are defined at 0.1 ppm (8 h time-weighted average) and 1 ppm (15-minute time-weighted average) due to the inherent dangers associated with benzene (Snyder et al., 1993). The presence of

benzene in ABHR formulations can be harmful to users' health and safety, as frequent use and the addition of water or a gelation agent (e.g. carbomer) can dramatically extend exposure time and skin absorption (*CDC | Facts About Benzene*, 2019). Long-term exposure to benzene has a substantial effect on the blood and bone marrow, resulting in anemia (*CDC | Facts About Benzene*, 2019). As a result, ABHRs that use technical-grade ethanol should be closely monitored to avoid continuous exposure to this hazardous chemical (*CDC | Facts About Benzene*, 2019).

According to MedPage Today, online Pharmacy Valisure is warning about compromised batches of a pandemic staple. Valisure filed a citizen petition with the FDA, requesting an urgent recall of the implicated batches and a modification of the advice to include a benzene exposure limit. Hand sanitizer is regulated by the FDA as a drug product (Henderson, 2021).

Any alcohol (ethanol) or IPA containing more than 630 ppm methanol is in violation of the FDA temporary guideline and could be regarded as proof of contamination or substitution. Under the FD&C Act, hand sanitizers containing methanol-contaminated alcohol (ethanol) or IPA are considered adulterated. Such alcohol (ethanol) or IPA substance should be disposed of, and the company should report the material and its source to the FDA (McCue, n.d.).

When absorbed transdermally, inhaled, or ingested, methanol in ABHS products is harmful to consumers. The latter can happen to children (accidental ingestion) and alcoholics (deliberate ingestion) who use ABHS as a substitute for alcohol (Abuga et al., 2021).

The synthetic alcohols available are very expensive. To get over these problems, some manufactures, avoid using ethyl alcohol and instead use toxic methyl alcohol as a replacement (sold at half the price). Unscrupulous manufactures could use branded container packing for marketers to sell spurious methanol-based sanitizers according The CGIS's press release states (Hakim, 2020).

Acetal was the most prevalent impurity, it was detected in 6 of the analyzed samples. The addition of two moles of ethanol and one mole of acetaldehyde to an acid catalyst produces 1,1-diethoxyethane (acetal) and water. In the presence of ethanol and acidic catalysts, reversible conversion of acetal to acetaldehyde can also occur. Acetal has been used as a solvent, as a chemical synthesis intermediary for protecting the carbonyl group in ketones and aldehydes, and as a fragrance ingredient (Capeletti et al., 2000). The FDA stated in an announcement that "acetal can irritate the upper respiratory tract, eyes, and skin" (Ellis, n.d.). As already mentioned, acetal may be derived from acetaldehyde, implying that acetaldehyde may be present in the analyzed samples. During the fermentation process, acetal and acetaldehyde can be converted in the presence of methanol, ethanol, or an acidic catalyst (Capeletti et al., 2000). According to recent research, acetaldehyde is a probable human carcinogen and the primary molecule involved in fetal alcohol spectrum disorder (Tse, Purdy, et al., 2021). Acetaldehyde is a hazardous and suspected carcinogen (the International Agency for Research on Cancer classifies it as Group 2B). Although acetaldehyde adsorption at these concentrations is unlikely to reach acute hazardous levels, regular use of ABHRs can cause percutaneous toxicity in children (Tse, Nelson, et al., 2021).

For ABHRs, the new manufactures are using non-traditional packaging. For example, hand sanitizers were delivered in a carbonated aluminum beverage container. After several weeks of storage at ambient temperature, the product gradually rusted and broke the container. Carbon dioxide is an acidic gas that can affect the chemical composition of ABHR by disrupting equilibrium reactions that produce ethers, acetals, and esters (Tse, Nelson, et al., 2021).

