Analysis of ethanol content in commercially available Hand sanitizers in Oman using Gas Chromatography- A comparative study

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ABSTRACT: The demand and usage of alcohol-based hand sanitizers have immensely increased worldwide in recent times as a protective measure against COVID-19 and other infectious diseases. To ensure that the public and health workers get hand sanitizers with an alcohol percentage within the label stated range, quality control of these products must be carried out by the governmental authorities. The study was designed to determine the ethanol content of eight commercially available and commonly used hand sanitizer gels available in the Omani market. Eight hand sanitizer gels were collected from various community pharmacies and commercial stores in Oman. The Gas Chromatography-Flame Ionisation Detection (GC-FID) method was employed to analyse ethanol content as it is one of the easiest, most reliable and most accurate methods for the determination of volatile content. Various physical properties of the samples such as pH, density, and refractive index, were also evaluated for comparison purpose. Sample prices were also compared from a pharmacoeconomic point of view. The majority of the hand sanitizers fell within the WHO recommendation range, with ethanol levels in the range of 66% to 76%, except A-7, which was below the recommended concentration for pathogenic microbial inactivation. The five of the samples tested showed ethanol contents below the labelled claim, whereas two sanitizers, A-1 and A-2, had ethanol contents above the labelled claim. The hand sanitizers differed significantly in price and certain physical properties which are likely to influence consumer preferences. Although the hand sanitizer products met the WHO requirements for alcohol content, the authors recommend that the regulatory authority should do a frequent testing of the samples available in the market to ensure quality and safety of these products.

1. INTRODUCTION

Alcohol-based hand sanitizers are a useful alternative to hand washing with soap and can prevent the spread of infectious diseases. Health organisations such as the Centres for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend the use of alcohol-based hand sanitizers in healthcare and the community to maintain good hand hygiene, as these can effectively reduce the microbial load on hands (CDC, 2023; WHO, 2023). Nevertheless, alternative options exist for sanitation purposes, including non-alcohol-based (NAB) sanitisation products.

COVID-19, a highly contagious viral illness, has had a significant influence on global demand for personal hygiene products such as soaps, hand soap, hand sanitizers, tissue papers, and other items. The production and sales of alcohol-based hand sanitizers surged during the COVID-19 pandemic and that demand remains high to this day (Glockler et al., 2022). The preference for alcohol-based sanitizers over non-alcohol-based (NAB) sanitization products among the public stems from several factors. These include their broad spectrum of activity against various pathogens, swift antimicrobial action, ease of use due to minimal or no residue left on surfaces post-application, and adherence to regulatory standards and guidelines set forth by the US Food and Drug Administration (FDA) and the WHO to assess their compliance. However, the flammability and potential for skin irritation, skin dryness, dermatitis, etc., associated with ethanol are notable limitations of alcohol-based

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sanitizers (Abuga & Nyamweya, 2021a; Kampf et al., 2020). The WHO also recommended alcohol-based sanitizers as the primary method for maintaining hand hygiene to mitigate the transmission of the COVID-19 pandemic, owing to their superior effectiveness and convenience (Abuga & Nyamweya, 2021a; Golin et al., 2020). A research study conducted in the US demonstrated the ability of commercially available foam and gel alcohol-based sanitizers (ABHS) to effectively neutralise the SARS-CoV-2 virus (Leslie et al., 2021). Because the effectiveness of alcohol-based hand sanitizers depends on the ethanol or isopropanol content, the CDC recommends the use of alcohol-based hand sanitizers greater than 60% ethanol or 70% isopropanol as the preferred form of hand hygiene, whereas the WHO recommends the strength of 80% ethanol or 75% isopropanol as standard for hand hygiene care. However, the quality and safety of commercially available hand sanitizers is a major concern for the regulatory authorities because many studies reported that the ethanol content of hand sanitizers in tested samples varies both within and between countries (Selam, 2020; Selam et al., 2022).

