ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2024.21

The Nigerian Journal of PHARMACY
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927
AS MEN OF HONOUR WE JOIN HANDS

Alcohol-Based Hand Sanitizers: Analysis for Content of Ethanol and Contaminants

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ARTICLE INFO

Article history:

Received 19th July 2024
Revised 8th October 2024
Accepted 9th October 2024
Online Published

Keywords:

Sanitizers;
Ethanol;
Contaminants;
GC-FID;
Compliance;
Safety

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ABSTRACT

Background: Alcohol-Based hand sanitizers (ABHS) are used as hand hygiene products because of the broad-spectrum activity of ethanol (and some other alcohols) against pathogenic microorganisms. They are effective in curbing the spread of infectious diseases such as Covid-19 and other viral, bacterial and fungi infections but the ethanol concentration has to be within a specified range. Some ABHS may contain toxic contaminants and the most prevalent ones are methanol and acetaldehyde. There is therefore a need for routine testing of the products for the content of ethanol and contaminants to ensure effectiveness and safety.

Methods: Thirteen samples of commonly available ethanol-based hand sanitizers were sourced randomly for shops and supermarkets in the Lagos metropolis of Lagos State, Nigeria. The samples were analysed for the content of ethanol, methanol and acetaldehyde using gas chromatography coupled with flame ionization detector (GC-FID). Values obtained were compared with label claims of the manufacturers for ethanol and with regulatory limits for the contaminants.

Results: The study reveals that 69% of the samples were within the WHO recommended range of 60-95 % v/v of ethanol concentration. Ethanol content was not stated on the labels of two of the samples and seven samples did not have NAFDAC numbers on their labels. One of the samples was contaminated with methanol and the concentration was above regulatory limits. Acetaldehyde was not found in any of the samples.

Conclusion: Some of the alcohol-based hand sanitizers in Lagos market are sub-standard, containing ethanol in concentrations below recommended range. There is also a potential toxicity risk posed by a small percentage that contains contaminants like methanol. This indicates a need for more regulatory control of the production, distribution and sale of sanitizer products for effectiveness and public safety.

Introduction

Alcohol-based hand sanitizers (ABHS) are the most widely used hand hygiene products worldwide[1]. They became indispensable following the outbreak of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2; also known as COVID-19) in 2019 and its attainment of the status of a pandemic in year 2020. They are used for their antimicrobial activity to keep hands free from pathogenic microorganisms and stem the spread of COVID-19 and other infectious diseases[1,2]. Their widespread usage in public places and shared facilities led to a surge in demand for the products and their raw materials. Even after the pandemic was declared over, ABHS remained in use for hand hygiene and prevention of community transmission of infectious diseases and are consequently still in the market.

Non-alcohol-based hand sanitizers (NABHS) are alcohol-free as they are formulated using other antimicrobial agents such as quaternary ammonium compounds like benzalkonium chloride, chlorhexidine and phenolic compounds like 2,4,4'–trichloro-2'-hydroxydiphenyl ether (triclosan). They are also used as hand hygiene products due to their biocidal effects but they are less popular than ABHS[3,4]

Ethanol is the main active ingredient of ABHS. It acts by denaturing the proteins in cell plasma membranes, thus exhibiting a broad spectrum of activity against bacteria, fungi and viruses[2,5]. Other alcohols that may be used include isopropanol and n-propanol but ethanol causes less irritation to the skin than the other alcohols[6]. The concentration range of alcohol for optimum germicidal activity is 60-95%, with activity increasing with concentration[7]. However, efficacy is reduced at concentrations above 95% because enough water is required for protein denaturation and the contact time of product on hands is reduced due to quicker evaporation of products of higher concentrations[8].

