QUALITY OF COMMERCIAL ALCOHOL-BASED HAND SANITIZERS IN KAMPALA: A NON-ADDRESSED ISSUE IN COVID-19 PREVENTION IN UGANDA

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Declaration

This thesis is my original work and has not been presented for thesis research examination in any other university, including the University of Nairobi, or another award elsewhere.

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Dedication

I am thankful to the Almighty God for his grace and blessings in undertaking this course thus far.

I dedicate this thesis to my dearest loving wife and prayer warrior, Esther Achan Okidi, for her steadfast support, patience, and encouragement, and my two little angels, my daughter Pirwot Janize Perline Okidi and my son Parwot Jayra Praise Okidi, for the many questions: “Where are you?”, “why?” and “when are you coming?” as well as beautiful waves of laughter and smiles over the telephone. You have been my driving force, my passion.

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ABHR	: Alcohol-based hand rubs
ABHS	: Alcohol-based hand sanitizer
ATR-FTIR	: Attenuated total reflectance Fourier-transform infrared spectroscopy
BC	: Benzalkonium chloride
CDC	: Centres for Disease Control and Prevention
COVID-19	: Coronavirus Disease 2019
EAS	: East African standard
FDA	: Food and Drug Administration
g	: Gram
GC	: Gas chromatography
GC-FID	: Gas chromatography - flame ionization detector (GC-FID)
GC-MS	: Gas chromatography-tandem mass spectrometry (GC-MS)
GMP	: Good Manufacturing Practices
IPA	: Isopropyl alcohol
KEBS	: Kenya Bureau of Standards
L	: Litre
LC-UV	: Liquid chromatography with ultraviolet absorbance detection
mL	: Millilitres
NCIP	: Novel coronavirus-infected pneumonia
NDA	: National Drug Authority
ppm	: Parts per million

qNMR	: Quantitative nuclear magnetic resonance spectroscopy
RSD	: Relative standard deviation
UNBS	: Uganda National Bureau of Standards
US EAS	: Uganda Standard East African standard
USP	: United States Pharmacopeia
v/v	: Volume by volume
WHO	: World Health Organization
µg	: Microgram
µL	: Microlitre

ABSTRACT

Introduction: The identification and worldwide spread of the new coronavirus disease 2019 (COVID-19) is a global public health emergency. The World Health Organization recommended alcohol-based hand sanitizers to prevent human-to-human transmission of coronavirus. The quality of hand sanitizers is of global concern.

Objective: This study aimed to conduct a qualitative and quantitative evaluation of commercial alcohol-based hand sanitizers in Kampala, Uganda.

Methods: Commercial products (130) in the market were sampled from five Divisions of Kampala city. The samples were assessed for appearance, packaging, labelling, and quality mark conformity. In addition, the pH of the samples was measured. Gas chromatography coupled with mass spectrometry and with flame ionization detectors were used for qualitative and quantitative analysis of the alcohol-based hand sanitizers, respectively.

Results and Discussion: Only 15 samples (12%) met all the specifications for appearance, packaging, labelling, and regulation characteristics assessed. Alcohol was detected in 128 samples (98%). The specific alcohols detected were as follows: Ethanol (86%), isopropyl alcohol (4%), and ethanol/isopropyl alcohol admixture (3%). Two samples contained no alcohol. Isopropyl alcohol was found as a denaturant in only one sample contrary to the label claims in seven samples. Seven samples contained methanol as an impurity. Twenty-two samples had divergent alcohol types from those declared on the label. Seventy-eight samples had alcohol content within the requisite range of 60-95% v/v. Forty-two had less than 60% v/v alcohol, and one contained more than 95% v/v. Five samples indicated methanol substitution, containing methanol solely, and two methanol contamination with methanol content above limits. A majority of the alcohol concentrations found in the study did not agree with the concentrations indicated on the labels. Sixty-seven samples did not comply with the specifications for pH.

Conclusion: Substandard and falsified alcohol-based hand sanitizers that contain harmful ingredients such as methanol are in circulation in the Kampala City Divisions.

Recommendation: There is a need to strengthen the regulatory institution and improve surveillance mechanisms to ensure compliance with set standards in manufacturing alcohol-based hand sanitizers in Uganda.

CHAPTER ONE: INTRODUCTION

1.1 Background

In 2019, a new coronavirus was reported in hospitalized patients in Wuhan, China. It presented as an acute respiratory syndrome-2 and an air-borne pathogen that could also spread through touching contaminated surfaces, characterized as a "novel coronavirus-infected pneumonia" (NCIP) (Zhu *et al*., 2020). This also led to the declaration of the disease as a worldwide pandemic and public health emergency by the World Health Organization (WHO) (WHO, 2021a), with Uganda reporting her first case of coronavirus disease 2019 (COVID-19) on 21st March 2020, in an asymptomatic traveller from Dubai. By April 15th, 2020, 54 patients had been reported in the country (Migisha *et al*., 2020).

Chakraborty and Maity (2020) reported that COVID-19 pandemic turned out to be the most critical challenge faced by humankind since the Second World War. As a result, the entire human population experienced vast health, economic, environmental, and social challenges. To help prevent the transmission of the disease, many nations recommended social distancing, avoiding body contacts such as handshakes, hand washing or use of sanitizers in the absence of hand washing. In addition, surface washing or sanitization like door knobs, massive testing and treating of patients, quarantining suspected persons through contact tracing, and instituting complete or partial lockdowns were also recommended, of which Uganda was not exceptional. Later, several COVID-19 vaccines essential for controlling COVID-19 were developed to induce strong immunity to the virus and reduce hospitalization and death (Sette and Crotty, 2021). The Centres for Disease Control and Prevention (CDC) recommended that children ages five years and older and eligible adults be vaccinated against COVID-19 irrespective of previous infections (CDC, 2021a). Vaccines are reported to protect one from viral infections and eliminate or reduce transmission within the affected population (Luyten and Beutels, 2016). It is worth noting that vaccines can lower the treatment costs of infectious diseases.

Hand sanitizers offer a fast and suitable means to eliminate pathogens from the hands when water and soap are inaccessible. As a result, they often protect and prevent the passage of bacteria, viruses, and other infectious pathogens (Golin *et al*., 2020). Sanitizers are formulated as liquids, gels, foams, sprays, dispensers, or wipes. When applied and rubbed on hands, sanitizers

kill infective microorganisms (Greenaway *et al*., 2018; Singh *et al*., 2020). Hand sanitizers are alcohol-based (ABHS) or alcohol-free based on the active ingredients used (Jing *et al*., 2020). The alcohol type, grade and concentration are key to product quality and efficacy against pathogenic microorganisms (Singh *et al*., 2020). For alcohol-based hand sanitizers, the CDC specifies that they should be formulated with at least 60% alcohol, which can either be ethanol or isopropanol (CDC, 2021b; Singh *et al*., 2020)

The World Health Organisation also defines different combinations of formulations of ABHS containing ethanol, isopropanol, hydrogen peroxides, and water (WHO, 2010). Non-alcohol-based hand sanitizers formulated with 0.12% (w/w) benzalkonium chloride (BC) are also effective in eliminating infectious microorganisms on surfaces (Bondurant *et al*., 2019).

Packaging and labelling of the ABHS are considered secondary to the quality of the ABHS formulation. Polyethylene terephthalate (PET) plastic packaging and glass bottles with leak-proof tops were recommended to be safe for the packaging of hand sanitizers (Blaxhall, 2020; WHO, 2010). However, the packaging of ABHS in aluminium beer cans may lead to container corrosion (Nyamweya and Abuga, 2020; Thomson and Bullied, 2020).

Different regulatory agencies specify regulatory parameters and production requirements of ABHS, including classification for quality control purposes (Dicken *et al*., 2020). For example, the US-FDA categorizes hand sanitizers as drugs and regulates them under biocidal products as per the European community for safety and efficacy concerns (US-FDA, 2021a). Locally, the Uganda National Bureau of Standards (UNBS) is mandated with the regulation of hand sanitizers (UNBS, 2013). The specific product quality tests include alcohol content, pH, and bactericidal efficacy. The general requirements are on appearance, smell, packaging, and labelling. The uniqueness of the UNBS standard compared to Food and Drug Administration (US-FDA) guidelines is that UNBS has included n-propanol as one of the permitted alcohols in addition to ethanol and isopropanol allowed by US-FDA at a minimum content of 60% v/v (UNBS, 2013).

Using hand sanitizers without virucidal activity or containing toxic ingredients correlates with a sense of insecurity and risks thereafter. Toxic substances render the formulation harmful and not suitable for human use (Matatiele *et al.,* 2021; Tse *et al.,* 2021a; Yip *et al.,* 2020). For example, ABHS products adulterated with methanol and other impurities like acetaldehyde, ethyl acetate,

and 1-propanol are of toxicity concern (Tse *et al.,* 2021a). Disabilities following methanol exposure through transdermal absorption has also been noted (Ashurst and Nappe, 2021).

Following the global medical crisis coupled with recommendations on the use of hand sanitizers in the absence of hand washing with water and soap to prevent and eliminate coronavirus transmission by health agencies worldwide, hand sanitizer demand increased considerably across the globe (Dicken *et al*., 2020).

The COVID-2019 epidemic offered manufacturing opportunities for pharmaceutical companies and many inexperienced manufacturers that began producing hand sanitizers unconventionally. In addition, several chemical industries, breweries and perfumeries also switched to hand sanitiser production (Bomgardner *et al*., 2020). Hence, the risk of introducing additional contaminants into the ABHS products (Abuga *et al*., 2021; Nyamweya and Abuga, 2020; Tse *et al*., 2021a). This led to their widespread usage, and initially, in 2020 there were sudden shortages in the supply of ABHS worldwide, including in Uganda (Berardi *et al*., 2020a; Tse *et al.*, 2021a).

In Uganda, as of July 8^{th,} 2020, in the early stages of COVID-19, the UNBS registered 136 manufacturers producing 182 brands of sanitizers (UNBS, 2020a). In the wake of the second wave of COVID-19 infections, UNBS, as of July 6^{th,} 2021, had 132 brands registered during the annual renewal of the license to manufacture sanitizers and disinfectants (UNBS, 2021). The decline in brands could have probably been due to several recommendations by WHO, national health agencies, and presidential addresses in Uganda in particular, where hand washing with soap and water using the correct technique for 20 seconds was emphasized as the preferred measure for hand hygiene during COVID-19 prevention (Wood, 2021). In Tanzania, such a decline in the manufacture of ABHS was attributed to government recommendations of a return to regular routines following several announcements of a reduction in the number of cases and the declaration of some areas as virus free. The impact was a decreased demand for ABHS (Halfan, 2020). This apparent decrease in demand was also noted in Uganda (Ojambo, 2021).

The UNBS specified a method for determining alcohol content described in the specification, Uganda Standard East African Standard (US EAS) 104, adapted from the East African Standard (EAS), which does not cover non-alcohol-based hand sanitizers. Furthermore, the method described a pycnometer, a non-specific technique, to determine alcohol content based on the

difference in specific gravity of alcohol-water mixtures at a particular temperature (UNBS, 2013).

Based on previous studies conducted in Kenya, USA, and South Africa, it was recommended that suitable analytical methods for ABHS be employed with strict requirements in quality control (Abuga *et al.,* 2021; Jie, 2020; Matatiele *et al.,* 2021). Furthermore, specific test methods, like gas chromatography (GC), are suggested to determine constituent alcohols and other volatiles (Abuga *et al*., 2021). The gas chromatography method validation demonstrated the suitability of the GC for alcohol analysis in hand sanitizers. Hence, GC plays an essential role in the quality control of ABHS (Jie, 2020).

There are no scientific studies that have evaluated the quality aspects of locally available ABHS in Uganda. Therefore, to ultimately safeguard consumers, this study will seek to evaluate the quality of commercial ABHS brands sold in the Ugandan market, Kampala.

1.2 Statement of the problem

Following the upsurge of COVID-19 in 2020, the WHO and the CDC recommended regular hand washing with water and soap and the use of sanitizers to prevent the risk of transmission and infection of the coronavirus disease (CDC, 2021a; Pradhan *et al.*, 2020; WHO, 2021a). The subsequent recommendations and ease of use of ABHS contributed to the dramatic rising demand and market for hand sanitizer products across the world against an unprepared manufacturing landscape (Berardi, *et al.*, 2020a). Consequently, this led to the introduction into the market of a large number of brands of ABHS with efficacy and safety challenges. There have been instances where products are substandard and/or falsified (Matatiele *et al*., 2021). Notably, some hand sanitizers were found to contain high-risk toxic substances namely: ethyl acetate, which causes skin defatting, and acetaldehyde, which is carcinogenic and teratogenic. Methanol and 1-propanol. In methanol use as an ethanol substitute by some ABHS manufacturers, numerous deaths and significant blindness have been reported when individuals unknowingly or knowingly consumed such ABHSs in Kenya (Gekonge, 2021; Waithera, 2020). In addition, there have been expressed concerns about ABHS products with packaging having appealing colouring or markings attractive to children leading to accidental ingestion (Joseph *et al*., 2011; US-FDA, 2020). More than 700 fatalities and disabilities were registered due to the ingestion of methanol-contaminated sanitizers in Iran (Aljazeera, 2020) and the USA (Fazio, 2020). The US-FDA

recalled several hand sanitizers with catastrophic amounts of impurities, such as methanol, n-propanol, benzene, acetal, and acetaldehyde (US-FDA, 2021b).

The UNBS has cautioned the public against buying certain blacklisted sanitizer brands of questionable quality reported to have failed alcohol content specification (UNBS, 2020b). There are, however, no scientific studies that have evaluated the quality of locally available hand sanitizers in Uganda. Therefore, assessing the quality of commercial ABHS products in circulation in the Kampala against UNBS specification offers the information necessary to identify substandard and falsified products and provide key information for efficient control by regulatory agencies to guarantee product quality.

1.3 Study justification

There is need for careful design and formulation of ABHS to provide the desired quality, effectiveness, and safety (Abuga *et al*., 2021; Jairoun *et al*., 2021). This project presents a unique approach to a non-addressed issue in COVID-19 prevention by focusing on assessing the quality of ABHS sold in Kampala.

While there are several published reports of studies in the literature from Italy, South Africa, Ethiopia, Kenya, and other countries that have addressed quality aspects of ABHS, there are no scientific studies done in Uganda. The only report on the quality of ABHS in Uganda is a newspaper article by UNBS where 15 sanitizer brands were blacklisted after failing to pass quality tests (UNBS, 2020b).

A study in Italy indicated that tested ABHS products for quality should fulfil the regulative need of their class as either biocide or cosmetic. However, a few cosmetic hand sanitizers with undeclared alcohol content were found to contain ethanol well below the concentrations suggested for disease prevention, hence falling below disinfection standards (Berardi *et al*., 2020b).

Another study in South Africa noted substandard ABHS available in the market. Some contained toxic ingredients like ethyl acetate, isobutanol, and methanol (Matatiele *et al*., 2021). In Ethiopia, the quality assessment of various ABHS in the Addis Ababa market demonstrated substandard ABHS products, with 70% of them falling below the WHO limit for alcoholic concentration. In

addition, the nature and origin of several brands of ABHS were unknown since most were in the market without requisite labelling (Selam, 2020).

Substandard and unlicensed ABHS products were found in circulation in the Kenyan market. For example, 89% of the brands stated alcohol as an ingredient, yet 62% did not display the specific alcohol used (Nyamweya and Abuga, 2020). Several products also contained many unknown volatiles, indicating poor quality. Gas chromatography with mass spectrometry was suggested to fully characterize unspecified volatile impurities in several products under investigation (Abuga *et al*., 2021). In Uganda, the UNBS, (2013) instant hand sanitizers standard, US EAS 789:2013, refers to a pycnometer as the technique used to determine the alcohol content in alcoholic beverages (US EAS 104: 2014). However, this method uses the specific density of alcohol to establish the alcohol content, and it cannot verify the different types of alcohol in the samples.

Gas chromatography-flame ionization detector (GC-FID) provides a reliable and straightforward method for identification and accurate quantification of active ingredients and impurities in alcohol-based hand sanitizers, while gas chromatography-mass spectrometry (GC-MS) was used for qualitative analysis of the hand sanitizer samples to identify unknown impurities (Bedner *et al.,* 2021). This study, therefore, aims to establish the quality of commercial ABHS in the five divisions of Kampala city, Uganda, using GC-MS and GC-FID, highly specific techniques for volatile constituents.

1.4 Study significance and utility

The results of this project may be beneficial to the government of Uganda in the provision of the necessary data to inform policy and enable enforcement of strict market control of ABHS products, improve the quality of products in circulation, and protect public health against unregistered harmful products. Furthermore, it would lead to sensitization and liaison between the various stakeholders. It may also lead to the sensitization of communities, and eventually, consumers against the risks of using substandard and falsified ABHS products. The results of this project could provide the Government of Uganda with information that shall potentially assist UNBS in revising UNBS specification for instant hand sanitizers. In addition, it might provide a good method for use by Ugandan researchers, and regulatory laboratories for determining the content of specific volatiles in alcohol-based hand sanitizers.

1.5 Research questions

i. Do the commercial ABHS in Kampala, Uganda, conform to UNBS specifications for ABHS in terms of appearance, packaging, labelling, UNBS standardization mark of registration, and pH?

ii. What are the alcohols and impurities, and their content in commercial ABHS in Kampala, Uganda?

1.6 Objectives of the study

1.6.1 General objective

To evaluate the quality of locally commercially available alcohol-based hand sanitizers in Kampala, Uganda.

1.6.2 Specific objectives

i. To assess commercial alcohol-based hand sanitizers in Kampala, Uganda, for conformity with Uganda National Bureau of Standards specification with respect to appearance, packaging, labelling, presence of UNBS standardization mark, and pH.

ii. To identify the alcohols and impurities in commercial alcohol-based hand sanitizers in Kampala, Uganda.

iii. To quantify the alcohols and impurities in commercial alcohol-based hand sanitizers in Kampala, Uganda.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of COVID-19

2.1.1 Symptoms and diagnosis of COVID-19

A novel coronavirus was first identified in December 2019, in Wuhan, China, followed by a rapidly spreading pandemic globally. Coronavirus disease 2019, also known as COVID-19, was on its outbreak, considered a new human coronavirus, namely severe acute respiratory syndrome coronavirus-2 (Huang *et al*., 2020). The virus is now known to be transmitted from a patient's mouth or nose in droplets as one coughs, sneezes, speaks, sings, or breathes. Infected persons may experience mild to moderate respiratory illness symptoms and recovery without any particular treatment, although others may become critically sick, requiring special medical interventions. It has been noted that old age and patients with pre-existing medical conditions like cardiovascular disease, diabetes, chronic respiratory disease, or cancer are more likely to develop serious illnesses (WHO, 2021a). The coronavirus disease 2019, a respiratory infection, presents with symptoms of dry cough, fever, severe headache, and tiredness, and symptoms range from mild to severe respiratory diseases and critical illness resulting in organ dysfunction, like cardiac failure, kidney failure, liver dysfunction, lung dysfunction, arrhythmia, and some death (Gordon *et al*., 2020; Kumar, 2020).

Some recent techniques were developed and implemented to diagnose COVID-19. The development of accurate, sensitive, and specific point-of-care devices has been advantageous in detecting human coronavirus and tracing infected persons at the early stages of the infection (Taleghani and Taghipour, 2021). Real-time polymerase chain reaction is a molecular technique used to diagnose new cases of COVID-19 and monitor treatment outcomes. In contrast, the immunoassay test is an additional tool for mass screening and confirming the molecular assay (Mathuria *et al*., 2020). In addition, organ dysfunctions linked to coronavirus disease can be identified using computed tomography scans and X-rays (Taleghani and Taghipour, 2021).

2.1.2 Preventive measures of COVID-19

The WHO and CDC recommend an array of control measures. These comprise regular hand washing with water and soap or use of hand sanitizers, social distancing, respiratory hygiene (covering nose and mouth while sneezing or coughing and wearing a face mask), proper

ventilation of indoor spaces, self-isolating until recovery if feeling ill and finally vaccination with COVID-19 vaccines (CDC, 2021a; Pradhan *et al.*, 2020; WHO, 2021a).

Several COVID-19 vaccines essential for controlling human coronavirus infections have been developed and promoted, and they are capable of inducing long-term immunity towards the virus and preventing hospitalization and death (Sette and Crotty, 2021). It is reported that COVID-19 vaccines with 50% efficacy reduce the acquisition and transmission of the virus, provide protection from serious short- and long-term complications, and prevent children and adults from severe illness and death (CDC, 2021b; WHO, 2021c). However, due to inequity in the timely supply and the desperate demand for safe and efficacious COVID-19 vaccines (Chung *et al*., 2021), hand hygiene remains one of the mainstays preventing COVID-19 spread.

2.2 Overview on use of hand sanitizers

Alcohols or benzalkonium chloride acting as anti-microbial compounds are the active ingredients in hand sanitizers (Golin *et al*., 2020). Alcohol-based hand sanitizers are suggested for regular use. In contrast, alcohol-free sanitizers are less preferred to ABHS because they have poorer efficacy and a narrower antimicrobial spectrum (Todd *et al*., 2010).

Both soap and alcohol disinfectants have been shown to remove microbial contaminants on skin surfaces. They dissolve microbial lipid membranes and hence disable the microorganism. Therefore, if water is inaccessible, an alcoholic disinfectant with at least 60% alcohol is an alternative for microbial infection prevention. Alcohol-based hand sanitizers have been highly efficacious in deactivating coronavirus (Prajapati *et al*., 2022) and have been used for many years to prevent various infectious diseases globally (Pidot *et al*., 2018). However, previous literature reports that frequent use of ABHS is linked to development of anti-microbial resistance and enhancement of the possibility of other viral diseases. There are also toxic and serious health risks to human health as well as the environment (Mahmood *et al*., 2020).

Efficacy of ABHS is influenced by various parameters, including the technique used for the application of sanitizers, the rate of volatilization of the alcohol in various preparations, the alcohol concentration and duration of the active substance when in contact with the hands during sanitization (Pasquini *et al*., 2020). Applying a sufficient ABHS (60 to 85% concentrations) and rubbing for 25-30 seconds demonstrated 99.99% killing of microorganisms on hands (Rotter, 1999).

2.2.1 Composition of hand sanitizers

Hand sanitizers can be categorized as alcohol-based or alcohol-free based on the active ingredients used (Jing *et al*., 2020). With regard to ABHS, the US-FDA, CDC, and WHO recommend 60-95 % v/v ethanol or isopropanol mixed with distilled water (Barrett and Babl, 2015; CDC, 2020; US-FDA, 2021c; WHO, 2009), and in some cases in combination with other non-alcoholic antiseptic agents (Todd *et al.*, 2010). In addition to alcohol, other ingredients that may be included are humectants (like glycerine), moisturizers such as vitamin E or aloe vera extract, thickening agents (mostly carbomers), pH adjusting agents (like triethanolamine, tromethamine), viscosity enhancers, fragrances, preservatives, colourants, and denaturants such as acetone, according to formulation type (Berardi *et al*., 2020a; Todd *et al*., 2010). The WHO specifies that ABHS should be formulated with either ethanol (80% v/v) or 2-propanol (75% v/v) mixed with glycerine (1.45% v/v), hydrogen peroxide (0.125% v/v), and sterile water, for household or local production of ABHS (WHO, 2010). There are reports of alcohol-based solutions with other vital ingredients such as octenidine dihydrochloride (0.1%), phenoxyethanol (2%), hexamethylene biguanide, alcohol denat and aminomethyl propanol (Langer *et al*., 2004).

Non-alcohol-based (alcohol-free) hand sanitizers formulated with benzalkonium chloride (BC) as an alternative to the ABHS have been proven effective in killing germs. Unlike alcohols, benzalkonium chloride (BC) and other active ingredients in alcohol-free sanitizers are not volatile, so anti-microbial activity can persist for extended periods. Benzalkonium chloride (BC) 0.10% (w/w) has been reported to be safe and effective with less likelihood of causing skin irritation (Aodah *et al*., 2021).

In Uganda, UNBS recommends using ethanol and/or isopropanol, n-propanol in the production of ABHS at a concentration of not less than 60% (UNBS, 2013). Manufacturers are also encouraged by other research articles to consider alcohol content of 70–80% (v/v) during the formulation of ABHS. This is because products with less than 60% alcohol reduce the efficacy and increase the risk of evaporation of the active ingredient during processing, transportation, storage, or use of ABHS (Jairoun *et al*., 2021).

2.2.2 Mode of action of hand sanitizers

Alcohol in the presence of water kills microorganisms by disrupting cell membrane permeability hence leakage of cytoplasm, denaturation of proteins, and in the end, cell lysis (Gold *et al*., 2021). The broad-spectrum germicidal activity of ABHS against fast-growing bacteria, viruses, and fungi involves the dissolution of the lipid membrane and denaturation of proteins, disrupting the virus membrane and inhibiting metabolism (Singh *et al*., 2020).

Non-alcohol hand sanitizers have shown comparable activity against certain viruses, fungi, and protozoans. Chlorhexidine, chloroxylenol, iodine/iodophors, triclosan, and alkyl benzalkonium chloride, among other agents, have anti-microbial activity used for hygienic hand washing (WHO, 2006).

Benzalkonium chloride is primarily used as the main ingredient in formulating alcohol-free hand sanitizer, though it is ineffective against non-enveloped viruses. The mechanisms of action involve reduction of the membrane's fluidity, creating hydrophilic gaps in the membrane and disruption of the physical and biochemical properties of membrane bilayer, and subsequently disturbing protein function. This depends on the cationic "head group" and alkyl chain "tail" component of benzalkonium chloride (Wessels and Ingmer, 2013).

Despite its corrosive nature, low concentration hydrogen peroxide eliminates contaminating bacterial spores in formulations. However, it is considered an inactive ingredient in hand sanitization preparations (WHO, 2010).