Ethyl acetate was detected in one study sample (sample 9) with a concentration of 215.31 ug/ml, not exceeding the limit set by the FDA authorities. Ethyl acetate is the result of alcohol acyl- transferases action. This can happen through ethanol-catalyzed acetic acid esterification, enzyme-catalyzed acetic acid esterification, or ester synthesis within the cell prior to diffusion into the solution. Ethyl acetate is an industrial solvent that is formed by an ester of ethanol and acetic acid (e.g. paints, plasticizers, denaturant, etc.). Although moderate toxicity has been documented by intraperitoneal, subcutaneous, and oral routes, acute toxicity is uncommon. Ethyl acetate vapors (400 ppm) can cause eye, nose, and throat irritation, as well as headaches, nausea, vomiting, lethargy, and unconsciousness (Tse, Purdy, et al., 2021). Many recalls has been made recently due to high levels of ethyl acetate owing to the use of technical-grade ethanol in ABHR formulation (*Recall of Certain Hand Sanitizers That May Pose Health Risks (Part 1 - June 17, 2020 to March 24, 2021)*, 2021).

Acetone was detected in sample 9 with a concentration of 5.22 ug/ml (within the limit set by FDA guidances). Approximately 80% of absorbed isopropanol is metabolized by the liver using first-order kinetics, and the enzyme alcohol dehydrogenase breaks it down into acetone. This could be a possible source for the presence of acetone in the sample.

Isopropanol metabolism to acetone was traditionally assumed to be the cause of CNS depression; however, further research found that isopropanol was the main culprit (Ashurst & Nappe, 2021). Acetone buildup may contribute to extended activity and toxicity with long-term exposure (Ashurst & Nappe, 2021).

5.2 Alcohol percentage determination

ABHS solutions with alcohol concentrations of less than 60% provide inadequate antisepsis, putting users at risk of infection. This is made worse by the false sense of security and trust that unwary people often have in the products they utilize. (Abuga et al., 2021). 29% of the analyzed samples contained an ethanol percentage higher than 60%. The precise assessment of ABHS alcohol (active ingredient) level is a critical quality control test that can also serve as a surrogate for efficacy. Low-quality raw materials may contribute to undesirable impurity profiles in the final product, which may contain impurities such as benzene, acetaldehyde, acetal and ethyl acetate. Cohen et al. discovered that in order to manufacture alcohol that matches FDA impurity limitations, non-traditional high purity ethanol manufacturing plants may require infrastructural and process improvements (Abuga et al., 2021).

To get the final required concentration, one should check the alcohol content and make the appropriate volume modifications. Controlling the alcohol concentration of the final solution can be done with an alcoholmeter (Information et al., 2009).

5.3 Interactions between detected impurities

In this study, hand sanitizers containing more than one impurity were present. One hand sanitizer sample (sample 9) contained a combination of four impurities (ethyl acetate, 1-

Propanol, benzene, and acetone). Another hand sanitizer sample (sample 10) comprised a mixture of two impurities (methanol, and 1,1-diethoxyethane).

Johansson & Ingelman-Sundberg have shown that ethanol has a synergistic effect on benzene toxicity. The rate of benzene metabolism was found to be 20-65 times higher in liver microsomes from ethanol- or acetone-treated rats than in microsomes from control animals. The findings suggest that benzene is metabolized mostly by the ethanol-inducible P-450 form in liver microsomes, and that ethanol's induction of this isozyme may explain why ethanol has a synergistic effect on benzene toxicity (Johansson & Ingelman-Sundberg, 1988). In the liver, the Cytochrome P450 2E1 converts benzene to its metabolic form benzene oxide, which can further metabolize to various other intermediates like o-benzoquinone and pbenzoquinone, which are the major metabolites including benzene toxicity (Joshi & Adhikari, 2019). Benzene affects the bone marrow and causes anemia, leukopenia, and thrombocytopenia depending on the dose received; further exposure for a longer period of time causes aplasia and pancytopenia (Joshi & Adhikari, 2019). Due to benzene's extreme toxicity, it has been substituted with relatively safe xylene and toluene, both of which have hematological toxicity (Joshi & Adhikari, 2019).

Furthermore, hemiacetals are formed when aldehydes (e.g. 1,1-diethoxyethane) combine with alcohols (e.g. methanol). Most of the acetals are not toxic. A full acetal is formed by reacting with a second alcohol molecule. To preserve aldehyde groups before performing additional processes on the molecule, they are frequently changed to acetal groups (*Acetals, Ketals, Hemiacetals, and Hemiketals | CAMEO Chemicals | NOAA*, n.d.).