The efficacy of ABHS may be affected by many factors including the concentration of the active ingredient (ethanol), formulation, volume used and contact time; contact time of 20 -30 seconds and product volume of 2 - 3 mL are usually recommended for optimum biocidal activity[8,9]. Apart from efficacy, safety is an important factor in the use of ABHS. The raw materials for producing these sanitizers have to comply with the specifications of Food Chemicals Codex (FCC) or the United States Pharmacopeia (USP). They are regulated by governmental agencies to ensure minimum presence of impurities. Pharmaceutical or food-grade ethanol are the recommended grades for ABHS production as they comply with FCC and USP specifications. Technical-grade ethanol may be used in special circumstances such as increased demands during public health emergencies but it often contains higher concentrations of impurities, the most common being methanol, ethyl acetate and acetaldehyde[10,11]. Other impurities may be introduced during formulation and packaging.

The brands of ABHS circulating in the Nigerian market are either in gel form or sprays (liquid form). Depending on the concentration of ethanol and frequency of use, these products can cause potential dermal absorption and inhalation hazards; oral exposure is possible only by accidental ingestion. Hence the CDC recommends that in private homes and routine community settings, ABHS should be used only when there is no easy access to soap and water[12]. However, studies have shown cutaneous absorption of ethanol from AHBS but not at pharmacologically relevant levels, even with repeated and prolonged use, meaning that the ethanol content in the recommended range is generally safe for consumers[13,14].

Impurities in low quality raw materials eventually transform into contaminants in the final products. Therefore, the quality of ABHS involves not only the alcohol content but also the level of contaminants. Some contaminants are known to be highly toxic while others are carcinogenic and teratogenic. Methanol can cause skin irritation and abrasion, as well as ocular, metabolic and neurologic toxicity. Chronic exposure to methanol can cause defatting of the skin while its metabolite, formic acid can cause organ damage[10,15]. Accidental ingestion of ABHS containing methanol can lead to severe adverse effects including headache, nausea, vomiting, blurred vision, abdominal pain, decreased consciousness seizures, and on occasions, death[16,17,18]. Acetaldehyde, another potential contaminant in ABHS is a likely human carcinogen, in addition to its teratogenic and mutagenic effects in animal models[15]. It is therefore of utmost importance that the production of ABHS comply with regulatory requirements to ensure consumer safety and efficacy of products, thereby safeguarding public health.

Some studies on the quality of hand sanitizers in Nigeria have analysed samples from North Central Nigeria and the Federal Capital Territory and some have focused on anti-bacterial effectiveness and effect of viscosity[19,20,21] The objective of this study was to analyse brands of commonly available ABHS in Lagos market for the content of ethanol, methanol and acetaldehyde and compare values with manufacturers' claims and regulatory limits.

Materials and Methods

Equipment and Reagents

Agilent GC 7820A coupled with a split/splitless manual injector/Flame Ionization Detector/Agilent Chemstation software, a bonded crosslinked Agilent J&W DB-624 (6 % cyanopropyl phenyl and 94 % polydimethylsiloxane) column, micropipette (10 - 200 µl) and tips, 10 µL syringe, absolute ethanol (99.99%) from EMSURE ACS grade, HPLC Grade acetonitrile and methanol (99.9%) from Lichrosolv (Germany), standard acetaldehyde (99.5%) from EMSURE ACS Reagent Ph Eur., distilled water.

Collection and Preparation of Samples

Samples of thirteen commonly available brands of alcohol-based hand sanitizers (ABHS) were sourced randomly from shops and supermarkets in the Lagos metropolis of Lagos State, Nigeria. Three of the samples were in liquid form (spray) and ten were in gel form. They were all within their shelf lives and all were ethanol-based. The liquid sanitizers were code-labelled SL1 to SL3 and gel brands SG1 to SG10. Their label information was observed and recorded before storing at room temperature until use.

Samples were prepared for analysis using the methods of Tse et al[22]. Each sample (4 mL) was weighed into a 100 mL volumetric flask and 50 mL of distilled water was added followed by mechanical shaking. Acetonitrile standard solution (10 mL) was added and the mixture was made up to the mark with distilled water. The gel samples were filtered before passing all sample solutions through Nylon 0.45 µm syringe filters prior to injection into the gas chromatograph.