2.2.3 Efficacy of hand sanitizers

To be generally considered safe and effective, ABHS are specified to contain at least 60 % v/v of either ethanol or 2-propanol, as well as minimal amounts of harmful impurities as per FDA regulation guidance (CDC, 2021a; Singh *et al.,* 2020). Isopropanol used in ABHS formulations has superior activity against bacteria. In contrast, ethanol is known to have increased potency against viruses. In addition, isopropanol has better efficacy than ethanol against coronavirus due to its relatively higher lipophilicity. The degree of the effect, though, depends on the alcohol content and physical properties of the specific pathogen (Singh *et al*., 2020). However, isopropanol and ethanol are considered ineffective against bacterial spores in formulations. Hence, the addition of hydrogen peroxide (3%) in formulations eliminates contaminating bacterial spores (WHO, 2009).

The efficacy of ABHS depends on several parameters, including alcohol type and content, the quantity applied on hands, contact time, formulation, other ingredients, viral contamination load, and application technique (Abuga and Nyamweya, 2021; Todd *et al*., 2010). The volume of alcohol and contact time increases the effectiveness of ABHS. Gel-based hand sanitizers are more efficacious against enveloped viruses than foam-based preparations with fast drying time. Applying at least 3 ml of alcohol and rubbing for around 45-50 seconds has been recommended (Singh *et al*., 2020). The CDC and WHO recommend the application of the formulation to the palm of one hand (or to cover all surfaces of both hands) and rubbing until the hands are dry for 30-60 seconds. However, different formulations offer variations in product volumes to apply to the hands (CDC, 2020; WHO, 2009).

2.2.4 Packaging and labelling of hand sanitizers

Packaging and labelling are critical components considered for product safety, efficacy and delivery. For example, several ABHS are packaged in plastic containers. Consumers of products must have the necessary information on ingredients in a product that is well labelled. Proper labelling avoids exposure to certain chemicals that may cause sensitivities and allergies and for consistent compliance and traceability of products (Nyamweya *et al*., 2021; Nyamweya and Abuga, 2020).

Locally, UNBS recommends using suitable well-closed containers or inert packages and resistant to environmental factors during normal handling, transportation, and storage. In addition, the labelling requires that the container or package is marked legibly and indelibly with specific information and cautionary warnings (UNBS, 2013).

Following a survey conducted on ABHS in Nairobi by Nyamweya and Abuga (2020), several non-conformities were observed in packaging, labeling, and other established regulatory standards with the ABHS samples. They included a lack of listed ingredients on the label, incomplete ingredient information, unconventional abbreviations or trade names, inappropriate labelling for the alcohol type and content, and some were categorized as falsified.

2.3 Regulation of hand sanitizers

Worldwide, individual countries have specific regulations that govern the different regulation parameters and production of ABHS, including classification. At the height of the COVID-19 pandemic, the development of such regulations helped meet the urgent demand for safe and effective hand sanitizers, following an acute shortage of hand sanitizers considered crucial for the safety and prevention of the spread of the SAR-CoV-2 virus (Dicken *et al*., 2020).

2.3.1 Standards of hand sanitizers

In the USA, hand sanitizers are categorized as drugs and regulated for safety and efficacy by the Food and Drug Administration (FDA) (US-FDA, 2021a). The manufacturers of hand sanitizer are expected to obtain market authorization from the FDA and with the formulation of products using standard ingredients and testing criteria verified by the USP. As below, there was also an assembly of standards into a single collection by the USP to guide hand sanitizer manufacturers during the coronavirus disease crisis (USP, 2021).

The USP standards and monographs provided the following specifications and acceptance criteria (USP, 2020a) in Table 1.

Table 1: USP specifications and acceptance criteria of ingredients used in the manufacture of ABHS

Ingredient	Acceptance criteria
Ethanol	NLT 94.9% and NMT 96.0%, by volume
Isopropyl alcohol	NLT 99.0%
Glycerine	NLT 99.0% and NMT 101.0%
Hydrogen Peroxide	NLT 29.0% and NMT 32.0%, by weight
A suitable preservative or preservatives	NMT 0.05%
Purified water	Obtained by a suitable process

NLT: not less than, NMT: not more than

There has also been the release of the USP collection of monographs and standards providing limits of impurities in pure alcohol, as shown in Table 2 (USP, 2020a).

Table 2: USP limits on impurities in pure alcohol used in the manufacture of ABHS

Name	Acceptance criteria, NMT (μL/L)
Methanol	200
Acetaldehyde and acetal	10, expressed as acetaldehyde
Benzene	2
Sum of all other impurities	300

NMT: not more than

The UNBS is the qualified regulatory authority in Uganda mandated with the powers to register and regulate ABHS according to the 'instant hand sanitizers standards' (Uganda Standard EAS 789:2013) adapted from the East African Standard for purposes of cross-border trade and use of ABHS (UNBS, 2013). Therefore, manufacturers of ABHS in Uganda must obtain market authorization from UNBS after demonstrating compliance with instant hand sanitizer standards.

The UNBS general requirements include appearance, smell, packaging such as well-closed, inert and strong containers or packages including closures, labelling legibly and indelibly with appropriate information like identity, quantity, and responsibility, and cautionary warnings, and finally, possession of UNBS quality mark (UNBS, 2013). Table 3 describes the specific quality requirements for instant hand sanitizers in US EAS 789:2013.

Table 3: UNBS quality specifications for instant hand sanitizer

Characteristic	Requirement	Test method
Alcohol content (ethanol and/or isopropanol, n-propanol), %, v/v, minimum	60.0	S 104 (EAS 104)
pH (neat)	6-8	-
Bactericidal efficacy	To pass test	-

S: Standard, EAS: East African Standard

The above UNBS 'instant hand sanitizers specification' differs from that of the US-FDA in that it allows for the manufacture of ABHS with n-propanol as the alternative to alcohol. However, n-propanol is not specified for incorporation in ABHS formulation in the United States. This is

because of n-propanol-associated toxicities, central nervous system depression, fatalities following accidental ingestion, and irritation and infrequent dermatological adverse reactions following skin or eye contact (US-FDA, 2021b).

2.3.2 Production of hand sanitizers

The coronavirus pandemic led to escalated demand for ABHS. To meet the public demand, there were modifications in procedures for the manufacture of ABHS by various regulatory agencies during the public health emergency, which enabled the industry's flexibility (Tse *et al*., 2021a; US-FDA, 2021d). As a result, many manufacturers increased their ABHS production, and others shifted from the production of certain items to ABHS (European Commission, 2020).

The surge in the ABHS market during the pandemic prompted increased competition in ABHS production, joined by inexperienced manufacturers who may have introduced additional contaminants into the product. In addition, many manufacturers may have used technical grade ethanol as a raw material instead of pharmaceutical/food grade alcohols. However, it is noted that technical grade ethanol (TGE) is permitted for use in the manufacture of ABHS by health agencies in Canada and the USA. It is considered to be of lower risk for general use and allowable in containing the spread of disease. However, TGE may have higher levels of certain impurities and contaminants incorporated during the formulation and packaging of ABHS with potential adverse toxicological effects (Tse *et al.,* 2021a).

Alcohols like ethanol used in the manufacture of ABHS can be obtained from the fermentation of biomass such as cereal grains or synthetically. Synthetic ethanol manufacture requires high energy for compressing ethylene and water over phosphoric acid catalysts, making it expensive (Hidzir *et al*., 2014; Kim *et al*., 2009). Therefore, ethanol manufacture for ABHS production is dominantly obtained through fermentation which is cheaper and renewable (for example using cereal grains), without toxic substances like petroleum-based olefins (Pascault *et al*., 2012).

Regulatory authorities are more vigilant in ensuring that raw materials used to manufacture hand sanitizer stick to official monographs or modified regulatory guidelines, limiting individual impurities. This ensures that raw materials are of high quality and that undesirable contaminants are minimized in the products (Tse *et al.,* 2021a).

Berardi *et al*. (2020a) suggested various preparation methods for ABHS gels. They include the direct addition method, inverse addition method, and other methods like the "hot/cold" technique used for high-viscosity solutions, which tend to form lumps. In addition, the combination (hybrid) of direct and reverse addition techniques can also be used.

The WHO recommends that hand rub formulations' manufacture and storage facilities be suitable. Local or central manufacturers without specialized air-conditioned and ventilated rooms are not mandated to produce quantities exceeding 50 litres. The production facilities are expected to directly dilute highly concentrated ethanol to the recommended concentration since pure ethanol is highly flammable (WHO, 2010).

2.3.3 Recalls of hand sanitizers

In the United States of America (USA), FDA recalled several hand sanitizers with detrimental contaminants, such as methanol, n-propanol, benzene, acetal and acetaldehyde and with inadequate levels of alcohol required for infection control (US-FDA, 2021b). In Canada, there were several reports on recalls and advice about certain brands of ABHS by Health Canada, which did not meet regulatory requirements for several reasons. The focus was on unauthorized use of technical grade ethanol, products containing unauthorized ingredients, labelling and/or packaging problems, false claims on efficacy, and the presence of impurities such as methanol, ethyl acetate and acetaldehyde (Health Canada, 2021; Nicol, 2021).

Health Sciences Authority (HSA), Singapore, recalled 18 batches of ABHS products from the market. The recall followed the detection of elevated levels of acetaldehyde and/or methanol in the hand sanitizers above the pharmaceutical pharmacopoeia limit set (HSA, 2021). There was a recall of ABHS manufactured in Turkey in various nations, including France, by the National Agency for Food and Drug Administration and Control (NAFDAC) for containing ethanol content less than 42%. The ethanol content was considered inadequate, increasing the risk of infection by the end users (NAFDAC, 2020). In addition, substandard hand sanitizers were reported in the Australian market. The ABHS formulations were found to contain alcohol concentrations of as low as 23%, necessitating a recall from the market (CHOICE, 2020).

In Central Africa, several hand sanitizers were recalled from the market due to poor quality. For example, in Rwanda, the ABHS were found to contain incorrect alcohol content and were banned from the market by Rwanda Food and Drugs Authority (FDA) (Byishimo, 2020), while

in Zambia, 13 brands of hand sanitizers were recalled from the market following failed protection and safety standards by the Zambia Bureau of Standards (ZAMRA, 2020).

Ethiopian Standards Agency recalled numerous ABHS from the market for failure to meet product quality specifications. For example, 70% of the products were sub-potent, and 100% failed the hydrogen peroxide content limit. In addition, most of the products were sold without labels making the nature of ingredients and sources unknown (Selam, 2020). Following the first COVID-19 case in Kenya, numerous substandard ABHS were found in the market and recalled (Ngina, 2020).

According to Ugandan media reports on April 3, 2020, the UNBS had cautioned the public not to buy 15 blacklisted sanitizer brands, having failed to pass mandatory laboratory tests like alcohol concentration, pH, and bactericidal efficacy. The public was encouraged to report the presence on the market of the 15 brands to enable the enforcement team to put them off the market (UNBS, 2020b).

2.4 Quality control of hand sanitizers

As part of good manufacturing practices (GMP), quality control requires manufacturers to regularly conduct audits of suppliers and test all raw materials before production and the finished products. Quality control of alcohol concentrates acquired from local production is conducted as pre-production and post-production analyses. This involves verifying alcohol type and concentration and adjusting the preparation formulation volume to secure a final recommended concentration (WHO, 2010).

For quality control purposes, the alcoholmeter determines the actual alcohol content in the final use ABHS formulation. However, GC which is a high level quality control technique can also be used to control the alcohol content of the final use ABHS.

According to the European Pharmacopoeia specifications, filtration can be used to screen for possible contamination (including spores) (WHO, 2010).

2.4.1 Quality of hand sanitizers in the market

Previous studies in other countries noted a significant rate of recurrence of substandard, unlicensed, and sub potent ABHS in circulation, with a number of them not stating the specific

alcohol used in manufacture (Abuga *et al.*, 2021; Matatiele *et al.*, 2021; Nyamweya and Abuga, 2020).

In Canada, an analysis of 42 alcohol-based hand rubs for nine common impurities to establish compliance with Health Canada interim guidelines found that 11 samples contained acetaldehyde, with concentrations 3.3 times higher than the set permitted limits. Seventeen samples exceeded the USP standards and monographs for combined acetal and acetaldehyde. However, methanol contents fell below the USP set limit of 200 µL/L (Tse *et al*., 2021b).

A study in Italy found that, of the tested for quality, all ABHS products fulfilled the regulatory need for their class: biocide or cosmetic. However, some alcohol-based hand rubs (approximately 43%) tested had ethanolic content falling below specified disinfection standards of 60 to 95% v/v (Berardi *et al*., 2020b).

In South Africa, of the 94 samples of hand sanitizer evaluated, 40 of the sanitizers had alcohol concentration not declared on the label. Only one sample was appropriately labelled as alcohol-free. Fifty-six per cent of the products sampled contained the specified alcohol concentration. In comparison, 44% were substandard and most likely sub-potent, with some containing toxic ingredients. It was also found that 30% of the analyzed sanitizers had alcohol concentrations exceeding 80% v/v, which does not render increased activity since water is necessary for the disinfectant activity of alcohol (Matatiele *et al*., 2021).

In Kenya, 74 samples were evaluated, and the alcohol content was presented as the sum of the two permitted alcohols; ethanol or isopropanol. It indicated that only 10.8% had alcohol content greater than 60%. In contrast, 40.5% of the samples met methanol limits, while the remaining samples were found to have either methanol substitution or methanol limits above the 630 ppm threshold. Some 18 samples (24.3%) contained different alcohols other than those indicated on the label. Concerning pH, 59.5% of the samples met pH specifications for ABHS (Abuga *et al*., 2021).

Nyamweya and Abuga (2020) reported the following excipients used in ABHS; glycerine as the most widely used humectant, added to keep hands moist, pH adjusting agents such as triethanolamine necessary also to promote gelation and adjust formulation thickness,

perfumes/fragrances, aloe, and colouring agents. Carbomers are most widely used as thickening agents in ABHS to achieve optimum viscosity.

However, there are no scientific studies done in Uganda. Except, the report by UNBS on media cautioning the public on purchasing 15 blacklisted sanitizer brands for failing to pass mandatory laboratory tests (UNBS, 2020b).

2.4.2 Impurities and adulteration in hand sanitizers

Various impurities can be naturally co-produced with ethanol during the manufacture of alcohol following the fermentation of biomass and distillation (Onuki *et al*., 2016). In most cases, the impurities are co-distilled as azeotropes alongside ethanol, complicating the purification processes of ethanol. In addition, the contaminants found in the formulation are promoted by several parameters, including the origin and amount of nitrogen, process conditions such as pH, yeast amount, biological and distillation processes (Capeletti *et al*., 2000; Hazelwood *et al*., 2008). Among the impurities co-produced and co-distilled with ethanol are acetates, aldehydes, butanols, amyl alcohols, pentanols, propanols, and methanol (Onuki *et al*., 2016).

It has been reported that some sanitizers contain, in addition to permitted alcohols, several toxic ingredients, like ethyl acetate, methanol, and 1-propanol. Alcohol-based hand sanitizer products adulterated intentionally with methanol, and other impurities are especially of concern due to their higher toxicity and serious adverse health consequences and/or death (Gekonge, 2021; Matatiele *et al.,* 2021; Tse *et al.,* 2021a; Yip *et al.,* 2020). In some incidences, ABHS manufacturers substitute ethanol as a raw material with methanol which they find attainable and relatively cheap compared to the recommended alcohols (Gekonge, 2021; Tse *et al.,* 2021a).

Consumers of ABHS susceptible to contact dermatitis and allergic reactions should be aware of the widespread use of fragrances in ABHS products (Nyamweya *et al.*, 2021). There has also been a registered report of a person who suffered burns after exposure of hands wetted with hand sanitizer over the flame. In addition, highly concentrated ABHS has been reported to be ignited in their packaging, during the application, or once spread on hands, leading to thermal injuries (O'Leary and Price, 2011).

2.4.3 Qualitative and quantitative analytical methods for hand sanitizers

For the intended product quality and effective killing of pathogenic microorganisms, correct identification and quantification of the active ingredients in an ABHS formulation is a critical quality assessment test and representative of product efficacy (Singh *et al.*, 2020). Based on previous studies in the literature, it is recommended that suitable analytical methods for ABHS integrated with strict requirements be used. Specific analytical methods like gas chromatography are suggested to determine alcohol types and other volatiles and establish interim impurities limits (Abuga *et al*., 2021; Matatiele *et al*., 2021).

In response to the pandemic, FDA developed and validated a temporary method to evaluate the quality of finished ABHS ready for the market. The method can analyze ethanol or isopropanol and establish potential injurious impurities in ABHS formulations (US-FDA, 2020). A gas chromatography-flame ionization detector method identifies and accurately quantifies active ingredients and impurities. In contrast, GC-MS is used to qualitatively analyze the hand sanitizer samples to identify any unknown contaminants. The use of a pycnometer is one of the other methods suggested for measuring the density of hand sanitizers as recommended by the FDA (Bedner *et al*., 2021).

The US-FDA developed interim impurities levels in ppm referred to as the FDA guidance for level 1 impurities and level 2 impurities. Level 1 impurities should not be more than methanol 630, benzene 2, acetaldehyde 50, and acetal 50 ppm, while level 2 impurities should not be more than acetone 4400, n-propanol 1000, ethyl acetate 2200, 2-butanol 6200, isobutanol 21700, 1-butanol 1000, 3-methyl-1-butanol 4100 and amyl alcohol 4100 ppm (US-FDA, 2020).

The determined interim impurity levels by the FDA could be tolerated for a relatively short period and used as permitted limits in finished ABHS formulations, necessitating the use of ABHS with cautions during the COVID-19 pandemic, preventing shortages of ABHS in the market. Level 1 impurities were subjected to quantitative analysis, while the limit test technique was used for level 2 stated impurities (US-FDA, 2020).

In the US, it is recommended that hand sanitizers be analyzed using GC-FID. The column chosen is DB-WAX column (30 m× 530 µm i.d, 1 µm film thickness), and helium as the carrier gas at a flow rate of 7 mL/min, with a continuous flow mode. During GC-FID analysis of the samples, acetonitrile 5% (v/v) was used as an internal standard. The alcohol calibration standards

included five types of alcohols (like methanol, ethanol, isopropyl alcohol, n-propanol, and glycerine) prepared within 1 to 4% (v/v). From the chromatogram obtained, methanol was retained first, followed by isopropanol before ethanol. It was noted that the resolution to internal standard and peak shape of the alcohols exceeded the USP standard requirement (Jie, 2020).

A Canadian study analyzed samples of alcohol-based hand rubs for common impurities using GC-FID. The gelled alcohol-based hand rubs were subjected to a rapid solvent extraction while the liquid samples were filtered before GC-FID analysis. An Agilent 7890 GC-FID was utilized for analysis, and a 30-m Agilent J&W DB-624 Ultra Inert column (0.32 mm, 1.80 25 μm film thickness) for separation. Helium was used as the carrier gas. The gas chromatography inlet was set in a split mode (40:1) at 140 ºC. The method was able to profile the impurities under investigation successfully (Tse *et al*., 2021b).

In Italy, a study conducted to quantify the alcohol content in commercially available ABHS employed GC-FID. Gas chromatography was set with a split-splitless injector mode and Supelcowax column (60 m× 0.25 mm i.d, 0.25 μm film thickness), using helium at 1 ml/min as a carrier gas. As a result, the determination of alcohol content was successful, with good precision and linearity (Berardi, *et al.,* 2020b).

In South Africa, samples of ABHS in the market were analyzed by Headspace GC-FID. Supelcowax column (30 m × 0.25 mm i.d, and film thickness of 0.5 μm). Alcohol type and content, including impurities, were established successfully (Matatiele *et al.,* 2021).

In Kenya, Abuga et al. (2021) carried out an assay for alcohol content using GC by determining ethanol, isopropyl alcohol, and methanol content. A Shimadzu GC-FID was utilized with ZB-WAX plus column (60 m × 0.25 mm i.d, film thickness 0.25 μm) and helium as carrier gas. The method could determine various unknown impurities in the ABHS products, requiring investigation with other techniques like GC-MS capable of characterizing volatiles.

A Brazilian study demonstrated that an alcoholmeter could be used for preliminary analysis of ABHS as a reachable, faster, and cheaper method. However, an alcoholmeter could only be used to quantify alcohol content in the ABHS formulation. In addition, yield stress and acidification of the carbopol-containing samples appeared as limiting factors for using an alcoholmeter (Estevão *et al.,* 2021).

In Uganda, the UNBS specification, US EAS 104:2014, recommends specific requirements, including product quality tests of alcohol content, pH (range 6–8), and bactericidal efficacy (UNBS, 2013). The technique used for determining alcohol content, principally ethanol, is the same used for alcoholic beverages. However, it does not cover non-alcohol based hand sanitizers. The technique determines alcohol content using a pycnometer by determining the difference in specific gravity of the alcohol-water mixtures at a particular temperature (UNBS, 2013). However, this technique is incapable of stating the type of alcohol used in the production of ABHS.

CHAPTER THREE: METHODOLOGY

3.1 Study design, site, samples and sampling

3.1.1 Study design

This study involved a laboratory-based experimental design.

3.1.2 Study site

The study samples were purchased from the five Divisions of Kampala: Kampala Central, Kawempe, Rubaga, Makindye, and Nakawa (KCCA, 2019). The choosing of Kampala is likely to represent the entire country since most products are manufactured or imported and/or distributed through Kampala. Therefore, the Kampala study site targeted the on-transit population and different social classes. Samples from these five Divisions of Kampala represented a range of brands and formulations found in the country, allowing the project results to be generalized to similar assumed brands marketed throughout Uganda. The laboratory analysis of the study samples was carried out in the Drug Analysis and Research Unit (DARU) laboratories, Department of Pharmaceutical Chemistry, Pharmaceutics and Pharmacognosy, Faculty of Health Sciences, University of Nairobi, Kenya.

3.1.3 Study samples

The target samples were gels and liquids in the smallest available packs of 30 ml to 200 ml of commercial ABHS brands available at retail outlets at room temperature range and commonly purchased for personal use by the general population in Kampala. The samples were kept in their original container in a refrigerator at a storage temperature of 5±3 ^{0}C and humidity of 65±5%, away from direct light until analysis to prevent the possibility of evaporation of the volatiles.

3.1.4 Sampling

Convenience or incidental sampling was conducted within Kampala. The points of sale chosen for sampling as the sampling frame were randomly selected pharmacies, drug shops, supermarkets, cosmetic shops (“kiosks”) and hawkers throughout the targeted areas of the city. Samples of ABHS were collected from each Division over two months. This was done from 1st March to 30th April 2022. The samples were transported under ambient temperatures with leak-tight and securely closed packaging, secured against shifting to protect them from damage.

3.2 Reagents and materials

Reagents used were obtained from recognised distributors within Nairobi. They included HPLC grade acetonitrile (Carlo Erba reagents S.A.S, Dasit Group Limited, France), absolute ethanol (Scharlab S.L., Sentmenat, Spain), analytical grade isopropyl alcohol (Finar Limited, Ahmedabad, India), analytical grade methanol (Finar Limited, Ahmedabad, India), and glycerine (Finar Limited, Ahmedabad, India) used as the standard references solvents for GC analysis. Water was distilled in glass apparatus in the laboratory.

3.3 Equipment

A Jenway® 3510 pH meter (Bibby Scientific Ltd, Stone, UK) was used in pH determination; while a Shimadzu GC-2010 plus (Shimadzu Corporation, Tokyo, Japan) with a mass spectrometer (MS) and with flame ionization detector (FID) (Shimadzu Corporation, Tokyo, Japan) using the GC solution software version 2.42 (Shimadzu Corporation, Tokyo, Japan) were used for identification and quantification of the volatile components, respectively; A ZB wax plus column with the dimensions 60 m by ID 0.25 mm with a film thickness of 0.25 µm (Phenomenex, Torrance, CA, USA) was used for the chromatographic separation. Analytical instruments were optimized and validated and/or calibrated before use.

3.4 Procedures

3.4.1 Brands

The different brands of ABHS were classified as per the type or form irrespective of the producers.

3.4.2 Appearance, packaging and labelling evaluation

The samples were evaluated for appearance, packaging, labelling, and other regulations by subjecting the samples to the visual assessment regarding UNBS standardization requirements.

3.4.3 Preparation of solutions for analysis

The preparation of the standard stock solutions and test stock solutions were handled as per the methods described by Abuga *et al.* (2021) and Jie (2020) with minor modifications. Test solutions were filtered through PTFE 0.22 µm microfilters (Nantong Filter-Bio Membrane Co., Jiangsu, China) before injection.

Preparation of the standards

A 10% v/v acetonitrile stock solution was made by diluting one millilitre of acetonitrile in a 10 ml volumetric flask and made up to volume using distilled water to give the internal standard for GC-MS/FID.

A mixed standard stock solution of each reference standard reagent, namely methanol, isopropyl alcohol, ethanol, acetonitrile and glycerine for quality control, was prepared by diluting 1 ml of each standard solvent to 10 ml using distilled water. The test solution of the mixed standard was prepared by mixing 300 µl of the resultant solution with 500 µl acetonitrile internal standard solution, then making to a final volume of 1000 µl (3% v/v solution) with water prior to injection. The vial was capped, and the contents were mixed thoroughly before analysis.

Preparation of the sample solutions

To prepare sample stock solution, 1 ml of the neat sample was diluted with distilled water in a 10 ml volumetric flask to the mark. The test solution was then prepared by mixing 300 µl from the sample stock solution and 500 µl from acetonitrile internal standard stock solution, then making to a final volume of 1000 µl (3% v/v solution) with water prior to injection within the range of 1.8 to 2.85% (v/v). The vial was capped and contents mixed thoroughly before analysis. Care was taken to prevent the loss of volatile components.