Chapter 6

Conclusion

Hand washing with water and soap is considered the gold standard for hand hygiene and reducing the spread of infectious diseases. Nevertheless, hand sanitizers (also known as hand antiseptic or hand rub) are indicated in the absence of water and soap. Lebanon, like many other nations across the world, has facilitated legislation to make it simpler for local enterprises to quickly create alcohol-based hand sanitizers in response to the massive increase in demand for hand sanitizers during the SARS-CoV-2 outbreak.

However, people manufacturing hand sanitizers should still adhere to WHO and FDA rules and avoid using low-quality alcohol that may include harmful chemicals.

In the present study, 10 hand sanitizer samples out of 31 were found to contain impurities listed in the FDA guidance including: 2-methyl-1-propanol, ethyl acetate, 1-Propanol, benzene, acetone, methanol, 1,1-diethoxyethane (Acetal). Regarding the alcohol content of the tested hand sanitizers, only 29% of the samples were compliant with the CDC recommendations (ethanol content higher than 60%).

The contaminants that were found and quantified in most of the ABHRs suggest that potential toxicity is low. Owing to the high availability of ABHR on the market, testing should be done on a regular basis to verify product compliance and customer safety.

Some limitations were faced in this study including the incapability of performing an external calibration curve for the detected impurities. The lack of material constituted a barrier in this research, therefore the determined concentrations for the impurities were

estimated based the concentration of the internal standard employed. This issue could open a door for further studies and experiments, leading to the conduction of a method using external calibration curve. Another drawback was the use of a column other than the one employed in the validated FDA method (followed in these experiments). The evaporation of the ethanol from the samples could also be another issue faced in the study.

References

A LEAN Approach for the Determination of Residual Solvents Using Headspace Gas Chromatography with Relative Response Factors. (n.d.). Chromatography Online. Retrieved September 16, 2021, from https://www.chromatographyonline.com/view/lean-approach-determination-residual-solvents-using-headspace-gas-chromatography-relative-response-f

Abuga, K., & Nyamweya, N. (2021). Alcohol-Based Hand Sanitizers in COVID-19 Prevention: A Multidimensional Perspective. *Pharmacy: Journal of Pharmacy Education and Practice*, *9*(1). https://doi.org/10.3390/pharmacy9010064

Abuga, K., Nyamweya, N., & King'ondu, O. (2021). *Quality of Alcohol Based Hand Sanitizers Marketed in the Nairobi Metropolis* [Preprint]. CHEMISTRY. https://doi.org/10.20944/preprints202109.0427.v1

Acetals, Ketals, Hemiacetals, and Hemiketals | CAMEO Chemicals | NOAA. (n.d.). Retrieved December 16, 2021, from https://cameochemicals.noaa.gov/react/70

Ahn, D.-G., Shin, H.-J., Kim, M.-H., Lee, S., Kim, H.-S., Myoung, J., & Kim, B.-T. K. and S.-J. (2020). *Current Status of Epidemiology, Diagnosis, Therapeutics, and Vaccines for Novel Coronavirus Disease 2019 (COVID-19)*. *30*(3), 313–324. https://doi.org/10.4014/jmb.2003.03011

Alzyood, M., Jackson, D., Aveyard, H., & Brooke, J. (2020). COVID-19 reinforces the importance of handwashing. *Journal of Clinical Nursing*, 10.1111/jocn.15313. https://doi.org/10.1111/jocn.15313

An Introduction To Headspace Sampling In Gas Chromatography Fundamentals And Theory. (n.d.). 35.

Analysis of Impurities in Alcohol-Based Hand Sanitizers by GC-MS | SHIMADZU (Shimadzu Corporation). (n.d.). Retrieved August 10, 2021, from https://www.shimadzu.com/an/literature/an_m315_en.html

Ashurst, J. V., & Nappe, T. M. (2021). Isopropanol Toxicity. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK493181/

Bednarek, R. S., Nassereddin, A., & Ramsey, M. L. (2022). Skin Antiseptics. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK507853/

Bedner, M., Murray, J. A., Urbas, A. A., MacCrehan, W. A., & Wilson, W. B. (2021). *A Comparison of Measurement Methods for Alcohol-Based Hand Sanitizers*. National Institute of Standards and Technology. https://doi.org/10.6028/NIST.IR.8342

