EVALUATION OF THE QUALITY OF COMMERCIAL ALCOHOL-BASED HAND SANITIZERS IN THE NAIROBI METROPOLITAN AREA, KENYA

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A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Pharmacy in Pharmaceutical Analysis of the University of Nairobi.

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2023

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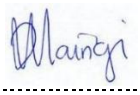
Signature  Date 14th April 2023

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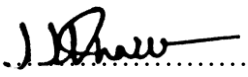
Signature  Date 14.04.2023

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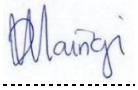
Signature 

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DEDICATION

I dedicate this thesis to my parents Peter M. Gateri and Loise W. Maingi for their tireless support ever since I started my studies in September 2020. Their words of encouragement have been my source of motivation.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABHS	Alcohol Based Hand Sanitizers
CDC	Centers for Disease Control and Prevention
ºC	Centigrade degrees
EAS	East African Standard
EtOH	Ethanol
FCC	Food Chemical Codex
FDA	Food and Drug Administration
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
IPA	Isopropyl alcohol
ID	Internal Diameter
KEBS	Kenya Bureau of Standards
KS	Kenya Standard
NIST	National Institute of Standards and Technology
NMT	Not More Than
OIML	International Organization of Legal Metrology
PEG	Polyethylene glycol
ppm	parts per million
SARS CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2

USD	United States Dollar
USP	United States Pharmacopeia
WHO	World Health Organization

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DEFINITION OF TERMS

Alcohol content	Sum total of the permitted alcohols in the product; ethanol, isopropyl alcohol and n-propanol[1]
Assay	A quantitative measure of amount of component in a sample[2]
Carcinogenic	A substance capable of causing cancer[3]
Fuel/technical grade ethanol	Ethanol that is purposely for cleaning, industrial use and as a solvent in non-food products which however is not recommended for food, drug or medical purposes[4]
Humectant	A moisturizing agent commonly found in products that come into contact with the skin[5]
Methanol contamination	Methanol is detected as a minor component in addition to the permitted alcohols[6]
Methanol substitution	Methanol is identified as the main alcoholic component[6]
Surfactant	A compound that facilitates the formation of emulsions between liquids of different polarities[7]
Volatile impurities	Undesirable residual solvents that are easily vaporize[8]

[1] KEBS (2014b) *KS EAS 789_2013*. Available at: https://www.kebs.org/index.php?option=com_content&view=article&id=671&Itemid=558 (Accessed: 26 November 2021).

[2] ICH (2006) 'Q 2 (R1) Validation of Analytical Procedures: Text and Methodology'. Available at: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf.

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ABSTRACT

The impact of the Coronavirus disease 2019 (COVID-19) has been widespread and devastating with the World Health Organization (WHO) attributing six million deaths to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by August 2022. Since a definitive cure has yet to be developed, the focus remains on controlling the transmission of the SARS-CoV-2 virus. Frequent hand washing is considered the gold standard in controlling the spread of the SARS-CoV-2 virus as well as other pathogens. Alcohol-based hand sanitizers (ABHS) are an effective alternative to the use of soap and water in maintaining hand hygiene. The recommended alcohol concentration range in ABHS is 60% - 95% v/v.

The main objective of this study was to evaluate the quality of commercially available alcohol-based hand sanitizers in the Nairobi Metropolitan area. The products were assessed on whether they met the specifications of the regulating authority; the Kenya Bureau of Standards (KEBS), including appearance, packaging and labelling requirements, alcohol content, and pH. In addition to this the identity of the volatiles present was determined.

A total of 122 commercially available ABHS were purchased through incidental sampling in the smallest pack size available at retail outlets. On a visual inspection, 77 (63%) samples met the packaging and labelling specifications. Identification of the volatiles present was performed using gas chromatography-mass spectrometry (GC-MS) where methanol was detected in 26 (21.3%) samples that were locally manufactured. From GC-MS analysis, about 61 (50%) samples analyzed showed that the volatiles identified corresponded to those indicated on the label.

Quantification of the alcohol content was performed by gas chromatography coupled with a flame ionization detector (GC-FID) whereof 54 (44.3%) samples were found to have alcohol in the recommended range of 60% - 95% v/v. Thus, 68 (55.7%) products had alcohol content that could not exert the desired microbicidal activity upon use. In 7 (5.7%) samples, none of the permitted alcohols were detected suggesting they could be substandard/falsified products. Methanol contamination was detected in 16 (13.1%) samples while methanol substitution was detected in 10 (8.2%) samples.

In conclusion, 10 (8.2%) samples all locally manufactured complied with the KEBS specification. These results demonstrate the need for more specific methods in the identification and quantification of the alcohol content as well as impurities in ABHS compared to the existing non-specific methods described in the KEBS specification; KS EAS 789:2013. Therefore, there is a need to emphasize on good manufacturing practices, especially to the local manufacturers with regard to the sourcing of raw materials used in the manufacture of ABHS.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Hand hygiene describes the activities that ensure effective hand cleansing. They can be broadly classified into; the use of hand rubs and the use of soap and water (WHO, 2009a). Proper hand hygiene leads to removal or killing of microorganisms on the hand surface, thus reducing incidences of gastrointestinal, respiratory as well as skin infections (Bloomfield *et al.*, 2007).

Hand hygiene can be achieved through hand washing with soap and water or by the use of sanitizers. When dealing with soiled hands the use of soap and water is preferable as reported by the Centers for Disease Control and Prevention (CDC) compared to the use of sanitizers which may be rendered ineffective (CDC, 2021; Singh, 2020). Hand sanitizers provide a quick and convenient method of eliminating bacteria from visibly clean hands. Alcohol-based hand sanitizers are effective in eliminating viruses such as the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) virus but they are ineffective against gastroenteritis where the use of soap and water remains the gold standard (Bloomfield *et al.*, 2007).

The positive impact of positive hand hygiene cannot be trivialized as seen in the reduction of respiratory infections such as colds by 16-21% in the general population (CDC, 2020a). A study conducted in a primary school in Kibera slums showed that the provision of hand sanitizers caused a decline in rhinorrhea by 23% (Pickering *et al.*, 2013). This demonstrates that the use of hand sanitizers is effective in areas where access to running water is problematic (CDC, 2020a).

1.2 Covid-19 Pandemic

The respiratory disease known as COVID-19 developed into a global pandemic where the causative agent was determined to be the SARS CoV-2 virus. By August 2022, a total of six million lives had been lost as a result of this pandemic (WHO, 2021a). A number of measures were put in place to limit the spread of the SARS-CoV-2 virus such as the use of face masks in public areas, and the avoidance of public gatherings while businesses and schools were advised to adapt their operations to limit physical interactions. Additionally, restrictions were put in place

with regard to domestic and international travel. The use of disinfectants on common surfaces and hand sanitizers was encouraged to control the spread of the virus (WHO, 2021a).

The United States Food and Drug Administration (US-FDA) encouraged the use of alcohol-based hand sanitizers as a means of containing the spread of the SARS-CoV-2 (FDA, 2021a). Subsequently, there was increased demand for ABHS products (Dive, 2021). Several factors contributed to the increased usage of these products. These include convenience to the user, improved hand hygiene compliance, a convenient alternative in areas with limited access to running water, reduced risk of developing resistance as well as comparable efficacy to hand washing using soap and water (Nwobodo *et al.*, 2020; WHO, 2009b).

In Kenya, Alcohol-Based Hand Sanitizers (ABHS) fall within the purview of the Kenya Bureau of Standards (KEBS) and 534 brands had been registered by the year 2020 (KEBS, 2020). The specifications for their production are detailed in KEBS standard, KS EAS 789:2013.

1.3 Classification and composition of hand sanitizers

Hand sanitizers are classified as alcohol-based or alcohol-free on the basis of active ingredient used (Jing *et al.*, 2020). The technique used in the application of these sanitizers influences their efficacy. An adequate amount of ABHS is applied to the surface of the hands followed by a rubbing motion for at least 20 seconds (CDC, 2020b).

Alcohol-free hand sanitizers contain disinfectants such as benzalkonium chloride or antimicrobials such as triclosan which are fast-acting. These products are commercially available as water-based foams and act by interfering with the cytoplasmic membrane of microbes leading to leakage of low molecular weight cytoplasmic constituents (Kumar *et al.*, 2021). Their use is not recommended in hospital settings due to their limited spectrum of activity as well as the special foaming mechanism required in the dispenser (Zogics, 2020).

With regard to alcohol-based hand sanitizers (ABHS), the CDC specifies that they should contain at least a 60% v/v alcohol content (CDC, 2021). ABHS are available as solutions of low viscosity, wipes, gels, and foams with gels being the most readily available commercial formulation (Kumar *et al.*, 2021).

In the year 2020, the FDA released temporary guidelines that specified the permitted alcohols for ABHS formulation as either ethanol or isopropyl alcohol in addition to other components such as humectants and pH adjusting agents (US-FDA, 2020). Sterilized water acts as a carrier for all the constituents. In certain cases it may not be possible to obtain sterilized water therefore, tap water may be used provided it is boiled and subsequently cooled and is free from any visible particulate matter (US-FDA, 2020). In most cases, ethanol is preferred following its superior virucidal activity and better skin tolerance compared to isopropanol (Berardi *et al.*, 2020).

An important distinction was made by the FDA, in a question and answer platform that when reference is made to a hand sanitizer containing alcohol it specifically refers to ethanol despite the fact that *iso*propyl alcohol could also be used in formulating ABHS (FDA, 2021b). The optimal concentration to elicit bactericidal activity is 60-95% v/v (Reynolds *et al.*, 2006). Absolute alcohol is a dehydrating agent whose bactericidal activity is significantly less than that of aqueous alcohol thus underscoring the importance of water in the formulation of ABHS.

KEBS has included *n*-propanol as one of the permitted alcohols in addition to ethanol and *iso*propyl alcohol at a minimum concentration of 60% v/v (KEBS, 2014b). Hydrogen peroxide, a constituent in ABHS functions as a sporicidal agent at a concentration range of 3% to 6% (Kumar *et al.*, 2021; WHO, 2009b). Glycerol is also a constituent in these hand sanitizers acting as a humectant to counter the drying effect of alcohol use on the skin (Abuga and Nyamweya, 2021). Interestingly, the presence of glycerin has been shown to counter the antimicrobial activity of alcohols. Therefore a balance between antimicrobial efficacy and skin tolerance is achieved at a glycerin concentration range of 0.5% to 0.73% in the formulation (Berardi *et al.*, 2020; Menegueti *et al.*, 2019).

Moisturizing compounds such as aloe and vitamin E enhance the commercial appeal of the product. Thickening agents such as carbomers are added to increase viscosity in order to reduce spillages. They are incorporated in gel-based formulations to reduce the rate of alcohol evaporation and increase the spread to the contaminating microorganisms (Berardi *et al.*, 2020).

1.4 Mechanism of Action

Alcohols exert their bactericidal activity by causing the denaturation of cell membrane proteins. In the presence of alcohols, cell membrane proteins clump together causing interference with the cell membrane integrity leading to cell death. It has also been observed that proteins are denatured more rapidly by alcohols in the presence of water (Singh *et al.,* 2020).

This mechanism of action is applicable to SARS-CoV-2 which is an enveloped virus composed of a lipid bilayer held together by a combination of hydrogen bonds and hydrophobic interactions. Alcohols are composed of a polar and a non-polar region and are therefore able to disrupt the lipid bilayer essentially dissolving it (Golin *et al.*, 2020). This mechanism of action has been supported by the evidence of denaturation of *Escherichia coli* dehydrogenases by alcohols. By inhibiting the production of the metabolites necessary for cell division bacteriostatic activity is achieved (Hasan *et al.*, 2020).

To a certain extent, alcohols can be considered sporicidal by affecting the enzymatic action required for germination to take place. This effect is temporary since on removal of the alcohol the enzymatic activity resumes. This reversible sporicidal activity of alcohols has led to their preference as surface disinfectants as well as skin antiseptics (McDonnell *et al.,* 1999). Due to its toxicity and minimal antimicrobial activity, methanol is rarely used as a disinfectant (Hasan *et al.*, 2020).

The bactericidal activity of ABHS does not increase with the increased concentration of alcohol. The reason for this is that the more concentrated the alcohol concentration there is increased volatility. This results in decreased efficacy due to a diminished contact time as well as the potential adverse skin reactions. Additionally water is also required for protein denaturation (Tse *et al.*, 2021).

At a concentration of 60% to 80% v/v ethanol inactivates all the lipophilic viruses; herpes and influenza viruses as well as most of the hydrophilic viruses; rhinovirus, enterovirus, and adenovirus (CDC, 2019). However, alcohols are ineffective against poliovirus and Hepatitis A virus.

1.5 Regulatory Requirements

An overwhelming demand for ABHS resulted from the WHO recommendation on their use as a preventative measure against the spread of the SARS-CoV-2 virus that causes COVID-19 (Dive, 2021). This recommendation followed evidence of inactivation of the SARS-CoV-2 by alcohols present in ABHS (Kratzel *et al.*, 2020). In an unprecedented move, the US-FDA permitted the production of alcohol-based sanitizers by firms not previously involved in their manufacture. The FDA procedures for the production of an aqueous solution to be used as ABHS were made public (Table 1.1), with an additional note that they do not apply to the production of gels, foams, or aerosolized sprays (US-FDA, 2020). The FDA specifications on the raw materials to be used in ABHS formulations are described in Table 1.1.

Table 1.1: FDA specifications for ABHS raw materials

Ingredient	Specification
Alcohol	Not less than 94.9 % ethanol by volume or USP grade *iso*propyl alcohol
Glycerin	USP grade or FCC glycerin
Hydrogen peroxide	USP grade
Sterile water	Prepared by boiling or distillation

FCC = Food Chemical Codex; USP = United States Pharmacopeia

The alcohol used in the production process can be obtained from a fermentation and distillation process that is used in the production of alcoholic consumables provided it meets the specifications for impurities. Alcohols can also be manufactured following a synthetic process and can therefore be used in the production process provided it meets the USP/FCC specifications.