3.4.4 System suitability and method validation of gas chromatography

Gas chromatography system suitability testing involved the checking of retention time repeatability, plate number, tailing factor and resolution. It involved the use of a column with a different length, internal diameter and film thickness from that of Jie (2020) method. There was a modification of the oven temperature program and prolonging the total program time, to allow for the identification and quantification of late-eluting glycerine among other working standards with chromatographic parameters exceeding USP method standard requirements. This was carried out using reference standard reagents, ZB-WAX plus column for separation and FID for detection, to obtain suitable chromatographic conditions for the analytical method.

Method validation was carried out according to the International Committee of Harmonization (ICH) Q2(R1) guidelines (ICH, 2005). The following validation parameters were studied;

precision (repeatability and intermediate precision), linearity of detector response (LODR) and accuracy (percent recovery).

Repeatability was examined by measuring repeatability on six replicate injections of the reference test solution at 100% of the test concentration. The percent relative standard deviation (% RSD) of the peak area ratios of the different alcohol standards were calculated as the standard deviation expressed as a percentage of the sample mean.

Intermediate precision was investigated by assaying three different vials of the same preparation at 100% of the test solution. Two injections of each vial were made, and the % RSD of the peak area ratios of the different alcohol standards across the system for each of the multiple test preparations were calculated.

The linearity of detector response (LODR) was studied in the 20-117 μg/ml concentration range. Five solutions of the alcohol standards were prepared to correspond to 20%, 47%, 70%, 93%, and 117% of the nominal analytical concentration (100 μg/ml). The regression equation was found by plotting the peak area (y) versus the concentration of the reference standards (x) expressed in μg/ml and the coefficient of determination (R^2) computed.

The accuracy of the method was determined by fortifying a sample known to contain ethanol 51.9% v/v with known amounts of the reference standard such as ethanol as described by Shabir *et al.* (2007), and studied at four concentration levels, that is, 51.9%, 61.9%, 71.9%, and 81.9 % v/v at 10% concentration interval. Two vials of each concentration level were prepared and injected in triplicate. Mean recoveries for the samples analyzed were calculated and checked for closeness to the actual known values. The recovery data were expressed as an average percent of triplicate injections.

3.4.5 Gas chromatography qualitative and quantitative analysis

The identification and quantification of alcohols and impurities were handled as per the method described by Abuga *et al.* (2021) and Jie (2020) with minor modifications in comparison with the chromatographic conditions obtained from the USP method.

Active ingredients, glycerine and impurities identification

The following chromatographic conditions obtained as per the USP method of detection of volatiles were used with modifications following system suitability tests and method validation

(US-FDA, 2020). The volatiles in ABHS were analyzed by GC-MS operated using a GC-MS solution software with helium as the carrier gas at 3.0 ml/min flow rate, pulsed split mode of 20:1, injection volume of 0.2 µl. ZB-WAX plus capillary column with the dimensions 60 m by ID 0.25 mm with a film thickness of 0.25 µm was used for separation. The oven temperature was programmed from 45 ºC to 240 ºC, with the initial temperature of 45 ºC maintained for 7 min, followed by a gradient of 240 ºC at 30 ºC/min for 6 min and 240 ºC at 35 ºC/min for 7 min. Total run time of 26.5 min. The mass selector was maintained at an ion source temperature of 200 ºC, and electron impact (EI) mass spectra were obtained at the acceleration energy of 70 eV. Fragment ions were analyzed in the full scan mode over a 20-300 m/z mass range. The filament delay time was set at 0 min.

Active ingredients, glycerine and impurities quantification

The chromatographic conditions used were obtained from that of Abuga *et al.* (2021) and Jie (2020) with minor modifications following system suitability tests and method validation. Gas chromatography with a flame ionization detector operated using GC solution software was utilized in the quantification of volatiles in ABHS. First, the injector was set in pulsed split mode of 20:1, injection volume of 0.2 µl. Then, the injection port and detector temperature were selected at 250 °C. Helium was used as a carrier gas at a 3.0 ml/min flow rate. A ZB-WAZ plus capillary column with the dimensions 60 m by ID 0.25 mm with a film thickness of 0.25 µm and temperature gradient of 4 5ºC (7 min), 240 ºC at 30 ºC/min for 6 min, and 240 ºC at 35 ºC/min for 7 min was used for separation. Total run time of 26.5 min.

3.4.6 pH determination

The Jenway® 3510 pH meter was calibrated using standard pH 7.00, 4.00, and 10.00 solutions and used to determine the pH of the neat samples.

3.5 Data processing, analysis and presentation

Microsoft® Excel® program was used to analyze appearance, packaging, labelling, UNBS standardization mark conformity, GC results and pH results. The results for assessment of appearance, packaging, labelling, and UNBS standardization mark conformity regarding the UNBS specifications for ABHS were presented as percentages of total of the samples evaluated.

The alcohols and impurities were identified by comparing peak mass spectral data and retention time matching of +/- 0.1 minute with those of the standards and reference spectra published by library-MS databases, considering the similarity index of higher confidence (that is, close to 100 %). Quantification of the alcohol and impurities was calculated by comparison of peak area ratios of the sample components and working reference standards to the internal standard, correcting the result for standard purity and the dilution factor. Obtained data were summarised in tables and graphs, the mean±standard deviation and percentage as the descriptive statistics were used to describe the data obtained. The alcohol content was reported as % label claim. Uganda National Bureau of Standards specification falling within WHO limit for alcoholic concentration (60–95 %v/v) and pH (6-8) was used to declare the quality of the ABHS.

3.6 Ethical considerations

Since this was a study involving the collection of ABHS samples from the Kampala area in Uganda and analyzing them in another country (Kenya), the authority was sought from National Drug Authority Uganda for the exportation of the samples to Nairobi (Kenya) and Malaba port clearance was done. The name of the different brands sampled and the collection points was not disclosed during data acquisition and analysis of the results to maintain confidentiality. Samples were coded for blind analysis. The proposal was presented to the Board of Research and Postgraduate Studies, University of Nairobi, for approval before study.

3.7 Results dissemination plan

The study findings are in a masters thesis and will also be disseminated in peer-reviewed journals and presented at scientific and professional conferences and symposia as oral and/or poster presentations. The study results will provide information to the regulatory authority and be used to alert the scientific community about the substandard and falsified ABHS products containing inappropriate concentrations of alcohol and toxic impurities in the market. The study's results will also be used as a baseline study for further studies.

3.8 Study limitations

Considering the purchase cost of multiple batches of a brand and the need to obtain as many different brands as possible, only a single sample per brand was sampled from outlets.

This study was limited to ABHS commercially available at retail outlets.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Appearance, packaging, labelling, and regulation requirements

A total of 130 brands of ABHS were collected from the five Divisions of Kampala over two months, with sample UGS 27 being the most popularly used. This sample was found in 70 (36.6%) retail outlets. One hundred ninety-one (191) retail outlets were randomly visited around Kampala from 1st March to 30th April 2022 while sampling and/or recording the brand(s) of ABHS found therein.

Figure 1 shows the compliance of the brands to appearance, packaging, labelling, and other regulations on ABHS regarding UNBS standardization requirements.

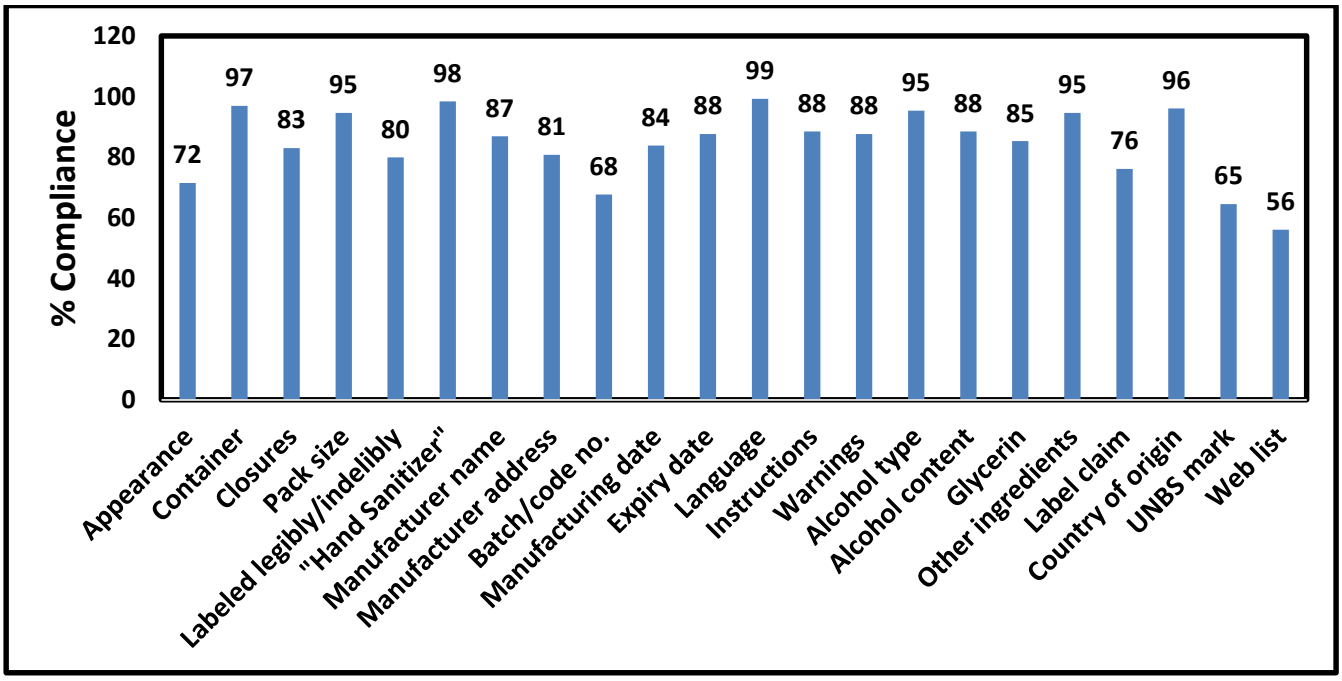


Figure 1: Compliance of ABHS brands to regulatory requirements

4.1.1 Form and appearance

The UNBS specifies, among other general requirements, that "hand sanitizer shall be clear, colorless and in the form of liquid or gel" (UNBS, 2013).

The majority of the 130 brands in this study were liquids (73%, n=95), and slightly over a quarter (27%, n=35) were gels. However, only eight of these samples were clearly labelled “gel.” This study’s results illustrated that there were more liquid (n=95) than gel (n=35) ABHS formulations around Kampala at the time of the study.

Most samples (72%, n=93) complied with the specification for appearance. However, a few samples (5%, n=6) were colored comprising of one liquid and five gels. The samples were in definite colors of blue, pink, brown, and green. Almost a quarter (22%, n=29) of the samples appeared cloudy with precipitates or clear with visible particles. Most (n=24) of these samples with visible precipitate of particles were liquid formulations and only five were gels with blue-colored insoluble particles. The gel formulations demonstrated varying viscosities, with twelve samples flowing within 2 seconds of container inversion while sixteen flowed within 5 seconds and seven not at all in the 2-5 seconds time interval (Nyamweya and Abuga, 2020). In addition, two samples referred to as "gel," with one labelled "organic gel," were free-flowing liquids, contrary to the claim.

4.1.2 Packaging and pack sizes

The UNBS recommends that ABHS be packaged in suitably well-closed containers. The container and closures should be chemically inert with the sanitizer and strong enough to offer protection during normal handling, transportation, and storage (UNBS, 2013).

Generally, the majority of samples (84%, n=109) were packaged in suitable containers with appropriate closures while the other 21 samples (16%) were out of specifications. Regarding the plastic containers used for packaging, 126 samples (97%) were packaged in clear polyethylene terephthalate (PET), while 4 samples (3%) were contained in opaque high-density polyethylene (HDPE). Overall, many closures (58%, n=75) had spray pumps, 30 (23%) disc top caps, 24 (18%) flip top caps, and only one (1%) was a trigger spray pump. However, packaging irregularities were noted with liquid containers and closures. Of these liquids, 3 (2%) samples had leaking closures, and 19 (15%) were in the wrong containers and closures, with 7 having flip-top caps and 12 disc-top closures, offering minimal protection during normal handling, transportation and storage (UNBS, 2013).

The pack sizes of ABHS ranged from 30-200 ml, with the most common (40%, n=52) fill volume of 60 ml. Two had a pack size of 30 ml, and only one had a pack size of 200 ml. Other significant pack sizes were 50 ml (n=27), 53 ml (n=1), 65 ml (n=5), 75 ml (n=2), 80 ml (n=2), 100 ml (n=24), or 120 ml (n=7). Six (5%) ABHS samples had no net contents labelled on the packaging or container, while one had a non-matching label and pack size. The sample was labelled 500 ml yet packaged in a 60 ml plastic container.

Polyethylene terephthalate (PET) plastic was preferred for the packaging of hand sanitizers because of its transparent nature enabling viewing of the product through the container. These PET plastics can either be recycled or rinsed out and reused. Both plastic and glass bottles with leak-proof tops were recommended for the packaging of hand sanitizers. Screw-cap tops, disc-tops, or flip-top closures were encouraged (Blaxhall, 2020; WHO, 2010).

There was need for use of appropriate packaging or container, including closures, as justified in literature by the corrosion and rupture of one of the products that were packaged in an aluminium can after a few weeks of storage at room temperature (Tse *et al*., 2021b).

4.1.3 Labelling

Hand sanitizers are required to be legibly and indelibly marked with accurate information (UNBS, 2013).

Eighty percent (n=104) of the samples were legibly and indelibly labelled. The majority (98%, n=128) of samples were labelled correctly as a "hand sanitizer", except for two samples, one of which had a faint label and one that was not labelled (Figure 1).

Furthermore, most samples (87%, n=113) had the manufacturer name stated, while 81% (n=105) had the manufacturer address. About 6% (n=8) of the samples indicated only the manufacturer name without a physical address. Four samples indicated only the mobile phone number. Two samples had the batch number, manufacturing date and expiry date erased, while one had covered them with an overlaid label.

The general use instructions were in English (99%). One sample had the language of instructions in Arabic and English, while one had it in Chinese and English. Only one sample did not bear any label to indicate instructions. The hand sanitizer should also be labelled with warnings such as "Do not allow the sanitizer to come into contact with eyes", "Keep Out of Reach of Children", "If swallowed, contact a doctor", and "Highly flammable, keep away from fire or flame" UNBS (2013). Few samples (12%, n=16) had no cautionary warning. Thirty-one (24%) of the samples with warnings stated were partially compliant, with only 1, 2, or 3 of the 4 required warnings, while 64% (n = 83) were fully compliant. Among the non-compliant samples, one was labelled "Don't ingest or inhale," which is not an accurate warning.

Several label issues included cut-off print portions, faint, obscured, overlaid, poor inking, tiny wordings, peeling off, and erased or missing labels. It is worth noting that appropriate labelling is a fundamental quality requirement. The label facilitates better identification and understanding of the product, with more user confidence and trust built on it and its distinguished benefit (Nyamweya and Abuga, 2020).

4.1.4 Ingredients and label claim

Considering the indication of the alcohol type, content, and other ingredients on packaging or container of ABHS, 6 (5%) samples did not specify the alcohol used. Eight (6%) samples that indicated denatured alcohol as the active ingredient had specified the denaturants, with 7 samples stating isopropyl alcohol and one sample stated phenoxyethanol. Indicating the alcohol type on the label eases the management of any accidental or intentional ABHS ingestion. However, one sample stated “Ethoxylated fatty alcohols” and another “Cetyl alcohol” as the active ingredient. One sample had the wrong spelling “Althyl alcohol” printed on the label as the active ingredient. The alcohol content stated ranged from 60% to 85%. The alcohol type and content are vital aspects of the perceived quality of ABHS.

The mislabelling issue illustrated in this study is similar to the results noted in the literature, where Kenyan and Canadian samples contained different alcohol types from those printed on the label (Abuga *et al*., 2021; Health Canada, 2021)

The other common ingredients listed in 95% (n=123) of samples included; hydrogen peroxide, triethanolamine, carbomer, fragrances (perfume), flavour, colours, tocopheryl acetate (vitamin E), aloe, and glycerine. The least common included propylene glycol, monopropylene glycol, dimethicone, sodium sulphate, betaine, coconut diethanolamide, diethyl phthalate, isopropyl myristate, allantoin, phosphoric acid, perhydrol, 1,2,3-trihydroxyipropane, inter-chlorodimethyl phenol, alkyl acrylate cross polymer, triethylamine, lanolin, sodium lauryl ether sulphate, alkyl dimethyl benzyl ammonium chloride, water, polymethyl siloxane, carbopol, methyl paraben, propyl paraben, peppermint, strawberry essential oil, cocoa alkyl, cinnamon, peppermint, kigelia, carbopol and lemon.

Seven samples had no inactive ingredients listed, and some had incomplete information about the ingredients with non-standardized abbreviations. Manufacturers of ABHS are mandated to indicate a complete list of ingredients on the label for user information since some of the

ingredients are potential allergens to specific individuals, preventing harm or providing other reasons for not using (Nyamweya *et al*., 2021).

A majority (n=99, 76%) of samples had label claims concerning efficacy expressed as a percentage of the microbial kill. The values labelled on the containers or packaging were 99%, 99.9%, 99.99%, or 100%. However, one sample had a wrong label claim stating "99.9% without water" instead of "99.9% killing of germs". Protein denaturation by alcohol is known to be promoted in the presence of water (Gold *et al*., 2021). The 99.99% killing of microorganisms claim has been demonstrated in other studies to be factual (Rotter, 1999). Therefore, experimental data must validate these values to safeguard the users' false sense of security.

4.1.5 Regulatory requirements

The country of origin is an important aspect to consider when regulating products in the market. And for product quality control purposes, UNBS certifies a product's quality and grants the manufacturer a permit to affix the "UNBS Quality Mark," which should be stamped on the product itself or the packaging. The product is also required to appear on the annual UNBS website list of brands authorized by the respective companies to produce locally (UNBS, 2013).

Several samples (86%, n=112) were locally manufactured in Uganda, while 13 (10%) indicated that they were imported from United Kingdom, China, People's Republic of China, Indonesia, Turkey, United Arabs Emirates, South Africa, and Kenya. Five samples did not state the country of origin. However, none of the imported products had a "UNBS Quality Mark", nor did they appear on the UNBS list of certified ABHS.

Although 75% (n=84) of the locally manufactured samples had the quality mark, only 31% (n=35) appeared on the list of certified ABHS for July 2021. However, considering the locally manufactured ABHS products listed for July 2020 and 2021 bearing the quality mark, 65% (n=73) complied. This demonstrated the possibility of substandard and counterfeit ABHS products in the market (Nyamweya and Abuga, 2020). The UNBS mark is intended to assure the customers that the ABHS conforms to the required standards of good quality.

4.2 Identification and assay using gas chromatography

4.2.1 System suitability and method validation of gas chromatography

System suitability of gas chromatography flame ionization detector

Gas chromatography system performance was checked using five alcohol standards (methanol, isopropyl alcohol, ethanol, acetonitrile, and glycerine) to obtain suitable chromatographic conditions for the analytical method, as shown in Table 4.

Table 4: Analytical conditions for GC-FID

GC-FID conditions	
Split inlet	250 °C, split ratio 20:1
Injection volume	0.2 µl
Carrier gas	Helium
Column flow rate	1.36 ml/min, constant flow mode
Oven	45ºC (7min), 240 ºC at 30 ºC/min for 6 min and 240 ºC at 35 ºC/min for 7 min
FID	250 °C, air: 400 ml/min, fuel gas (H_2): 40 ml/min, constant make up: 30 ml/min
MS	Ion source 200 ºC, acceleration energy 70 eV, full scan mode over a 20-300 m/z
Column	ZB-WAX plus, dimensions 60 m by ID 0.25 mm, 0.25 µm film thickness
Total run time	26.5 min

The instrument and method suitability was investigated regarding retention time repeatability, theoretical plate number, peak tailing factor, and alcohol peak resolution. As a result, the following optimal chromatographic parameters were obtained, as illustrated in Table 5.

Table 5: Chromatographic parameters for GC-FID suitability for ABHS analysis

Standard	Mean retention time (min)	Retention time % RSD (n=6)	Theoretical plate	Peak tailing factor	Resolution
Methanol	6.3	0.026	>36,000	1.8	0
2-Propanol	7.1	0.041	>49,000	0.0	>5.8 MeOH/P
Ethanol	7.2	0.042	>50,000	0.0	>1.1 P/EtOH
Acetonitrile	8.5	0.033	>180,000	1.1	>12.0 EtOH/ACNL
Glycerine	21.5	0.050	>24,000	0.9	>41.7 ACNL/GLY

MeOH: Methanol, P: 2-Propanol, EtOH: Ethanol, ACNL: Acetonitrile, GLY: Glycerine

The ICH guideline and USP <611> standard recommends the following; retention time % RSD ≤2%, retention time (t_R) ± 0.1 min, theoretical plate number (N) >2,000, peak tailing factor (As) <2, and peak resolution (Rs) >4 (ICH, 2005; USP, 2020b).

The obtained results demonstrated that the system was suitable for analyzing alcohol and volatile impurities. However, the peak resolution between ethanol and 2-propanol was 1.1 without baseline separation. The effect on the accuracy of quantification of ethanol and 2-propanol (isopropyl alcohol, IPA) was not recorded since they rarely co-exist in the same ABHS formulation. If they co-existed, a peak resolution of 1.1 was adequate to produce accurate quantification, as reported by other authors Jie (2020). Methanol and 2-propanol were observed to elute before ethanol and acetonitrile, while glycerine was eluted last. The retention time variation of all the alcohol standards was +/- 0.01 min. The inclusion of glycerine among the standards was intended to show how the system could produce a good peak and permit the detection of late-eluting and sticky alcohols such as glycerine (Jie, 2020), recommended as one of the ingredients in the WHO formulation for ABHS.

Method validation of gas chromatography with flame ionization detector

The proposed GC-FID analytical method for the quantification of alcohols and volatile impurities was examined, and the following validation parameters computed from obtained data, (Table 6).

Table 6: Validation parameters for system performance suitability evaluation

Parameter	**Alcohol**		
	Methanol	**2-Propanol**	**Ethanol**
Repeatability (% RSD)	1.9	3.1	3.7
Intermediate precision (% RSD)	1.8	3.3	2.7
Linearity of detector response (R^2)	0.994	0.997	0.991
Accuracy (% average)	**Ethanol**; 100, 102.6, 99.3, and 100.4		

The ICH defines precision as the degree of agreement among a series of measurements obtained from multiple sampling, often expressed as the percent relative standard deviation (% RSD) of

replicate measurements and linearity of detector response (LODR) as the range of concentrations of analyte for which the procedure provides a test result that is in direct correlation to the amount of analyte in the sample. Accuracy was described as the closeness of agreement between the value obtained by the method and the true value (ICH, 2005). The % RSD is the standard deviation expressed as a percentage of the sample mean.

The ICH Q2 (R1), USP <611> and US-FDA recommend the following; an RSD of sample peak areas of no more than 2.0% and 4.0% for repeatability and intermediate precision, respectively, linearity of detector response with R^2 >0.999 (coefficient of determination), and accuracy (recovery) of 100±2% (ICH, 2005; US-FDA, 1994; USP, 2020b).

The average repeatability and intermediate precision on methanol, 2-propanol and ethanol are lower than 4%. The coefficients of determination (R^2) for the alcohol standards ranged from 0.991 to 0.997. The quantitation accuracy (recovery) was carried out on a sample of ABHS containing ethanol as the only active ingredient, and the mean recoveries ranged from 99.3% to 102.6%. The system performance of the GC-FID method produced acceptable validation characteristics. Therefore, it was considered reliable and fit for the assay of alcohols and volatile impurities (Jie, 2020).

System suitability test is a critical aspect of the analysis required to reduce the resources needed for the method validation. In addition, both system suitability check and method validation prove that the analytical method can be successfully adapted for its intended purpose (Shabir *et al.*, 200). Figure 2 shows a typical gas chromatogram.