For example, several studies conducted during the COVID-19 pandemic revealed that a good number of hand sanitizers on the market had lower ethanol content than specified limits. Thus the US FDA issued product alerts for several hand sanitizer products from domestic and international manufacturers (FDA, 2022). A study done in South Africa found that a large proportion of hand sanitizers had ethanol content below the required limit (Govender et al., 2022). Jairoun et al., reported hand sanitizers with alcohol concentrations below 60%, and some of which contained methanol as an impurity (Jairoun et al., 2021).

Despite the importance of hand sanitizers and the health risks associated with low ethanol content, no study has yet assessed the ethanol content of commercially available hand sanitizers in Oman. The Gas chromatography- Flame ionization detector technique (GC-FID) is one of the rapid and effective methods for the determination of the alcohol contents in hand-based sanitizers. Therefore, this study aims to determine the alcohol content in hand sanitizers using gas chromatography to compare the ethanol content of different brands available in the Omani market.

2. MATERIALS AND METHODS

2.1. Sample collection

Eight hand sanitizers were collected from different local pharmacies and commercial stores in Muscat and Salalah in the Sultanate of Oman. These samples were coded (A-1 to A-8) to hide the identity of manufacturers and avoid any conflict of interest. Table 1 presents descriptions of the eight hand sanitizers chosen for the study.

2.2. Determination of physical properties

Hand sanitizers were evaluated for the following physicochemical properties for comparison purpose.

2.2.1. pH measurement

The acidity of hand sanitizers was measured by using a calibrated digital pH/temperature laboratory bench meter (Mi151, Milwaukee, Romania) at the room temperature.

2.2.2. Density measurement

A benchtop Abbe 60/DR refractometer (Bellingham + Stanley Limited, UK) was used to measure RI, a physical property, to know how fast light propagates through the hand sanitizers at room temperature. The RI was then calculated at 20°C by using the following equation:

\[ \text{RI at } 20^\circ \text{C} = ((T-20) \times 0.00045) + \text{RI observed at the room temperature (T)}, \]

where T refers to the room temperature at which RI was measured.

2.2.3. Refractive index (RI measurement)

A benchtop Abbe 60/DR refractometer (Bellingham + Stanley Limited, UK) was used to measure RI, a physical property, to know how fast light propagates through the hand sanitizers at room temperature. The RI was then calculated at 20°C by using the following equation:

\[ \text{RI at } 20^\circ \text{C} = ((T-20) \times 0.00045) + \text{RI observed at the room temperature (T)}, \]

where T refers to the room temperature at which RI was measured.

2.3. Determination of ethanol content in hand sanitizers

Gas chromatography- Flame ionization detector technique was used to test the ethanol content of the hand sanitizers. Briefly, the PerkinElmer 600 GC system fitted with Rtx-5MS capillary column (50 m x 320 μm ID x 0.50 μm) was coupled to a PerkinElmer flame ionization detector. Inert gas helium of 99.9999% high purity was used as a carrier gas. The column flow was adjusted to the constant flow of 1.5 mL/min gas, while the total flow was seen to be 77.4 mL/min. The injection temperature as well as the column oven temperature were set to 200°C at a pressure of 10.82 psi with the FID temperature being set to 260°C. The program's progress was started at 40°C with a 1-minute lag time at this temperature and then a 5°C increase in temperature every minute to reach the set 200°C. Two reference standards were used to quantify the specific peaks at which 99.8% ethanol and the solvent (acetonitrile or deionized distilled water) peak appear in the chromatogram. The sample is analysed in duplicate to calculate the average % of ethanol content in hand sanitizers.