Preparation of Standard Solutions

A stock standard solution of ethanol (10% v/v) was prepared and serially diluted with distilled water to 5, 2, 1, 0.5 and 0.2 % v/v ethanol standard solutions. Acetonitrile (0.1% v/v) was added as an internal standard to each of the standard solutions for preparation of the standard calibration curve. From a 1% v/v stock solution of methanol, 0.5, 0.2, 0.1, 0.05 and 0.02 % containing 0.1% w/v of acetonitrile were prepared for calibration curve. Standard calibration solutions of acetaldehyde containing 0.5, 0.2, 0.1, 0.05 and 0.02 % with 0.1% v/v of acetonitrile were also prepared.

GC Analysis

Analysis was carried out using GC with flame ionization detector following the USP Method IIb[23] for alcohol determination and Tse et al method for contaminants with slight modification[22,24]. The gas chromatographic conditions were as follows: The inlet temperature was set at 140C with split ratio of 40:1; Nitrogen gas was used as the carrier gas at a flow rate of 2.5 mL/min; the stationary phase was composed of 6 % cyanopropyl phenyl and 94 % polydimethylsiloxane; the oven temperature was set initially at 40C for the first 5 minutes then ramped to 225 C at a rate of 20C/min, and then kept at 225C for another 2.5 min; an equilibration time of 5 minutes was set between injections; the FID temperature was set at 250C; hydrogen and air flow were set at 30 mL/min and 300 mL/min respectively; the total run time was 16.75min.

Samples and calibration standards solutions (1 µL each) were injected manually into the GC using a 10 µL syringe. Each solution was run in triplicates and the mean and standard deviation calculated.

Statistical Analysis

Descriptive statistics using tables, means and percentages were used to analyse the data obtained. Area ratios of ethanol and contaminants in the samples were expressed as mean ± standard deviation. Concentrations of ethanol were compared with label claims of the manufacturers and the values for contaminants were compared with regulatory limits using Student t-test at $p < 0.05$.

Results

The results of this study show that the ethanol content of the samples ranged from 30 to 86 % v/v as against the manufacturers' claims of 62 to 80% v/v on the labels. For two of the sample, ethanol content was not stated on the label. Four of the samples did not meet the WHO recommended range of 60-95% v/v for ethanol content.

Seven of the samples did not have NAFDAC numbers on their labels and one of them contained methanol in concentrations above regulatory limits. Acetaldehyde was not detected in any of the samples. In summary, 69% of the samples complied with regulatory standard for ethanol content and were also free from contaminants.

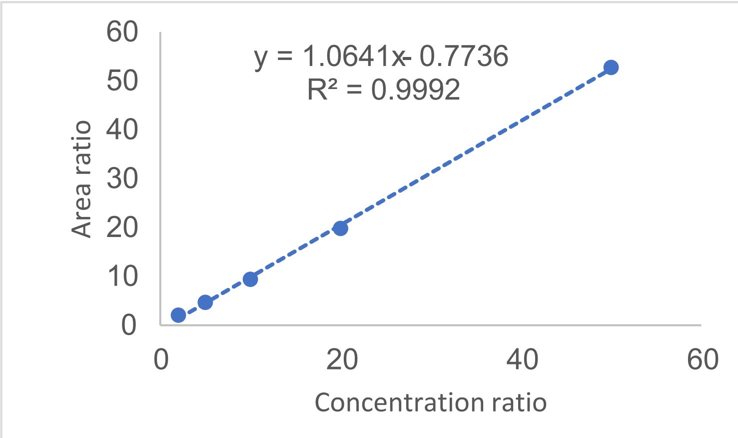
The code names of the ABHS samples studied and their observed label information are presented in Table 1. Three of the samples were in liquid/spray form and ten were in gel form. All samples were ethanol-based.