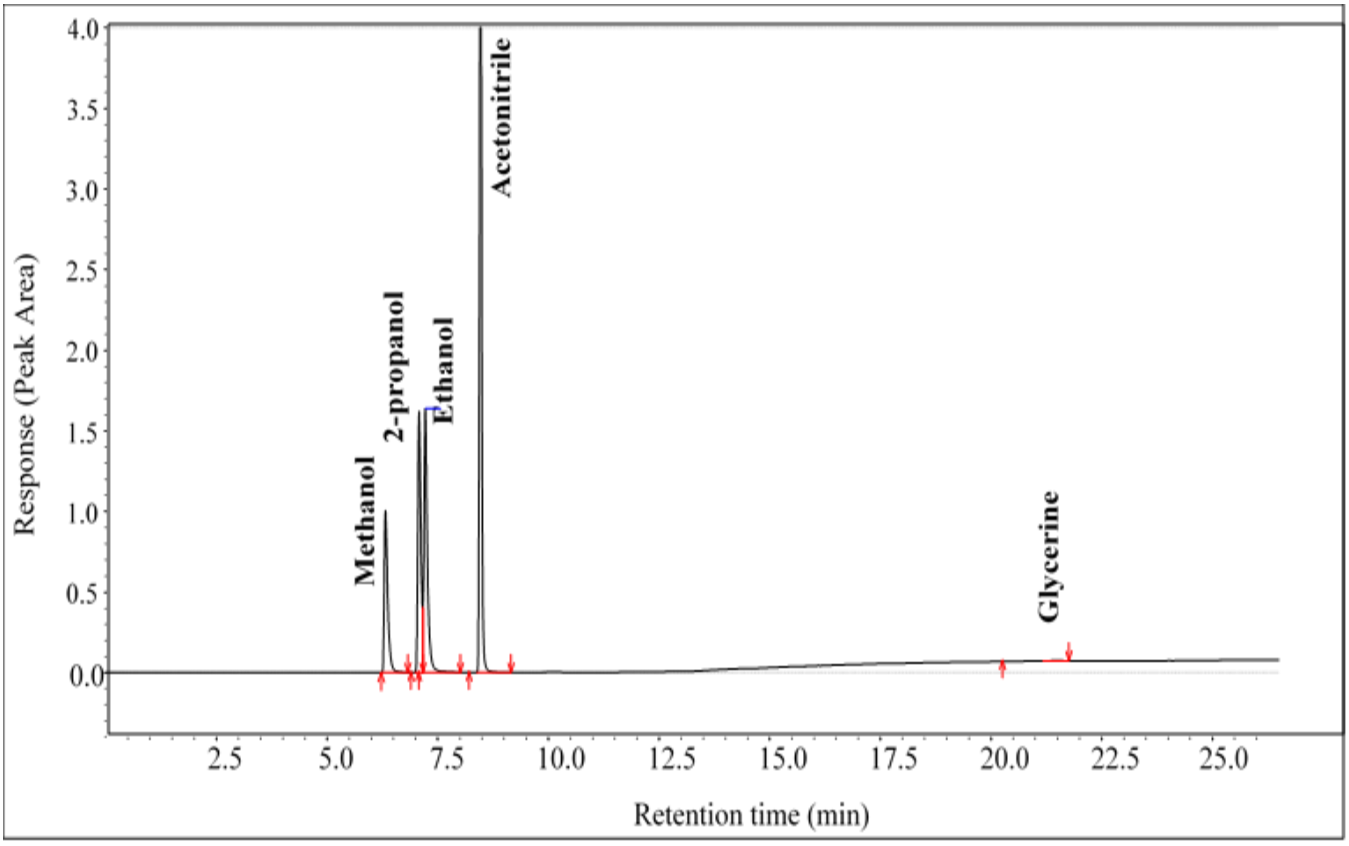


Figure 2: A typical chromatogram of the five alcohol standards

4.2.2 Identification of active ingredients, glycerine and impurities

This analytical technique focused on identification of the main components and possible volatile impurities in ABHS as given in Appendix 1. The results of GC-MS profiling of volatiles were also shown in Figure 3. This study has established the presence of substandard and falsified formulations in the Kampala market.

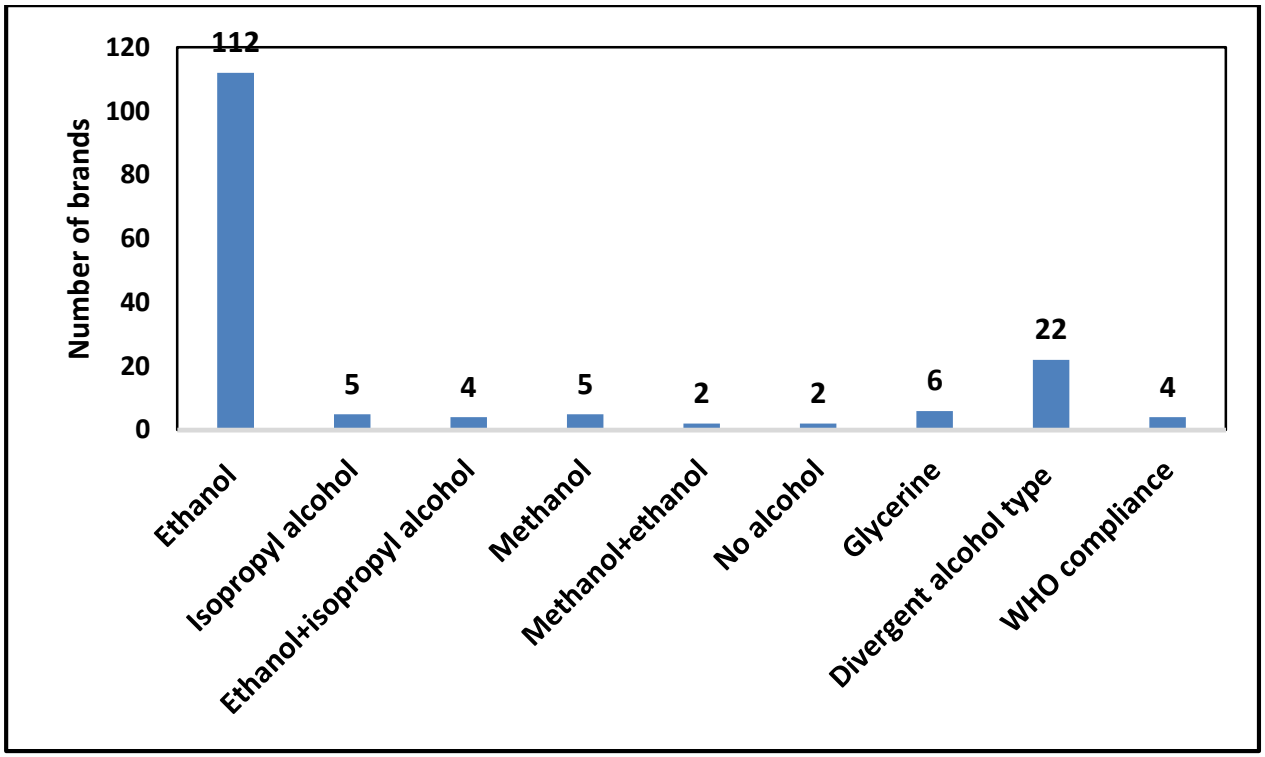


Figure 3: The GC-MS profiling of alcohols in ABHS samples

Active ingredients

The WHO recommends ethanol and isopropyl alcohol as the permitted alcohols (WHO, 2010). Ethanol (86%, n=112), 2-propanol (4%, n=5), and ethanol/2-propanol admixture (3%, n=4) referred to as the two permitted alcohols in the formulation of ABHS, were detected in several samples as shown in Figure 3 and Appendix 1. Seven samples were found to either contain purely methanol or contaminated with methanol. Two samples were found to contain no active ingredient, as demonstrated in the given chromatogram (Figure 4). One gel was imported from China, and the other liquid was locally manufactured. In the case of ten samples which stated the use of denatured alcohol, only one was identified with isopropyl alcohol as the denaturant while ethanol was the major ingredient. Alcohol denaturants are added to ABHS to offer an unpleasant taste and hence deter ingestion. Isopropyl alcohol and methanol or denatonium benzoate in low concentrations below the set threshold limits are used by manufacturers of ABHS as denaturants (Gacuiga *et al.*, 2022; Langer *et al.*, 2004).

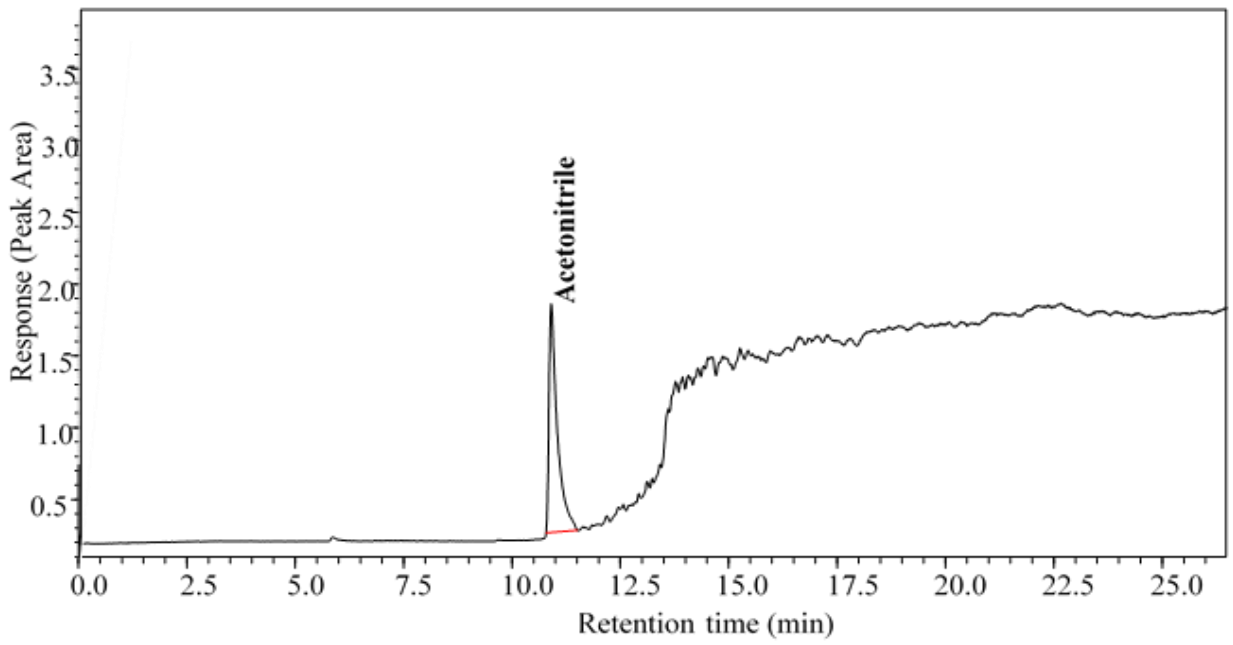


Figure 4: A typical chromatogram of ABHS with no active ingredient (alcohol) or glycerine

Impurities

The presence of methanol and n-propanol in ABHS is considered an impurity or contaminant and harmful for human use by the US-FDA. The warning for n-propanol was targeted towards prevention/reduction of misuse as drinkable alcohol substitutes rather than as a result of its toxicity following use as hand sanitizer (US-FDA, 2021b). In contrast, the UNBS, and Kenya Bureau of Standards (KEBS) have included n-propanol as one of the permitted alcohols in addition to ethanol and isopropanol at a minimum content of 60% v/v (KEBS, 2014; UNBS, 2013). In addition, other parts of the world like India (Central Drug Standard and Control Organization) and United Kingdom (Health and Safety Executive) have also accepted n-propanol as an active ingredient in the formulation of ABHS (Rahi *et al.*, 2021).

The non-recommended methanol was detected in 7 samples. Five samples (4%) had pure methanol substitution with methanol as the only alcohol present in the samples, as shown in Figure 5. The methanol substitution was for ethanol seen with 3 products and isopropyl alcohol with 2 products. As shown in Figure 6, two samples had both methanol and ethanol, an indication of methanol contamination. All seven samples found to contain methanol were locally manufactured.

The USP specifies that less than 200 ppm (0.020% v/v) is the allowable interim limit of methanol in ABHS (USP, 2020a) and US-FDA states methanol as a “level 1 impurity” with an adjusted interim limit of less than 630 ppm (0.063% v/v) (US-FDA, 2020). However, the UNBS specification does not include methanol as one of the permitted alcohols used in manufacturing ABHS (UNBS, 2013). From the literature, the presence of methanol in low concentration could be used as a denaturant in the ABHS (Gacuiga *et al.*, 2022). The establishment of the methanol content is critical because its metabolites, formaldehyde and formic acid, are known to be toxic (Cook and Brooke, 2021; Dear *et al*., 2020).

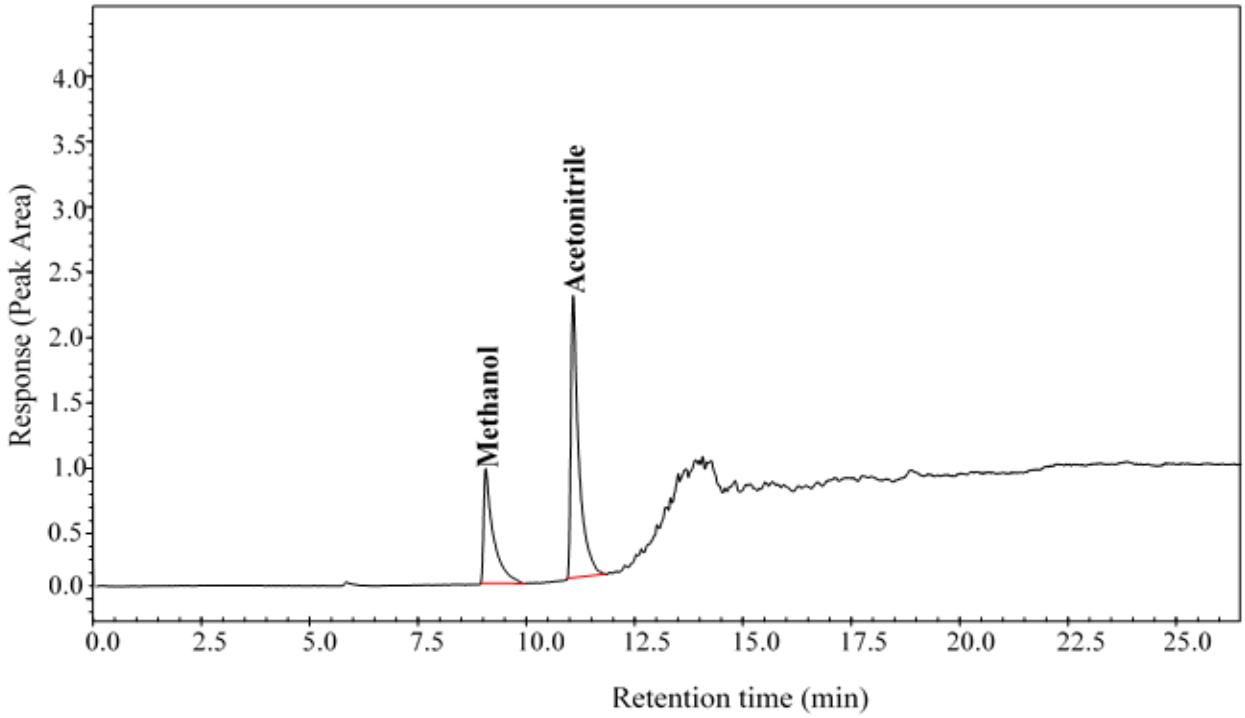


Figure 5: A typical chromatogram of ABHS with methanol substitution

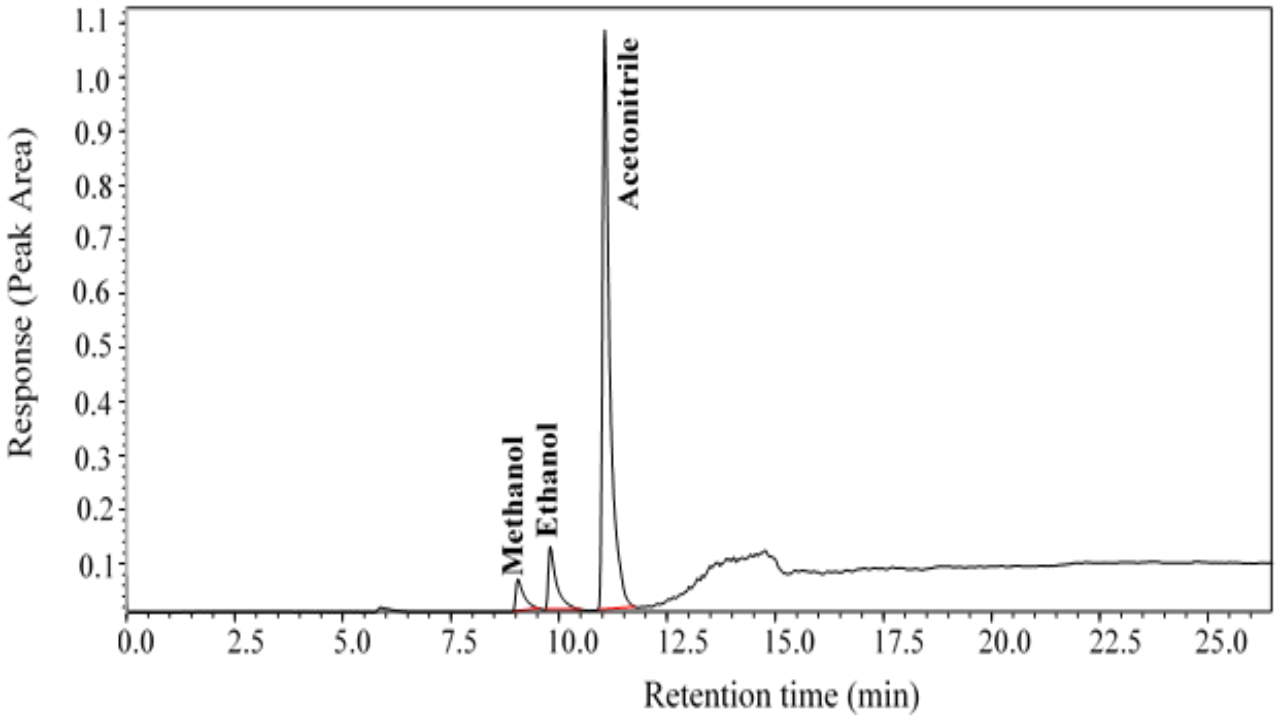


Figure 6: A chromatogram of ABHS with methanol contamination (methanol and ethanol)

Substandard and falsified samples

The study identified 22 samples of ABHS with divergent alcohol types from those declared on the label. Ethanol (n=7), isopropyl alcohol (n=10), cetyl alcohol (n=2), and methanol (n=3) expressed on the label as the active ingredient(s) or among other actives were not detected. Two samples found to contain no active ingredient were labelled with ethyl alcohol or ethoxylated fatty alcohols as the active ingredient.

Ten samples stated that denatured alcohol was the active ingredient, with 8 specifying denaturants such as isopropyl alcohol (n=7) and phenoxyethanol (n=1). Two samples did not specify the denaturant used. However, only one of the samples had the denaturant identified. Seven samples did not specify the alcohol type used by only stating “alcohol” on the label, and 2 samples had no active ingredient declared on the label, but all were found to contain ethanol.

Notably, 111 samples (85%) had glycerine labelled as one of the ingredients. Interestingly, only 6 samples comprising 3 liquids and 3 gels were identified to contain glycerine, of which one had not stated glycerine on its label. Of the 6 samples with glycerine, three were locally manufactured, one was imported from South Africa, and two had no country of origin stated.

Two of the 6 samples with glycerine were in combination with methanol, as shown in Appendix 1. Figure 7 shows a chromatogram of glycerine and methanol.

In addition, there was a sample with the wrong spelling of the type of alcohol, “Alkyly alcohol”, and ethanol was the identified alcohol.

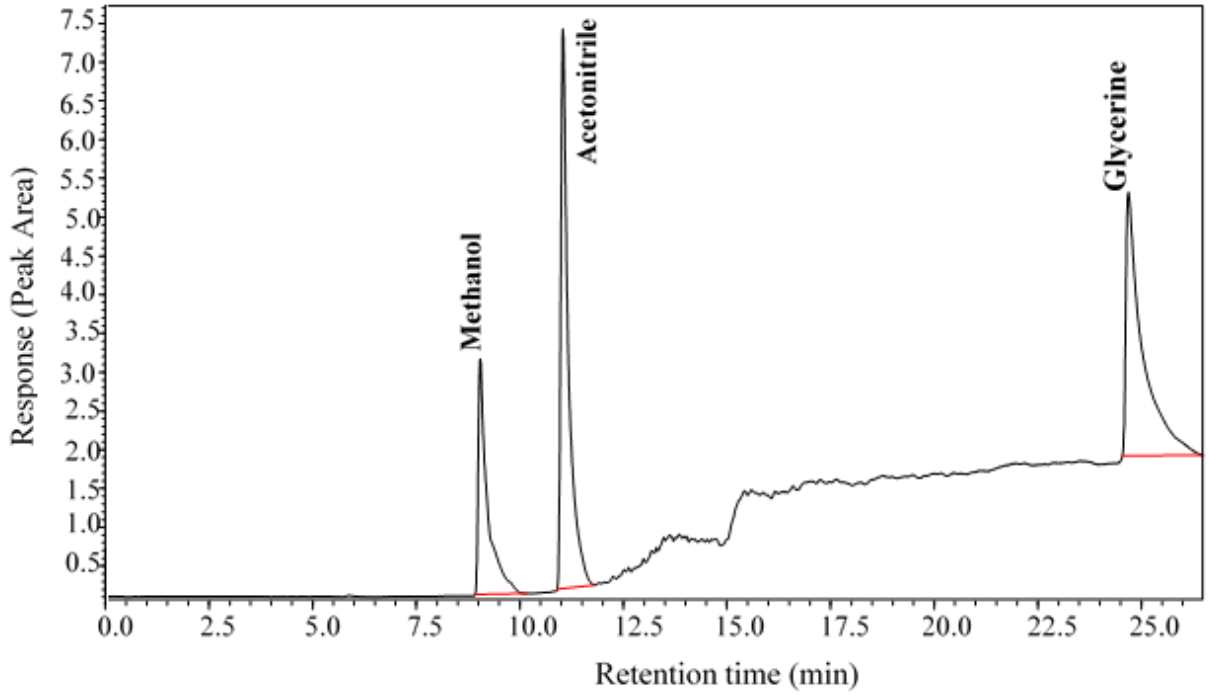


Figure 7: A typical chromatogram of ABHS with methanol and glycerine

4.2.3 Content of active ingredients, glycerine and impurities

The GC-FID method focused on quantification of the identified permitted alcohols and possible volatile impurities in ABHS using methanol 99.8% v/v, 2-propanol 99.5% v/v, ethanol 99.9% v/v, glycerine 99.5% v/v and acetonitrile as standards. The results are shown in Appendix 2 and Table 7.

Content of active ingredients

The UNBS specification for instant hand sanitizers requires alcohol content of no less than 60% (UNBS, 2013), while WHO recommends 60-95% v/v ethanol or isopropyl alcohol with glycerine (1.45% v/v), hydrogen peroxide (0.125% v/v), and sterile water (WHO, 2009).

The total alcohol strength ranged from 9.33% v/v to 98.95% v/v, with an average alcohol content of 63±15% (mean± standard deviation %) as shown in Table 7. Seventy-eight samples (60.0%)

complied with WHO specifications for alcohol content with an average alcohol content of 69±8% as shown in Table 7. In 43 samples, the alcohol concentration was found to fall outside the acceptance criteria of 60-95% v/v. Forty-two samples (32.3%) contained less than 60% v/v alcohol, with mean content of 48±13%. Of the 42 samples with less than 60% alcohol, 32 samples were locally manufactured, six were imported, with five from China and one from the United Kingdom, and four samples had not stated the country of origin. Only one contained 98.95% v/v sum of the permitted alcohols, exceeding 95% v/v, and it was locally manufactured. Both GC-MS and GC-FID analyses indicated that two brands of ABHS had no alcohol.

From literature, ethanol has been shown to have better activity against viruses, whereas isopropyl alcohol demonstrated better bactericidal activity (Gold *et al.,* 2021). In addition, ethanol 70-95% v/v has been reported to display a more potent and broader virucidal activity covering several clinically relevant viruses (Kampf, 2018), whereas isopropyl alcohol 60-100% v/v demonstrated better bacterial and fungal inhibition activity. Therefore, formulations of ABHS with an alcohol content of 85%-95% have been recommended for improved antimicrobial spectrum (Thaddeus *et al*., 2018).

Hand sanitizers formulated with an alcohol content of less than 60% demonstrated reduced efficacy, increasing the risk of transmission of infection (Jairoun *et al*., 2021; Prajapati *et al*., 2022). In comparison, high alcohol concentration was known to render the preparation less effective since microbial proteins are not easily denatured without water (Gold *et al*., 2021).

The results indicate a need to validate the antimicrobial efficacy of ABHS as the pandemic abates and laxity is witnessed by the manufacturers, regulatory bodies, and consumers (Chojnacki *et al*., 2021). However, the efficacy and quality of ABHS have been reported to be influenced more by factors like the rate of volatilization from the preparation rather than alcohol type and concentration. Hence the need to utilize methods like near-infrared spectroscopy (NIRS) which can determine the rate of volatilization alongside qualitative and quantitative analysis at a faster rate and lower cost in several dosage forms (Pasquini *et al*., 2020).

Glycerine content

Few of the ABHS (n=6, 4.6%) comprising three liquid and three gel formulations contained glycerine as the humectant in a concentration ranging from 4.55% v/v to 57.43% v/v with average glycerine content of 18.05±22%, as shown in Table 7. None of the ABHS samples with

glycerine complied with the WHO current specification of 1.45% v/v glycerine (WHO, 2010) and the suggested concentration range of 0.5% to 0.73% v/v demonstrated to be adequate (Berardi *et al.,* 2020a).

Hand sanitizers are commonly formulated with glycerine as the humectant. The moisturizing effect is more significant with increasing concentration. However, a balance between the effect and efficacy of ABHS has to be struck (Wood, 2021). Adding glycerine to the formulation lowers the antimicrobial activity of alcohol, especially 2-propanol, due to reduced alcohol diffusion with increasing viscosity. Hence the need to be used with caution in the preparation of ABHS (Thaddeus *et al*., 2018). Nevertheless, excellent antimicrobial activity was demonstrated with ABHS formulated with WHO specifications of either ethanol (80% v/v) or isopropanol (75% v/v) mixed with glycerine (1.45% v/v) (Chojnacki *et al*., 2021). A concentration range of 0.5% to 0.73% v/v has been demonstrated to be adequate (Berardi *et al.,* 2020a).

Methanol content

The USP has set the interim limit of methanol at 200 ppm or 200 μL/L or 0.020% v/v (USP, 2020a), and US-FDA adjusted the interim methanol limit to less than 630 ppm (0.063% v/v or 630 μL/L) (US-FDA, 2020).

Methanol content in the seven ABHS samples (5.4%) previously identified by GC-MS ranged from 25.7% v/v to 98.0% v/v. The samples were liquid formulations with an average methanol content of 70.4±26% (Table 7). Five samples were found to contain solely methanol, an indication of methanol substitution, and two with a concentration above the interim limits set by USP and US-FDA were in combination with ethanol. None of the samples with methanol complied with the USP interim limits and US-FDA adjusted interim limits.

Despite the numerous cases of acute methanol poisoning reported worldwide following ingestion of ABHS tainted with unlisted methanol as an active ingredient intentionally (Ashurst and Nappe, 2021; Chan and Chan, 2018), this study detected methanol at levels above the USP and US-FDA limits in the ABHS brands collected (US-FDA, 2020; USP, 2020a).