3. RESULTS AND DISCUSSION

3.1. General information on hand sanitizers

The gel form of eight alcohol-based hand sanitizers was collected from Muscat and Salalah, the two major cities of Oman. These samples were made in five countries: two each in Oman (A-1 and A-4), Jordan (A-6 and A-7) and Turkey (A-5 and A-8), while a piece in China (A-2) and UAE (A-3). The ethanol content of seven hand sanitizers range as stated on their label, is from 70-76% v/v, whereas the sample from UAE was found to be without an ethanol percentage label claim. The A-6 (Jordan) and A-2 (China) samples were claimed to...
contain 76% and 75% ethanol, while the label of the rest of the samples showed a content of 70%. The price of hand sanitizers varied from 1.82-4.08 USD. Surprisingly, both the locally made sanitizers (A-1 and A-4) were the most expensive (4.08 USD each), followed by A-2 (3.9 USD; China). The Jordanian samples A-6 and A-7 were the cheapest, with a price of 1.82 and 2.09 USD, almost half the price of the Omani samples. Label of samples revealed that almost all samples contain water and glycerine as a humectant. The label details, including price and country of origin of eight hand sanitizers, are presented in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Price in OMR (USD)</th>
<th>Country of Origin</th>
<th>Active Ingredient</th>
<th>Name of first few excipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1.569 (4.08)</td>
<td>Sultanate of Oman</td>
<td>Ethyl alcohol 70% v/v</td>
<td>Aqua, Mono propylene glycol, Glycerine, Acrylates / C10-30 alkyl acrylate cross polymer, Triethanolamine / aminomethyl propanol</td>
</tr>
<tr>
<td>A2</td>
<td>1.5 (3.9)</td>
<td>China</td>
<td>75% Ethanol</td>
<td>Acrylic acid, polymers, Triolamine, Water</td>
</tr>
<tr>
<td>A3</td>
<td>1.00 (2.6)</td>
<td>United Arab Emirates</td>
<td>Ethyl alcohol</td>
<td>Aqua, Acrylates / C10-30 alkyl acrylate cross polymer, Glycerine, Triethanolamine</td>
</tr>
<tr>
<td>A4</td>
<td>1.57 (4.08)</td>
<td>Sultanate of Oman</td>
<td>Ethyl alcohol 70%</td>
<td>Carbomer, Colour and perfume Aqua</td>
</tr>
<tr>
<td>A5</td>
<td>1.05 (2.73)</td>
<td>Turkey</td>
<td>Ethanol 70% v/v</td>
<td>Aqua, Glycerine, Carbomer, Perfume</td>
</tr>
<tr>
<td>A6</td>
<td>0.700 (1.82)</td>
<td>Jordan</td>
<td>Ethyl alcohol 76% v/v</td>
<td>Water (aqua), Glycerine, Isopropyl Myristate, Acrylates / C10-30 alkyl acrylate cross polymer</td>
</tr>
<tr>
<td>A7</td>
<td>0.805 (2.09)</td>
<td>Jordan</td>
<td>Ethyl alcohol 70%</td>
<td>Water, Carbopol, Triethanolamine, Glycerine</td>
</tr>
<tr>
<td>A8</td>
<td>0.945 (2.45)</td>
<td>Turkey</td>
<td>Ethanol 70% v/v</td>
<td>Aqua, Glycerine, Carbomer, Perfume</td>
</tr>
</tbody>
</table>

### 3.2. Physical properties of hand sanitizers

The physical properties of hand sanitizers, such as pH, density, and RI, are presented in Table 2. The pH of hand sanitizers was observed in the range of 6.44-8.06, which is overall consistent with the pH of 100% ethanol, which is very slightly basic at 7.33 (Kosaric et al., 2011). The fluctuation in pH of these hand sanitizers is mainly due to the additives and different percentages of ethanol present. The product with the highest pH was A-2 at 8.06 followed by A-5 (7.42) and A-6 (7.24), which are slightly alkaline but all the other five sanitizers lean towards acidity. This factor can be a purposeful choice made by the manufacturers of these sanitizers, as acidity is shown to denature microbial enzymes. However, the level of acidity isn’t high enough to cause severe skin irritation, making these products acceptable to the general public. The near neutrality of these hand sanitizers is imperative to the efficacy and acceptability in the market (Selam et al., 2022).