Tables 1: Code names and label information of the samples

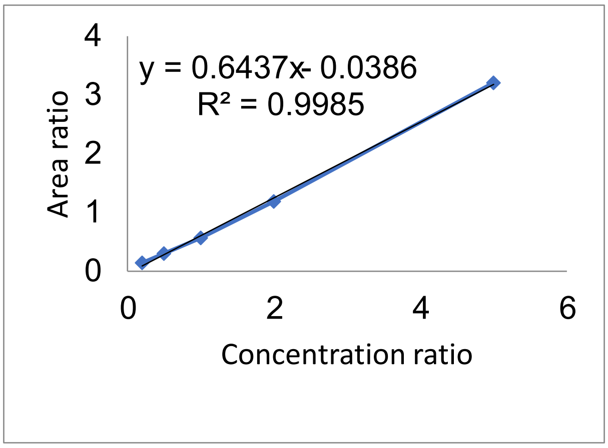
S/N		Sample Code	Sanitizer Form	NAFDAC NO.	Label Claim for Ethanol (% v/v)
1.	2SURE	SL1	Spray	03-7476	70
2.	STERIHANS	SL2	Spray	-	70
3.	THEP	SL3	Spray	-	70
4.	2SURE	SG1	Gel	03-7477	70
5.	ARCHY’S	SG2	Gel	-	62
6.	CHAIS	SG3	Gel	-	70
7.	DETTOL	SG4	Gel	-	Not Stated
8.	GERM-X	SG5	Gel	-	62
9.	LIFEBUOY	SG6	Gel	03-7340	70
10.	SIMPLY LOVELY	SG7	Gel	03-8013	75
11.	STERIHANS	SG8	Gel	-	80
12.	SUNSHINE	SG9	Gel	03-7494	70
13.	WIND	SG10	Gel	03-3275	Not Stated

Standard solutions of ethanol, methanol and acetonitrile were run and used to plot standard calibration curves as presented in Figure 1. The line equations were used to calculate the concentrations of ethanol, methanol and acetonitrile in the samples and the results are shown in Table 2. Figure 2 presents the chromatograms of four of the samples.

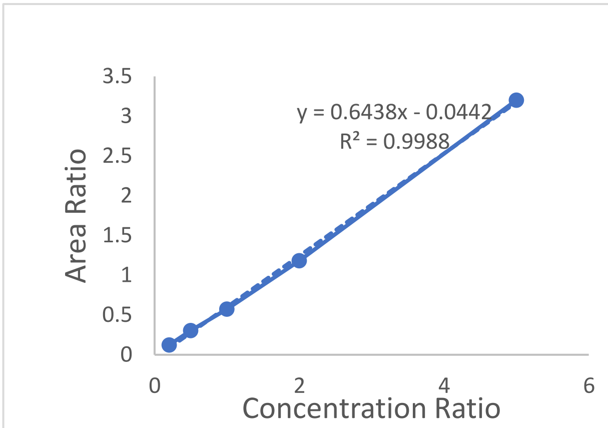
Figure 3 presents a comparison between the concentrations of ethanol in the samples with the label claims of the manufacturers while Figure 4 is a display of the proportion of compliant and non-compliant sanitizer samples.



A - Ethanol



B - Methanol



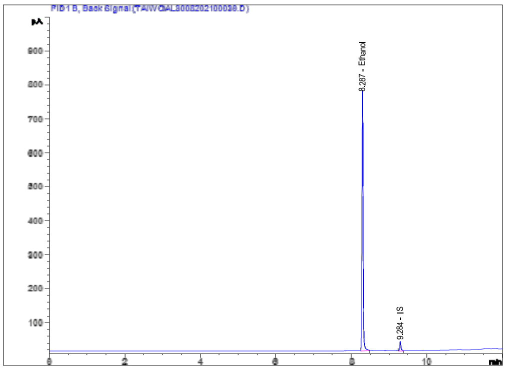
C - Acetaldehyde

Figure 1: Standard calibration curves for ethanol, methanol and acetaldehyde.