Of the seven samples that failed the methanol limit, two were found on the UNBS website as certified brands with a UNBS quality mark at the time of the study (UNBS, 2021). This indicated that unsafe ABHS with undeclared methanol were in circulation in the market.

Methanol toxicity due to accidental ingestion by children and intentional consumption of ABHS as a substitute for alcohol (ethanol) is known to result in several disabilities like blindness and even death (Aljazeera, 2020; Fazio, 2020; Gekonge, 2021). Methanol-induced desquamation and dermatitis demonstrate skin absorption following prolonged exposure to methanol-containing ABHS (Chan and Chan, 2018; Rundle *et al*., 2020). There is a need for sensitization of the public on the health risks associated with using ABHS adulterated with high methanol content.

Table 7: Descriptive statistics of the ABHS composition

ABHS content characteristics	**Number of brands**	**Mean± standard deviation (% v/v)**
Total alcohol	128	63.0±15
Sum of permitted alcohol (ethanol + isopropyl alcohol)	121	61.9±14
Sum of permitted alcohol between 60-95	78	69.1±8
Sum of permitted alcohol less than 60	42	47.6±13
Sum of permitted alcohol more than 95	1	99.0±0
Glycerine	6	18.0±22
Methanol substitution	7	70.4±26
Sum of permitted alcohol in liquid samples	87	65.5±12
Sum of permitted alcohol in gel samples	34	52.8±17
No alcohol	2	0.0

The percentage of the sum of permitted alcohol (ethanol+isopropyl alcohol) in liquid samples was 66±12% v/v and 53±17% v/v in the gel samples. At the same time, the percentage of sum of permitted alcohol (ethanol+isopropyl alcohol) in all samples was 62±14% v/v, slightly less than the total alcohol content, including methanol of 63±15% v/v.

However, the net content of alcohol for ABHS samples stated on the labels ranged from 60% to 85%. Therefore, the alcohol concentrations found in the study did not agree with the concentrations indicated on the labels.

4.3 pH determination

The UNBS recommends a pH range of 6-8 for hand sanitizers (UNBS, 2013). The pH of the neat samples of ABHS ranged between 2.6 and 8.9, as shown in Figure 8 and Appendix 2, measured within the product temperature range of 19.0 ^{0}C–22.7 ^{0}C, which is commonly encountered during the handling and storage of ABHS by customers. Figure 8 below shows the number of ABHS products within the different pH ranges.

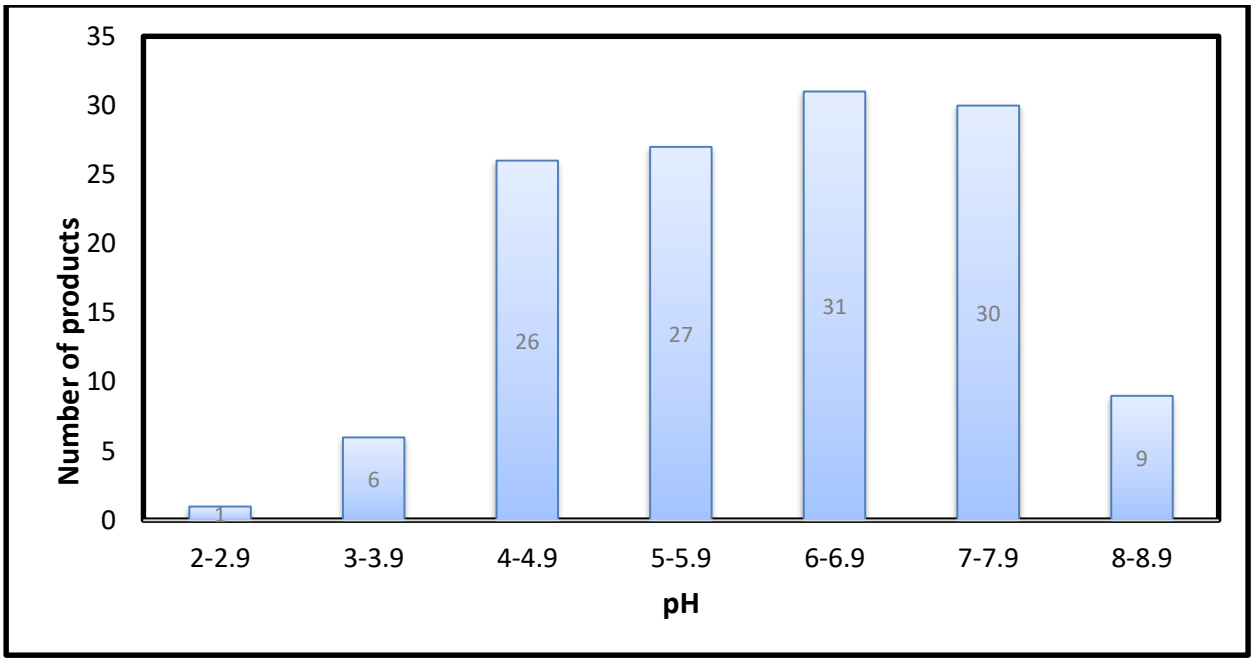


Figure 8: pH ranges of the ABHS products

Forty-eight percent (n=63) of the samples of ABHS had a pH between 6 and 8, with 36 liquid and 27 gel formulations. Slightly above half (52%, n=67) failed the pH test as per the UNBS pH specification range (UNBS, 2013).

Among the formulations of ABHS which failed the test, 88% (n=59) were liquid, and 12% (n=8) gel. It is worth noting that most (n=60) of the ABHS were slightly acidic with a pH <6, while a few (n=7) were slightly basic with a pH >8 among those that failed the test. The non-compliance with the pH specification could be associated with the presence of the listed excipients most likely used in the formulation, such as triethanolamine, phosphoric acid, tocopheryl acetate, polymethyl siloxane, dimethicone, 1,2,3-trihydroxyipropane, and diethyl phthalate which are pH modifying agents (Abuga and Nyamweya, 2021).

However, most ABHS that complied with the pH specification had sodium sulphate, coconut diethanolamide, triethanolamine, alkyl acrylate cross polymer, inter-chloro dimethyl phenol, betaine, triethylamine, sodium lauryl ether sulphate, and alkyl dimethyl benzyl ammonium chloride among others as the excipients. The levels of these excipients could have greatly contributed to the differences in the acidity and alkalinity of the ABHS formulation. The skin surface is acidic, with pH values below 5.0 that is reported to offer better skin functionality, such as resistance to irritant dermatitis, less scaling and increased hydration. The application of sanitizers with lower or higher pH tends to lower or increase the skin pH, respectively. However, the skin pH returns to the "natural" pH after some hours (Lambers *et al.,* 2006).

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 General discussion

The most common brands were found to be locally manufactured. Sample UGS 27 was popularly used following the commissioning of the manufacturing unit with support from the president of Uganda at the beginning of the COVID-19 pandemic and illustrated as the first locally produced ABHS in Uganda (Saraya, 2020). Published efficacy studies conducted on UGS 27, and media reports by National Drug Authority (NDA) Uganda noting UGS 1 and UGS 27, among others, demonstrated their good quality, unlike other brands (Fred *et al.*, 2020; NDA, 2020). The most common sample on the market, UGS 27, passed most of the qualitative and quantitative tests, except pH, which was slightly lower than the specified lower limit by UNBS, and had no glycerine.

Overall, only 15 samples (12%) met all the requirements for appearance, packaging, labelling, and UNBS quality mark specifications. These presented several ABHS brands in the market with unknown nature and origin. The results of this study are comparable to those in literature from other countries. For example, studies in Kenya and Ethiopia reported several non-conformities observed in appearance, packaging, labelling, and established regulatory standards with the ABHS samples. The nature and origin of most brands were unknown and referred to as substandard and falsified (Nyamweya and Abuga, 2020; Selam, 2020).

Some samples (n=6, 4.6%) had both the UNBS mark and NDA Uganda mark, posing a risk of introducing substandard and falsified ABHS in the market. One of the 6 samples did not appear on the UNBS website, and one had an alcohol content of less than 60% v/v.

At the time of this study, NDA Uganda was not the mandated body to certify ABHS, despite the confusion at the beginning of the COVID-19 pandemic. However, the NDA Uganda may carry out quality control tests and pharmacovigilance studies on the product in the market due to the built-in capability and commitment to safeguarding the public against products with health hazards (NDPA Act, 1993).

The UNBS and NDA Uganda have previously published media reports on the list of unsafe ABHS brands produced by unscrupulous manufacturers. They have warned the public against

using such substandard and falsified products (NDA, 2020; UNBS, 2020b). Unfortunately, a few of those brands were still in circulation without the "UNBS Quality Mark." Substandard and falsified ABHS products predispose individual users to adverse events and compromise efforts of regulatory agencies regarding controlling COVID-19 and other pandemics (Tse *et al.*, 2021a). Therefore, the safety of the uninformed customers lies in the hands of the manufacturer and regulator.

Two brands of ABHS found to contain no active ingredient like alcohol or glycerine neither had a "UNBS Quality Mark" nor appeared on the UNBS list of certified products (UNBS, 2013). One gel and the other liquid formulation were purchased from a supermarket and a pharmacy, respectively. Of the six samples (5%) which contained glycerine, only one had a UNBS quality mark, but no country of origin on the label.

Three of the seven samples identified to contain methanol as an impurity did not bear the UNBS quality mark, implying they were not certified by UNBS to be fit for human use. In addition, several ingredients that could interfere with analysis were listed on the packaging or containers by other researchers (Tse *et al*., 2021b); however, they were not observed with GC-MS in this study. Studies in South Africa and Kenya detected several impurities such as methanol, ethyl acetate, isobutanol, 1-propanol, and 3-methyl-butanol in ABHS (Abuga *et al*., 2021; Matatiele *et al*., 2021). By contrast, GC-MS in this study did not detect the named impurities, except methanol.

Considering ABHS samples with alcohol concentrations less than 60% v/v, twenty-one had a UNBS quality mark, and 21 also did not. Five appeared on the UNBS website without a certification mark printed on the label. The sample with an alcohol content above 95% v/v had no UNBS quality mark. Interestingly, one sample without any label had ethanol as an active ingredient and was within the recommended range of 60%-95% (UNBS, 2013; WHO, 2009). Methanol substitution was indicated in five samples, while two samples had methanol in combination with ethanol and had methanol concentrations exceeding the limits set by USP and US-FDA (US-FDA, 2020; USP, 2020a).

In South Africa and Kenya, the studies found ABHS samples containing less than 60% v/v ethanol or isopropyl alcohol, methanol substitution or methanol content above limits, and some failed pH range (Abuga *et al*., 2021; Matatiele *et al*., 2021). In contrast, Italian research indicated

that ABHS samples satisfied the regulatory requirements specific to the class to which they belong. At the same time, cosmetic hand sanitizers had alcoholic strength below the disinfection standard (Berardi *et al*., 2020b). A Canadian study demonstrated that all samples analyzed except one complied with alcoholic strength and methanol limits, and 26 % did not satisfy acetaldehyde limits (Tse *et al*., 2021b).

For quality purposes, ABHS should be formulated using pharmaceutical-grade/food-grade ethanol and evaluated against the USP ethanol monograph, which requires an assay of methanol, benzene, acetal, and acetaldehyde in addition to a sum of all other organic compounds detected by GC (USP, 2020a). However, the USP and Health Canada recommended using technical grade ethanol with an interim limit of methanol (≤200 ppm) and the sum of all other impurities not exceeding 300 ppm in response to the COVID-19 pandemic (Canada, 2020; USP, 2020a). The rest of the other organic impurities were not detected in this study. Some ABHS manufacturers could have used technical-grade ethanol due to its availability and lower cost of production, though with known higher levels of impurities (Tse *et al*., 2021b). Other manufacturers chose to use pure methanol as a substitute for permitted alcohols (ethanol and 2-propanol), the source of which was to be ascertained. In Uganda, Saraya company limited, one of the leading manufacturers of ABHS, stated the use of ethanol obtained through further processing of molasses extracted after sugarcane to produce ABHS. Furthermore, "SARAYA" also indicated sourcing ethanol from a popularly consumed locally produced gin drink referred to as "Waragi" (Saraya, 2020).

Irrespective of the vaccination rate, ABHS will remain the first-line defence for COVID-19 and other upcoming infectious diseases transmitted through contact (Pidot *et al*., 2018). Hence, the need for producing quality ABHS as per current good manufacturing practices.

5.2 Conclusion

Overall, only 15 samples (12%) met all the requirements for appearance, packaging, labelling, and UNBS quality mark specifications. Over 93 samples (72%) complied with the specification for appearance, and 104 samples (80%) were packaged in suitable containers with appropriate closures. Several label irregularities included cut-off print portions, faint, obscured, overlaid, poor inking, tiny wordings, peeling off, and erased or missing labels. A majority of samples

(86%, n=112) were locally manufactured, 13 samples (10%) were imported and 5 (4%) samples did not state the country of origin.

It is worth noting that only four samples (3%) were manufactured with the WHO specified reagents such as ethanol or 2-propanol as the active ingredient and glycerine as the humectant. Ethanol was detected in 112 samples (86%), 2-propanol in 5 samples (4%) and 4 samples (3%) had ethanol/2-propanol admixture. Two samples were found to contain no active ingredient, with one imported and the other locally manufactured. Only 6 (4.6%) samples of ABHS were identified to contain glycerine as the humectant, however, with glycerine content exceeding WHO specification. This study also detected methanol with levels above the USP and US-FDA limits in 7 ABHS brands collected. Of these 7 samples, 5 indicated methanol substitution, while 2 samples demonstrated methanol contamination. All 7 samples identified with methanol were locally manufactured.

Finally, 78 samples (60.0%) complied with the WHO recommended content of permitted alcohol of 60-95% v/v. However, at the same time, many (n=52, 40.0%) were substandard and/or falsified formulations, primarily due to no alcohol or less alcohol or excess alcohol content and methanol content exceeding the interim limits in the ABHS. Forty-three of the samples failed the alcohol concentration range. Forty-two samples (32.3%) contained less than 60% v/v alcohol, and 32 samples were locally manufactured; Only one contained more than 95% v/v alcohol and was also locally manufactured. A percentage of forty-eight (n=63) of the samples passed the pH range, while 52% (n=67) failed the UNBS pH specification range.

This study demonstrates that substandard and falsified ABHS products with undeclared methanol content were in circulation in the Kampala divisions during the COVID-19 pandemic when the need was considerably increased, and regulations were relaxed for manufacturers to escalate the production of ABHS to meet the sudden surge in demand as observed in countries globally.

Since most products are manufactured or imported/distributed through Kampala, the results will likely represent quality concerns across the country. Pharmacovigilance studies in other regions need to be carried out to assess the quality of ABHS and provide information for policy improvement and sensitization of the public against substandard and falsified products in the market.

5.3 Recommendations

5.3.1 Recommendations for policy and practice

1. Besides the "UNBS Quality Mark," this study demonstrates a need for a product permit number. Such a number can be monitored via a functional instant online up-to-date system to verify and report certified ABHS products in circulation.
2. There is need to strengthen the regulatory institution and improve surveillance mechanisms to ensure compliance with set standards in manufacturing ABHS in Uganda. In liaison with all other stakeholders mechanisms must be put in place to detect and report substandard or counterfeit products.
3. The testing of ABHS quality should employ current and validated test methods like GC-MS/FID other than the non-specific method, a pycnometer, currently recommended by UNBS.

5.3.2 Recommendations for further work

1. It is proposed that UNBS specification for "instant hand sanitizers" be revised to "specification for alcohol-based hand sanitizers" to be more specific and be evaluated to accommodate more strict requirements like the specification of allowable grades of ethanol or 2-propanol and their quality control standard, the definition of limits for impurities, and the need for compulsory testing of raw materials by manufacturers.
2. This is a further implication for the need to revise the East African standards for regulation of ABHS and strengthen it for purposes of cross-border trade among the member states.

REFERENCES

Abuga, K., and Nyamweya, N. (2021). Alcohol-Based Hand Sanitizers in COVID-19 Prevention: A Multidimensional Perspective. *Pharmacy*, *9*(1), 64. https://doi.org/10.3390/pharmacy9010064

Abuga, K., Nyamweya, N., and 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

Aljazeera. (2020). Over 700 dead after drinking alcohol to cure coronavirus.

Aodah, A. H., Bakr, A. A., Booq, R. Y., Rahman, M. J., Alzahrani, D. A., Alsulami, K. A., Alshaya, H. A., Alsuabeyl, M. S., Alyamani, E. J., and Tawfik, E. A. (2021). Preparation and evaluation of benzalkonium chloride hand sanitizer as a potential alternative for alcohol-based hand gels. *Saudi Pharmaceutical Journal*, *29*(8), Article 8. https://doi.org/10.1016/j.jsps.2021.06.002

Ashurst, J. V., and Nappe, T. M. (2021). Methanol Toxicity. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK482121/

Barrett, M. J., and Babl, F. E. (2015). Alcohol-based hand sanitiser: A potentially fatal toy. *Med. J. Aust*, *203*(1), 43–44.

Bedner, M., Jacolin A., M., Aaron A., U., William A., M., and Walter B., W. (2021). A Comparison of Measurement Methods for Alcohol-Based Hand Sanitizers. National Institute of Standards and Technology. 63.

Berardi, A., Perinelli, D. R., Merchant, H. A., Bisharat, L., Basheti, I. A., Bonacucina, G., Cespi, M., and Palmieri, G. F. (2020a). Hand sanitisers amid CoViD-19: A critical review of alcohol-based products on the market and formulation approaches to respond to increasing demand. *International Journal of Pharmaceutics*, *584*, 119431. https://doi.org/10.1016/j.ijpharm.2020.119431

Berardi, A., Cenci-Goga, B., Grispoldi, L., Cossignani, L., and Perinelli, D. R. (2020b). Analysis of Commercial Hand Sanitisers amid CoViD-19: Are We Getting the Products that We Need? *AAPS PharmSciTech*, *21*(7), 286. https://doi.org/10.1208/s12249-020-01818-6

Blaxhall, A. (2020). Firms struggling over hand sanitiser packaging material. *European Pharmaceutical Review*. https://www.europeanpharmaceuticalreview.com/article/120001/firms-struggling-over-hand-sanitiser-packaging-material/

Bomgardner, M., Mullin, R., and Scott, A. (2020). Specialty chemicals stepping up to the sanitizer shortage. *Chemical & Engineering News*, *98*(11), 12–12.

Bondurant, S. W., Duley, C. M., and Harbell, J. W. (2019). Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1,

2, and 4 hours after application. *American Journal of Infection Control*, *47*(8), 928–932. https://doi.org/10.1016/j.ajic.2019.01.004

Byishimo, B. (2020, October 27). Rwanda: FDA Recalls “Pure” hand sanitizer over poor quality. *The New Times*. https://allafrica.com/stories/202010270076.html

Canada, H. (2020, October 2). Technical-grade ethanol for the manufacture of hand sanitizers and hard-surface disinfectants during the COVID-19 pandemic*: Risk assessment summary report* [Guidance]. https://www.canada.ca/en/health-canada/services/drugs-health-products/natural-non-prescription/legislation-guidelines/covid19-technical-grade-ethanol-hand-sanitizer/risk-assessment-summary-report.html

Capeletti, M. R., Balzano, L., de la Puente, G., Laborde, M., and Sedran, U. (2000). Synthesis of acetal (1, 1-diethoxyethane) from ethanol and acetaldehyde over acidic catalysts. *Applied Catalysis A: General*, *198*(1–2), L1–L4.

CDC. (2020, September 10). Show Me the Science – When & How to Use Hand Sanitizer in Community Settings | Handwashing | *CDC*. https://www.cdc.gov/handwashing/show-me-the-science-hand-sanitizer.html

CDC. (2021a, November 5). Benefits of Getting a COVID-19 Vaccine. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/vaccine-benefits.html

CDC. (2021b, August 10). Hand Sanitizer Use Out and About. Centers for Disease Control and Prevention. https://www.cdc.gov/handwashing/hand-sanitizer-use.html

Chakraborty, I., and Maity, P. (2020). COVID-19 outbreak: Migration, effects on society, global environment and prevention. *Science of The Total Environment*, *728*, 138882. https://doi.org/10.1016/j.scitotenv.2020.138882

Chan, A. P., and Chan, T. Y. (2018). Methanol as an unlisted ingredient in supposedly alcohol-based hand rub can pose serious health risk. *International Journal of Environmental Research and Public Health*, *15*(7), 1440.

CHOICE. (2020). “White Knight hand sanitizer recalled by ACCC",-Schülke & Mayr GmbH. https://www.schuelke.com/sg-en/unsere-expertise/Recall-of-hand-sanitizer.php

Chojnacki, M., Dobrotka, C., Osborn, R., Johnson, W., Young, M., Meyer, B., Laskey, E., Wozniak, R. A. F., Dewhurst, S., and Dunman, P. M. (2021). Evaluating the Antimicrobial Properties of Commercial Hand Sanitizers. *MSphere*, *6*(2), e00062-21. https://doi.org/10.1128/mSphere.00062-21

Chung, J. Y., Thone, M. N., and Kwon, Y. J. (2021). COVID-19 vaccines: The status and perspectives in delivery points of view. *Advanced Drug Delivery Reviews*, *170*, 1–25. https://doi.org/10.1016/j.addr.2020.12.011

Cook, M. A., and Brooke, N. (2021). Event-Based Surveillance of Poisonings and Potentially Hazardous Exposures over 12 Months of the COVID-19 Pandemic. *International Journal of Environmental Research and Public Health*, *18*(21). https://doi.org/10.3390/ijerph182111133

Dear, K., Grayson, L., and Nixon, R. (2020). Potential methanol toxicity and the importance of using a standardised alcohol-based hand rub formulation in the era of COVID-19. *Antimicrobial Resistance and Infection Control*, *9*(1), 1–3. https://doi.org/10.1186/S13756-020-00788-5/TABLES/1

Dicken, R. D., Gallagher, T., and Perks, S. (2020). Overcoming the Regulatory Hurdles for the Production of Hand Sanitizer for Public Health Protection: The UK and US Academic Perspective. *ACS Chemical Health & Safety*, *27*(4), 209–213. https://doi.org/10.1021/acs.chas.0c00065

Estevão, P. L. S., Colodi, F. G., Carmo, L. C. L. do, Santos, M. de F. C., Barison, A., D'Oca, C. R. M., Nagata, N., and Freitas, R. A. de. (2021). Alcoholmeter as a simple and accessible way for Ethanol determination in Alcohol-Based Hand Sanitizers. *Journal of the Brazilian Chemical Society*, *32*, 1239–1248. https://doi.org/10.21577/0103-5053.20210026

European Commission. (2020). *E*uropean commission. Guidance on the applicable legislation for leave-on hand cleaners and hand disinfectants (gel, solution, etc.) [Internet]. 2020. *Available from: Https://ec.europa.eu/docsroom/documents/40523.*

Fazio, M. (2020). Die in New Mexico after drinking hand sanitizer, officials say. *The New York Times*.

Fred, T., Sophia, K., Alex, S., Emmanuel, B., Tom, L., and Lucas, A. (2020). Comparison of antibacterial efficacy of locally produced alcohol based hand sanitizer and commonly available commercial hand sanitizer used in healthcare facilities in Uganda. *Open Access Library Journal*, *7*(4), 1–13.

Gacuiga, M. W., Wanjau, R. N., Murigi, M. K., Mbugua, G. W., Ndiritu, A. M., and Gikonyo, N. K. (2022). Alcohols and Denaturants Hand Rub Sanitizers Used to Curb COVID-19 Pandemic in Kiambu County, Kenya. *International Journal of Scientific Research in Chemical Sciences*, *9*(3), 1–5.

Gekonge, D. (2021, February 21). 5 Students Go Blind, 1 Dead After Consuming Hand Sanitizer. Teacher.Co.Ke. https://teacher.co.ke/5-students-go-blind-1-dead-after-consuming-hand-sanitizer/

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

Golin, A. P., Choi, D., and 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

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., O'Meara, M. J., Rezelj, V. V., Guo, J. Z., Swaney, D. L., Tummino, T. A., Hüttenhain, R., Kaake, R. M., Richards, A. L., Tutuncuoglu, B., Foussard, H., Batra, J., Haas, K., Modak, M., … Krogan, N. J. (2020). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*, *583*(7816), 459–468. https://doi.org/10.1038/s41586-020-2286-9

Greenaway, R. E., Ormandy, K., Fellows, C., and Hollowood, T. (2018). Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. *Journal of Hospital Infection*, *100*(2), 195–201. https://doi.org/10.1016/j.jhin.2018.07.011

Halfan, H. M. (2020, September 29). The rise and fall of sanitiser and face mask business in Tanzania. *Africa at LSE*. https://blogs.lse.ac.uk/africaatlse/2020/09/29/rise-and-fall-face-masks-hand-sanitiser-business-health-tanzania-hygiene-covid19/

Hazelwood, L. A., Daran, J.-M., Van Maris, A. J., Pronk, J. T., and Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on Saccharomyces cerevisiae metabolism. *Applied and Environmental Microbiology*, *74*(8), 2259–2266.

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

Hidzir, N. S., Som, A. S., and Abdullah, Z. (2014). Ethanol production via direct hydration of ethylene: A review. *International Conference on Global Sustainability and Chemical Engineering (ICGSE)*.

HSA. (2021, April). Recall of hand sanitisers (Feb to April 2021). HSA. https://www.hsa.gov.sg/announcements/product-recall/recall-of-hand-sanitisers-(feb-to-april-2021)

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, *395*(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

ICH, I. H. T. (2005). Validation of Analytical Procedures: Text and Methodology Q2 (R1)(2005) International Conference on Harmonization. *Geneva, Switzerland*.