Benzene Found in Multiple Hand Sanitizer Brands. (2021, March 25). https://www.medpagetoday.com/special-reports/exclusives/91806

Brownstein, A. M. (2015). Chapter 5—Isobutanol. In A. M. Brownstein (Ed.), *Renewable Motor Fuels* (pp. 47–56). Butterworth-Heinemann. https://doi.org/10.1016/B978-0-12-800970-3.00005-4

Capeletti, M. R., Balzano, L., de la Puente, G., Laborde, M., & Sedran, U. (2000). Synthesis of acetal (1,1-diethoxyethane) from ethanol and acetaldehyde over acidic catalysts. *Applied Catalysis A: General*, *198*(1), L1–L4. https://doi.org/10.1016/S0926-860X(99)00502-5

CDC. (2020, February 11). *COVID-19 and Your Health*. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/hand-sanitizer.html

CDC | Facts About Benzene. (2019, May 15). https://emergency.cdc.gov/agent/benzene/basics/facts.asp

Center for Drug Evaluation and Research. (2020, June 29). *FDA advises consumers not to use hand sanitizer products manufactured by Eskbiochem*. U.S. Food and Drug Administration. https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-consumers-not-use-hand-sanitizer-products-manufactured-eskbiochem

Commissioner, O. of the. (2020a, March 24). *FDA issues final rule on safety and effectiveness of consumer hand sanitizers*. FDA; FDA. https://www.fda.gov/news-events/press-announcements/fda-issues-final-rule-safety-and-effectiveness-consumer-hand-sanitizers

Commissioner, O. of the. (2020b). 5 Things to Know About Triclosan. *FDA*. https://www.fda.gov/consumers/consumer-updates/5-things-know-about-triclosan

Commissioner, O. of the. (2020c). Is Your Hand Sanitizer on FDA's List of Products You Should Not Use? *FDA*. https://www.fda.gov/consumers/consumer-updates/your-hand-sanitizer-fdas-list-products-you-should-not-use

Dai, L., Quiroga, A., Zhang, K., Runes, H., Yazzie, D., Mistry, K., Chetwyn, N., & Dong, M. (2010). A Generic Headspace GC Method for Residual Solvents in Pharmaceuticals: Benefits, Rationale, and Adaptations for New Chemical Entities. *LCGC North Am.*, *28*, 54, 56–66.

Determination of Ethanol and Isopropanol Content in Hand Sanitizers Using Nitrogen Carrier Gas. (n.d.). 4.

Dhama, K., Patel, S. K., Kumar, R., Masand, R., Rana, J., Yatoo, Mohd. I., Tiwari, R., Sharun, K., Mohapatra, R. K., Natesan, S., Dhawan, M., Ahmad, T., Emran, T. B., Malik, Y. S., & Harapan, H. (2021). The role of disinfectants and sanitizers during COVID-19 pandemic: Advantages and deleterious effects on humans and the environment. *Environmental Science and Pollution Research International*, 1–18. https://doi.org/10.1007/s11356-021-14429-w

Direct Injection Gas Chromatography Mass Spectrometry (GC-MS) Method for the Detection of Listed Impurities in Hand Sanitizers. (n.d.). 11.

Eggers, M., Eickmann, M., Kowalski, K., Zorn, J., & Reimer, K. (2015). Povidone-iodine hand wash and hand rub products demonstrated excellent in vitro virucidal efficacy against Ebola virus and modified vaccinia virus Ankara, the new European test virus for enveloped viruses. *BMC Infectious Diseases*, *15*, 375. https://doi.org/10.1186/s12879-015-1111-9

Ellis, R. (n.d.). *FDA Issues Warning for ArtNaturals Hand Sanitizers*. WebMD. Retrieved November 11, 2021, from https://www.webmd.com/lung/news/20211007/fda-issues-warning-for-artnaturals-hand-sanitizers

Gold, N. A., Mirza, T. M., & Avva, U. (2021). Alcohol Sanitizer. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK513254/

Golin, A. P., Choi, D., & Ghahary, A. (2020). Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. *American Journal of Infection Control*, *48*(9), 1062–1067. https://doi.org/10.1016/j.ajic.2020.06.182