Density is an important physical parameter to determine the identity of the tested substance. We can assume that the lower the density of hand sanitizer, the lower the ethanol content, since ethanol has a lower density than water (Gumala et al., 2020). According to the FDA guidelines, hand sanitizers should have a density of approximately 0.85 g/mL (FDA, 2020). In the current study, the density of samples ranged from 0.8664-0.9368 g/mL, where the Omani sample, A-1, had the highest and the Turkey sample, A-7, showed the lowest density. Because the density of all samples is nearly close to 0.85, it could be inferred that all the hand sanitizers comply with the standards.

The refractive index (RI) directly measures the purity or composition of liquids and depends on their density, temperature, and concentration. Ethanol 70% solution (% w/w) has an RI of 1.3652 at 20°C, which is consistent with the obtained results (1.3640-1.3677) given in Table 2 (Wohlfarth, 2017).

According to the WHO guidelines, an optimum hand sanitizer has an ethanol content between 65-70% (WHO, 2009). Each of the products chosen had a specific claim, and the current study aimed to determine if they met this claim and followed the WHO recommendation. Various methods reported in the literature for detecting volatile impurities and assessing the quality and ethanol content of ABHS products include mid-infrared, near-infrared, and Fourier-transform infrared spectroscopy, as well as headspace gas chromatography-mass spectrometry (GC-MS) or GC-FID (Abrigo et al., 2022; Alam et al., 2023; Costa et al., 2022; Fonseca et al., 2020). In the current study, ethanol content in the hand sanitizers was determined using GC-FID. The gas chromatograms of analysed samples are illustrated in Figure 1. The results in Table 2, which are computed from the gas chromatograms, show that ethanol content of all the hand sanitizers fell within the range of the WHO recommendation, with a range of 66% to 76%. However, five samples (A-4 to A-8) contained less ethanol content than the label claimed. Only A-1 (Oman) and A-2 (China) hand sanitizers had ethanol contents above the claim at 73% and 76%, respectively. The product A-7 (Jordan) had the least amount of ethanol (65%), lower than the recommended concentration for viral or bacterial inactivation. A study conducted on the ethanol content of seven off-the-shelf hand sanitizer gels, including two biocides and five cosmetics from the Italian market, using gas chromatography,
concluded that products with the highest ethanol content have greater antibacterial activity (Berardi et al., 2020). Nisbar et al. employed the GC-MS technique to examine 69 ABHS samples for ethanol or isopropyl alcohol (IPA) content. Out of these samples, 14 samples contained lower alcohol content than indicated on the label, while 4 samples showed the presence of a high amount (5.3 to 19.4%) of methanol impurity (Nisbar et al., 2023).

Matatiele and colleagues, similar to our study, investigated 94 randomly selected ABHS in the Johannesburg region, employing GC-FID to assess alcohol. Among these, three formulations did not contain alcohol, while the remaining products contained ethanol, 2-propanol, or 1-propanol, either individually or in combination. Notably, 37 (41%) of the hand sanitizers contained alcohol concentrations below 60%. Additionally, ethyl acetate, isobutanol, and other non-recommended alcohols, such as methanol and 3-methylbutanol, were also identified (Matatiele et al., 2022). In a separate study also conducted in South Africa, de Bruin et al. detected estrogenic activity in 29 out of 60 hand sanitizers, while none of the products met the labelling requirements. Interestingly, while 50 alcohol-based hand sanitizers (ABHS) contained alcohol concentrations of ≥60% v/v, some were found to contain skin irritants and substances potentially hazardous to both human health and the environment (De Bruin et al., 2024).

However, according to Abuga and Nyamweya, beyond alcohol content, numerous other factors play an important role in determining the effectiveness of hand sanitizer products. They proposed a three-dimensional tetrahedron model to manufacture an ABHS. The type and concentration of alcohol, alongside the excipients, formulation, and manufacturing practices, are crucial cornerstones of their model (Abuga & Nyamweya, 2021b). Many studies advocated for the utilization of high-quality sanitizer products (Stafford et al., 2023).