Jairoun, A. A., Al-Hemyari, S. S., and 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

Jie, Z. (2020, June 29). Hand sanitizer analysis using the Agilent 8860 GC configured with a flame ionization detector. https://www.readkong.com/page/hand-sanitizer-analysis-using-the-agilent-8860-gc-4213811

Jing, J. L., Pei Yi, T., Bose, R. J. C., McCarthy, J. R., Tharmalingam, N., and Madheswaran, T. (2020). Hand sanitizers: A review on formulation aspects, adverse effects, and regulations. *International Journal of Environmental Research and Public Health*, *17*(9). https://doi.org/10.3390/ijerph17093326

Joseph, M. M., Zeretzke, C., Reader, S., and Sollee, D. R. (2011). Acute ethanol poisoning in a 6-year-old girl following ingestion of alcohol-based hand sanitizer at school. *World Journal of Emergency Medicine*, *2*(3), 232–233. PubMed. https://doi.org/10.5847/wjem.j.1920-8642.2011.03.014

Kampf, G. (2018). Efficacy of ethanol against viruses in hand disinfection. *The Journal of Hospital Infection*, *98*(4), 331–338. https://doi.org/10.1016/j.jhin.2017.08.025

KCCA. (2019). Statistical abstract for Kampala city, About the Authority—Kampala Capital City Authority | For a better City. https://www.kcca.go.ug/about-the-authority.

KEBS. (2014). Kenya Bureau of Standards Instant Hand Sanitizers, KS EAS 789:2013.

Kim, S.-S., Kim, J., Shin, S.-C., and Agblevor, F. A. (2009). Distinction between Bioethanol and Synthetic Ethanol in a Mixture of Gasoline Using Low Level Liquid Scintillation Counting. *Chemistry Letters*, *38*(8), 850–851.

Kumar, S. (2020). *COVID-19:* A Drug Repurposing and Biomarker identification by using Comprehensive Gene-Disease Associations through Protein-Protein Interaction Network Analysis. https://doi.org/10.20944/preprints202003.0440.v1

Lambers, H., Piessens, S., Bloem, A., Pronk, H., and Finkel, P. (2006). Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science*, *28*(5), 359–370. https://doi.org/10.1111/j.1467-2494.2006.00344.x

Langer, S., Salakdeh, M. S., Goertz, O., Steinau, H., Steinstraesser, L., and Homann, H. (2004). The impact of topical antiseptics on skin microcirculation. *European Journal of Medical Research*, *9*(9), 449–454.

Luyten, J., and Beutels, P. (2016). The social value of vaccination programs: Beyond cost-effectiveness. *Health Affairs*, *35*(2), 212–218.

Mahmood, A., Eqan, M., Pervez, S., Alghamdi, H. A., Tabinda, A. B., Yasar, A., Brindhadevi, K., and Pugazhendhi, A. (2020). COVID-19 and frequent use of hand sanitizers; human health and environmental hazards by exposure pathways. *Science of the Total Environment*, *742*, undefined-undefined. https://doi.org/10.1016/j.scitotenv.2020.140561

Matatiele, P., Southon, B., Dabula, B., Marageni, T., Poongavanum, P., and Kgarebe, B. (2021). Monitoring Quality of Alcohol-Based Hand Sanitizers Used in Johannesburg Area During the Covid-19 Pandemic. [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-612413/v1

Mathuria, J. P., Yadav, R., and Rajkumar. (2020). Laboratory diagnosis of SARS-CoV-2—A review of current methods. *Journal of Infection and Public Health*, *13*(7), 901–905. https://doi.org/10.1016/j.jiph.2020.06.005

Migisha, R., Kwesiga, B., Mirembe, B. B., Amanya, G., Kabwama, S. N., Kadobera, D., Bulage, L., Nsereko, G., Wadunde, I., Tindyebwa, T., Lubwama, B., Kagirita, A. A., Kayiwa, J. T., Lutwama, J. J., Boore, A. L., Harris, J. R., Bosa, H. K., and Ario, A. R. (2020). Early cases of SARS-CoV-2 infection in Uganda: Epidemiology and lessons learned from risk-based testing approaches – March-April 2020. *Globalization and Health*, *16*(1), 114. https://doi.org/10.1186/s12992-020-00643-7

NAFDAC. (2020, October 27). Public Alert No. 024/2020-European Union Rapid Alert System Recall Symex Hand Sanitizers – NAFDAC. https://www.nafdac.gov.ng/public-alert-no-024-2020-european-union-rapid-alert-system-recall-symex-hand-sanitizers/

NDA. (2020). 56% of sanitiser brands in Uganda fail NDA efficacy test. The Observer - Uganda. https://observer.ug/news/headlines/64209-56-of-sanitiser-brands-in-uganda-s-market-fail-nda-test

NDPA Act. (1993). National Drug Policy and Authority Act. | Principal Legislation. https://www.ugandalaws.com/principal-legislation/national-drug-policy-and-authority-act

Ngina, F. (2020). Here are the eight brands of sanitizers banned by KEBS. The Standard. https://www.standardmedia.co.ke/ureport/article/2001371751/here-are-the-eight-brands-of-sanitizers-banned-by-kebs

Nicol, A.-M. (2021). Recalls highlight importance of hand sanitizer safety. *Environmental Health Review*, *64*(1), 6–10.

Nyamweya, N. N., and Abuga, K. O. (2020). A Survey of Alcohol-Based Hand Sanitizers in Nairobi: Packaging, Labelling and Regulatory Compliance. *East and Central African Journal of Pharmaceutical Sciences*, *23*(2), Article 2.

Nyamweya, N. N., Lumb, P. N., Mujyarugamba, J. C., and Abuga, K. O. (2021). Inactive ingredients used in Alcohol-Based Hand Sanitizers marketed in the Nairobi Metropolitan Area. *Pharmaceutical Journal of Kenya 25:17–20*, *25*.

Ojambo, F. (2021, July 30). Uganda Relaxes Lockdown to Ease Economic Fallout From Pandemic. *Bloomberg.Com*. https://www.bloomberg.com/news/articles/2021-07-30/uganda-relaxes-lockdown-to-ease-economic-fallout-from-pandemic

O'Leary, F. M., and Price, G. J. (2011). Alcohol hand gel—A potential fire hazard. *Journal of Plastic, Reconstructive & Aesthetic Surgery: JPRAS*, *64*(1), 131–132. https://doi.org/10.1016/j.bjps.2010.03.026

Onuki, S., Koziel, J. A., Jenks, W. S., Cai, L., Grewell, D., and van Leeuwen, J. H. (2016). Taking ethanol quality beyond fuel grade: A review. *Journal of the Institute of Brewing*, *122*(4), 588–598.

Pascault, J.-P., Höfer, R., and Fuertes, P. (2012). Mono-, di-, and oligosaccharides as precursors for polymer synthesis.

Pasquini, C., Hespanhol, M. C., Cruz, K. A. M. L., and Pereira, A. F. (2020). Monitoring the quality of ethanol-based hand sanitizers by low-cost near-infrared spectroscopy. *Microchemical Journal*, *159*, 105421. https://doi.org/10.1016/j.microc.2020.105421

Pidot, S. J., Gao, W., Buultjens, A. H., Monk, I. R., Guerillot, R., Carter, G. P., Lee, J. Y., Lam, M. M., Grayson, M. L., and Ballard, S. A. (2018). Increasing tolerance of hospital Enterococcus faecium to handwash alcohols. *Science Translational Medicine*, *10*(452), eaar6115.

Pradhan, D., Biswasroy, P., Kumar Naik, P., Ghosh, G., and Rath, G. (2020). A Review of Current Interventions for COVID-19 Prevention. *Archives of Medical Research*, *51*(5), 363–374. https://doi.org/10.1016/j.arcmed.2020.04.020

Prajapati, P., Desai, H., and Chandarana, C. (2022). Hand sanitizers as a preventive measure in COVID-19 pandemic, its characteristics, and harmful effects: A review. *Journal of the Egyptian Public Health Association*, *97*(1), 6. https://doi.org/10.1186/s42506-021-00094-x.

Rahi, S., Kumar, A., and Rana, A. (2021). Hand Sanitizer: Regulatory Perspective in Different Countries. International Journal of Pharmaceutical Sciences Review and Research, 71(2). https://doi.org/10.47583/ijpsrr.2021.v71i02.001

Rotter, M. (1999). Hand washing and hand disinfection [Chapter 87]. *Hospital Epidemiology and Infection Control*.

Rundle, C. W., Presley, C. L., Militello, M., Barber, C., Powell, D. L., Jacob, S. E., Atwater, A. R., Watsky, K. L., Yu, J., and Dunnick, C. A. (2020). Hand hygiene during COVID-19: Recommendations from the American Contact Dermatitis Society. *Journal of the American Academy of Dermatology*, *83*(6), 1730–1737.

Saraya. (2020, July 9). Hand Washing, Coronavirus and How we Became the First Producers of Alc. https://saraya.world/news/hand-washing-coronavirus-and-how-we-became-the-first-producers-of-alcohol-sanitizer-in-uganda

Selam, M. N. (2020). Hand sanitizers marketed in the streets of addis ababa, ethiopia, in the era of covid-19: A quality concern. *Risk Management and Healthcare Policy*, *13*, 2483–2487. https://doi.org/10.2147/RMHP.S284007

Sette, A., and Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*, *184*(4), 861–880. https://doi.org/10.1016/j.cell.2021.01.007

Shabir, G. A., John Lough, W., Arain, S. A., and Bradshaw, T. K. (2007). Evaluation and Application of Best Practice in Analytical Method Validation. *Journal of Liquid*

Chromatography and Related Technologies, *30*(3), 311–333. https://doi.org/10.1080/10826070601084753

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

Taleghani, N., and Taghipour, F. (2021). Diagnosis of COVID-19 for controlling the pandemic: A review of the state-of-the-art. *Biosensors and Bioelectronics*, *174*, 112830. https://doi.org/10.1016/j.bios.2020.112830

Thaddeus, N. I., Francis, E. C., Jane, O. O., Obumneme, A. C., and Okechukwu, E. C. (2018). Effects of some common additives on the antimicrobial activities of alcohol-based hand sanitizers. *Asian Pacific Journal of Tropical Medicine*, *11*(3), 222. https://doi.org/10.4103/1995-7645.228437

Thomson, E. L., and Bullied, A. R. (2020). Corrosion resistance of aluminum beer cans containing hand sanitizer. *Journal of the American Society of Brewing Chemists*, *78*(4), 279–283. https://doi.org/10.1080/03610470.2020.1784634

Todd, E. C. D., Michaels, B. S., Holah, J., Smith, D., Greig, J. D., and Bartleson, C. A. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 10. Alcohol-based antiseptics for hand disinfection and a comparison of their effectiveness with soaps. *Journal of Food Protection*, *73*(11), 2128–2140. https://doi.org/10.4315/0362-028X-73.11.2128

Tse, T. J., Purdy, S. K., Shen, J., Nelson, F. B., Mustafa, R., Wiens, D. J., and Reaney, M. J. T. (2021a). 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

Tse, T. J., Nelson, F. B., and Reaney, M. J. (2021b). 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.

UNBS. (2013). Uganda National Bureau of Standards Instant Hand Sanitizers, US EAS 789:2013.

UNBS. (2020a). Uganda National Bureau of Standards. https://unbs.go.ug/news-highlights.php?news=149&read

UNBS. (2020b). UNBS blacklists 15 sanitizer brands after failed lab tests. https://observer.ug/news/headlines/64126-unbs-blacklists-15-sanitizer-brands-after-failed-lab-tests

UNBS. (2021). Uganda National Bureau of Standards. https://www.unbs.go.ug/news-highlights.php?news=187&read

US-FDA. (1994). Validation of chromatographic methods. Center for Drug Evaluation and Research (CDER), Washington, 2.

US-FDA. (2020). Direct injection gas chromatography mass spectrometry (GC-MS) method for the detection of listed impurities in hand sanitizers. 11.

US-FDA. (2020, September 2). COVID-19 Update: FDA warns consumers about hand sanitizer packaged in food and drink containers. FDA; FDA. https://www.fda.gov/news-events/press-announcements/covid-19-update-fda-warns-consumers-about-hand-sanitizer-packaged-food-and-drink-containers

US-FDA. (2021a). Topical antiseptic products: Hand sanitizers and antibacterial soaps. *FDA*. https://www.fda.gov/drugs/information-drug-class/topical-antiseptic-products-hand-sanitizers-and-antibacterial-soaps

US-FDA. (2021b). FDA updates on hand sanitizers consumers should not use. *FDA*. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-hand-sanitizers-consumers-should-not-use

US-FDA. (2021c, February 11). Temporary policy for manufacture of alcohol for incorporation into alcohol-based hand sanitizer products during the public health emergency (COVID-19) guidance for industry. U.S. Food and Drug Administration; FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/temporary-policy-manufacture-alcohol-incorporation-alcohol-based-hand-sanitizer-products-during

US-FDA. (2021d). Hand Sanitizers | COVID-19. *FDA*. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/hand-sanitizers-covid-19

USP. (2020a). United States Pharmacopeia Excerpted USP-NF and FCC Standards: A Hand sanitizer resource. *Https://www.usp.org/sites/default/files/usp/document/healthquality-safety/usp-hand-sanitizer-ingredients.pdf*

USP. (2020b). USP <611> Alcohol determination. http://www.uspbpep.com/usp29/v29240/usp29nf24s0_c611.html

USP. (2021, February 3). U.S. Pharmacopeia (USP) expands alcohol-based hand sanitizer support in response to quality incidents reported during the COVID-19 pandemic. https://www.usp.org/news/usp-expands-alcohol-based-hand-sanitizer-support

Waithera. (2020, May 17). Five Murang'a youths who drank sanitiser to be rehabilitated. The Star. https://www.the-star.co.ke/counties/central/2020-05-17-five-muranga-youths-who-drank-sanitiser-to-be-rehabilitated/

Wessels, S., and Ingmer, H. (2013). Modes of action of three disinfectant active substances: A review. *Regulatory Toxicology and Pharmacology*, *67*(3), 456–467. https://doi.org/10.1016/j.yrtph.2013.09.006

WHO. (2006). WHO guidelines on hand hygiene in health care (advanced draft): Global safety challenge 2005-2006: clean care is safer care (WHO/EIP/SPO/QPS/05.2 Rev.1). World Health Organization. https://apps.who.int/iris/handle/10665/69323

WHO. (2009). WHO guidelines on hand hygiene in health care: First global patient safety challenge clean care is safer care. World Health Organization. http://www.ncbi.nlm.nih.gov/books/NBK144013/

WHO. (2009). Guidelines' preparation process. 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/NBK144024/

WHO. (2010, May 7). Guide to local production: WHO-recommended handrub formulations. https://www.who.int/publications-detail-redirect/WHO-IER-PSP-2010.5

WHO. (2021a). Timeline: WHO's COVID-19 response. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline

WHO. (2021b). COVID-19 advice—Protect yourself and others | WHO Western Pacific. https://www.who.int/westernpacific/emergencies/covid-19/information/transmission-protective-measures

WHO. (2021c, February 26). What is COVID-19 vaccine efficacy? WHO | Regional Office for Africa. https://www.afro.who.int/news/what-covid-19-vaccine-efficacy

Wood, H. (2021, April 29). Which is better: Alcohol or non-alcohol hand sanitiser? https://www.initial.com/blog/hand-hygiene/which-is-better-alcohol-or-non-alcohol-hand-sanitiser

Yip, L., Bixler, D., Brooks, D. E., Clarke, K. R., Datta, S. D., Dudley, S., Komatsu, K. K., Lind, J. N., Mayette, A., Melgar, M., Pindyck, T., Schmit, K. M., Seifert, S. A., Shirazi, F. M., Smolinske, S. C., Warrick, B. J., and Chang, A. (2020). Serious adverse health events, including death, associated with ingesting alcohol-based hand sanitizers containing methanol—Arizona and New Mexico, May–June 2020. *Morbidity and Mortality Weekly Report*, *69*(32), 1070–1073. https://doi.org/10.15585/mmwr.mm6932e1

ZAMRA. (2020, June 19). 13 Hand sanitizer brands face recall ~. https://www.znbc.co.zm/news/13-hand-sanitizer-brands-face-recall/

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G. F., Tan, W., and China Novel Coronavirus Investigating and Research Team. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *The New England Journal of Medicine*, *382*(8), 727–733. https://doi.org/10.1056/NEJMoa2001017

APPENDICES

Appendix 1: Active ingredient and glycerine identified in hand sanitizers in Kampala

Sample code	Active ingredient on label	Active ingredient identified	Glycerine stated	Glycerine identified
UGS 1	Ethanol	Ethanol	SD	ND
UGS 2	Ethyl alcohol	Ethanol	SD	ND
UGS 3	Ethyl alcohol	Ethanol	SD	ND
UGS 4	Ethanol	Ethanol	SD	ND
UGS 5	Alcohol	Ethanol	SD	DECTECTED
UGS 6	Alcohol (Ethanol, IPA)	Methanol, ethanol	SD	ND
UGS 7	Alcohol	Ethanol	SD	ND
UGS 8	Ethanol	Methanol	SD	ND
UGS 9	Ethanol	Ethanol	SD	ND
UGS 10	Ethanol	Ethanol	SD	ND
UGS 11	Ethyl alcohol, (Denaturant. IPA 3.3%)	Ethanol	N/S	ND
UGS 12	Ethyl alcohol, (Denaturant IPA 3.3%)	Ethanol	N/S	DETECTED
UGS 13	Alcohol (Ethyl alcohol, IPA)	Ethanol	SD	ND
UGS 14	Ethanol	Ethanol	SD	ND
UGS 15	(Methanol, IPA, ethanol)	Ethanol	SD	ND
UGS 16	Ethyl alcohol	Ethanol	SD	ND
UGS 17	Ethanol	Ethanol	SD	ND
UGS 18	Ethanol	Methanol	SD	ND
UGS 19	IPA	Ethanol	SD	ND
UGS 20	Ethyl alcohol	Ethanol	SD	ND
UGS 21	Ethanol	Ethanol	SD	ND
UGS 22	Ethanol	Ethanol	SD	ND
UGS 23	Alcohol	Ethanol	SD	ND
UGS 24	Ethanol	Methanol, ethanol	N/S	ND
UGS 25	Ethyl alcohol	Ethanol	SD	ND
UGS 26	Ethanol	Ethanol	SD	ND
UGS 27	Ethanol	Ethanol	SD	ND
UGS 28	Ethanol	Ethanol	SD	ND
UGS 29	Ethyl alcohol	Ethanol	N/S	ND
UGS 30	Ethyl alcohol	Ethanol	SD	ND
UGS 31	IPA	Ethanol	SD	ND
UGS 32	Ethyl alcohol	Ethanol	SD	ND
UGS 33	Ethyl alcohol	Ethanol	SD	ND

Sample code	Active ingredient on label	Active ingredient identified	Glycerine stated	Glycerine identified
UGS 34	Ethanol	Ethanol	SD	ND
UGS 35	Denatured alcohol	IPA, ethanol	SD	ND
UGS 36	Ethyl alcohol	Ethanol	SD	ND
UGS 37	Ethyl alcohol	Ethanol	SD	ND
UGS 38	Ethyl alcohol	Ethanol	SD	ND
UGS 39	Ethanol, (IPA 1%)	Ethanol	SD	ND
UGS 40	Ethyl alcohol	Ethanol	SD	ND
UGS 41	Ethyl alcohol	Ethanol	SD	ND
UGS 42	Ethanol	IPA	SD	ND
UGS 43	IPA	Methanol	SD	DETECTED
UGS 44	Alcohol denatured	Ethanol	N/S	ND
UGS 45	Ethyl alcohol	ND	N/S	ND
UGS 46	Ethyl alcohol	Ethanol	SD	ND
UGS 47	Ethyl alcohol	Ethanol	SD	ND
UGS 48	Alcohol	Ethanol	SD	ND
UGS 49	Alcohol	Ethanol	SD	ND
UGS 50	Ethanol	Ethanol	SD	ND
UGS 51	Ethanol	Ethanol	SD	ND
UGS 52	Ethanol	Ethanol	SD	ND
UGS 53	Ethanol	Ethanol	SD	ND
UGS 54	Ethanol	Ethanol	SD	ND
UGS 55	Ethanol	Ethanol	SD	ND
UGS 56	Ethyl alcohol	Methanol	SD	DETECTED
UGS 57	Ethanol	Ethanol	SD	ND
UGS 58	Ethanol	Ethanol	SD	ND
UGS 59	Ethanol	Ethanol	SD	ND
UGS 60	Ethyl alcohol, (Det. IPA 3.3%)	Ethanol	N/S	ND
UGS 61	Ethyl alcohol, (Det. IPA 3.3%)	Ethanol	N/S	ND
UGS 62	Ethyl alcohol	Ethanol	SD	ND
UGS 63	Ethanol	Ethanol	SD	ND
UGS 64	Cetyl alcohol	IPA	SD	DETECTED
UGS 65	Ethanol (Cetyl alcohol)	Ethanol	SD	ND
UGS 66	Ethanol	Ethanol	SD	ND
UGS 67	IPA	IPA, ethanol	SD	ND
UGS 68	Ethyl alcohol	Ethanol	SD	ND
UGS 69	Ethyl alcohol (Methanol, IPA)	Ethanol	SD	ND
UGS 70	Athyl alcohol	Ethanol	SD	ND
UGS 71	Ethanol	Ethanol	SD	ND

Sample code	Active ingredient on label	Active ingredient identified	Glycerine stated	Glycerine identified
UGS 72	Alcohol, (Phenoxyethanol 0.2%)	Ethanol	N/S	ND
UGS 73	Ethyl alcohol, IPA	Ethanol	SD	ND
UGS 74	Ethyl alcohol	Ethanol	SD	ND
UGS 75	Ethyl alcohol	Ethanol	SD	ND
UGS 76	Ethanol	Ethanol	SD	ND
UGS 77	Ethoxylated fatty alcohols	ND	N/S	ND
UGS 78	Alcohol	Ethanol	SD	ND
UGS 79	Ethyl alcohol	Ethanol	SD	ND
UGS 80	Ethanol	Ethanol	N/S	ND
UGS 81	Alcohol (Ethanol)	IPA	N/S	ND
UGS 82	Ethanol	Ethanol	SD	ND
UGS 83	Ethanol	Ethanol	SD	ND
UGS 84	IPA	Ethanol	SD	ND
UGS 85	Ethanol	Ethanol	SD	ND
UGS 86	IPA	IPA	N/S	ND
UGS 87	Ethanol	Ethanol	SD	ND
UGS 88	Alcohol	Ethanol	SD	ND
UGS 89	IPA	IPA	SD	ND
UGS 90	Ethyl alcohol	Ethanol	SD	ND
UGS 91	Ethanol	Ethanol	SD	ND
UGS 92	Ethanol	Ethanol	SD	ND
UGS 93	Ethanol	Ethanol	SD	ND
UGS 94	Ethanol	Ethanol	SD	ND
UGS 95	N/S	Ethanol	N/S	ND
UGS 96	Ethanol	Ethanol	SD	ND
UGS 97	Ethyl alcohol	Ethanol	SD	ND
UGS 98	Ethanol	Ethanol	SD	ND
UGS 99	Ethanol	Ethanol	SD	ND
UGS 100	Ethanol	Ethanol	SD	ND
UGS 101	Ethanol	Ethanol	SD	ND
UGS 102	Ethanol	Ethanol	SD	ND
UGS 103	Ethanol	Ethanol	SD	ND
UGS 104	Alcohol (Denatured alcohol)	Ethanol	SD	ND
UGS 105	Ethanol (Propyl alcohol)	Ethanol	SD	DETECTED
UGS 106	Alcohol (Ethanol/IPA)	Methanol	SD	ND
UGS 107	Ethanol	Ethanol	N/S	ND
UGS 108	Alcohol (Det. alcohol, IPA)	Ethanol	SD	ND
UGS 109	Ethyl alcohol (IPA 3.3)	Ethanol	N/S	ND
UGS 110	Ethyl alcohol	Ethanol	SD	ND

Sample code	Active ingredient on label	Active ingredient identified	Glycerine stated	Glycerine identified
UGS 111	IPA	Ethanol	SD	ND
UGS 112	Ethanol	Ethanol	SD	ND
UGS 113	Ethanol, IPA	Ethanol	SD	ND
UGS 114	Ethyl alcohol	Ethanol	SD	ND
UGS 115	Ethyl alcohol	Ethanol	SD	ND
UGS 116	Ethanol	Ethanol	N/S	ND
UGS 117	Ethyl alcohol	Ethanol	SD	ND
UGS 118	Ethanol	Ethanol	SD	ND
UGS 119	Ethyl alcohol	Ethanol	SD	ND
UGS 120	Ethyl alcohol	IPA, ethanol	SD	ND
UGS 121	Methyl alcohol, Ethanol	Ethanol	N/S	ND
UGS 122	Ethanol	Ethanol	SD	ND
UGS 123	Ethyl alcohol	Ethanol	SD	ND
UGS 124	Ethyl alcohol	Ethanol	SD	ND
UGS 125	Ethanol	Ethanol	SD	ND
UGS 126	Ethyl alcohol	IPA, ethanol	SD	ND
UGS 127	Ethanol	Ethanol	SD	ND
UGS 128	N/S	Ethanol	N/S	ND
UGS 129	Ethanol	Ethanol	SD	ND
UGS 130	Ethanol	Ethanol	SD	ND

N/S: not stated, **SD:** stated, **ND:** not detected, **IPA:** isopropyl alcohol

Appendix 2: Content of active ingredient and glycerine, and pH values of hand sanitizers in Kampala