The FDA specifications on the composition of ABHS are similar to the WHO recommendations (FDA, 2021b). This includes ethanol (80% v/v) or *iso*propyl alcohol (75% v/v) aqueous solution, glycerin (1.45% v/v), hydrogen peroxide (0.125% v/v) and sterilized water. The FDA recommends that no other active ingredients should be added to improve aesthetic characteristics

such as smell and taste as this may increase the risk of accidental ingestion, especially in young children (Rayar *et al.*, 2013). These products may also negatively affect the quality of the final product.

Following the increased demand for ABHS, the US-FDA released temporary guidelines related to the manufacture of ABHS in 2020 (US-FDA, 2020) in which the use of fuel/technical grade ethanol for the manufacture of ABHS was approved. As a result, fuel-producing industries were used as a source of alcohol in the manufacture of sanitizers provided they met the USP/FCC specifications. Screening for impurities specific to the manufacturing environment was necessary in addition to those specified in the USP. Arising from the use of fuel/technical grade ethanol for the manufacture of ABHS, the limits for methanol content were adjusted to 630 ppm (0.063 % v/v), from 200 ppm (0.02% v/v).

In 2021, the FDA suspended the temporary guidelines related to the manufacture of ABHS which was a result of the increased supply of ABHS by the conventional manufacturers that were able to meet market demand. Thus, the use of fuel/technical grade ethanol for the manufacture of ABHS was prohibited and the limit for methanol present in ABHS revised to 200 ppm (FDA, 2021a). The limits for other possible impurities and residual solvents are described in Table 1.2.

The presence of acetaldehyde in technical-grade ethanol has always been of concern due to its known carcinogenic potential. To this end, the FDA determined that its presence in NMT 50 ppm can be tolerated especially over a shorter time as in the application of a hand sanitizer for which the contact time should be about 20 seconds (CDC, 2020a). According to the USP, the limits for content of *iso*propyl alcohol is not less than 99.0% (USP 29, 2006b) while that for ethanol is not more than 96.0% by volume (USP 29, 2006a).

Table 1.2: Limits of potential impurities and residual solvents in ABHS

Impurity	Limit
Methanol	NMT 200 ppm
Benzene	NMT 2 ppm
Acetaldehyde	NMT 50 ppm
Acetal (1,1-diethoxyethane)	NMT 50 ppm
Acetone	NMT 4400 ppm
n-propanol	NMT 1000 ppm
Ethyl acetate	NMT 2200 ppm
Sec-butanol	NMT 6200 ppm
Iso-butanol	NMT 21700 ppm
Iso-amyl alcohol	NMT 4100 ppm
Amyl alcohol	NMT 4100 ppm
n-butanol	NMT 4100 ppm
Sum of all other impurities	NMT 300 ppm

NMT = Not More Than; ppm = parts per million

An important step that is recommended by the FDA is the denaturation of alcohol intended for ABHS production. This is the process of enriching the alcohol with an unpalatable additive thus making it unsuitable for human consumption. The denaturation process can either be carried out during the manufacturing stage or during the compounding stage. The recommended substances to be used as denaturing agents include denatonium benzoate, sucrose octaacetate, *tert*-butyl alcohol or, trimethyl citrate 3% w/w (Abuga and Nyamweya, 2021).

ABHS in the Kenyan market are regulated by KEBS, according to the specifications for instant hand sanitizers; KS EAS 789:2013. This specification only applies to alcohol-based hand sanitizers and allows for the use of *n*-propanol as an alcohol alternative in addition to ethanol and *iso*propyl alcohol. The quality requirements specified in KS EAS 789:2013 are described in Table 1.3 (KEBS, 2014b).

Table 1.3: KEBS specifications for ABHS

Characteristic	Requirement	Test method
Alcohol content (IPA/EtOH/*n*-propanol)	60.0	EAS 104
pH	6-8	EAS 789
Bactericidal efficacy	Pass	EAS 789

EAS = East African Standard; EtOH = Ethanol; IPA = Isopropyl alcohol

In the KEBS specification, KS EAS 789:2013 for instant hand sanitizers the method for determination of alcohol content cross-refers to the KS EAS 104:2014 which is the method for determination of alcohol content in alcoholic beverages (KEBS, 2014a). In the latter, ethanol content is determined by specific gravity using a pyknometer / densitometer or a special hydrometer used by the International Organization of Legal Metrology (OIML).

The packaging requirements are also specified such as the use of a well-sealed container that does not interact with the constituents in any step of manufacture, transportation, and storage. Labelling should indicate the name of the product, manufacture details, net content, ingredients present, the manufacturing and expiry date, the country of manufacture, and warning information (KEBS, 2014b).

To determine the disinfecting efficacy of the ABHS the specification describes a microbiological method which tests the ability of specified bacteria to grow in the presence of the sanitizer. The growth of the challenge microorganisms; *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Staphylococcus aureus* is supported by the growth media; Wright and Mundy Broth with Dextrose. These microorganisms are introduced in serially diluted sanitizer at 0.5, 1 and 1.5 times the recommended use-dilution of the ABHS. After a prescribed period, a sample is obtained and introduced into a recovery media. A sample is considered to have passed the efficacy test based on the extent of bacterial growth in the two cultured samples. Freshly

prepared sanitizer and a freshly prepared inoculum should be used in each instance and at the recommended dilution.

1.6 Quality Control

According to the WHO, quality control describes the measures taken to ensure that raw materials, intermediates, packaging materials, and finished products conform to the established specifications for identity, strength, and purity (WHO, 2014). They include the setting of specifications, sampling, testing, and analytical clearance.

The KS EAS 789:2013 specification for instant hand sanitizer details the recommended alcohol content (not less than 60% v/v), a pH range (6-8), and a method to determine the bactericidal activity (KEBS, 2014b). The specification does not indicate any limits for methanol or any of the other toxic contaminants that are likely to be present.

For the determination of alcohol content, the specification for instant hand sanitizers KS EAS 789:2013 (KEBS, 2014b) refers to another specification KS EAS 104:2014 (KEBS, 2014a). The method describes the use of a pyknometer to determine the difference in the specific gravity of the aqueous alcohols. The drawback of this method is that there is a negligible difference in the specific gravity of alcohols which makes them practically indistinguishable. Therefore substitution may occur, and one cannot distinguish between the permitted alcohols and methanol that may be present in adulterated products (Abuga, Nyamweya, *et al.*, 2021). This potentially exposes members of the public to toxic contaminants such as methanol when substituted for ethanol or *iso*propyl alcohol (Holzman *et al.*, 2021).

Good Manufacturing Practices (GMP) dictate that manufacturers should use high-quality raw materials of the desired quality for ABHS production. To meet the high demand for ABHS products, non-conventional ethanol-producing plants such as fuel producers were used as sources for raw materials. Therefore, limits were set for methanol, acetaldehyde, and acetal (Cohen *et al.*, 2021). The environmental conditions in which ABHS production takes place should also be sanitary to reduce the risk of contamination.

Specific batch release tests should be carried out before the release of the final product. This involves assessing the manufacturing process as well as batch testing to ensure adherence to

defined release procedures (WHO, 2014). Upon compliance with the batch release tests, a certificate of analysis is then issued by a qualified person indicating compliance with GMP. Once these processes are completed the final step involves the batch release step which involves a change of status to saleable stock. It is the responsibility of the regulating authority, KEBS to periodically assess the manufacturers on whether they comply with GMP guidelines as mandated by the Standards Act Chapter 496 (Standards Act, 1974).

1.7 Previous studies

The demand for ABHS has led to a renewed interest in critically assessing the quality of products in circulation. An analytical method combining Gas Chromatography with Mass Spectrometry (GC-MS) has been developed and validated for the quality assessment of ABHS products (WHO, 2021c). This method specifies the percentage alcohol content of ethanol and *iso*propyl alcohol as well as defines the limits of common impurities (WHO, 2021c). Building on the application of gas chromatography in the analysis of volatiles, a number of studies have been carried out where GC is coupled to mass spectrometry (MS) for identification of the volatiles present while a flame ionization detector (FID) has also been coupled to GC for quantification of the alcohols present.

A Canadian study used GC-FID analytical methodology to analyze commercially available hand sanitizers (Tse *et al.*, 2021). The ethanol content was estimated by comparing the chromatogram with that obtained from an absolute analytical grade ethanol blank. Seventeen samples (40.5%) exceeded the acetal and acetaldehyde limits as per USP specifications (USP 29, 2006a). The importance of proper packaging was highlighted 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.*, 2021).

Another study was conducted in South Africa to review the quality of ABHS available in the market using GC-FID. About 41 (44%) samples were found to be substandard and 11 (11.7%) samples contained toxic ingredients such as ethyl acetate and methanol (Matatiele *et al.*, 2021).

A study conducted in Kenya presented the alcohol content as the sum of the two permitted alcohols; ethanol and *iso*propyl alcohol. Eleven samples (14.9%) were found to have methanol substitution and 18 (24.3%) were found to contain different alcohols from what was indicated on

the label claim. This study also considered the pH of the ABHS and only about half of the samples; (44, 59.5%) complied with the pH specifications. The presence of excipients such as triethanolamine and acid-based polymers has been shown to affect the pH (Abuga, Nyamweya, *et al.*, 2021). Following these adverse findings, the regulating authority KEBS should mainstream efforts in post-market surveillance (PMS) of these products to detect falsified/ substandard products. These products in addition to being ineffective pose greater harm to the user as they may contain toxic contaminants such as methanol, acetaldehyde and benzene.

The impact of COVID-19 cannot be understated, since it has taught humanity the importance of hand hygiene in minimizing the spread of the virus. The lessons learnt should be promoted, hence the need to regularly monitor the quality of ABHS as they play a key role in maintaining proper hand hygiene.

1.8 Problem statement

A large number of ABHS brands were introduced into the market subsequent to the WHO recommendation that ABHS should be used to limit the transmission of the SARS-Cov-2 virus that causes COVID-19 (WHO, 2021b).

The presence of volatile impurities in the ABHS may portend serious health implications. A recent study conducted in the Nairobi Metropolitan Area showed that methanol was detected in 11 (14.9%) samples (Abuga and Nyamweya, 2021). This toxic alcohol is known to cause visual impairment in ingestion or chronic use on irritated skin (Chan *et al.*, 2018). The intentional misuse of ABHS in individuals suffering from substance abuse has resulted in serious health implications and even death especially when there is methanol substitution (Mohammad *et al.*, 2021).

Frequent use of these contaminated hand sanitizers leads to an increased concentration of volatile impurities in the bloodstream through transdermal absorption (Atolani *et al.*, 2020). The use of poor quality raw materials is a potential source of toxic solvents such as benzene and acetaldehyde which are potentially carcinogenic (US-FDA, 2020; Yip, 2020).

In the management of individuals with alcohol-use disorder, a disulfiram-ethanol reaction is likely following the trans-dermal absorption of the alcohol in ABHS. It is characterized by

nausea, vomiting, and flushing of the face. The amount absorbed through inhalation is significantly higher compared to transdermal absorption (Brewer *et al.*, 2020).

The unintentional ingestion of ABHS by children results in serious implications such as apnea, acidosis, and even coma (Rayar *et al.*, 2013). The presentation of ABHS in brightly colored bottles and an appealing smell makes these products attractive to children and increases the likelihood of their ingestion and consequent poisoning (Mahmood *et al.*, 2020).

In the past, 14 manufacturers were ordered to recall their products over a failure to meet the specifications (Mueni, 2021). An in-depth investigation by the regulator KEBS is necessary on a larger scale with respect to packaging, labelling, licensing as well as the alcohol content of these products.

1.9 Justification

It was reported in 2020 that 534 hand sanitizers were registered by KEBS (KEBS, 2020). A more recent official count is however unavailable. The specification KS EAS 789:2013 describes quality requirements for ABHS as well as the method for determination of efficacy which was made available to members of the public on the KEBS website during the COVID-19 pandemic (KEBS, 2014b). However, to determine the alcohol content in these products reference is made to the technique used to determine the alcohol content in alcoholic beverages KS EAS 104: 2014 (KEBS, 2014a). The technique uses the specific density of alcohol to establish the alcohol content, the limitation with the use of this technique is that the difference in the specific gravity of alcohols is negligible making it difficult to distinguish them (Wade, 2021). Therefore, there is need for more specific analytical methods to be developed.

There is need to collect data on relevant aspects of ABHS such as the presence of unregistered products in the market which could be counterfeit and therefore undermine the desired protection to users during the COVID-19 pandemic. The analytical technique applied in this study uses gas chromatography. Moreover, it is also important to determine the alcohol content in these products and whether it falls within acceptable limits as this impacts on their effectiveness. The presence of unwanted volatiles is of great importance as a matter of public safety based on their undesirable effects. The results obtained from this study will inform the regulatory authority to consider amending the specifications for the determination of the content of volatiles in ABHS.

Recent studies in the Nairobi metropolis identified several gaps which this study sought to address (Abuga, Nyamweya, *et al.*, 2021). There was a need for characterization of all the volatiles present in ABHS by GC-MS. The change in the market dynamics also brought out the need for a more recent study to be conducted to provide information on the current situation. The previous study focused on samples obtained from the Nairobi Metropolis resulting in 62 ABHS brands while the current study focused on a wider sampling area with a larger sample size.

1.10 Research question

Do commercial ABHS products marketed in the Nairobi metropolitan area meet regulatory and quality specifications?

1.11 Objectives

1.11.1 General objective

The main objective was to determine the quality of alcohol-based hand sanitizers marketed in the Nairobi metropolitan area.

1.11.2 Specific objectives

1. To establish whether the ABHS samples meet the KEBS specifications for packaging, labelling and pH.
2. To characterize the composition of ABHS products using gas chromatography-mass spectrometry.
3. To determine the volatile content of ABHS products using gas chromatography.

CHAPTER TWO
REGULATORY SPECIFICATIONS FOR HAND SANITIZERS

2.1 Introduction

The Kenya Bureau of Standards (KEBS) is the regulatory authority mandated with the control of ABHS under the specification for "Instant Hand Sanitizers" in KS EAS 789:2013. The specification is applicable for ABHS products found within the East African Community (EAC). The harmonization of goods and services requirements and putting in place standardized procedures eased the barriers to trade practices within the EAC. Manufacturers are required to display the standardization mark commonly denoted as SM on ABHS products. In addition to this, the SM permit number should be displayed below the SM mark to allow for verification of the product registration status as well as confirm the status of the product.