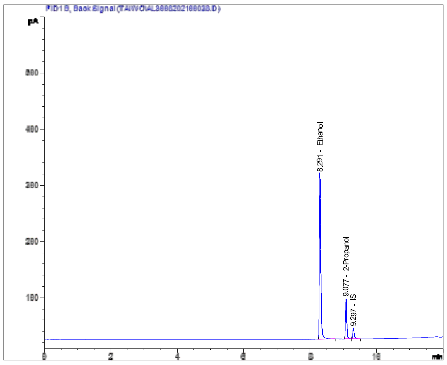
Table 2: Concentrations of ethanol, methanol and acetaldehyde in the samples

S/N	Sample Code	Mean Area Ratio (± SD) Ethanol	Methanol	Acetaldehyde	Concentration (% v/v) Ethanol	Methanol	Acetaldehyde
1.	SL1	27.96 ± 0.131	-	ND	85.5	-	ND
2.	SL2	27.96 ± 0.184	-	ND	85.5	-	ND
3.	SL3	9.28 ± 0.058	-	ND	30.0	-	ND
4.	SG1	28.04 ± 0.105	-	ND	85.7	-	ND
5.	SG2	20.50 ± 0.118	-	ND	65.2	-	ND
6.	SG3	10.50 ± 0.294	1.46 ± 1.520	ND	33.5	7.374	ND
7.	SG4	20.55 ± 0.262	-	ND	65.4	-	ND
8	SG5	12.48 ± 0.141	-	ND	40.0	-	ND
9.	SG6	24.72 ± 0.219	-	ND	75.0	-	ND
10.	SG7	15.78 ± 0.183	-	ND	49.6	-	ND
11.	SG8	28.19 ± 0.248	-	ND	86.0	-	ND

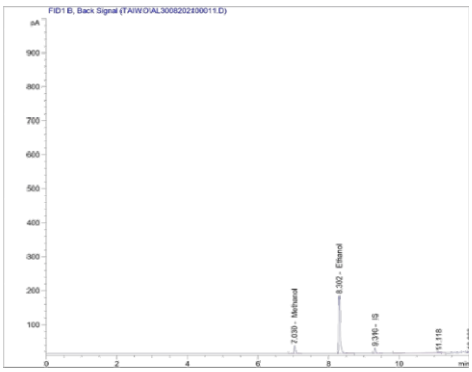
*ND = Not Detected



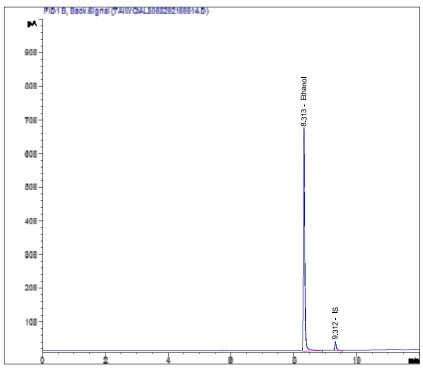
A – Sample SL2



C – Sample SG5



B – Sample SG2



D – Sample SG8

Figure 2: Chromatograms of four of the samples. (D)