Sample code	% alcohol on label	Content of alcohol (% v/v)						pH	Temperature (^{0}C) of sample recorded
		Glycerine	Methanol	IPA	Ethanol	Total alcohol	Total ethanol + IPA		
UGS 1	80	-	-	-	70.5	70.5	70.5	5.8	20.5
UGS 2	80	-	-	-	62.8	62.8	62.8	5.6	20.0
UGS 3	70	-	-	-	54.1	54.1	54.1	6.3	20.4
UGS 4	70	-	-	-	63.0	63.0	63.0	5.1	20.5
UGS 5	85	57.4	-	-	27.8	27.8	27.8	6.8	20.6
UGS 6	70	-	40.8	-	15.7	56.6	15.7	7.0	22.7
UGS 7	N/S	-	-	-	58.7	58.7	58.7	7.4	20.8
UGS 8	80	-	98.0	-	-	98.0	-	7.7	21.0
UGS 9	80	-	-	-	78.2	78.2	78.2	4.7	22.0
UGS 10	80	-	-	-	82.0	82.0	82.0	5.4	21.4
UGS 11	70	-	-	-	51.5	51.5	51.5	6.4	20.4
UGS 12	70	29.2	-	-	71.3	71.3	71.3	4.7	20.9
UGS 13	75	-	-	-	64.1	64.1	64.1	6.5	20.0
UGS 14	75	-	-	-	61.7	61.7	61.7	5.5	20.3
UGS 15	75	-	-	-	47.7	47.7	47.7	4.1	20.0
UGS 16	70	-	-	-	59.2	59.2	59.2	7.9	20.6
UGS 17	70	-	-	-	58.4	58.4	58.4	8.1	20.2
UGS 18	80	-	80.1	-	-	80.1	-	7.3	20.2
UGS 19	70	-	-	-	80.3	80.3	80.3	4.1	20.4
UGS 20	76.8	-	-	-	65.0	65.0	65.0	5.6	20.5
UGS 21	80	-	-	-	77.9	77.9	77.9	8.7	20.1
UGS 22	80	-	-	-	82.8	82.8	82.8	6.9	19.0
UGS 23	80	-	-	-	69.5	69.5	69.5	7.6	21.6
UGS 24	80	-	25.7	-	28.3	54.0	28.3	4.2	19.4
UGS 25	80	-	-	-	39.6	39.6	39.6	5.7	19.7
UGS 26	72	-	-	-	70.1	70.1	70.1	7.0	19.9
UGS 27	80	-	-	-	67.4	67.4	67.4	2.6	19.4
UGS 28	72.6	-	-	-	58.9	58.9	58.9	4.3	20.9
UGS 29	65	-	-	-	64.5	64.5	64.5	5.1	19.3
UGS 30	70	-	-	-	59.7	59.7	59.7	7.1	19.9
UGS 31	72.5	-	-	-	22.0	22.0	22.0	5.5	20.0
UGS 32	77	-	-	-	82.2	82.2	82.2	5.9	19.6
UGS 33	N/S	-	-	-	51.1	51.1	51.1	6.2	20.8
UGS 34	80	-	-	-	61.5	61.5	61.5	8.1	19.7

Sample code	% alcohol on label	Content of alcohol (% v/v)						pH	Temperature (^{0}C) of sample recorded
		Glycerine	Methanol	IPA	Ethanol	Total alcohol	Total ethanol + IPA		
UGS 35	80	-	-	6.2	32.9	39.1	39.1	6.9	21.9
UGS 36	80	-	-	-	79.9	79.9	79.9	5.1	19.9
UGS 37	75	-	-	-	67.9	67.9	67.9	7.5	20.2
UGS 38	75	-	-	-	67.4	67.4	67.4	6.8	19.5
UGS 39	70	-	-	-	62.3	62.3	62.3	7.7	19.8
UGS 40	80	-	-	-	61.5	61.5	61.5	7.8	19.9
UGS 41	75	-	-	-	54.5	54.5	54.5	6.3	19.9
UGS 42	70	-	-	89.2	-	89.2	89.2	8.3	19.4
UGS 43	N/S	4.5	83.0	-	-	83.0	-	8.9	19.7
UGS 44	N/S	-	-	-	69.4	69.4	69.4	7.3	19.5
UGS 45	62	-	-	-	-	-	-	5.6	20.0
UGS 46	70	-	-	-	54.7	54.7	54.7	6.5	19.5
UGS 47	62	-	-	-	9.3	9.3	9.3	7.9	19.8
UGS 48	75	-	-	-	55.5	55.5	55.5	7.4	19.5
UGS 49	N/S	-	-	-	51.9	51.9	51.9	7.1	19.7
UGS 50	80	-	-	-	64.8	64.8	64.8	5.0	20.2
UGS 51	80	-	-	-	49.2	49.2	49.2	6.4	20.0
UGS 52	70	-	-	-	64.8	64.8	64.8	5.4	20.5
UGS 53	76	-	-	-	70.6	70.6	70.6	6.1	20.6
UGS 54	70	-	-	-	61.9	61.9	61.9	6.8	20.0
UGS 55	80	-	-	-	46.1	46.1	46.1	7.9	20.0
UGS 56	N/S	4.9	84.8	-	-	84.8	-	5.7	20.0
UGS 57	75	-	-	-	65.7	65.7	65.7	4.4	20.2
UGS 58	80	-	-	-	50.7	50.7	50.7	8.3	20.0
UGS 59	70	-	-	-	72.4	72.4	72.4	8.7	20.1
UGS 60	70	-	-	-	80.5	80.5	80.5	4.9	20.2
UGS 61	70	-	-	-	76.3	76.3	76.3	4.7	20.0
UGS 62	70	-	-	-	79.6	79.6	79.6	4.7	20.1
UGS 63	70	-	-	-	75.0	75.0	75.0	5.2	20.8
UGS 64	N/S	5.3	-	46.4	-	46.4	46.4	4.5	20.7
UGS 65	64	-	-	-	66.2	66.2	66.2	6.0	20.3
UGS 66	70	-	-	-	62.1	62.1	62.1	5.0	20.4
UGS 67	N/S	-	-	25.5	73.5	99.0	131.1	4.9	20.6
UGS 68	N/S	-	-	-	68.3	68.3	68.3	8.0	19.4
UGS 69	70	-	-	-	39.1	39.1	39.1	6.8	20.5
UGS 70	70	-	-	-	49.2	49.2	49.2	8.0	20.6
UGS 71	75	-	-	-	56.7	56.7	56.7	5.0	20.4

Sample code	% alcohol on label	Content of alcohol (% v/v)						pH	Temperature (^{0}C) of sample recorded
		Glycerine	Methanol	IPA	Ethanol	Total alcohol	Total ethanol + IPA		
UGS 72	70	-	-	-	63.6	63.6	63.6	7.3	19.3
UGS 73	70	-	-	-	58.9	58.9	58.9	4.1	20.5
UGS 74	80	-	-	-	36.2	36.2	36.2	3.4	20.4
UGS 75	75	-	-	-	57.0	57.0	57.0	4.8	20.6
UGS 76	70	-	-	-	58.8	58.8	58.8	5.8	20.5
UGS 77	N/S	-	.-	-	-	-	-	7.2	20.6
UGS 78	70	-	-	-	68.1	68.1	68.1	5.8	20.5
UGS 79	80	-	-	-	60.3	60.3	60.3	4.9	20.6
UGS 80	75	-	-	-	59.0	59.0	59.0	5.6	19.3
UGS 81	70	-	-	56.5	-	56.5	56.5	7.4	19.0
UGS 82	60	-	-	-	67.0	67.0	67.0	3.8	20.5
UGS 83	75	-	-	-	70.4	70.4	70.4	4.9	20.6
UGS 84	75	-	-	-	71.8	71.8	71.8	4.7	20.4
UGS 85	75	-	-	-	63.0	63.0	63.0	7.4	20.3
UGS 86	60	-	-	57.3	-	57.3	57.3	7.3	19.7
UGS 87	65	-	-	-	60.5	60.5	60.5	6.3	19.5
UGS 88	60+	-	-	-	61.9	61.9	61.9	7.1	19.5
UGS 89	80	-	-	47.4	-	47.4	47.4	7.5	20.5
UGS 90	75	-	-	-	55.4	55.4	55.4	5.8	20.6
UGS 91	80	-	-	-	75.6	75.6	75.6	4.9	20.8
UGS 92	80	-	-	-	67.7	67.7	67.7	6.2	20.7
UGS 93	67	-	-	-	62.5	62.5	62.5	3.6	20.6
UGS 94	80	-	-	-	63.2	63.2	63.2	5.6	20.5
UGS 95	N/S	-	-	-	51.7	51.7	51.7	6.4	20.5
UGS 96	80	-	-	-	57.4	57.4	57.4	7.3	20.6
UGS 97	N/S	-	-	-	66.7	66.7	66.7	6.3	20.6
UGS 98	70	-	-	-	63.0	63.0	63.0	6.0	20.5
UGS 99	70	-	-	-	79.0	79.0	79.0	6.3	20.5
UGS 100	70	-	-	-	64.4	64.4	64.4	5.8	20.4
UGS 101	70	-	-	-	12.2	12.2	12.2	7.5	19.3
UGS 102	62	-	-	-	70.8	70.8	70.8	6.0	19.1
UGS 103	70	-	-	-	63.4	63.4	63.4	7.6	20.7
UGS 104	80	-	-	-	72.3	72.3	72.3	6.3	20.2
UGS 105	74.5	6.9	-	-	66.0	66.0	66.0	3.7	20.2
UGS 106	80	-	80.3	-	-	80.3	-	6.9	20.3
UGS 107	80	-	-	-	80.4	80.4	80.4	7.5	20.3
UGS 108	70	-	-	-	59.6	59.6	59.6	6.8	20.3

Sample code	% alcohol on label	Content of alcohol (% v/v)						pH	Temperature (^{0}C) of sample recorded
		Glycerine	Methanol	IPA	Ethanol	Total alcohol	Total ethanol + IPA		
UGS 109	70	-	-	-	48.0	48.0	48.0	6.9	19.9
UGS 110	70	-	-	-	83.3	83.3	83.3	4.5	20.3
UGS 111	70	-	-	-	95.1	95.1	95.1	5.3	20.2
UGS 112	80	-	-	-	71.4	71.4	71.4	4.2	20.2
UGS 113	75	-	-	-	70.2	70.2	70.2	3.9	20.2
UGS 114	70	-	-	-	53.2	53.2	53.2	4.0	20.1
UGS 115	75	-	-	-	67.6	67.6	67.6	7.6	20.0
UGS 116	70	-	-	-	61.2	61.2	61.2	4.5	20.0
UGS 117	70	-	-	-	66.5	66.5	66.5	4.8	19.6
UGS 118	N/S	-	-	-	62.2	62.2	62.2	6.8	20.4
UGS 119	70	-	-	-	62.3	62.3	62.3	6.6	19.7
UGS 120	75	-	-	7.6	55.7	63.3	63.3	6.6	19.8
UGS 121	80	-	-	-	76.5	76.5	76.5	6.4	19.9
UGS 122	80	-	-	-	55.6	55.6	55.6	7.3	19.3
UGS 123	80	-	-	-	48.5	48.5	48.5	5.2	20.2
UGS 124	70	-	-	-	21.7	21.7	21.7	6.0	21.3
UGS 125	75	-	-	-	62.9	62.9	62.9	4.5	20.1
UGS 126	N/S	-	-	3.7	29.4	33.1	33.1	7.2	19.9
UGS 127	70	-	-	-	65.4	65.4	65.4	3.6	19.8
UGS 128	N/S	-	-	-	71.5	71.5	71.5	4.8	19.9
UGS 129	80	-	-	-	65.0	65.0	65.0	4.6	20.0
UGS 130	80	-	-	-	67.4	67.4	67.4	5.4	19.7

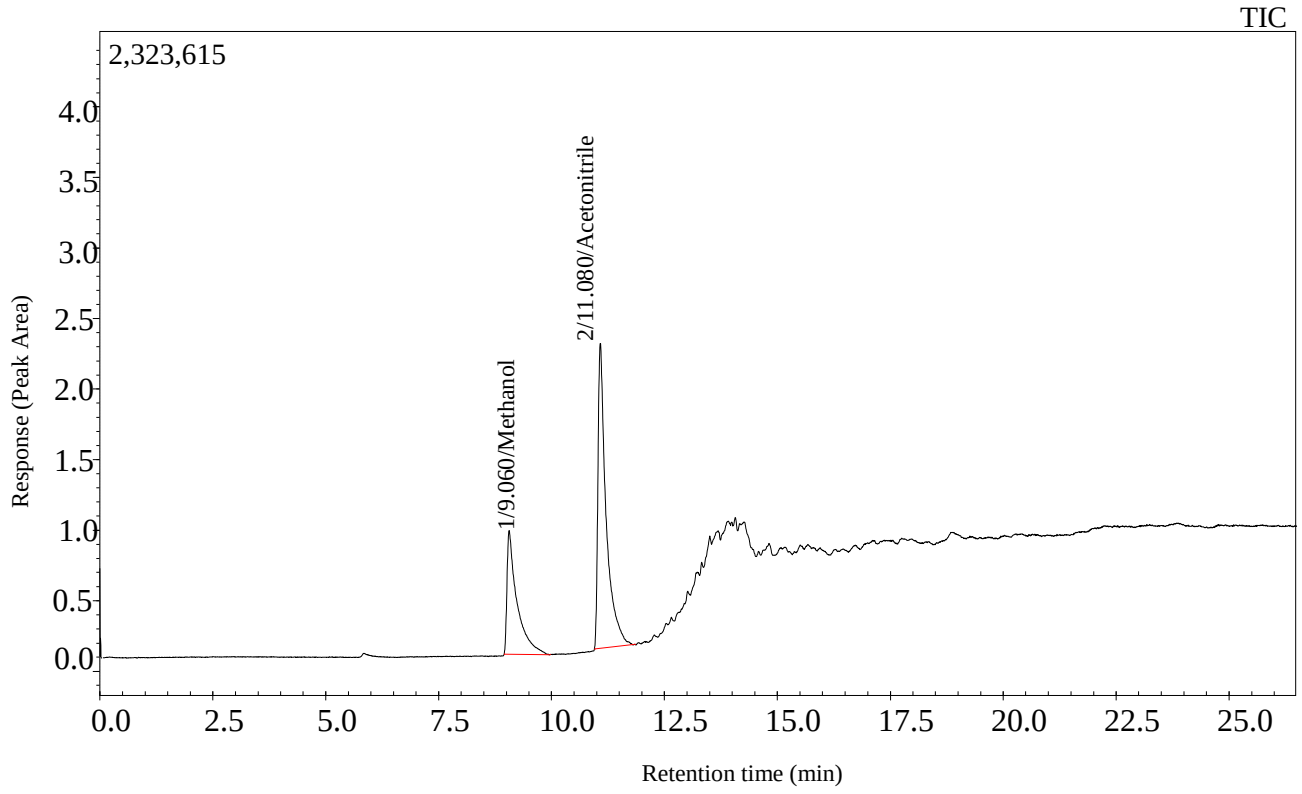
N/S: not stated, **IPA:** isopropyl alcohol

Appendix 3: A typical GC-MS spectral data for sample UGS 18

Qualitative Analysis Report

Sample Information

Analyzed by	: Admin
Analyzed	: 5/5/2022 3:24:53 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: ABHS
Sample ID	: UGS 18
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 20
Injection Volume	: 1.00
Data File	:C:\Users\user\Desktop\GCMS\MASTERS PROJECT\BATCH\ABHS_UGS 18_552022_3.qgd
Org Data File	:C:\Users\user\Desktop\GCMS\MASTERS PROJECT\BATCH\ABHS_UGS 18_552022_3.qgd
Method File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\METHOD\ABHS METHOD.qgm
Org Method File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\METHOD\ABHS METHOD.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\SURGICAL SPIRIT-12-07-2021.qgt
Modified by	: Admin
Modified	: 7/27/2022 3:15:13 PM



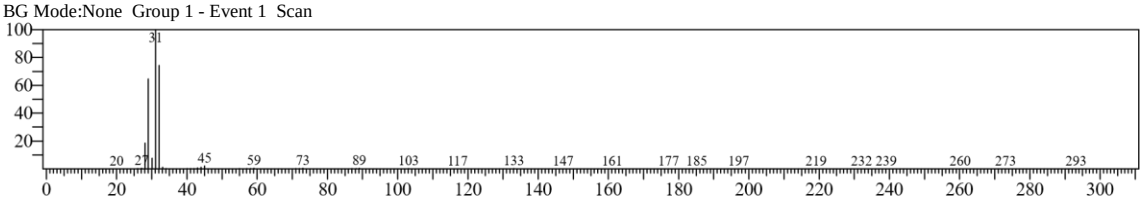
Library

<< Target >>

Line#:1 R.Time:9.060(Scan#:1813) MassPeaks:255

RawMode:Single 9.060(1813) BasePeak:31.05(358540)

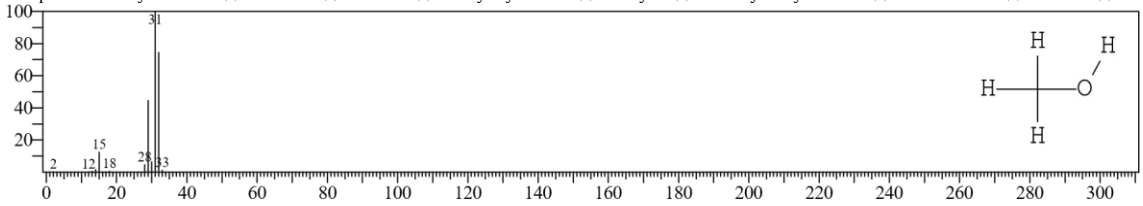
BG Mode:None Group 1 - Event 1 Scan



Hit#:1 Entry:16 Library:NIST11.lib

SI:95 Formula:CH4O CAS:67-56-1 MolWeight:32 RetIndex:0

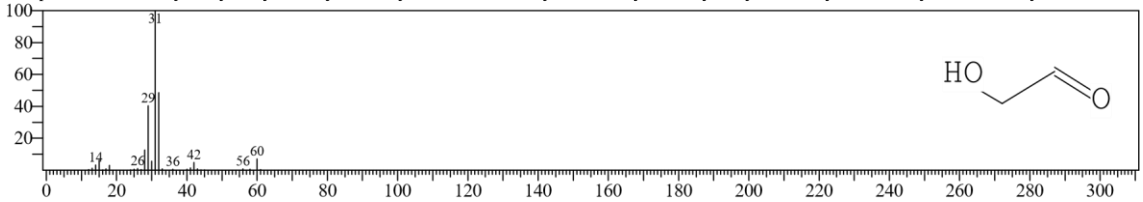
CompName:Methyl Alcohol $$ Methanol $$ Carbinol $$ Methyl hydroxide $$ Methylol $$ Monohydroxymethane $$ Wood alcohol $$ CH3OH $$ Colonia



Hit#:2 Entry:133 Library:NIST11.lib

SI:91 Formula:C2H4O2 CAS:141-46-8 MolWeight:60 RetIndex:651

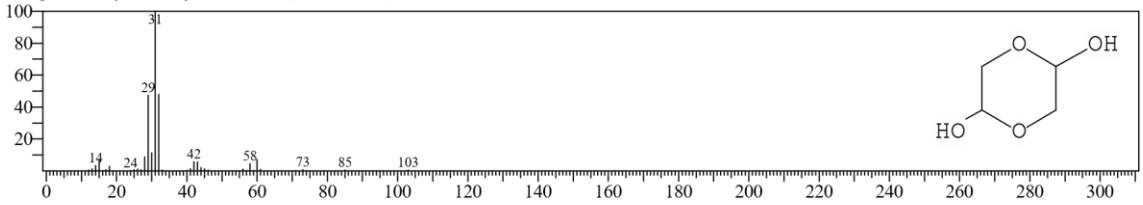
CompName:Acetaldehyde, hydroxy- $$ Glycolaldehyde $$ Diose $$ Glycolic aldehyde $$ Hydroxyacetaldehyde $$ Methylol formaldehyde $$ Monomethy



Hit#:3 Entry:5196 Library:NIST11.lib

SI:91 Formula:C4H8O4 CAS:23147-58-2 MolWeight:120 RetIndex:1074

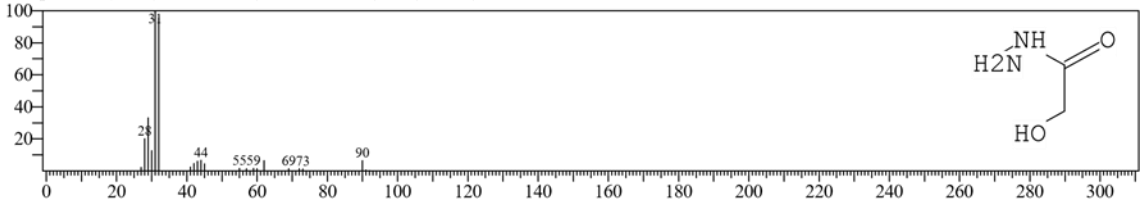
CompName:Glycolaldehyde dimer $$ 1,4-Dioxane-2,5-diol # $$



Hit#:4 Entry:1095 Library:NIST11.lib

SI:90 Formula:C2H6N2O2 CAS:3530-14-1 MolWeight:90 RetIndex:1106

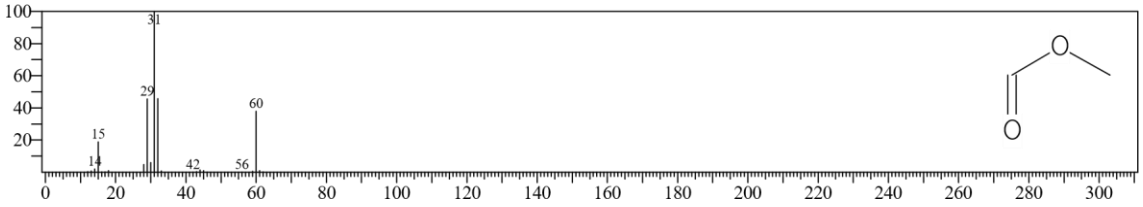
CompName:Hydroxyacetic acid, hydrazide $$ 2-Hydroxyacetohydrazide # $$



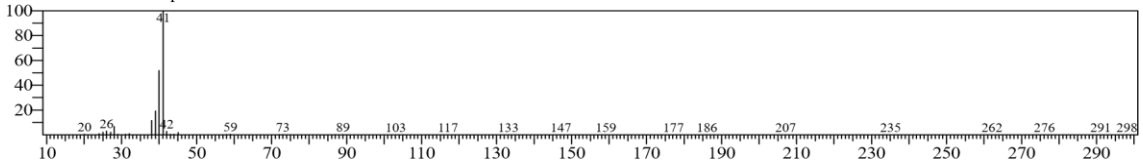
Hit#:5 Entry:132 Library:NIST11.lib

SI:89 Formula:C2H4O2 CAS:107-31-3 MolWeight:60 RetIndex:484

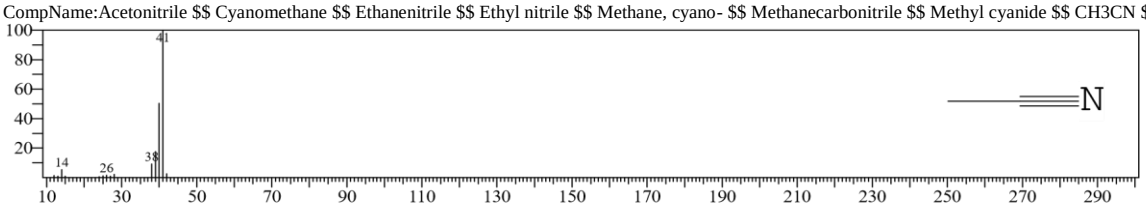
CompName:Methyl formate $$ Formic acid, methyl ester $$ Methyl methanoate $$ HCOOCH3 $$ Formiate de methyle $$ Methylester kyseliny mravenci $



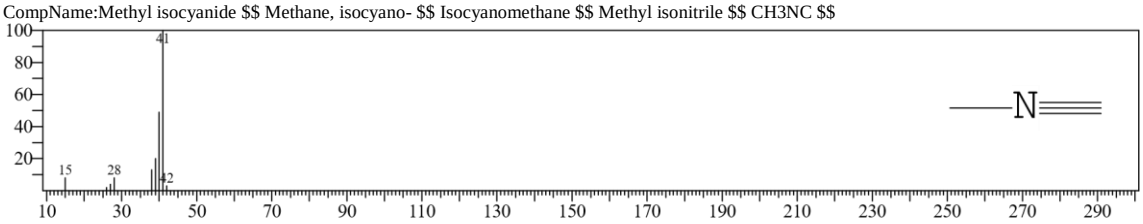
<< Target >>
Line#:2 R.Time:11.080(Scan#:2217) MassPeaks:258
RawMode:Single 11.080(2217) BasePeak:41.05(1106609)
BG Mode:None Group 1 - Event 1 Scan



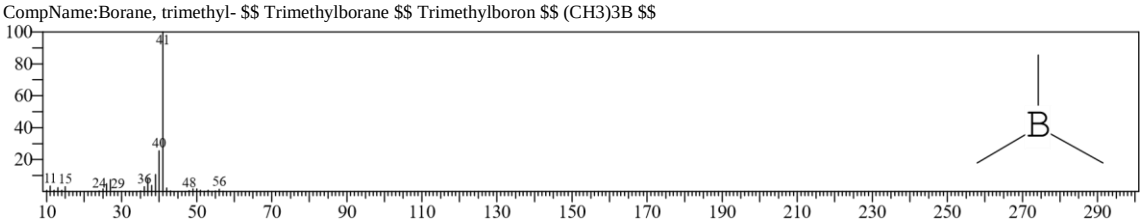
Hit#:1 Entry:31 Library:NIST11.lib
SI:96 Formula:C2H3N CAS:75-05-8 MolWeight:41 RetIndex:465
CompName:Acetonitrile $$ Cyanomethane $$ Ethanenitrile $$ Ethyl nitrile $$ Methane, cyano- $$ Methanecarbonitrile $$ Methyl cyanide $$ CH3CN $$ A