GÜNER, R., HASANOĞLU, İ., & AKTAŞ, F. (2020). COVID-19: Prevention and control measures in community. *Turkish Journal of Medical Sciences*, *50*(3), 571–577. https://doi.org/10.3906/sag-2004-146

Hakimi, A. A., & Armstrong, W. B. (2020). Hand Sanitizer in a Pandemic: Wrong Formulations in the Wrong Hands. *The Journal of Emergency Medicine*, *59*(5), 668–672. https://doi.org/10.1016/j.jemermed.2020.07.018

Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends in Immunology*, *41*(12), 1100–1115. https://doi.org/10.1016/j.it.2020.10.004

Inactivation of SARS-CoV-2 by commercially available alcohol-based hand sanitizers | Elsevier Enhanced Reader. (n.d.). https://doi.org/10.1016/j.ajic.2020.08.020

Information, N. C. for B., Pike, U. S. N. L. of M. 8600 R., MD, B., & Usa, 20894. (2009). WHO-recommended handrub formulations. In *WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care*. World Health Organization. https://www.ncbi.nlm.nih.gov/books/NBK144054/

Jairoun, A. A., Al-Hemyari, S. S., & Shahwan, M. (2021). The pandemic of COVID-19 and its implications for the purity and authenticity of alcohol-based hand sanitizers: The health risks associated with falsified sanitizers and

recommendations for regulatory and public health bodies. *Research in Social and Administrative Pharmacy*, *17*(1), 2050–2051. https://doi.org/10.1016/j.sapharm.2020.04.014

Jing, J. L. J., Pei Yi, T., Bose, R. J. C., McCarthy, J. R., Tharmalingam, N., & Madheswaran, T. (2020). Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations. *International Journal of Environmental Research and Public Health*, *17*(9), 3326. https://doi.org/10.3390/ijerph17093326

Johansson, I., & Ingelman-Sundberg, M. (1988). Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes. *Cancer Research*, *48*(19), 5387–5390.

Joshi, D. R., & Adhikari, N. (2019). An Overview on Common Organic Solvents and Their Toxicity. *Journal of Pharmaceutical Research International*, 1–18. https://doi.org/10.9734/jpri/2019/v28i330203

Kratzel, A. et al. (2020). *Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 by WHO-Recommended Hand Rub Formulations and Alcohols*. https://doi.org/10.3201/eid2607.200915

Keni, R., Alexander, A., Nayak, P. G., Mudgal, J., & Nandakumar, K. (2020). COVID-19: Emergence, Spread, Possible Treatments, and Global Burden. *Frontiers in Public Health*, *8*, 216. https://doi.org/10.3389/fpubh.2020.00216

Lanese, N. (2020, July 28). *Dozens of hand sanitizers contain a toxic ingredient. How do you know yours is safe?* Livescience.Com. https://www.livescience.com/methanol-tainted-hand-sanitizer-safety.html

Lebanon COVID: 588,578 Cases and 7,999 Deaths - Worldometer. (n.d.). Retrieved August 20, 2021, from https://www.worldometers.info/coronavirus/country/lebanon/

Lebanon: The latest coronavirus counts, charts and maps. (n.d.). *Reuters*. Retrieved August 20, 2021, from https://graphics.reuters.com/world-coronavirus-tracker-and-maps/countries-and-territories/lebanon/

Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. *Journal of Pharmaceutical Analysis*, *10*(2), 102–108. https://doi.org/10.1016/j.jpha.2020.03.001

Lotfi, M., Hamblin, M. R., & Rezaei, N. (2020). COVID-19: Transmission, prevention, and potential therapeutic opportunities. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *508*, 254–266. https://doi.org/10.1016/j.cca.2020.05.044

Makhni S, Umscheid CA, Soo J, et al. Hand Hygiene Compliance Rate During the COVID-19 Pandemic. *JAMA Intern Med.* 2021;181(7):1006–1008. doi:10.1001/jamainternmed.2021.1429

McCue, O.-J. (n.d.). *Temporary Policy for Preparation of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency (COVID-19)*. 20.