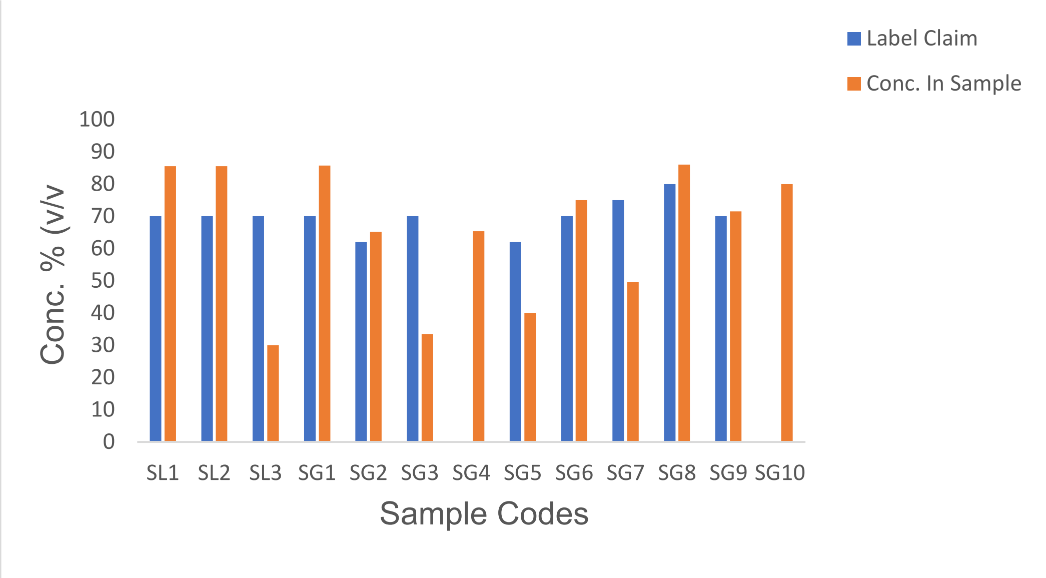


Figure 3: Concentrations of ethanol in samples compared to label claim

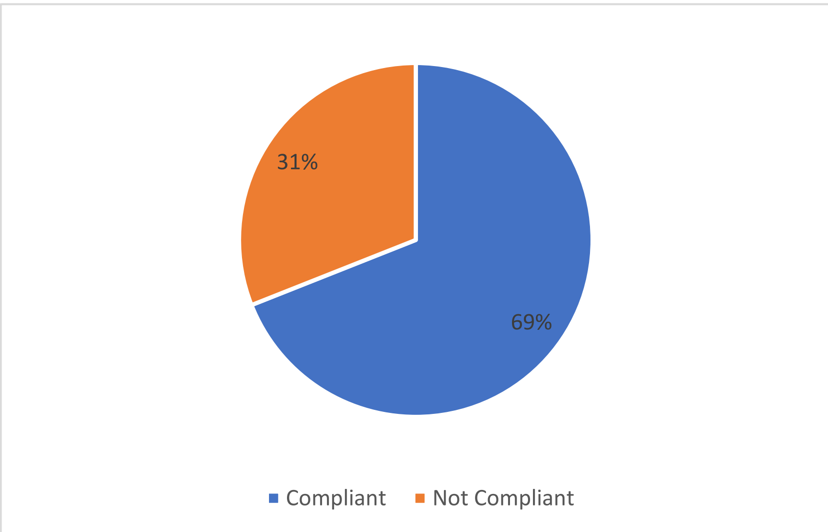


Figure 4: Chart showing proportions of compliant and non-compliant samples with regard to ethanol content

Discussion

Alcohol-based hand sanitizers (ABHS) are useful hand hygiene products required to curb the spread of infectious diseases such as Covid-19. The concentration of alcohol in ABHS has to be within the specified range to be effective and contaminants have to be at minimum level to be safe for use. Ethanol is the most commonly used alcohol in ABHS products. This study determined the concentration of ethanol in different brands of ABHS in Lagos market and quantified two common contaminants associated with the products.

The method of gas chromatography coupled to a flame ionization detector (GC-FID) used in this study was suitable giving linear standard calibration curves and regression coefficients of 0.9992, 0.9985 and 0.9988 for ethanol, methanol and acetaldehyde respectively. This technique has also been used by a number of researchers to measure ethanol and contaminants in ABHS[22,23]. The use of acetonitrile as internal standard in this study helped to eliminate errors and further improve accuracy.