2.2 Specifications

The specification KS EAS 789:2013 was developed by the East African Standards Committee which is composed of representatives from National Standards bodies within the partner states as well as representatives from private organizations and consumer protection bodies. They are subject to review and member states are advised to keep up to date with the most recent changes prior to their implementation. The specifications describe the appearance, packaging, labelling, pH, and alcohol content in ABHS (KEBS, 2014b).

2.2.1 Appearance

According to the KEBS specifications on appearance, ABHS should be of an acceptable odor, the consistency should be in the form of a liquid or a gel that is clear and colorless. The absence of an unacceptable odor is also emphasized as well as the conformation of the substances used to EAS 377 which describes the permitted substances and preservatives in cosmetics (KEBS, 2014b).

2.2.2 Packaging

The ABHS should be packaged in suitable containers that have an effective closure mechanism to prevent spillages or evaporation of the volatile components during handling, transportation, and storage. It is important that the container as well as the closure mechanism do not interact in any way with the constituents (KEBS, 2014b).

2.2.3 Labelling

According to the United States Food and Drug Administration (US-FDA) the label on a product has three major roles; to identify the product, to specify who the intended user is and to demonstrate how the product is to be used (FDA, 2020). The information on the Drug Facts Label was harmonized to make it easier for the consumer to understand.

The KS EAS 789:2013 specification spells out labelling requirements for instant hand sanitizers. For this purpose, instructions on the container should be clearly visible and legible. Pertinent information that should be indicated on the label includes; the name of the ABHS indicating that it is a hand sanitizer, the manufacturer name and address, the batch number, date of manufacture as well as the date of expiry. A list of the ingredients present in the ABHS, and the volume of the ABHS present in the container. Instructions on how the ABHS is to be used in the predominant language spoken in the country of sale. Cautionary instructions such as “do not ingest” or ‘highly flammable’’ are very important as they protect the users.

2.2.4 pH

KEBS specifies the desired pH range for ABHS products as 6-8. This range is close to the neutral pH which is important for products that are in contact with the skin. ABHS are dehydrating to the skin and can cause a change in the pH of the skin making the user more susceptible to bacteria or viruses (Proksch, 2018).

2.3 Materials and methods

2.3.1 Sampling

The incidental sampling technique was used in the purchase of ABHS samples. The ABHS samples were purchased during a one-month period spanning 15th December 2021 to 15th January 2022. The smallest pack size available at the point of purchase was selected. A total of

122 brands were purchased in the Nairobi Metropolitan region from supermarkets, shops, pharmacies, and cosmetic shops thereby selecting one brand per purchase point. However, for two brands, samples were obtained from separate locations based on observable differences in consistency and product label. The identity of ABHS was coded using a numerical system; S 001 to S 122 for blinding as well as eliminating any bias towards any manufacturer. A reconnaissance carried out prior to sampling established that there were enough brands within the sampling frame to support the study. Figure 2.1 is a map showing a snapshot of the different sampling sites.

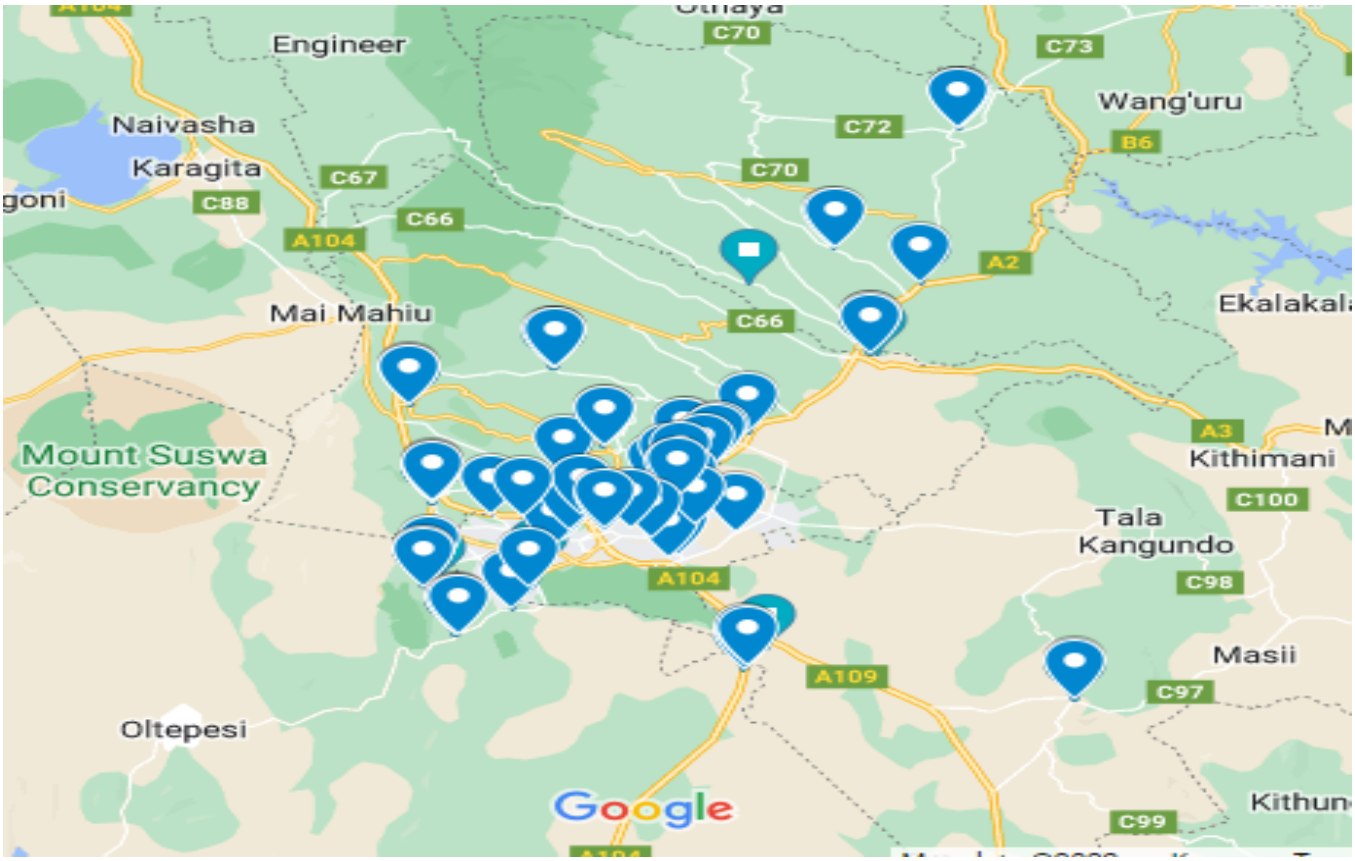

Figure 2.1: Map showing the sampling points within Nairobi metropolitan region

Available at: https://www.google.com/maps/d/u/0/edit?mid=1gtpAN5Zwd1Gv2tEg-hp4-mBzBI8Ux94U&usp=sharing

2.3.2 Compliance with KEBS specifications

The ABHS were subjected to a visual inspection to highlight product characteristics. These include appearance, consistency to determine whether it's a gel or a liquid, net contents, ingredients present, presence of batch number, manufacturing and expiry date, usage instructions, and cautionary warnings. Even though, the KEBS specification indicates that the ABHS should not have any disagreeable odor or smell this characteristic was not evaluated as it was considered to be subjective. The features were entered into a Microsoft Excel sheet for statistical analysis.

KEBS provided a procedure for verifying the SM permit number of each sample by sending a text message of the permit number in the format SM# ***** to the number 20023. The response received indicated details such as the product name, brand name, manufacturer name, the SM issuance date, SM expiry date, and lastly, the status of the SM as either the permit is valid or not valid (KEBS, 2021).

2.4 Results and discussion

Of the 122 ABHS samples, 15 (12.3%) were observed to be liquid in consistency. Their closure mechanism also supported the appropriate dispensing of a liquid ABHS. This shows that during the sampling period, gels were more readily available compared to liquids as they accounted for 105 (86.1%) of the total samples purchased. For two of the samples, the container was opaque thus making it impossible to determine the consistency of the product by visual inspection *in situ.*

With regard to the color and consistency of these samples, eight (6.6%) were found to be colored; light blue or light pink. On the other hand, four (3.3%) of them were found to be cloudy and contained flecks of particulate matter. About 77 (63%) samples met all the labelling and marking requirements such as the product name indicating that it is an ABHS, the manufacturer address, net content, the alcohol present, other ingredients present, instructions for use, manufacturing date, batch number and expiry date and cautionary instructions as shown in Figure 2.2. This compares well with an Ethiopian study where 73 (59.5%) samples complied with the packaging and labelling requirements (Wallelign *et al.*, 2022).

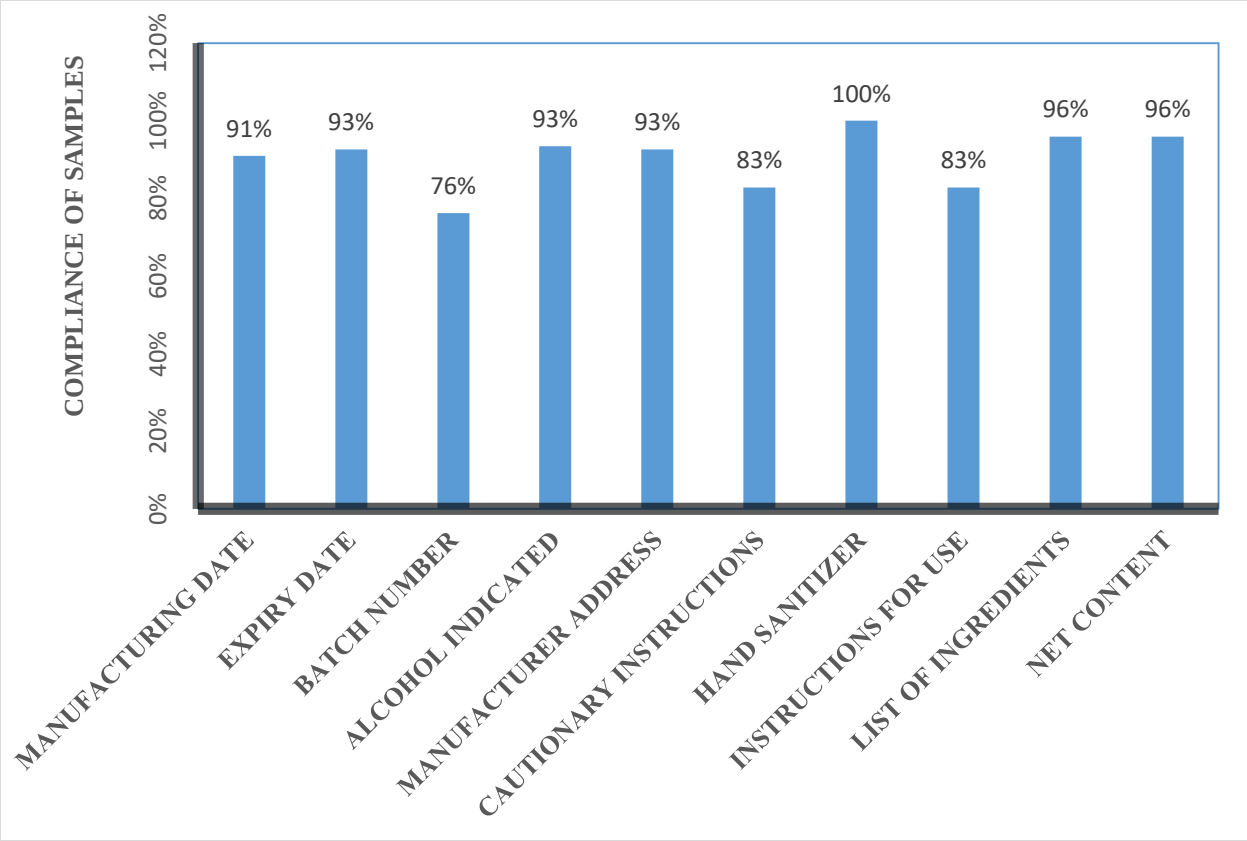

Figure 2.2: ABHS samples compliance with regulatory requirements

With regard to the labelling, two pairs of samples purchased from different locations were found to have similar brand names but on further inspection, the manufacturer details were different. It is important that the label indicate the type of alcohol present in the ABHS. Being the major active component, its presence is necessary for antimicrobial activity. Despite this, 8 (6.6%) samples did not specify the alcohol present.

In 17 (13.9%) samples the SM number was not indicated at all while in 14 (11.5%) samples the SM number was indicated but illegible. On the date of purchase, the registration status of the samples was verified by the regulator KEBS. In 23 of the samples that had an SM permit number, the response obtained was not valid while for two of the products there was actually no response. Furthermore, in two of the samples, the product name responses obtained were for hair conditioner and cheese product respectively. In two other samples the brand name obtained from the regulator did not match that on the label. This method of verifying the registration details of

the samples was found to be quite effective in detecting the presence of falsified/counterfeit products as well as identifying other details such as the permit status of a sample.

Eighty four (69.4%) samples were packaged in plastic material made of polyethylene terephthalate (PET) which can be recycled, rinsed out, and reused (Blaxhall, 2020) while one product was packaged in glass. In 37 (30.6%) samples the packaging material was not indicated even though it was evidently plastic.

The ABHS were packed in a wide range of volumes viz 35 ml, 50 ml, 65 ml, 100 ml, and 120 ml as shown in Appendix 1. The most common pack size was 50 ml as seen in 53 (45.2%) samples. In five products (4.1%) the volume was not indicated on the label or container. A disc top cap was found in 52 (42.6%) samples, 55 (45.1%) had a flip top cap as the closing mechanism while 12 (9.8%) were found to have spray pump closures.