Muralidar, S., Ambi, S. V., Sekaran, S., & Krishnan, U. M. (2020). The emergence of COVID-19 as a global pandemic: Understanding the epidemiology, immune response and potential therapeutic targets of SARS-CoV-2. *Biochimie*, *179*, 85–100. https://doi.org/10.1016/j.biochi.2020.09.018

Ogilvie, B. H., Solis-Leal, A., Lopez, J. B., Poole, B. D., Robison, R. A., & Berges, B. K. (2021). Alcohol-free hand sanitizer and other quaternary ammonium disinfectants quickly and effectively inactivate SARS-CoV-2. *Journal of Hospital Infection*, *108*, 142–145. https://doi.org/10.1016/j.jhin.2020.11.02

Penton, Z. E. (2002). Chapter 10 Headspace gas chromatography. In *Comprehensive Analytical Chemistry* (Vol. 37, pp. 279–296). Elsevier. https://doi.org/10.1016/S0166-526X(02)80047-2

Provisional Peer Reviewed Toxicity Values for Isobutanol (CASRN 78-83-1). (n.d.). 26.

Recall of certain hand sanitizers that may pose health risks (Part 1—June 17, 2020 to March 24, 2021). (2021, October 28). Government of Canada, Communications and Public Affairs Branch. https://recalls-rappels.canada.ca/en/alert-recall/recall-certain-hand-sanitizers-may-pose-health-risks-part-1-june-17-2020-march-24-2021

Research, C. for D. E. and. (2021a). Hand Sanitizers | COVID-19. *FDA*. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/hand-sanitizers-covid-19

Research, C. for D. E. and. (2021b, January 19). *Policy for Testing of Alcohol (Ethanol) and Isopropyl Alcohol for Methanol, Including During the Public Health Emergency (COVID-19)*. U.S. Food and Drug Administration; FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-testing-alcohol-ethanol-and-isopropyl-alcohol-methanol-including-during-public-health

Ritchie, H., Mathieu, E., Rodés-Guirao, L., Appel, C., Giattino, C., Ortiz-Ospina, E., Hasell, J., Macdonald, B., Beltekian, D., & Roser, M. (2020). Coronavirus Pandemic (COVID-19). *Our World in Data*. https://ourworldindata.org/covid-cases

Seto, Y. (1994). Determination of volatile substances in biological samples by headspace gas chromatography. *Journal of Chromatography A*, *674*(1), 25–62. https://doi.org/10.1016/0021-9673(94)85216-2

Singh, D., Joshi, K., Samuel, A., Patra, J., & Mahindroo, N. (2020). Alcohol-based hand sanitisers as first line of defence against SARS-CoV-2: A review of biology, chemistry and formulations. *Epidemiology and Infection*, *148*, e229. https://doi.org/10.1017/S0950268820002319

Snyder, R., Witz, G., & Goldstein, B. D. (1993). The toxicology of benzene. *Environmental Health Perspectives*, *100*, 293–306.

Struyf, T., Deeks, J. J., Dinnes, J., Takwoingi, Y., Davenport, C., Leeflang, M. M., Spijker, R., Hooft, L., Emperador, D., Dittrich, S., Domen, J., Horn, S. R. A., & Van den Bruel, A. (2020). Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. *The Cochrane Database of Systematic Reviews*, *2020*(7), CD013665. https://doi.org/10.1002/14651858.CD013665

Tse, T. J., Nelson, F. B., & Reaney, M. J. T. (2021). Analyses of Commercially Available Alcohol-Based Hand Rubs Formulated with Compliant and Non-Compliant Ethanol. *International Journal of Environmental Research and Public Health*, *18*(7), 3766. https://doi.org/10.3390/ijerph18073766

Tse, T. J., Purdy, S. K., Shen, J., Nelson, F. B., Mustafa, R., Wiens, D. J., & Reaney, M. J. T. (2021). Toxicology of alcohol-based hand rubs formulated with technical-grade ethanol. *Toxicology Reports*, *8*, 785–792. https://doi.org/10.1016/j.toxrep.2021.03.034

US EPA, O. (n.d.). *Benzene CASRN 71-43-2 | DTXSID3039242 | IRIS | US EPA, ORD*. Retrieved November 11, 2021, from https://cfpub.epa.gov/ncea/iris2/chemicallanding.cfm?substance_nmbr=276

US EPA, O. (2020, March 13). *About List N: Disinfectants for Coronavirus (COVID-19)* [Overviews and Factsheets]. https://www.epa.gov/coronavirus/about-list-n-disinfectants-coronavirus-covid-19-0