The results of this study revealed that nine out of the thirteen brands (69%) complied with the WHO recommendation of 60-95% v/v of alcohol for effectiveness. The concentrations ranged from 65 to 86% v/v. However, in a previous study in 2022, Obi *et al* found that 78% of alcohol-based hand sanitizers circulation in Federal Capital Territory (FCT), Nigeria contained less than 60% alcohol[19]. Seven samples were above the concentrations of ethanol stated on their labels but for SG4 and SG10, ethanol concentration was not stated on the label. The differences between the concentrations of ethanol found in samples SL1 and SL2 and the respective label claims of the products were significant ($p < 0.05$). These differences may arise due to measurement errors during the formulation process. However, since the alcohol content of these samples met the regulatory standard, their effectiveness as antimicrobial hand sanitizers is not compromised. Also, none of the samples exceeded the upper regulatory limit of 95% v/v ethanol. The reduced water content would have limited the ability of ethanol to denature the cell plasma membrane proteins, which would have rendered the sanitizer ineffective.

Four of the samples (31%) had concentrations of ethanol ranging from 30 to 50 % v/v. This is an improvement on the 78% reported in a previous study[19]. These low concentrations fell below the label claims of the respective products as well as the regulatory limit of 60-95% v/v, and the difference was significant for all the four ($p < 0.05$). A similar study has found that 41% of ABHS in the Johannesburg area of South Africa were substandard, containing less than 60% alcohol[24]. Three of the non-compliant samples did not display NAFDAC numbers on their labels, which may be an indication that they had not been approved for use at the time of the study. A total of seven samples (54%) had no NAFDAC numbers on their labels. This is similar to the report of Obi *et al* that 40% of alcohol-based hand sanitizers in the FCT had no evidence of regulatory control[19].

Methanol was detected in only one of the samples (0.8%). The amount detected was significantly higher than the FDA regulatory limit of 0.063% v/v. The contaminated sample (SG3) did not display a NAFDAC number and was also not compliant with regards to ethanol content. This indicates that it may not be under any form of regulatory control. Methanol contamination may arise from the use of substandard raw materials such as technical grade ethanol or some other errors during formulation. Technical grade ethanol has been shown to be more likely to contain methanol contaminant than food or pharmaceutical grade ethanol[10]. Methanol is a toxic organic substance which can be absorbed through the skin from hand sanitizers especially when used frequently. Transdermal exposure over time can lead to dose-dependent toxicity with symptoms such as central nervous system depression, headache, dizziness, nausea, lack of coordination, confusion, blurred vision, loss of sight and death[14,15].

Acetaldehyde was not detected in any of the samples studied. The contaminant was either not present or may have been present below detectable levels. Some studies have however, shown presence of acetaldehyde along with other contaminants in ABHS[22,25].

The presence of contaminants in ABHS exposes the users of the products to potential health hazards because of the toxic nature of the compounds. A study of a wider scope, involving more products from different parts of the country will however be required for a clearer picture of the quality of sanitizers available to the public in Nigeria.

Conclusions

This study revealed that 69% of alcohol-based hand sanitizers in Lagos market are in compliance with WHO requirement of 60-95% v/v alcohol concentration for biocidal activity, indicating their effectiveness. The concentration of ethanol in 31% of the samples ranged from 30 to 50% v/v. Such products are substandard and will not be effective disinfectants for use in hand hygiene. The alcohol content found in eleven of the samples did not match the label claims of the manufacturers while alcohol content was not stated on the labels of two of the samples.

Methanol, a toxic contaminant was detected in one of the samples in concentrations above the FDA limit for the contaminant. Such sanitizers constitute potential health hazards for the users of the products. Seven of the thirteen samples, including the contaminated sample had no NAFDAC numbers on their labels.

This study has contributed data to the quality of alcohol-based hand sanitizers circulating in Lagos, Nigeria. It also brings to fore, an urgent need for more market surveillance and routine testing of sanitizer products in circulation for the purpose of flushing out unregistered and substandard sanitizers in order to ensure efficacy and safety of the products for public use.

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