While the COVID-19 pandemic may have ended, it has left a lasting lesson on the critical importance of hand sanitization in mitigating the spread of infectious diseases. This global crisis has significantly altered public perception and transformed hygiene practices, with alcohol-based sanitizers emerging as indispensable tools in the fight against the coronavirus. The heightened awareness of the public persists even in the aftermath of the pandemic, as many continue to prioritize hand hygiene through the use of alcohol-based sanitizers. However, it is imperative to assess the continued relevance and safety of alcohol-based sanitizers in the post-pandemic era. According to Phillips et al., there was a notable 72.5% rise in exposures to alcohol-based sanitizers between 2019 and 2020, as per data compiled by Texas Poison Control Centers. Fortunately, the majority of these exposures did not necessitate medical attention, and there were no reported deaths among the cases documented in Texas for the specified period (Phillips et al., 2020).

In 2022, Manuel et al. conducted a comprehensive study to evaluate the efficacy and safety of ethanol-based sanitization products, particularly those of uncertain quality, and the utilization of bulk refillable dispensers amidst the COVID-19 pandemic. Their findings unveiled concerning revelations: a prevalent occurrence of substandard alcohol levels, noticeable visual impurities, and alarming concentrations of potentially hazardous chemical impurities in these products. This research unequivocally underscores the inherent risks linked with the use of subpar alcohol-based sanitizers and the common practice of bulk refilling (Manuel et al., 2022).

Another interesting study evaluated the quality of 310 marketed ABHS products registered with the FDA through GC-MS technique. Surprisingly, findings revealed that a significant portion, 104 products (33.5%), failed to meet the declared alcohol content as per their labels. Ethanol-based sanitizers fared worse in both overall assay and impurities, with a failure rate of 84.3%, compared to isopropanol-based counterparts, which showed substantially lower rates (11.2% and 6.2%, respectively). These results underscore the critical importance of stringent testing protocols in ensuring the production of high-quality sanitizer products (Stafford et al., 2023).

Table 2
Physicochemical properties and ethanol content of the samples

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Hand Sanitizer</th>
<th>pH</th>
<th>Density (g/mL)</th>
<th>Refractive Index (RI)</th>
<th>Ethanol content (% v/v)</th>
<th>Label Claim</th>
<th>Calculated (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>6.98</td>
<td>0.9368</td>
<td>1.3660</td>
<td>70</td>
<td>73 ± 4.29</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>8.06</td>
<td>0.9278</td>
<td>1.3677</td>
<td>75</td>
<td>76 ± 8.57</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A3</td>
<td>6.89</td>
<td>0.8804</td>
<td>1.3640</td>
<td>No claim</td>
<td>66 ± 5.71</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A4</td>
<td>6.44</td>
<td>0.8680</td>
<td>1.3650</td>
<td>70</td>
<td>66 ± 5.71</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A5</td>
<td>7.42</td>
<td>0.8926</td>
<td>1.3642</td>
<td>70</td>
<td>69 ± 1.43</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>A6</td>
<td>7.24</td>
<td>0.8916</td>
<td>1.3654</td>
<td>76</td>
<td>75 ± 7.14</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>A7</td>
<td>6.65</td>
<td>0.8664</td>
<td>1.3640</td>
<td>70</td>
<td>65±1.74</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>A8</td>
<td>6.86</td>
<td>0.9004</td>
<td>1.3641</td>
<td>70</td>
<td>68 ± 2.86</td>
<td></td>
</tr>
</tbody>
</table>