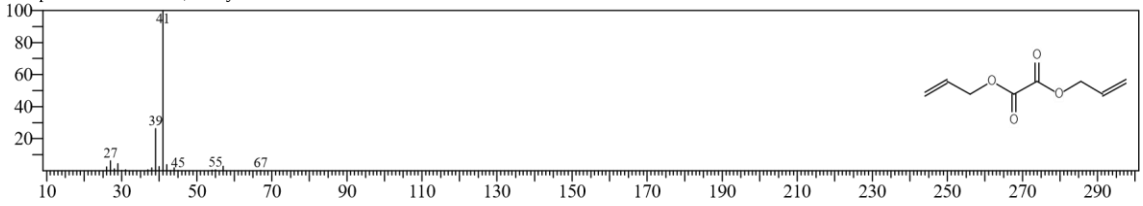
Hit#:2 Entry:32 Library:NIST11.lib
SI:96 Formula:C2H3N CAS:593-75-9 MolWeight:41 RetIndex:0
CompName:Methyl isocyanide $$ Methane, isocyano- $$ Isocyanomethane $$ Methyl isonitrile $$ CH3NC $$



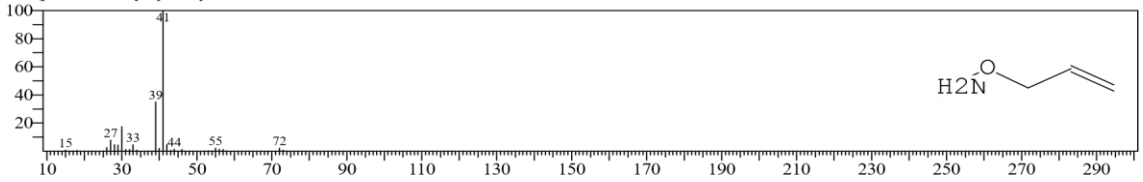
Hit#:3 Entry:92 Library:NIST11.lib
SI:87 Formula:C3H9B CAS:593-90-8 MolWeight:56 RetIndex:0
CompName:Borane, trimethyl- $$ Trimethylborane $$ Trimethylboron $$ (CH3)3B $$



Hit#:4 Entry:25524 Library:NIST11.lib
SI:86 Formula:C8H10O4 CAS:0-00-0 MolWeight:170 RetIndex:1131
CompName:Oxalic acid, diallyl ester



Hit#:5 Entry:338 Library:NIST11.lib
SI:82 Formula:C3H7NO CAS:6542-54-7 MolWeight:73 RetIndex:628
CompName:o-Allylhydroxylamine

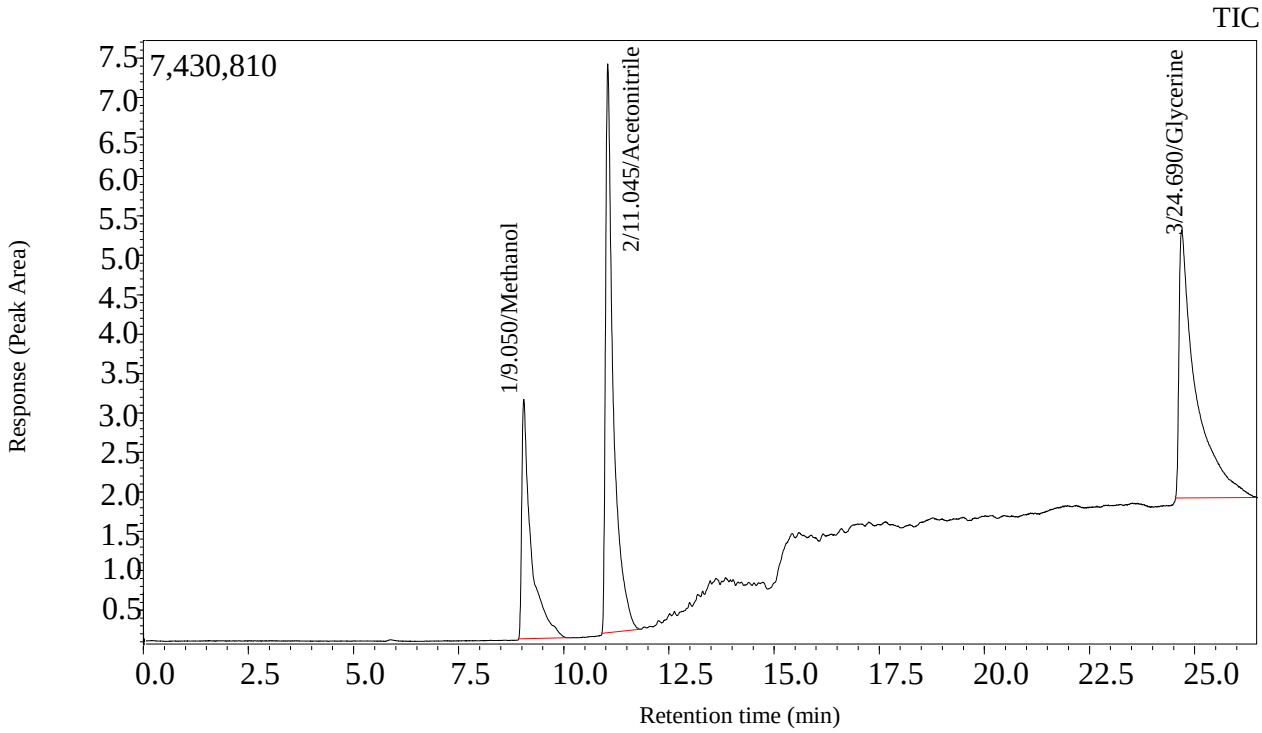


Appendix 4: A typical GC-MS spectral data for sample UGS 43

Qualitative Analysis Report

Sample Information

Analyzed by	: Admin
Analyzed	: 5/7/2022 5:36:09 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: ABHS
Sample ID	: UGS 43
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 61
Injection Volume	: 1.00
Data File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\BATCH\ABHS_UGS 43_562022_39.qgd
Org Data File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\BATCH\ABHS_UGS 43_562022_39.qgd
Method File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\METHOD\ABHS METHOD.qgm
Org Method File	: C:\Users\user\Desktop\GCMS\MASTERS PROJECT\METHOD\ABHS METHOD.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\SURGICAL SPIRIT-12-07-2021.qgt
Modified by	: Admin
Modified	: 7/27/2022 3:26:34 PM



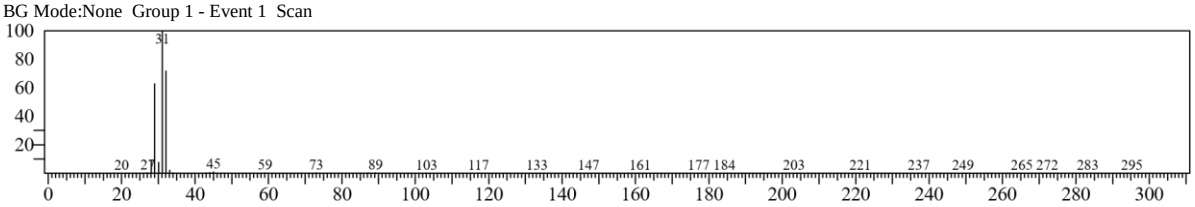
Library

<< Target >>

Line#:1 R.Time:9.050(Scan#:1811) MassPeaks:278

RawMode:Single 9.050(1811) BasePeak:31.05(1232821)

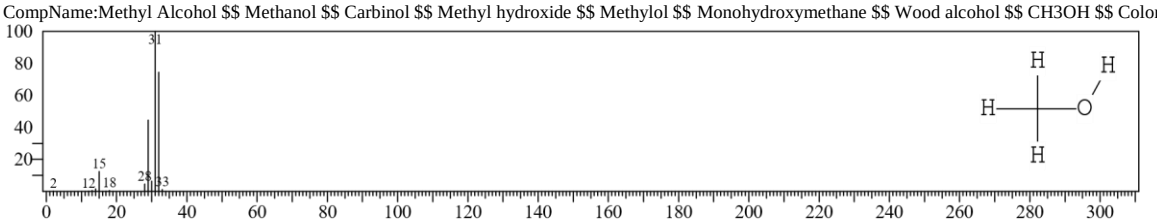
BG Mode:None Group 1 - Event 1 Scan



Hit#:1 Entry:16 Library:NIST11.lib

SI:96 Formula:CH4O CAS:67-56-1 MolWeight:32 RetIndex:0

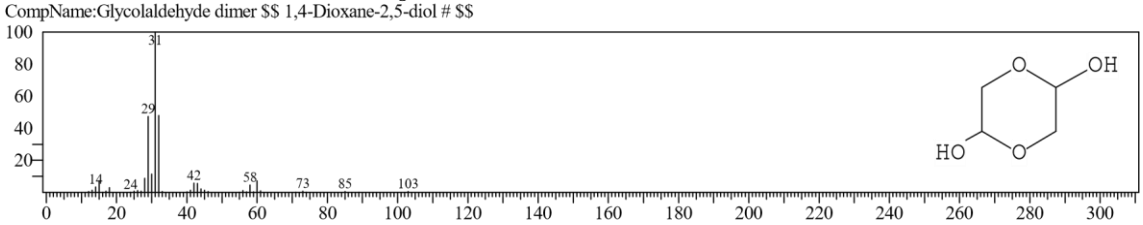
CompName:Methyl Alcohol $$ Methanol $$ Carbinol $$ Methyl hydroxide $$ Methylol $$ Monohydroxymethane $$ Wood alcohol $$ CH3OH $$ Colonia



Hit#:2 Entry:5196 Library:NIST11.lib

SI:92 Formula:C4H8O4 CAS:23147-58-2 MolWeight:120 RetIndex:1074

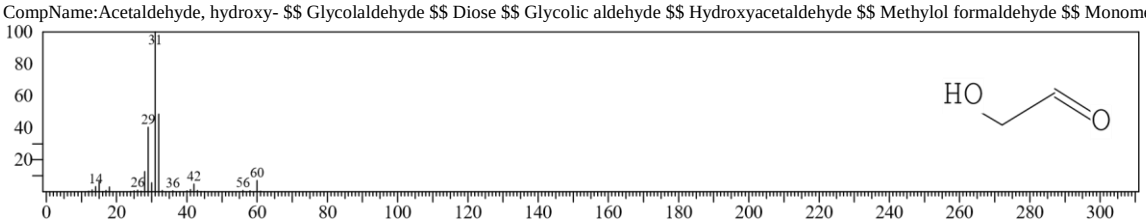
CompName:Glycolaldehyde dimer $$ 1,4-Dioxane-2,5-diol # $$



Hit#:3 Entry:133 Library:NIST11.lib

SI:92 Formula:C2H4O2 CAS:141-46-8 MolWeight:60 RetIndex:651

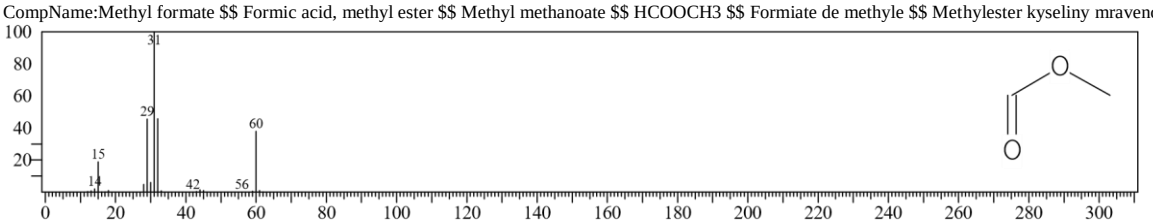
CompName:Acetaldehyde, hydroxy- $$ Glycolaldehyde $$ Diose $$ Glycolic aldehyde $$ Hydroxyacetaldehyde $$ Methylol formaldehyde $$ Monomethy



Hit#:4 Entry:132 Library:NIST11.lib

SI:90 Formula:C2H4O2 CAS:107-31-3 MolWeight:60 RetIndex:484

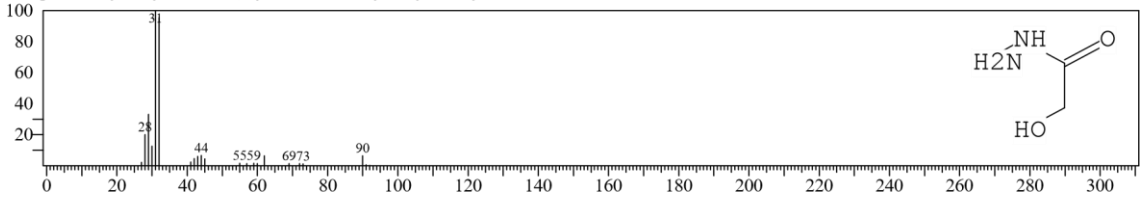
CompName:Methyl formate $$ Formic acid, methyl ester $$ Methyl methanoate $$ HCOOCH3 $$ Formiate de methyle $$ Methylester kyseliny mravenci $



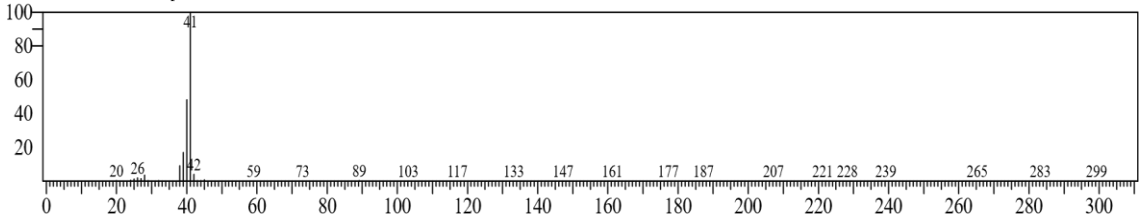
Hit#:5 Entry:1095 Library:NIST11.lib

SI:88 Formula:C2H6N2O2 CAS:3530-14-1 MolWeight:90 RetIndex:1106

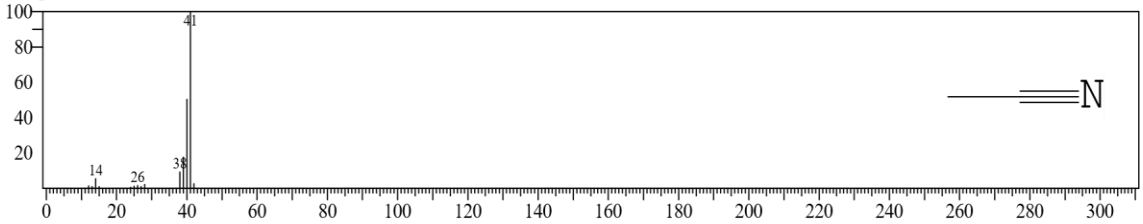
CompName:Hydroxyacetic acid, hydrazide $$ 2-Hydroxyacetohydrazide # $$



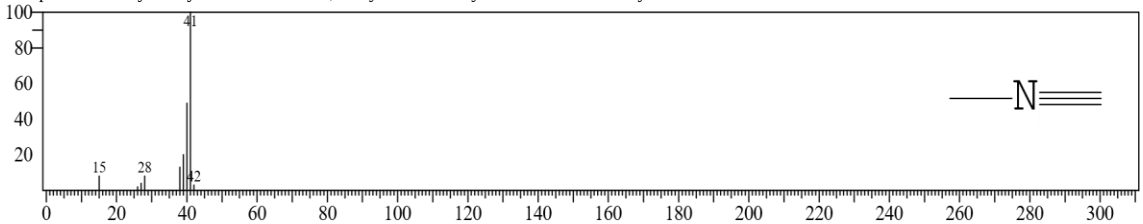
<< Target >>
Line#:2 R.Time:11.045(Scan#:2210) MassPeaks:279
RawMode:Single 11.045(2210) BasePeak:41.05(3917837)
BG Mode:None Group 1 - Event 1 Scan



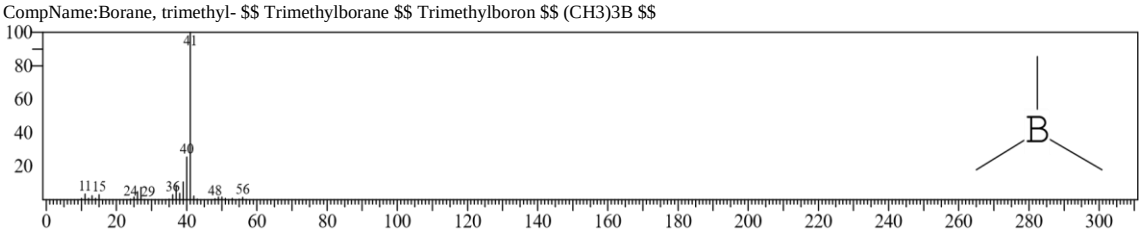
Hit#:1 Entry:31 Library:NIST11.lib
SI:98 Formula:C2H3N CAS:75-05-8 MolWeight:41 RetIndex:465
CompName:Acetonitrile $$ Cyanomethane $$ Ethanenitrile $$ Ethyl nitrile $$ Methane, cyano- $$ Methanecarbonitrile $$ Methyl cyanide $$ CH3CN $$ A



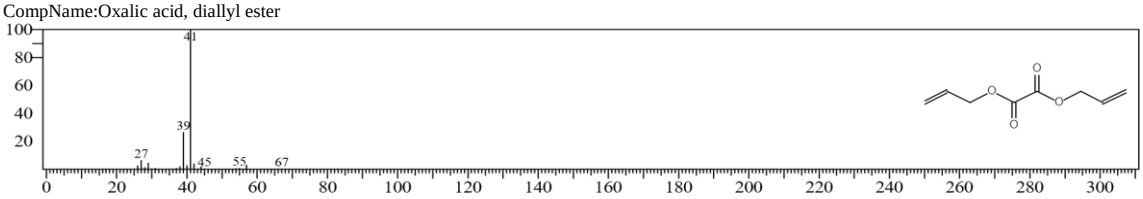
Hit#:2 Entry:32 Library:NIST11.lib
SI:96 Formula:C2H3N CAS:593-75-9 MolWeight:41 RetIndex:0
CompName:Methyl isocyanide $$ Methane, isocyano- $$ Isocyanomethane $$ Methyl isonitrile $$ CH3NC $$



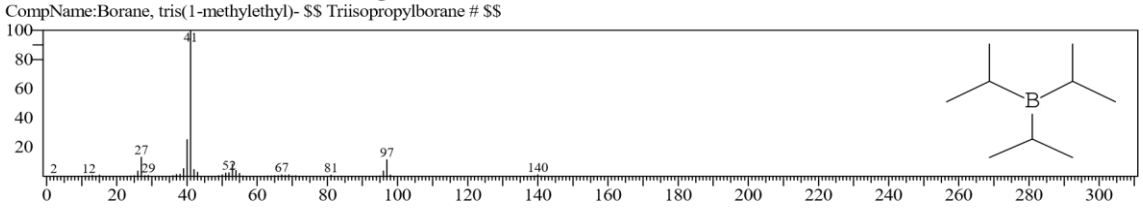
Hit#:3 Entry:92 Library:NIST11.lib
SI:89 Formula:C3H9B CAS:593-90-8 MolWeight:56 RetIndex:0
CompName:Borane, trimethyl- $$ Trimethylborane $$ Trimethylboron $$ (CH3)3B $$



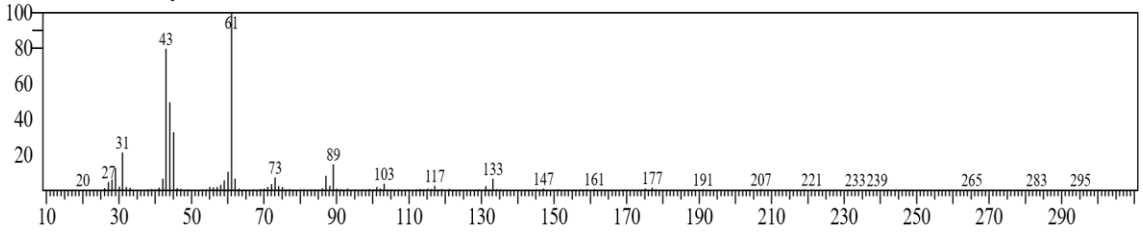
Hit#:4 Entry:25524 Library:NIST11.lib
SI:87 Formula:C8H10O4 CAS:0-00-0 MolWeight:170 RetIndex:1131
CompName:Oxalic acid, diallyl ester



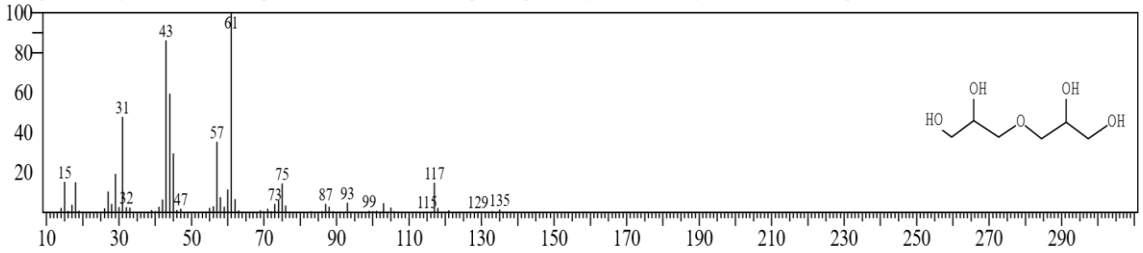
Hit#:5 Entry:11348 Library:NIST11.lib
SI:82 Formula:C9H21B CAS:1776-66-5 MolWeight:140 RetIndex:0
CompName:Borane, tris(1-methylethyl)- $$ Triisopropylborane # $$



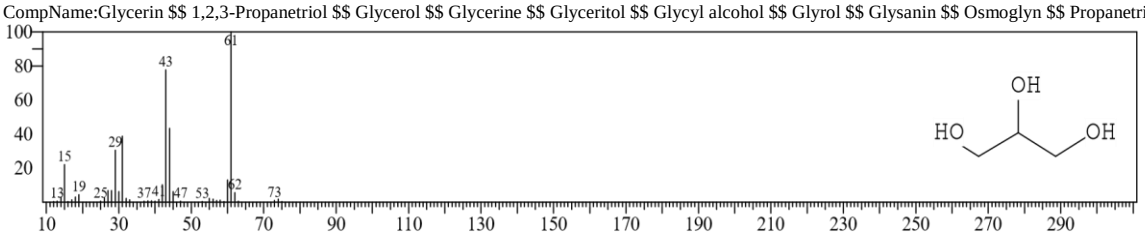
<< Target >>
Line#:3 R.Time:24.690(Scan#:4939) MassPeaks:281
RawMode:Single 24.690(4939) BasePeak:61.05(1236483)
BG Mode:None Group 1 - Event 1 Scan



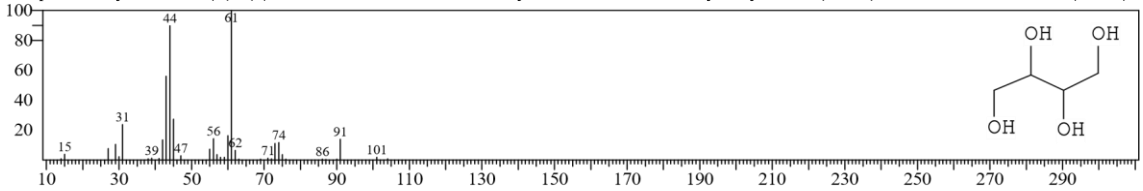
Hit#:1 Entry:23053 Library:NIST11.lib
SI:87 Formula:C6H14O5 CAS:627-82-7 MolWeight:166 RetIndex:1504
CompName:Diglycerol $$ 1,2-Propanediol, 3,3'-oxybis- $$ .alpha.,.alpha.'-Diglycerol $$ Diglycerine $$ 1,2-Propanediol, 3,3'-oxydi- $$ 3,3'-Oxydi-1,2-prop



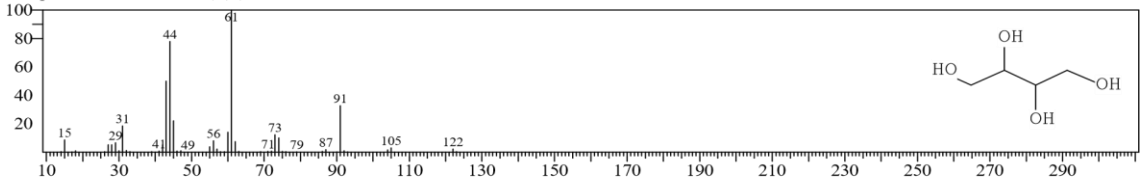
Hit#:2 Entry:1187 Library:NIST11.lib
SI:86 Formula:C3H8O3 CAS:56-81-5 MolWeight:92 RetIndex:967
CompName:Glycerin $$ 1,2,3-Propanetriol $$ Glycerol $$ Glycerine $$ Glyceritol $$ Glycyl alcohol $$ Glyrol $$ Glysanin $$ Osmoglyn $$ Propanetriol $$



Hit#:3 Entry:5490 Library:NIST11.lib
SI:84 Formula:C4H10O4 CAS:149-32-6 MolWeight:122 RetIndex:1229
CompName:Erythritol $$ 2(R),3(S)-1,2,3,4-Butanetetrol $$ meso-Erythritol $$ 1,2,3,4-Tetrahydroxybutane, (R*,S*)- $$ 1,2,3,4-Butanetetrol, (R*,S*)- $$ E



Hit#:4 Entry:5491 Library:NIST11.lib
SI:82 Formula:C4H10O4 CAS:6968-16-7 MolWeight:122 RetIndex:1229
CompName:dl-Threitol $$ 1,2,3,4-Butanetetrol # $$



Hit#:5 Entry:5492 Library:NIST11.lib
SI:82 Formula:C4H10O4 CAS:2319-57-5 MolWeight:122 RetIndex:1229
CompName:1,2,3,4-Butanetetrol, [S-(R*,R*)]- $$ Threitol, L- $$ l-Threitol $$ L-1,2,3,4-Butanetetraol $$