The samples were analyzed for prices during purchase. The 50 ml pack size being the most commonly sampled was found to retail at an average of KES 85.40 (USD 0.73) and a median price of KES 80 (USD 0.68). The average price of the 100 ml pack size was found to be KES 106 (USD 0.91) and a median price of KES 100 (USD 0.86). Comparing the results to those of the previous study conducted in the Kenyan market by Abuga and Nyamweya in 2021, the price of the 100ml pack size has significantly reduced (Exchange Rates, 2022). This could be attributed to the decreased demand for the ABHS following the relaxed preventative measures or an increased supply of ABHS in the market.

From the manufacturer details, it was observed that 93 (76.2%) samples were manufactured locally while 15 (12.3%) of them were imported. In 14 (11.5%) samples, the country of origin was not indicated. The implication is that the local manufacturers were able to meet the demand as shown by fewer imports.

Figure 2.3 shows the geographical distribution of the samples purchased within the regions of the Nairobi metropolitan area. Accordingly, the least number of samples were obtained from Machakos County. Ninety three (76.2%) samples were found to be locally manufactured.

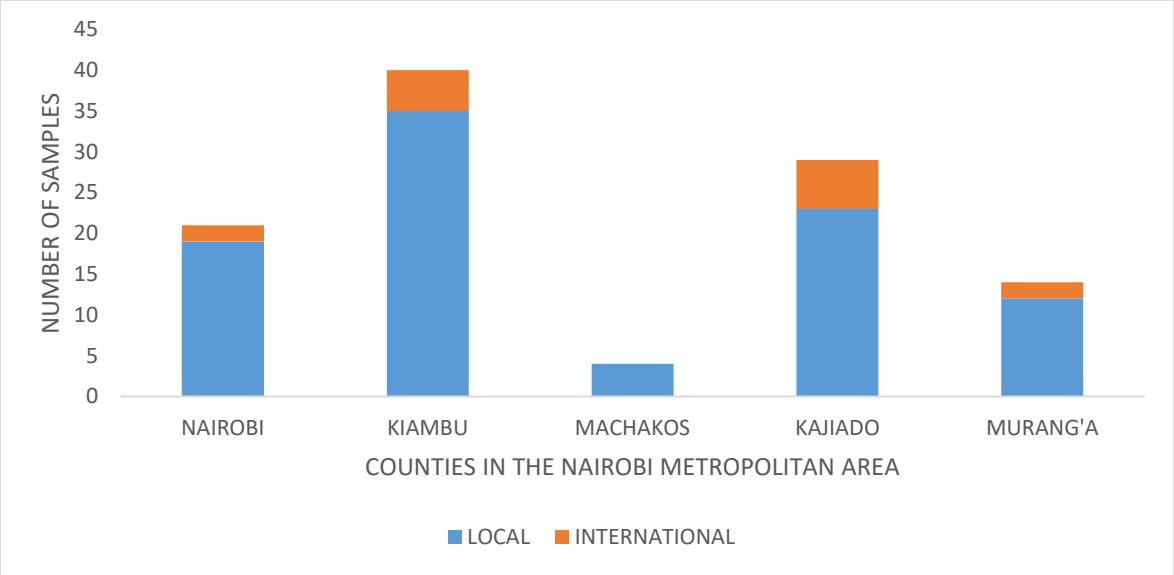

Figure 2.3 Geographical distribution of samples

The reduced risk of spillages of gels and moisturized hand feel are some of the reasons for their popularity compared to liquids (Greenaway *et al.*, 2018). The coloring observed in 7% of samples may be an attempt to make the product more attractive to consumers (Selam, 2020). However, they may contain allergens hence the need for more testing for safety (US-FDA, 2020).

The presence of all the details as described in the KEBS specification for labelling on a product instils a sense of confidence in the user. For instance, a list of the other ingredients present in the product helps to inform the user of the presence of possible irritants. The same applies to the warnings that should be indicated on the product in the case of accidental ingestion or contact with the eyes. These can help provide fast relief to the user prior to seeking comprehensive medical attention. Therefore, it is important that all the relevant details as specified by KEBS be indicated on the product label.

The presence of two samples with different manufacturer details but identical names may be indicative of the presence of falsified products in the market. The name of a product is meant to be distinctive and specific to that product (Company *et al.*, 2018). The presence of the standardization mark is also an important indicator of the registration status of a product. The 17 samples that did not have the SM mark or the SM number may be falsified. While for the 23

samples whose status was found to be “not valid”, it can be inferred that the regulator KEBS has not approved these products for use.

The pH of samples was measured using the Jenway® 3510 pH meter after calibration with standard buffer solutions of pH 4.0, 7.0, and 10.0 respectively. The pH measurement was carried out on neat samples and only in 109 (89.3%) samples were they found to be within the KEBS specified range of 6-8 as shown in Table 2.1. The pH meter was also equipped with a sensor which recorded the temperature of the sample while measuring the pH. The pH measurements were taken at room temperature to mimic conditions during use. In several samples, a narrower pH range (5-6.5) was indicated on the label. Table 2.1 also displays results on the alcohol content of the samples for which a detailed discussion is presented in Chapter 4.

Table 2.1: pH results of ABHS samples under study

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Glycerin content (% v/v)
			< 60	60-95	> 95		
S 1	8.0	21.9	57.2			25.1	-
S 2	7.9	20.5	9.7			69.7	-
S 3	5.1	20.6		72.5		-	-
S 4	8.0	19.9		67.4		-	-
S 5	5.9	20.0		69.4		-	-
S 6	8.0	19.6		59.8		-	-
S 7	7.9	20.5		63.2		-	-
S 8	4.7	20.2			100.3	-	-
S 9	5.4	20.6		68.3		-	-
S 10	8.2	20		91.1		-	-
S 11	8.3	20.8			97.5	-	-
S 12	6.9	19.9		86.4		-	-
S 13	5.5	19.7	54.9			-	-
S 14	6.7	20.6	55.9			-	-
S 15	6.6	20.6	59.0			-	-
S 16	8.3	20.3	12.8			-	-
S 17	5.3	20.7	18.5			-	-
S 18	7.3	20.9		88.1		-	-
S 19	5.7	20.5	54.6			-	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Glycerin content (% v/v)
			< 60	60-95	> 95		
S 20	8.8	20.6	20.1			62.5	-
S 21	6.8	20		79.9		-	-
S 22	4.6	19.4		71.2		-	-
S 23	7.9	20.2			-	74.7	-
S 24	7.2	19.4	54.0			-	-
S 25	8.2	20.4		66.1		-	-
S 26	8.1	19.7		61.2		-	-
S 27	6.3	20.9		60.4		-	-
S 28	6.9	20.0	34.5			-	-
S 29	5.3	21.6		71.5		-	-
S 30	6.3	19.5		68.4		-	-
S 31	7.9	20.1			-	-	-
S 32	7.2	20.2			-	17.5	-
S 33	8.1	20.8	12.3			71.7	-
S 34	8.3	19.5		74.3		-	-
S 35	7.0	20.0	6.4			-	0.2
S 36	7.7	19.5			-	88.6	-
S 37	7.2	20.3		86.5		-	-
S 38	5.3	21.4	49.7			38.2	-
S 39	5.4	20.5			-	-	-
S 41	5.4	20		75.1		-	-
S 42	7.9	20.8			-	-	-
S 43	7.6	19.3	47.4			19.2	-
S 44	5.7	19.9	3.0			28.6	-
S 45	6.1	20.4			-	19.9	-
S 46	7.6	20.5		69.6		-	-
S 47	6.6	19.8	40.9			-	-
S 48	5.9	20.4			-	12.7	-
S 49	7.8	20.6		79.3		-	-
S 50	6.0	20.3			95.8	-	-
S 51	4.0	20.4		83.1		-	-
S 52	8.3	20.6	33.3			-	0.3
S 53	8.1	20	55.4			-	-
S 54	6.3	20.7			-	63.9	-
S 55	6.2	19.5		66.9		-	-
S 56	5.7	20.4	53.5			-	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Glycerin content (% v/v)
			< 60	60-95	> 95		
S 57	5.6	20.6		-		72.5	-
S 58	8.9	20.5	59.4			-	-
S 59	7.8	19.5		72.3		-	-
S 60	6.5	20		79.8		-	-
S 61	6.9	20	55.7			-	-
S 62	5.9	20.6		63.9		-	-
S 63	7.4	22		-		78.8	-
S 64	5.4	19.9	16.8			26.0	-
S 65	7.0	20.5	42.7			-	-
S 66	6.1	19.7		60.6		-	-
S 67	8.7	20.5		76.3		-	-
S 68	6.1	19.4		78.3		-	-
S 69	5.6	19		92.6		46.8	-
S 70	5.3	19.3		64.1		-	-
S 71	6.8	20.0		-		-	-
S 72	7.4	20.5		70.8		-	-
S 73	6.1	21	51.9			-	-
S 74	5.7	19.1	58.8			-	-
S 75	5.6	19.6		60.1		-	-
S 76	6.3	20.8	51.1			-	-
S 77	5.7	20.2	56.7			-	-
S 78	6.5	20.5		62.6		-	-
S 79	7.9	19.7	48.6			34.5	-
S 80	6.8	20.5		83.3		-	-
S 81	6.0	20.6		94.2		-	-
S 82	7.8	19.3		74.5		-	-
S 83	6.5	20.4	42.6			-	-
S 84	7.1	20.5		75.6		-	-
S 85	5.8	20		67.6		18.3	-
S 86	7.0	19.4	54.9			-	-
S 87	7.6	20		70.3		-	-
S 88	7.1	20.2		93.7		-	-
S 89	7.1	20		72.6		-	-
S 90	6.2	19.8		77.2		-	-
S 91	6.7	20.2	54.0			-	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Glycerin content (% v/v)
			< 60	60-95	> 95		
S 92	5.5	20.2	53.4			-	-
S 93	7.0	19.8		67.5		-	-
S 94	7.5	20.2	59.4			-	-
S 95	6.8	19.9	55.6			-	-
S 96	6.9	20.2	21.5			-	-
S 97	6.6	20.4	10.4			10.3	-
S 98	7.7	20.4		68.6		-	-
S 99	6.9	20.3	50.7			8.0	-
S 100	7.0	20.6		66.8		-	-
S 101	7.2	20.1		64.0		-	-
S 102	7.4	19.3	47.0			-	-
S 103	5.5	19.5			-	67.4	-
S 104	7.6	19	23.8			49.6	-
S 105	6.5	20.6	51.4			-	-
S 106	7.0	22.7	56.3			-	-
S 107	5.7	19.9		60.3		-	-
S 108	7.3	20.2		60.4		-	-
S 109	7.0	20.3	34.1			-	-
S 110	5.7	20.7		62.5		-	-
S 111	6.5	20.1		59.9		-	-
S 112	6.0	20.3	38.9			34.9	-
S 113	7.4	20.6		89.6		-	-
S 114	7.1	20.3	58.1			-	-
S 115	6.0	20.4	53.5			19.6	-
S 116	7.3	19.7	58.8			-	-
S 117	6.8	20.2	12.3			53.4	-
S 118	7.3	20.5	30.5			-	-
S 119	5.5	19.7		66.7		-	-
S 120	6.8	19.9	31.6			-	-
S 121	5.7	19.3		63.6		-	-
S 122	6.9	20.1			-	-	-

- Not Detected TEMP-Temperature

Several pH-modifying agents were declared on the labels of 41 (33.6%) samples. Triethanolamine was the most commonly observed pH-adjusting agent in 38 (31.1%) samples.

Carbomers and other thickening agents require to be neutralized to achieve maximum thickening hence the need for these pH-modifying agents (Abuga and Nyamweya, 2021).

It is necessary for the regulatory authority, KEBS to step up measures on education to the public on the relevant details to look-out for in ABHS products. It would also be important to create awareness of the various ways in which one may be able to check on the registration status of a product prior to use.

CHAPTER THREE

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF HAND SANITIZERS

3.1 Introduction

Gas chromatography (GC) coupled with mass spectrometry (MS) is an analytical technique that combines the separation capabilities of GC with the detection power of MS. The coupling of these two techniques provides higher efficiency in the analysis of samples. Gas chromatography allows for the separation of the volatiles while mass spectrometry enables the identification of the specific compounds.

The USP monograph for ethanol describes specific tests for the identification, and control of volatile impurities within defined limits (USP 29, 2006a). The specific tests for the presence of volatile impurities describe the use of gas chromatography (GC). Coupling GC with MS allows for the identification of the volatiles based on the mass-to-charge ratio upon ionization. Identification of each compound was carried out based on mass spectral matching to the United Stated National Institute for Standards and Technology (NIST) mass spectral library.

3.2 Instrumentation

A Shimadzu GC-2010 plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) operated using GC solution software version 2.42 (Shimadzu Corporation, Kyoto, Japan) equipped with a mass spectrometric detector was used to identify and characterize the volatiles. A ZB wax plus column (Phenomenex, Torrance, CA, USA) of dimensions 60 m, an internal diameter of 0.25mm coated with polyethylene glycol (PEG) of a film thickness of 0.25 µm was used for chromatographic separation.

The chromatographic conditions were based on the method validated by Jie Zhang (Zhang, 2020). Minor modifications of the temperature program were made to permit identification and quantification of glycerin as outlined in Table 3.1.

Table 3.1: Gas Chromatographic conditions for ABHS analysis

GC Parameters	
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	45 ºC (7 min), 240 ºC at 30 ºC/min for 6 min and 240 ºC at 35 ºC/min for 7 min
Column	ZB-WAX plus, dimensions 60 m by ID 0.25 mm, 0.25 µm film thickness
Total run time	26.5 min

ID = Internal Diameter

3.3 Reagents

These were purchased from local suppliers in Nairobi City. HPLC grade acetonitrile (Carlo Erba reagents S.A.S, Dasit Group Limited, Val-de-Reuil, France) as the internal standard. Absolute ethanol (Scharlab S.L., Sentmenat, Spain), analytical grade isopropyl alcohol (Finar Limited, Ahmedabad, India), analytical grade methanol (Finar Limited, Ahmedabad, India), and glycerin (Finar Limited, Ahmedabad, India) were used as the standards for GC analysis. Freshly distilled water was prepared in the Drug Analysis & Research Unit (DARU) laboratory, University of Nairobi. Test solutions were filtered through PTFE (Polytetrafluoroethylene) 0.22 µm micro filters (Nantong Filter-Bio Membrane Co., Jiangsu, China) prior to analysis. The percentage potencies as indicated on the labels of each of the standards is shown in Table 3.2.