Villa, C., & Russo, E. (2021). Hydrogels in Hand Sanitizers. *Materials*, *14*(7), 1577. https://doi.org/10.3390/ma14071577

Vujasinović, M., Kočar, M., Kramer, K., Bunc, M., & Brvar, M. (2007). Poisoning with 1-propanol and 2-propanol. *Human & Experimental Toxicology*, *26*(12), 975–978. https://doi.org/10.1177/0960327107087794

Wilson, M., & Mowad, C. (2007). Chloroxylenol. *Dermatitis*, *18*(2), 120–121.

World Health Organization (Ed.). (2009). *WHO guidelines on hand hygiene in health care: First global patient safety challenge: clean care is safer care*. World Health Organization, Patient Safety.

Worrying levels of some impurities in hand sanitizers, chemists find. (n.d.). Chemical & Engineering News. Retrieved September 11, 2021, from https://cen.acs.org/acs-news/acs-meeting-news/Worrying-levels-impurities-hand-sanitizers/99/i14

Xiao, H., He, L., Tong, R., Yu, J., Chen, L., Zou, J., Li, J., Bian, Y., & Zhang, Y. (2014). Rapid and Sensitive Headspace Gas Chromatography–Mass Spectrometry Method for the Analysis of Ethanol in the Whole Blood. *Journal of Clinical Laboratory Analysis*, *28*(5), 386–390. https://doi.org/10.1002/jcla.21698

Appendix

Below is the table of hand sanitizers' samples used to carry out the research.

Samples	Composition as per label	Formulation	Volume (mL)	Prod. Date	Expiry Date
Sample 1	Denatured ethanol, water, glycerine, benzalkonium/o-phenylphenol, chloride	spray	60	11/10/2020	11/10/2023
Sample 2	Ethanol 70%, chlorhexidine digluconate, glycerol, water	spray	100	17/7/2020	17/7/2023
Sample 3	Ethyl alcohol 70%, deionized water, benzalkonium chloride	spray	110	1/4/2021	1/4/2023
Sample 4	Ethanol 70%, chlorhexidine digluconate, glycerol, water	spray	50	11/1/2020	11/10/2024
Sample 5	Alcohol denatured, aqua, glycerin, vaccinium oxycoccos fruit extract, acrylates/C10-30 alkyl acrylate crosspolymer, aminomethyl propanol, parfum citral, benzyl salicylate, coumarin, butylphenyl methylpropional, lialool, hexyl cinnamal, limonene	gel	240	1/4/2021	1/4/2023
Sample 6	Alcohol denatured, aqua, glycerin, vaccinium oxycoccos fruit extract, acrylates/C10-30 alkyl acrylate crosspolymer, aminomethyl propanol, parfum citral, benzyl salicylate, coumarin, butylphenyl methylpropional, lialool, hexyl cinnamal, limonene	gel	100	1/2/2021	1/2/2024
Sample 7	Alcohol denatured, aqua, glycerin, vaccinium	gel	550	1/5/2021	1/5/2024

	oxycoccos fruit extract, acrylates/C10-30 alkyl acrylate crosspolymer, aminomethyl propanol, parfum citral, benzyl salicylate, coumarin, butylphenyl methylpropional, lialool, hexyl cinnamal, limonene				
Sample 8	Ethyl alcohol 60%, alkyl dimethyl benzyl ammonium chloride 0.04%, moisturizer, aqua, parfum, hydroxycitronellal, benzyl benzoate, benzyl salicylate, cinnamyl alcohol	spray	60	1/3/2021	1/3/2024
Sample 9	N.A	spray	N.A	N.A	1/6/2023
Sample 10	Ethanol, aqua, glycerin, carbomer, aloe barbadenis leaf juice, isopropyl myristate, tocopheryl acetate, parfum, propylene glycol, aminoethyl propanol	gel	259	10/2/2021	9/2/2025
Sample 11	Ethanol (70 %, glycerine)	gel	1000	1/6/2021	1/6/2023
Sample 12	Distilled water, Vitamin E, Ethanol 70%	spray	80	1/1/2021	1/1/2024
Sample 13	Alcohol, aqua, propylene glycol, carbomer, triethanolamie, glycerin, citric acid, EDTA	gel	100	N.A	1/4/2023
Sample 14	Ethanol, Isopropanol 70%, water, acrylates/C30-10 Alkyl acrylate Crosspolymer, propylene glycol, oisturizers triethanolamine	gel	60	1/10/2020	1/10/2023
Sample 15	Alcohol Denat (70% v/v), aqua, glycerin, propylene	gel	80	29/4/2020	29/4/2023