Table 3.2: Percentage potency of solvent standards

STANDARDS	% POTENCY
Methanol (Finar Limited, Ahmedabad, India)	99.8
Isopropyl alcohol (Finar Limited, Ahmedabad, India)	99.5
Ethanol (Scharlab S.L., Sentmenat Spain)	99.9
Glycerin (Finar Limited, Ahemdabad, India)	99.5

3.3.1 Preparation of samples and standards

A stock solution of the internal standard was prepared by transferring 1.0 ml of HPLC grade acetonitrile in a 10.0 ml volumetric flask and diluting to the mark with distilled water. Thereafter, 500 µl was measured out using a micropipette and added to standard and sample preparations prior to analysis.

The standard stock solution was prepared by measuring out 1.0 ml of each of the standard solvents of methanol, isopropyl alcohol, ethanol and glycerin into a 10.0 ml volumetric flask and made to the mark with distilled water.

The test standard solution was prepared by measuring out 300 µl of the standard stock solution, 500 µl of the internal standard and 200 µl of distilled water.

Sample solutions were prepared by diluting 1.0 ml of the neat sample to 10.0 ml with distilled water. An aliquot equivalent to 300 µl was micropippeted, mixed with 500 µl of internal standard solution and diluted to 1.0 ml with distilled water.

3.4 Method validation

Validation can be described as the method of proving that the analytical method is capable of producing results that are valid, reproducible and reliable (Shabir *et al.*, 2007). The International Council for Harmonization (ICH) has developed guidelines for the validation of analytical procedures (ICH, 2010). There are specific parameters that should be assessed when optimizing an analytical method including precision, linearity of detector response and accuracy.

The precision of a procedure is described as the degree of agreement amongst multiple individual test results of the same sample under prescribed conditions. It is described in terms of standard deviation, variance, or the coefficient of variation following replicate runs. Linearity of detector response describes that at a range of concentrations of the analyte subjected to the above-mentioned chromatographic conditions, the observed result can be attributed to the analyte concentration present. The ICH defines accuracy as a measure of how close the observed results are to the true value (ICH, 2006).

3.4.1 Precision

To determine the injection precision, six injections of a single vial of the standard solution were made, and the results were evaluated for coefficient of variation which was calculated to be < 4%. The quantitation precision was determined by preparing three vials of the standard solution and performing two injections of each vial, the coefficient of variation was found to be ≤ 3%. These values were acceptable based on the limits described by Zhang (Zhang, 2020). In both cases, a minimum of six determinations were made as recommended by the USP (USP 29, 2006a). The results are expressed in Table 3.3.

Table 3.3: Summary of precision and linearity of detector response results

Volatile	Injection Precision (% RSD)	Quantitation precision (%RSD)	Coefficient of Determination (R^2)
Methanol	1.9	1.8	0.99
Isopropyl alcohol	3.1	3.3	1.00
Ethanol	3.8	2.7	0.99

3.4.2 Linearity of Detector Response

For linearity, five solutions were prepared within the range 20% - 120% of the nominal concentration (100 µg/ml) and subjected to GC assay. A plot of peak area vs concentration was used to determine linearity. The coefficient of determination (R^2) obtained from the regression curves was > 0.99 thus complying with the acceptance criteria (Shabir, 2004).

3.4.3 Accuracy

Accuracy was performed by spiking a sample whose alcohol content was already determined with a known amount of analyte (Shabir, 2004). On subjecting the spiked sample to similar chromatographic conditions as the un-spiked sample the results were compared and the amount of analyte added was quantified. The sample was spiked over a range of 50% to 150% of the assigned concentration.

The ethanol content in the un-spiked sample was calculated to be 51.9%. The un-spiked sample was designated a value of 100%. Ethanol standard (99.9% v/v) was added in the range of 10%, 20% and 30%. Two vials were prepared at each concentration range and injected in triplicate (Shabir, 2004). The results of the ethanol content of the spiked samples are shown in Table 3.4. The FDA specifies an accuracy criteria of 100% ±2% (FDA, 1994). These results showed the method is capable of producing accurate results.

Table 3.4: Ethanol content in the spiked and un-spiked samples.

	Assigned ethanol concentration	Nominal values	% Deviation
Un-spiked sample	51.9%	100%	-
Spiked samples	62.6%	110%	0.9
	71.5%	120%	0.4
	82.3%	130%	0.4

The other validation parameters such as robustness and limit of quantitation (LOQ) were not established in this study since the main objective was identification and assay. The working assumption is that the permitted alcohols will be present in significant amounts hence the limit of detection (LOD) and LOQ were not determined.

3.5 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

GC-MS was used in the identification and characterization of the volatiles profile of the ABHS. Once the volatile components have undergone separation, their identification is based on their

fragmentation patterns compared to the offline NIST database. The temperature program is shown in Table 3.5.

Table 3.5: Temperature program for GC analysis

RATE (per min.)	TEMPERATURE (ºC)	HOLD TIME (min)
-	45	7.0
30	240	6.0
35	240	7.0

The GC-MS Shimadzu uses electron impact for ionization at an acceleration energy of 70 eV. The ion source temperature of the mass selector was maintained at 200 °C. Analysis of the fragment ions was carried out in the full scan mode over a 20-300 m/z range with filament delay time set a 0 min.

3.5.1 RESULTS AND DISCUSSION

Prior to analysis system suitability tests were carried out for the chromatographic system using standard solutions to ensure that parameters such as the resolution and tailing factor fall within the USP specifications. The results are shown in Table 3.6.

Table 3.6: System suitability results

Analyte	Average RT (min)	Theoretical plate (N)	Tailing Factor (As)	Resolution (Rs)
Methanol	6.33	31547	1.71	0.00
IPA	7.09	40526	0.00	5.41
Ethanol	7.23	46697	0.00	1.04
Acetonitrile	8.48	76240	1.06	11.59
Glycerin	21.49	48431	0.69	55.34

RT - Retention time

The USP specifications for ethanol determination by gas chromatography recommends that the resolution (R) should be not less than 2.0 while the tailing factor should not exceed 2.0. Although the resolution of ethanol from isopropyl alcohol did not meet the criteria, the method was still considered appropriate as these are permitted alcohols in ABHS whereby the total content was used for evaluation.

Mass spectrometric analysis was carried out on the 122 samples whereby, four injections were made for each sample. The retention time and the similarity index from the NIST offline database were used for the identification of components. The results are recorded in Table 3.7.

A test standard solution was initially run to provide a base for comparison of retention times however, the similarity index was considered a more objective tool for identification. Once a sample was run manual peak integration was carried out, a peak was selected and scanned in the offline NIST database for identification of the compound based on the highest similarity index.

Table 3.7: Results of GC-MS analysis of ABHS samples

SAMPLE CODE	ALCOHOL IDENTIFIED THROUGH GC-MS	OTHER COMPOUNDS IDENTIFIED
S 1	Methanol, isopropyl alcohol, ethanol	-
S 2	Methanol, ethanol	-
S 3	Ethanol	-
S 4	Isopropyl alcohol, ethanol	-
S 5	Ethanol	-
S 6	Isopropyl alcohol, ethanol	-
S 7	Ethanol	-
S 8	Isopropyl alcohol	-
S 9	Isopropyl alcohol, ethanol	-
S 10	Isopropyl alcohol	-
S 11	Isopropyl alcohol	-
S 12	Ethanol	-
S 13	Ethanol	-
S 14	Ethanol	-
S 15	Ethanol	-
S 16	Isopropyl alcohol	-
S 17	Isopropyl alcohol	-
S 18	Isopropyl alcohol, ethanol	-

SAMPLE CODE	ALCOHOL IDENTIFIED THROUGH GC-MS	OTHER COMPOUNDS IDENTIFIED
S 19	Ethanol	-
S 20	Methanol, isopropyl alcohol	Heptaethylene glycol
S 21	Isopropyl alcohol	Nonaethylene glycol
S 22	Ethanol	Propylene glycol, heptaethylene glycol
S 23	-	Propylene glycol, undecaethylene glycol
S 24	Methanol	Dodecaethylene glycol
S 25	Isopropyl alcohol, ethanol	Undecaethylene glycol
S 26	Ethanol	Undecaethylene glycol
S 27	Ethanol	-
S 28	Ethanol	Undecaethylene glycol, octaethylene glycol monoether
S 29	Isopropyl alcohol	Dodecaethylene glycol
S 30	Ethanol	-
S 31	Ethanol	-
S 32	-	Hexaethylene glycol, dodecaethylene glycol, heptaethylene glycol monomethyl ether
S 33	Methanol	Propylene glycol
S 34	Methanol, ethanol	Undecaethylene glycol, heptaethylene glycol, octaethylene glycol monomethyl ether, dodecaethylene glycol
S 35	Ethanol	Docaethylene glycol, octaethylene glycol monoether
S 36	Ethanol	Glycerin
S 37	Methanol	-
S 38	Isopropyl alcohol	Hepatethylene glycol, dodecaethylene glycol,, octaethylene glycol monomethyl ether
S 39	Methanol, isopropyl alcohol	-
S 40	-	-
S 41	-	Glycerin
S 42	Ethanol	-
S 43	-	Propylene glycol
S 44	Methanol, isopropyl alcohol	Undecaethylene glycol, Heptaethylene glycol monoether, octaethylene glycol monoether,
S 45	Methanol, ethanol	-
S 46	Methanol	Propylene glycol
S 47	Ethanol	Hexaethylene glycol

SAMPLE CODE	ALCOHOL IDENTIFIED THROUGH GC-MS	OTHER COMPOUNDS IDENTIFIED
S 48	Isopropyl alcohol	Propylene glycol, nonaethylene glycol, undecaethylene glycol, octaethylene monomethyl ether, dodecaethylene ether
S 49	-	Propylene glycol, octaethylene monomethyl ether, undecaethylene glycol
S 50	Isopropyl alcohol	-
S 51	Isopropyl alcohol	-
S 52	Ethanol	-
S 53	Isopropyl alcohol	Glycerin
S 54	Ethanol	-
S 55	Methanol	-
S 56	Ethanol	Hexaethylene glycol
S 57	Ethanol	-
S 58	Methanol	Octaethylene glycol
S 59	Ethanol	-
S 60	Isopropyl alcohol	Undecaethylene glycol
S 61	Ethanol	Undecaethylene glycol
S 62	Ethanol	Undecaethylene glycol
S 63	Isopropyl alcohol	Heptaethylene glycol, undecaethylene glycol, hexaethylene glycol monoether, octaethylene glycol monoether, dodecaethylene glycol
S 64	Methanol	Octaethylene glycol, dodecaethylene glycol, undecaethylene glycol
S 65	Methanol, isopropyl alcohol	Octaethylene glycol monomethyl ether, undecaethylene glycol
S 66	Isopropyl alcohol	Octaethylene glycol monomethyl ether, dodecathylene glycol
S 67	Isopropyl alcohol	Octaethylene glycol monomethyl ether, undecaethylene glycol
S 68	Ethanol	Undecaethylene glycol
S 69	Isopropyl alcohol	Undecaethylene glycol
S 70	Methanol, ethanol	Undecaethylene glycol, dodecaethylene glycol
S 71	Ethanol	Undecaethylene glycol, octaethylene glycol monoether, dodecaethylene glycol
S 72	-	Undecaethylene glycol, heptaethylene glycol, octaethylene glycol monomethyl ether, dodecaethylene glycol
S 73	Isopropyl alcohol	Propylene glycol, octaethylene glycol monoether, undecaethylene glycol

SAMPLE CODE	ALCOHOL IDENTIFIED THROUGH GC-MS	OTHER COMPOUNDS IDENTIFIED
S 74	Ethanol	Undecaethylene glycol, dodecaethylene glycol
S 75	Isopropyl alcohol	Undecaethylene glycol
S 76	Ethanol	Nonethylene glycol, dodecaethylene glycol
S 77	Ethanol	Undecaethylene glycol
S 78	Isopropyl alcohol	Heptaethylene glycol, dodecathylene glycol
S 79	Ethanol	Undecaethylene glycol
S 80	-	Propylene glycol, undecaethylene glycol, dodecaethylene glycol
S 81	Ethanol	Undecaethylene glycol
S 82	Ethanol	Undecatheylene glycol, octaethylene glycol monomethyl ether
S 83	Ethanol	-
S 84	Ethanol	-
S 86	Methanol, isopropyl alcohol	-
S 87	Ethanol	-
S 88	Isopropyl alcohol, ethanol	-
S 89	Isopropyl alcohol	-
S 90	Isopropyl alcohol	-
S 91	Ethanol	-
S 92	Isopropyl alcohol	-
S 93	Ethanol	-
S 94	Ethanol	Dodecaethylene glycol
S 95	Ethanol	Undecaethylene glycol, octaethylene glycol monomethyl ether
S 97	Isopropyl alcohol	-
S 98	Methanol, isopropyl alcohol	-
S 99	Ethanol	-
S 100	Methanol, isopropyl alcohol	Nonaethylene glycol,
S 101	Ethanol	Nonaethylene glycol monoether
S 102	Ethanol	-
S 103	Isopropyl alcohol	Propylene glycol
S 104	Methanol	-
S 105	Methanol, isopropyl alcohol	Octaethylene glycol monomethyl ether, undecaethylene glycol, decaethylene glycol
S 106	Methanol	-
S 107	Ethanol	-
S 108	Ethanol	-

SAMPLE CODE	ALCOHOL IDENTIFIED THROUGH GC-MS	OTHER COMPOUNDS IDENTIFIED
S 109	Ethanol	-
S 110	Isopropyl alcohol	-
S 111	Ethanol	-
S 112	Ethanol	-
S 113	Methanol, isopropyl alcohol	-
S 114	Ethanol	-
S 115	Isopropyl alcohol	-
S 116	Methanol, isopropyl alcohol	-
S 117	Ethanol	Heptaethylene glycol
S 118	Methanol, isopropyl alcohol	-
S 119	Ethanol	-
S 120	Ethanol	-
S 121	Ethanol	-
S 122	Ethanol	-

- Not Detected

3.5.1.1 Permitted alcohols; ethanol and isopropyl alcohol

The permitted alcohols, ethanol and isopropyl alcohol were found in 105 (86.1%) samples. At this stage of analysis quantification of these permitted alcohols, methanol and glycerin was not performed. The labels of these samples indicate that in 28 (23%) of them were denatured using an alcohol. Denaturation is the process of introducing contaminants such as denatonium benzoate, sucrose octaacetate, isopropanol or 3% trimethyl citrate (w/w) to minimize the chances of accidental ingestion (Abuga and Nyamweya, 2021). The most common denaturant indicated on the labels was 3.3% isopropyl alcohol.

In 61 (50%) samples, the results obtained from MS analysis correspond to those claimed by the label. Conversely, 50% of the samples analyzed the label claim did not correspond to any of the peaks observed.

3.5.1.2 Impurities

Methanol contamination was observed in 16 (13.1%) samples. This is where methanol was identified along with the permitted alcohols. Figure 3.1 is a chromatogram depicting methanol contamination in S 118. The presence of methanol in ABHS poses harmful effects in instances of intentional and accidental ingestion (Rayar *et al.,* 2013). Inhalation and transdermal absorption

are other modes of absorption through which the negative effects of methanol are realized (Brewer *et al.*, 2020).

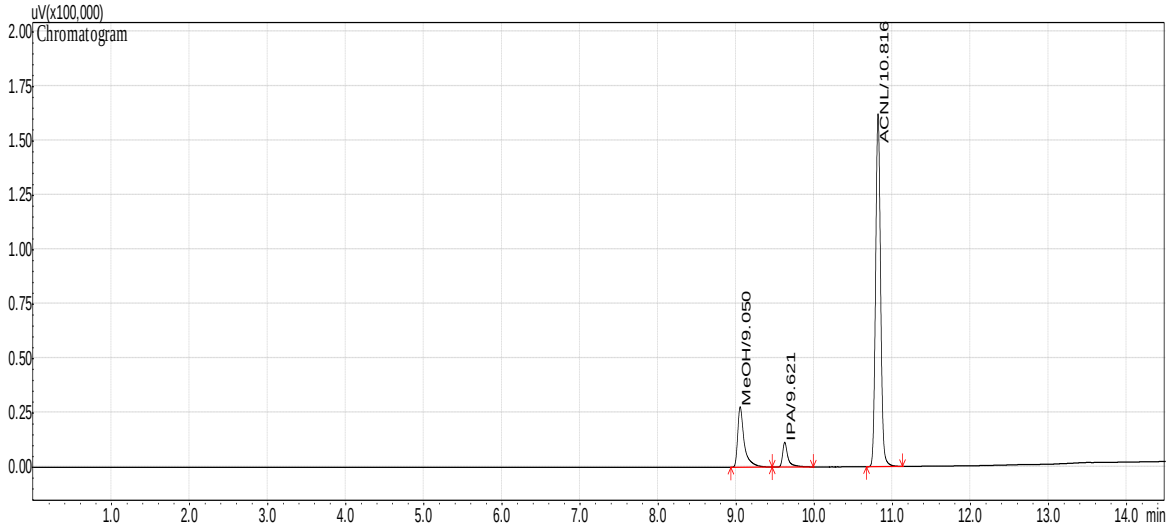

Figure 3.1: Chromatogram showing methanol contamination in S 118

The chromatogram in Figure 3.2 shows methanol substitution which was observed in 10 (8.2%) samples. This describes a situation where methanol was identified as the main alcohol present in the sample. Despite being an alcohol like ethanol and isopropyl alcohol, the use of methanol in ABHS is not recommended as per the KEBS specification (KEBS, 2014b). However, in other regions such as the United Kingdom the use of methanol in trade-specific formulations of denatured alcohols is permitted (Dear *et al.*, 2020). The presence of methanol in these samples was not expected as it was not indicated on the labels as a denaturant.

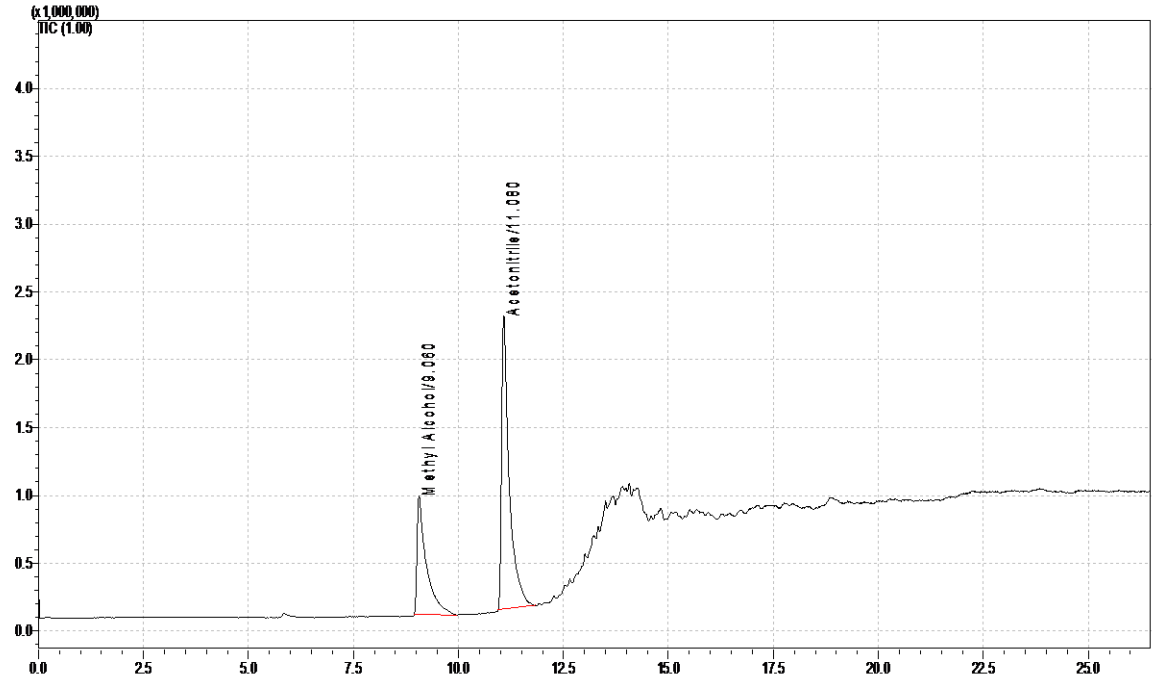

Figure 3.2: Chromatogram showing a sample with methanol substitution in S 36

Since methanol is not a permitted alcohol in ABHS, it is not expected that it would be listed on the product label. A pricing advantage may account for the use of methanol in place of ethanol in ABHS products. The market price of USP grade methanol (KES 300/L) is significantly less compared to the USP grade ethanol (KES 1200/L) that is specified for the production of ABHS. Contamination of raw materials could lead to the observed methanol contamination.

3.5.1.3 Humectants

In 7 (5.7%) samples, there was no alcohol detected. None of the permitted alcohols or methanol was detected as shown in Figure 3.3. This demonstrates deliberate commercialization of ABHS products that do not have any active ingredient present. These substandard/falsified products portend adverse consequences on unsuspecting users.

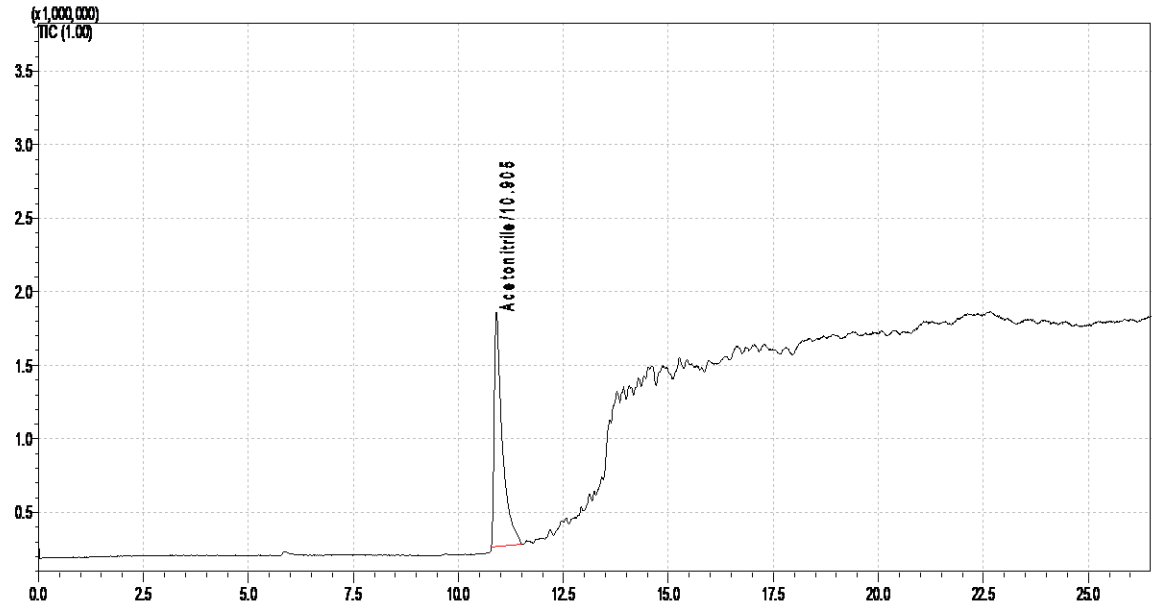

Figure 3.3: Chromatogram showing sample with no alcohol in S 39

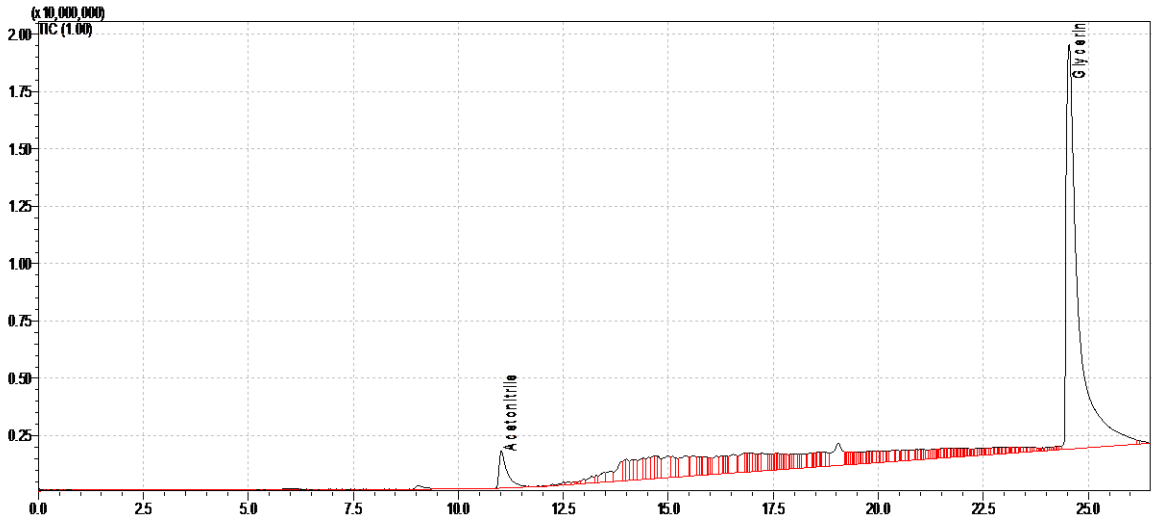

Figure 3.4: Chromatogram showing a sample with glycerin as the only compound in S 40

Glycerin was identified in three (2.5%) samples despite its presence being indicated on the labels of 88 (66.4%) samples. Although labelling on 22 (18%) samples claimed presence of propylene glycol, it was identified in only 11 (9%) samples. Glycerin and propylene glycol are likely added to these products as thickening agents to improve the viscosity and overall efficacy (Saha *et al.*, 2021). The viscosity of these ABHS has a positive effect on the efficacy and consumer

perception, by reducing the rate of alcohol evaporation leading to an increased contact time with the offending microorganism (Villa *et al.*, 2021).

Polyethylene glycol and its derivatives such as hepta-ethylene glycol, nona-ethylene glycol, octa-ethylene glycol, dimethicone and dodeca-ethylene glycol were observed in 52 (42.6%) samples. Their use in the cosmetic industry has been encouraged as a humectant, surfactant and an emulsifying agent to counter the drying effect of alcohols on the skin (Berardi *et al.*, 2020; Jang *et al.*, 2015). The presence of propylene glycol and its derivatives as identified from the MS results could also result from the leaching of the ZB wax column following its repeated usage.

GC-MS analysis allowed for the characterization of the 122 samples. None of the potentially carcinogenic compounds such as benzene and acetaldehyde were detected in contradistinction with previous studies conducted in other regions. For instance in a Canadian study acetaldehyde was detected in 11 samples in amounts that exceed the specified limits (Tse *et al.*, 2021). The alcohols identified are methanol and the permitted alcohols ethanol and *iso*propyl. *n*-propanol was not detected in any of the samples despite its use being permitted for the manufacture of ABHS in Kenya (KEBS, 2014b). Quantification of each of these volatiles required further analysis to determine if they comply with specifications.

In considering the external validity of the results from this study, it is important to highlight that they cannot be extrapolated to other regions in Kenya. This is due to differences in the market distribution of ABHS especially for border counties in which seepage of products from neighboring countries may occur.