	glycol, carbomer, benzalkonium chloride, parfum, benzyl salicylate, eugenol, geraniol, citronellol				
Sample 16	ethanol, isopropanol 70%, water, acrylates/C10-30 Alkyl Acrylate Crosspolymer, propylene glycol, moisturizers, triethanolamine, perfume	gel	60	N.A	1/12/2024
Sample 17	ethanol, diagnosed water, T.E.A, benzophenone4-carboner940, glycerin, EDTA4Na, propylene glycol, polysorbate, vitamin, fragrance, dye	gel	59	N.A	1/6/2023
Sample 18	alcohol denat., aqua, aloe barbadensis extract, glycerin, alkyl dimethyl benzyl ammonium chloride, urea, parfum, limonene	spray	50	23/6/2021	23/6/2023
Sample 19	alcohol denat., water, glycerin, vitamin E, carbomer, triehtanolamine	spray	65	1/7/2021	1/7/2024
Sample 20	N.A	gel	150	1/4/2021	1/4/2024
Sample 21	ethyl alcohol, alkyl dimethyl benzyl ammonium chloride, moisturizer, aqua, perfume	spray	125	1/3/2021	1/3/2025
Sample 22	ethyl alcohol 70% (v/v), aqua, propylene glycol, acrylate C10-30 Alkyl Acrylate Crosspolymer, aminomethyl propanol	gel	60	N.A	1/12/2023
Sample 23	ethanol 70% (v/v), aqua, glycerin, carbomer, Acrylates / C10 alkyl Benzophenone-3, fragrance yellow no.10, green no.5, red 7	gel	50	1/2/2020	1/2/2024
Sample 24	alcohol denat., aqua, acrylate/C10-30 Alkyl Acrylate Crosspolymer,	gel	50	14/6/2021	14/06/2024

	aloe barbadensis extract, glycerin, alkyl dimethyl benzyl ammonium chloride, 2-Amino-2-Methyl-1-Propanol, parfum, limonene				
Sample 25	alcohol denat 68% w/w	gel	450	N.A	5 years from production date
Sample 26	alcohol denat, aqua, glycerin, acrylate/C10-30 Alkyl Acrylate Crosspolymer, 2-Amino-2-Methyl-1-Propanol, aloe barbadensis extract, parfum, limonene	gel	750	15/05/2021	15/05/2024
Sample 27	aqua, tocopheryl acetate (vitamin E), carbomer, ethyl alcohol, isopropyl alcohol, glycerin, triethanolamine	gel	500	15/03/2020	15/03/2023
Sample 28	ethanol, aqua, carbomer, parfum, 2-Amino-2-Methyl-1-Propanol, glycerin, tocopheryl acetate, benzophenone	gel	59	N.A	N.A
Sample 29	denatured alcohol, aqua, carbomer, perfume, tea	gel	60	N.A	N.A
Sample 30	antimicrobial agent, rhickener, solvents, ethanol 65%, glycerin, water, vitamins, fragrance and colors	gel	40	N.A	N.A
Sample 31	eucalyptus essence, alcohol, aloevera gel, aqua, glycerin, tea tree oil, fragrance	liquid	80	N.A	N.A

Regression Statistics	
Multiple R	0.99620053
R Square	0.9924155
Adjusted R Square	0.98988733
Standard Error	0.02091676
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.17174198	0.17174198	392.5434286	0.000280977
Residual	3	0.00131253	0.00043751		
Total	4	0.17305451			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.64951329	0.047236691	13.7501863	0.000832409	0.499185063	0.79984153
X Variable 1	1.31050364	0.066144597	19.8127088	0.000280977	1.100002011	1.52100527

STD DEVIATION	0.10562445
DETECTION LIMIT	0.26597461
QUANTITATION LIMIT	0.80598365