CHAPTER FOUR

GAS CHROMATOGRAPHIC ASSAY OF ALCOHOL BASED HAND SANITIZERS

4.1 Introduction

The microbicidal effect of ABHS is a direct result of the alcohol content. Effective bactericidal activity is achieved at a range of 60% to 95% v/v alcohol concentration The CDC recommends an alcohol content of at least 60% v/v (CDC, 2020a). According to the Kenya Bureau of Standards (KEBS), ethanol, *iso*propyl alcohol and n-propanol are the alcohols permitted in formulating ABHS (KEBS, 2014b) at a minimum concentration of 60% v/v. Ethanol is preferred to isopropanol due to its superior virucidal activity and better skin tolerance (Berardi *et al.*, 2020)

The bactericidal activity of these aqueous alcohols is via the clumping of cell membrane proteins thus interfering with the integrity of the cell membrane. The protein denaturation takes place more rapidly in the presence of water (Singh *et al.*, 2020) thereby supporting the conclusion that an increase in the alcohol content does not translate to an increase in bactericidal activity (Reynolds *et al.*, 2006). In addition to its role as a humectant, glycerin also has an antimicrobial effect at a concentration range of 0.5% to 0.73% v/v (Menegueti *et al.*, 2019).

The importance of optimal alcohol content cannot be understated as sub-optimal alcohol content confers ineffective protection to the user. A study conducted in South Africa to determine the alcohol content in ABHS showed that in 44% of the samples the alcohol content was not optimal (Menegueti *et al.*, 2019). The presence of impurities such as methanol, acetal and acetaldehyde is also quite important as they can trickle down to the user following intentional or unintentional absorption. This is shown in the Canadian study where seventeen samples exceeded the acetal and acetaldehyde limits (Tse *et al.*, 2021).

4.2 Materials and Methods

4.2.1 Instrumentation

A Shimadzu GC-2010 plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) operated using GC solution software version 2.42 (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector was used to quantify the volatiles. A ZB wax plus column

(Phenomenex, Torrance, CA, USA) of dimensions 60 m, an internal diameter of 0.25mm coated with polyethylene glycol (PEG) of a film thickness of 0.25 µm was used for chromatographic separation.

The chromatographic conditions were based on the method validated by Jie Zhang (Zhang, 2020). Minor modifications of the temperature program were made to permit identification and quantification of glycerin. The chromatographic conditions are described in Table 4.1 (Zhang, 2020).

Table 4.1: GC-FID conditions for ABHS analysis

GC-FID Parameters	
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	45 ºC (7 min), 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, Hydrogen: 40 ml/min, constant make up: 30 ml/min
Column	ZB-WAX plus, dimensions 60 m by ID 0.25 mm, 0.25 µm film thickness
Total run time	26.5 min

4.2.2 Reagents

These were purchased from local suppliers in Nairobi City. HPLC grade acetonitrile (Carlo Erba reagents S.A.S, Dasit Group Limited, Val-de-Reuil, France) as the internal standard. 99.9% absolute ethanol (Scharlab S.L., Sentmenat, Spain), 99.5% analytical grade isopropyl alcohol (Finar Limited, Ahmedabad, India), 99.8% analytical grade methanol (Finar Limited, Ahmedabad, India) and 99.5% glycerin (Finar Limited, Ahmedabad, India) were used as the standards for GC analysis. Freshly distilled water was prepared in the Drug Analysis & Research Unit (DARU) laboratory, University of Nairobi. Test solutions were filtered through PTFE

(Polytetrafluoroethylene) 0.22 µm micro filters (Nantong Filter-Bio Membrane Co., Jiangsu, China) prior to analysis.

4.2.3 Preparation of samples and standards

A stock solution of the internal standard was prepared by transferring 1.0 ml of HPLC grade acetonitrile in a 10.0 ml volumetric flask and diluting to the mark with distilled water. Thereafter, 500 µl was measured out using a micropipette and added to standard and sample preparations prior to analysis.

The standard stock solution was prepared by measuring out 1.0 ml of each of the standards; methanol, isopropyl alcohol, ethanol and glycerin into a 10.0 ml volumetric flask and made to the mark with distilled water.

The standard solution was prepared by measuring out 300 µl of the standard stock solution, 500 µl of the internal standard and 200 µl of distilled water.

Sample solutions were prepared by diluting 1.0 ml of the neat sample to 10.0 ml with distilled water. An aliquot equivalent to 300 µl was micropippeted, mixed with 500 µl of internal standard solution and diluted to 1.0 ml with distilled water.

4.3 Results

Quantification of the alcohol content was carried out analogous to that used by Okaru et al. to determine the ethanol content in illicit drinks (Okaru *et al.*, 2017). The alcohol content was calculated by using an external standard with good precision. A comparison of the peak area ratios of the individual samples to the internal standard against the standards was carried out. The dilution factors were not considered as the standard and the sample were prepared in a similar manner causing them to cancel out. The results are shown in Table 4.2.

Table 4.2: Alcohol and glycerin content of ABHS samples

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Methanol content (ppm)	Glycerin content (% v/v)
			< 60	60-95	> 95			
S 1	8.0	21.9	57.2			25.1	251,000	-
S 2	7.9	20.5	9.7			69.6	696,000	-
S 3	5.1	20.6		72.5		-	-	-
S 4	8.0	19.9		67.4		-	-	-
S 5	5.9	20.0		69.4		-	-	-
S 6	8.0	19.6		59.8		-	-	-
S 7	7.9	20.5		63.2		-	-	-
S 8	4.7	20.2			100.3	-	-	-
S 9	5.4	20.6		68.3		-	-	-
S 10	8.2	20		91.1		-	-	-
S 11	8.3	20.8			97.5	-	-	-
S 12	6.9	19.9		86.4		-	-	-
S 13	5.5	19.7	54.9			-	-	-
S 14	6.7	20.6	55.9			-	-	-
S 15	6.6	20.6	59.0			-	-	-
S 16	8.3	20.3	12.8			-	-	-
S 17	5.3	20.7	18.5			-	-	-
S 18	7.3	20.9		88.1		-	-	-
S 19	5.7	20.5	54.6			-	-	-
S 20	8.8	20.6	20.1			62.5	625,000	-
S 21	6.8	20		79.9		-	-	-
S 22	4.6	19.4		71.2		-	-	-
S 23	7.9	20.2			-	74.6	746,000	-
S 24	7.2	19.4	54.1			-	-	-
S 25	8.2	20.4		66.1		-	-	-
S 26	8.1	19.7		61.2		-	-	-
S 27	6.3	20.9		60.4		-	-	-
S 28	6.9	20.0	34.5			-	-	-
S 29	5.3	21.6		71.5		-	-	-
S 30	6.3	19.5		68.4		-	-	-
S 31	7.9	20.1				-	-	-
S 32	7.2	20.2			-	17.5	175,000	-
S 33	8.1	20.8	12.3			71.7	717,000	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Methanol content (ppm)	Glycerin content (% v/v)
			< 60	60-95	> 95			
S 34	8.3	19.5		74.3		-	-	-
S 35	7.0	20.0	6.4			-	-	0.2
S 36	7.7	19.5		-		88.6	886,000	-
S 37	7.2	20.3		86.5		-	-	-
S 38	5.3	21.4	49.7			38.2	382,000	-
S 39	5.4	20.5		-		-	-	-
S 40	5.9	20.5		-		-	-	0.6
S 41	5.4	20.5		75.1		-	-	-
S 42	7.9	20.8		-		-	-	-
S 43	7.6	19.3	47.4			19.2	192,000	-
S 44	5.7	19.9	3.0			28.6	286,000	-
S 45	6.1	20.4		-		19.8	198,000	-
S 46	7.6	20.5		69.6		-	-	-
S 47	6.6	19.8	40.9			-	-	-
S 48	5.9	20.4		-		12.7	127,000	-
S 49	7.8	20.6		79.3		-	-	-
S 50	6.0	20.3			95.8	-	-	-
S 51	4.0	20.4		83.1		-	-	-
S 52	8.3	20.6	33.3			-	-	0.3
S 53	8.1	20	55.4			-	-	-
S 54	6.3	20.7		-		63.9	639,000	-
S 55	6.2	19.5		66.9		-	-	-
S 56	5.7	20.4	53.5			-	-	-
S 57	5.6	20.6		-		72.5	725,000	-
S 58	8.9	20.5	59.4			-	-	-
S 59	7.8	19.5		72.3		-	-	-
S 60	6.5	20		79.7		-	-	-
S 61	6.9	20	55.7			-	-	-
S 62	5.9	20.6		63.9		-	-	-
S 63	7.4	22.0		-		78.8	788,000	-
S 64	5.4	19.9	16.8			26.0	260,000	-
S 65	7.0	20.5	42.7			-	-	-
S 66	6.1	19.7		60.6		-	-	-
S 67	8.7	20.5		76.3		-	-	-
S 68	6.1	19.4		78.3		-	-	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Methanol content (ppm)	Glycerin content (% v/v)
			< 60	60-95	> 95			
S 69	5.6	19		92.6		46.8	468,000	-
S 70	5.3	19.3		64.1		-	-	-
S 71	6.8	20.0			-	-	-	-
S 72	7.4	20.5		70.8		-	-	-
S 73	6.1	21	51.9			-	-	-
S 74	5.7	19.1	58.8			-	-	-
S 75	5.6	19.6		60.1		-	-	-
S 76	6.3	20.8	51.1			-	-	-
S 77	5.7	20.2	56.7			-	-	-
S 78	6.5	20.5		62.6		-	-	-
S 79	7.9	19.7	48.6			34.5	345,000	-
S 80	6.8	20.5		83.3		-	-	-
S 81	6.0	20.6		94.2		-	-	-
S 82	7.8	19.3		74.5		-	-	-
S 83	6.5	20.4	42.6			-	-	-
S 84	7.1	20.5		75.6		-	-	-
S 85	5.8	20		67.6		18.3	183,000	-
S 86	7.0	19.4	54.9			-	-	-
S 87	7.6	20		70.3		-	-	-
S 88	7.1	20.2		93.7		-	-	-
S 89	7.1	20		72.6		-	-	-
S 90	6.2	19.8		77.2		-	-	-
S 91	6.7	20.2	54.0			-	-	-
S 92	5.5	20.2	53.6			-	-	-
S 93	7.0	19.8		67.5		-	-	-
S 94	7.5	20.2	59.4			-	-	-
S 95	6.8	19.9	55.6			-	-	-
S 96	6.9	20.2	21.5			-	-	-
S 97	6.6	20.4	10.4			10.3	103,000	-
S 98	7.7	20.4		68.6		-	-	-
S 99	6.9	20.3	50.7			8.0	80,000	-
S 100	7.0	20.6		66.8		-	-	-
S 101	7.2	20.1		64.0		-	-	-
S 102	7.4	19.3	47.0			-	-	-

SAMPLE CODE	pH	SAMPLE TEMP (ºC)	PERMITTED ALCOHOLS (% v/v)			Methanol Content (% v/v)	Methanol content (ppm)	Glycerin content (% v/v)
			< 60	60-95	> 95			
S 103	5.5	19.5		-		67.4	674,000	-
S 104	7.6	19	23.8			49.6	496,000	-
S 105	6.5	20.6	51.4			-	-	-
S 106	7.0	22.7	56.3			-	-	-
S 107	5.7	19.9		60.3		-	-	-
S 108	7.3	20.2		60.4		-	-	-
S 109	7.0	20.3	34.1			-	-	-
S 110	5.7	20.7		62.5		-	-	-
S 111	6.5	20.1		59.9		-	-	-
S 112	6.0	20.3	38.9			34.9	349,000	-
S 113	7.4	20.6		89.6		-	-	-
S 114	7.1	20.3	58.1			-	-	-
S 115	6.0	20.4	53.5			19.6	196,000	-
S 116	7.3	19.7	58.8			-	-	-
S 117	6.8	20.2	12.3			53.4	534,000	-
S 118	7.3	20.5	30.5			-	-	-
S 119	5.5	19.7		66.7		-	-	-
S 120	6.8	19.9	31.6			-	-	-
S 121	5.7	19.3		63.6		-	-	-
S 122	6.9	20.1			-	-	-	-

- Not Detected ABHS-Alcohol Based Hand Sanitizer TEMP-Temperature

4.3.1 Methanol content

Methanol contamination was observed in 16 (13.1%) samples while methanol substitution was recorded in 10 (8.2%) samples. The content was found to exceed the current FDA limits of 200 ppm (0.02% v/v) (FDA, 2021a). The KEBS specification does not set any limits on the methanol content in ABHS (KEBS, 2014b). Comparing these results with those obtained from a previous study carried out in the Kenyan market, the cases of methanol contamination have slightly decreased from 14.9% to 13.1% (Abuga, Nyamweya, *et al.*, 2021).

A recent study carried out at the Department of Chemistry, Kenyatta University used a Fourier Transform Infra-Red (FTIR) spectrophotometer to analyze ABHS. Based on the results, methanol contamination was observed in 41.7% of the samples. At the time of analysis using FTIR on ABHS only two of the samples exceeded the FDA temporary limits of 630 ppm

(0.063% v/v). Methanol substitution was not detected in any of the samples (Gacuiga *et al.*, 2022).

The CDC reported 15 cases of methanol toxicity associated with the intentional ingestion of ABHS in individuals with alcohol-use disorder over a two-month period in the year 2020. The ABHS were ingested to substitute ethanol. The observable effects included seizures, permanent vision loss and four patients died (Yip, 2020). Cases of unintentional ingestion are common in children where the amount ingested could be just a drop from the bottle or from licking the amount dispensed onto the hands. In such scenarios, the effects will range from mild stomach irritation causing nausea and vomiting (Rayar *et al.*, 2013).

4.3.2 Permitted alcohols

The KEBS specification describes the permitted alcohols used in the formulation of ABHS as ethanol and or *iso*propanol or *n*-propanol (KEBS, 2014b). The specification also sets the limit for alcohol content as ≥ 60% v/v. The results obtained are summarized in Table 4.3 where the mean alcohol content of samples within the permitted alcohol range was seen to be 72.2% v/v.

Table 4.3: Descriptive summary of ABHS analytical results

Alcohol content (% v/v)	Count	Mean ± SD (% v/v)
A. Sum of permitted alcohols (EtOH/ IPA)		
< 60	50	41.5 ± 17.6
60 – 95	54	72.2 ± 9.8
>95	3	97.9 ± 2.3
B. Methanol	26	42.8 ± 24.9
C. Glycerin	3	0.4 ± 0.2

In 50 (41%) samples the sum of permitted alcohols was less than the specified limit. The alcohol content in 67% of these 50 samples was found to be < 55% v/v. Samples with an alcohol content that is less than the specified amounts confer a false sense of confidence to the user as they are unable to meet the microbicidal activity. Fifty seven samples (46.7%) complied with assay limits

(≥ 60% v/v). These results present a worrying situation for the consumers in the Kenyan market similar to those in Ethiopia where a study carried out in 2020 showed that 70% of the samples analyzed failed to comply with the FDA specifications (Selam, 2020). Conversely in a Canadian study 41 (97.6%) samples had an ethanol concentration sufficient for virucidal activity (Tse *et al.*, 2021).

The FDA specifications defined a range of the alcohol content in ABHS (60% - 95% v/v) (Reynolds *et al.*, 2006). This range of alcohol concentration has been found to provide effective microbicidal cover, especially against the SARS-CoV-2 virus. In three samples (2.5%), the alcohol concentration was found to be more than 95% v/v. These products may not be effective following the reduced contact time on the hands surface as well as the presence of water required for the bactericidal effect of the alcohols to be actualized.

4.3.3 Glycerin and propylene glycol content

The KEBS does not make any specifications on glycerin in ABHS. Its primary role in the composition of ABHS is to act as a humectant to counter the drying effect of alcohols on the skin surface thus improving tolerance (Abuga, Nyamweya, *et al.*, 2021). A delicate balance in its concentration has to be attained because glycerin has been shown to counter the antimicrobial effect of alcohols. The recommended concentration range of 0.5% - 0.73% v/v has been shown to be ideal (Berardi *et al.*, 2020; Menegueti *et al.*, 2019). Glycerin was quantified in only three (2.4%) samples whereby only one sample had glycerin content within the specified range (0.61% v/v).

Propylene glycol (PEG), a thickening agent was identified in 11 (9%) samples. Its content was expressed as glycerin since a reference standard was unavailable. By increasing the viscosity of ABHS, there is prolonged contact time on the hand surface which enhances the microbicidal effect (Villa *et al.*, 2021). The PEG content was 4.09% v/v which falls within the recommended content range of 2% -5% v/v (Berardi *et al.*, 2020).

CHAPTER FIVE

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The main objective of this study was to determine the quality of commercially available ABHS against limits specified by KEBS. This considers aspects of the appearance, labelling, pH as well as alcohol composition and content of these products. With regard to the geographical profiling of the samples, 93 (76.2%) were indicated to be manufactured locally.

Only 10 (8.2%) samples, all locally manufactured met all the specifications described. Therefore, 112 (92%) samples could be considered substandard and falsified products. Seventy seven (63.1%) samples met the packaging and labelling specifications and more than half of these samples were of local origin. The importance of the presence of the relevant information cannot be understated especially the cautionary instructions. The use of ABHS which contain the non-permitted alcohols can also have dangerous effects such as lowered blood sugar and in extreme cases seizures or coma in children (Soloway, 2021).

Methanol substitution was identified in 10 (8.2%) samples, all of which were locally manufactured. A similar finding was observed in the samples with respect to methanol contamination where 16 (13.1%) samples were locally manufactured.

Of the 54 (44.2%) samples that were found to be within the specified range of alcohol content (60% - 95% v/v), 37 (68.5%) were locally manufactured. The 50 (41%) samples whose alcohol content was found to be < 60% v/v, 41 (72%) were locally manufactured. All seven of the samples that were found to be completely devoid of any of the permitted alcohol were locally manufactured. This presents a worrisome trend amongst the locally available manufacturers as these products were marketed and labelled as ABHS while they are of inferior quality. Two of these potentially falsified products were found to have a Standardization mark and their permit status indicated as valid at the time of sampling. This could indicate a possible error during the manufacturing process. However, in five of the remaining samples, the Standardization mark

was not visible at the time of sampling suggesting that they could be substandard/falsified products.

5. 2 Recommendation

Based on these findings, recommendations can be made to the regulatory authority KEBS, specifically on the technique used in determining the alcohol content. The KEBS specified method for identification and quantification of alcohol content is non-specific (KEBS, 2014a). The negligible difference in the specific gravity of alcohols leads to an inability in distinguishing between the permitted alcohols and contaminants such as methanol (Wade, 2021). Thus, there is a need for more sensitive and specific analytical methods developed in the hand sanitizer specifications. The specific analytical method could be applied for the identification and quantification of the alcohols. In the analytical method described in this study, GC was coupled to an MS/FID detector for identification and quantification of the volatiles as well as contaminants present. The method was also able to provide a profile of the other constituents such as glycerin, propylene glycol and polyethylene glycol.

Despite this study being focused on assessing the quality of ABHS, the analytical method used can be applied in the quantification of alcohol content in alcoholic beverages and the identification of contaminants. For instance, the harmful effects of methanol would be more pronounced based on the amount of alcoholic beverages consumed and the absorption rate (Pressman *et al.*, 2020).

KEBS should put into place a more stringent standardization process to be able to capture ABHS in the market that have a different coding system which did not allow for verification of details. This also underscores the need for post market surveillance to capture the presence of counterfeits or falsified products.

Additionally, a stricter safeguard of the manufacturing practices, especially by the local manufacturers in the sourcing of raw materials is required. The use of contaminated raw materials could result in the methanol contamination observed in 16 samples. Alcohols are highly volatile therefore it is important to ensure that the containers and dispensing mechanism do not allow for evaporation of the alcohol to sub-optimal levels thus yielding ineffective

products in the market. There is a need for more studies to be carried out to determine the robustness of the GC method used in this study and its widespread applicability.

5.3 Study Limitations

This study was limited by convenient sampling at retail shops, supermarkets and pharmacies. It is also possible that the quality of ABHS may be affected based on the environmental conditions at the point of purchase. The study did not take into consideration the environmental conditions at the time of purchase and how they affect the quality of ABHS.

Commercial ABHS are not the only hand sanitizer products available to the public. During the COVID-19 pandemic, individual institutions such as hospitals and schools produced ABHS for internal use. This study did not sample these products for quality testing.

Since only one batch per ABHS product was sampled for analysis, batch variations could not be assessed. Furthermore, the regulator (KEBS) and individual manufacturers were not contacted for verification of products suspected to be counterfeit.

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APPENDICES

Appendix 1: Analysis of volume, country of origin and SM status of ABHS

SAMPLE CODE	COUNTRY	PRICE IN KES	VOLUME (ml)	SM NUMBER	SM STATUS
S 1	NOT INDICATED	150	120	42035	NOT VALID
S 2	LOCAL	150	100	43073	VALID
S 3	INTERNATIONAL, UGANDA	150	100	NOT INDICATED	
S 4	LOCAL	150	100	42295	NOT VALID
S 5	LOCAL	150	120	41706	VALID
S 6	LOCAL	170	120	47018	VALID
S 7	INTERNATIONAL, UNITED ARAB EMIRATES	100	40	NOT INDICATED	
S 8	LOCAL	250	120	41555	VALID
S 9	LOCAL	150	65	NOT VISIBLE	
S 10	LOCAL	150	65	NOT VISIBLE	
S 11	LOCAL	100	NOT INDICATED	41722	VALID
S 12	LOCAL	135	60	41655	VALID
S 13	LOCAL	150	50	41988	NOT VALID
S 14	LOCAL	60	65	42036	NOT VALID
S 15	LOCAL	50	65	34404	VALID
S 16	NOT INDICATED	100	50	NOT INDICATED	
S 17	LOCAL	100	50	41888	VALID
S 18	LOCAL	100	50	41697	NOT VALID
S 19	LOCAL	120	100	10594	NOT VALID
S 20	LOCAL	100	100	41747	NOT VALID
S 21	LOCAL	50	50	43408	VALID
S 22	LOCAL	100	60	41856	VALID
S 23	LOCAL	125	50	22761	NO RESPONSE FROM KEBS
S 24	NOT INDICATED	85	50	41814	VALID
S 25	INTERNATIONAL, UNITED KINGDOM	80	60	NOT VISIBLE	
S 26	NOT INDICATED	70	60	NOT VISIBLE	
S 27	LOCAL	100	50	NOT VISIBLE	
S 28	LOCAL	100	50	25528	VALID
S 29	LOCAL	100	100	NOT VISIBLE	

SAMPLE CODE	COUNTRY	PRICE IN KES	VOLUME (ml)	SM NUMBER	SM STATUS
S 30	LOCAL	32	65	41971	VALID
S 31	LOCAL	60	50	28669	VALID
S 32	LOCAL	100	50	NOT VISIBLE	
S 33	LOCAL	50	50	41827	VALID
S 34	LOCAL	100	100	41769	VALID
S 35	LOCAL	100	100	49862	NO RESPONSE FROM KEBS
S 36	LOCAL	150	NOT INDICATED	41782	NOT VALID
S 37	LOCAL	100	50	42091	NOT VALID
S 38	LOCAL	200	100	42031	VALID
S 39	LOCAL	100	100	41957	VALID
S 40	LOCAL	180	100	39575	VALID
S 41	NOT INDICATED	50	50	NOT INDICATED	
S 42	LOCAL	80	100	15073	VALID
S 43	NOT INDICATED	50	50	NOT VISIBLE	
S 44	NOT INDICATED	60	50	NOT INDICATED	
S 45	NOT INDICATED	150	100	NOT INDICATED	
S 46	LOCAL	100	50	39217	NOT VALID
S 47	LOCAL	80	50	41558	VALID
S 48	LOCAL	150	100	32973	VALID
S 49	LOCAL	100	50	33588	VALID
S 50	LOCAL	100	100	NOT VISIBLE	
S 51	LOCAL	100	50	NOT VISIBLE	
S 52	NOT INDICATED	70	50	46099	NOT VALID
S 53	LOCAL	65	50	40795	NOT VALID
S 54	INTERNATIONAL, UGANDA	100	60	NOT INDICATED	
S 55	LOCAL	50	30	29130	VALID
S 56	LOCAL	100	60	45066	VALID
S 57	INTERNATIONAL, UGANDA	100	60	NOT INDICATED	
S 58	LOCAL	100	50	46836	VALID
S 59	LOCAL	100	100	42908	VALID
S 60	LOCAL	85	100	44416	NOT VALID
S 61	LOCAL	100	60	35174	VALID

SAMPLE CODE	COUNTRY	PRICE IN KES	VOLUME (ml)	SM NUMBER	SM STATUS
S 62	INTERNATIONAL, CHINA	100	50	NOT INDICATED	
S 63	LOCAL	120	50	42031	VALID
S 64	LOCAL	55	50	41785	VALID
S 65	LOCAL	100	65	46054	NOT VALID
S 66	LOCAL	59	50	41755	VALID
S 67	LOCAL	50	50	44884	VALID
S 68	LOCAL	100	100	41742	VALID
S 69	LOCAL	220	100	1670	NOT VALID
S 70	LOCAL	100	60	23058	VALID
S 71	INTERNATIONAL, TURKEY	65	50	NOT INDICATED	
S 72	LOCAL	150	65	42604	NOT VALID
S 73	LOCAL	50	50	44237	VALID
S 74	LOCAL	65	50	40901	VALID
S 75	LOCAL	100	100	41861	NOT VALID
S 76	LOCAL	150	NOT INDICATED	NOT INDICATED	
S 77	NOT INDICATED	180	50	NOT VISIBLE	
S 78	LOCAL	100	50	NOT VISIBLE	
S 79	INTERNATIONAL, CHINA	200	100	UCR202002122498	
S 80	LOCAL	150	NOT INDICATED	42153	VALID
S 81	LOCAL	100	NOT INDICATED	43639	VALID
S 82	LOCAL	100	50	43350	VALID
S 83	LOCAL	100	60	45090	VALID
S 84	LOCAL	100	65	2834	VALID
S 85	LOCAL	150	50	23122	VALID
S 86	LOCAL	80	50	43156	VALID
S 87	NOT INDICATED	300	100	NOT VISIBLE	
S 88	LOCAL	100	50	44198	NOT VALID
S 89	LOCAL	110	100	29256	VALID
S 90	LOCAL	100	60	41831	VALID
S 91	LOCAL	75	50	34757	VALID
S 92	LOCAL	100	50	34524	NOT VALID

SAMPLE CODE	COUNTRY	PRICE IN KES	VOLLUME (ml)	SM NUMBER	SM STATUS
S 93	LOCAL	70	65	41784	NOT VALID
S 94	INTERNATIONAL UNITED KINGDOM	100	60	39505	VALID
S 95	LOCAL	100	60	25932	VALID
S 96	INTERNATIONAL, UNITED KINGDOM	80	60	42284	VALID
S 97	LOCAL	100	50	NOT INDICATED	
S 98	LOCAL	50	50	28431	VALID
S 99	LOCAL	50	50	42693	VALID
S 100	LOCAL	105	60	41696	VALID
S 101	INTERNATIONAL, INDIA	100	100	NOT INDICATED	
S 102	LOCAL	30	30	47521	VALID
S 103	LOCAL	65	50	27188	VALID
S 104	LOCAL	50	50	43359	NOT VALID
S 105	LOCAL	100	100	41686	VALID
S 106	LOCAL	55	50	46438	VALID
S 107	LOCAL	200	60	12024	NOT VALID
S 108	INTERNATIONAL, CHINA	150	50	UCR202002171326	
S 109	LOCAL	45	50	23122	VALID
S 110	LOCAL	50	50	45316	NOT VALID
S 111	INTERNATIONAL, CHINA	250	100	UCR201901512323	
S 112	LOCAL	100	50	42171	VALID
S 113	LOCAL	50	50	NOT VISIBLE	
S 114	NOT INDICATED	87	65	41665	VALID
S 115	LOCAL	60	60	43071	VALID
S 116	LOCAL	150	100	NOT INDICATED	
S 117	INTERNATIONAL, TURKEY	65	50	NOT INDICATED	
S 118	LOCAL	50	50	43409	NOT VALID
S 119	LOCAL	230	65	NOTINDICATED	
S 120	LOCAL	200	50	41840	VALID
S 121	NOT INDICATED	100	100	NOT VISIBLE	
S 122	INTERNATIONAL, UNITED KINGDOM	80	60		

Appendix 2: Photographs of the ABHS samples used in the study