Many studies advocated for the utilization of ABHS in the fight against COVID-19. It was pointed out that the efficacy of ABHS depends upon various factors, including but not limited to their proper application, applied volume, contact time, viral load, manufacturing techniques, selection of active ingredients, handling procedures, and ensuring the safe deployment of hand sanitizers against the targeted pathogen (Saha et al., 2021; Singh et al., 2020).
In another study carried out in the United States, researchers examined ethanol and organic impurities in 31 children’s ABHS products. The findings revealed that seven of these products surpassed the impurity thresholds established by the FDA’s recommended interim limits. Notably, benzene levels reached up to 9.14 ppm, while acetaldehyde and acetal concentrations reached as high as 134.12 ppm and 75.60 ppm, respectively. Furthermore, the total measured alcohol content varied significantly, ranging from 52% to 98% across all tested hand sanitizers. This range extended from 39% below to 31% above the labeled concentration (Gloekler et al., 2022).

Corcoran et al., recently published findings from a single-center, retrospective review examining pediatric (<19 years) hand sanitizer ingestions at their Wisconsin poison center in the US spanning from May 1, 2020, to January 28, 2022. During this period, they received 801 calls related to hand sanitizer exposure, leading to 140 children being referred to a healthcare facility due to hand sanitizer ingestion. Among these cases, 88 (63%) underwent measurement of methanol and/or ethanol concentrations. Interestingly, no child exhibited detectable methanol concentration, while 78 underwent ethanol testing, with 12 showing detectable ethanol concentration (Corcoran et al., 2023).

Therefore, there is a pressing need to design and develop quality formulations that prioritize safety by minimizing flammability risks, ensuring efficacy in antimicrobial action, and mitigating potential skin irritations and other adverse effects associated with the frequent use of alcohol-based sanitizers (Abuga & Nyamweya, 2021a; Jing et al., 2020).

Furthermore, user awareness on safe handling of ABHS could help in preventing their potential deleterious effects such as allergies, skin irritation, lung injury, fire hazards, and toxicities (Saha et al., 2021).

4. CONCLUSION

During COVID-19, hand sanitizer consumption increased worldwide, and alcohol-based hand sanitizers (ABHS) are still in high demand even after the pandemic is over. Hence, increased demand for hand sanitizers has underscored the importance of ensuring their effectiveness and safety. According to widely accepted health regulatory bodies, hand sanitizers should contain a percentage of alcohol greater than 65% to be considered effective. Since gels are highly accepted due to their effectiveness against pathogenic microorganisms and their ease of use, it is important to ensure that the product benefits their purchase. Hence, we tested the content of eight ABHS gels sold in Oman. Results of the current study revealed that all the hand sanitizer products met the WHO criteria for alcohol content and fell on the same price range. However, variation in some physical properties such as pH was observed. The higher pH values may be due to the higher percentage of the ingredients and additives beyond the defined limits. In our opinion, factors like viscosity and fragrance only influence the choice of the consumer but can further increase the cost and may compromise with the efficacy of hand sanitizers. It is worth noting that only two out of the eight hand sanitizers tested in our study met their labelled claims. Considering that these hand sanitizer products are readily available over the counter (OTC), it highlights the importance of regular evaluation of their alcohol content, which is an active ingredient. The health regulatory authority in Oman took proactive measures by withdrawing five other sanitizer brands from the market due to their substandard quality. Therefore, it is recommended to implement similar nationwide campaigns by regulatory bodies to assess the content of locally available sanitizers, to uphold their quality and safety standards.

Furthermore, there is an urgent need for ongoing research and development aimed at formulating hand sanitizers that not only effectively fight against pathogens but also mitigate risks such as flammability and skin irritations associated with frequent use. By prioritizing both safety and efficacy in the formulation and ensuring stringent regulatory oversight, it can be guaranteed that hand sanitizers remain a dependable tool for safeguarding public health and maintaining confidence in the efficacy of hand sanitization practices.
CONFLICTS OF INTEREST

The authors declare no competing or financial conflict of interest.

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JNAS - Collection and/or assembly of data, MJJA, SAK, NAAL
Data analysis and interpretation, SAH - Writing the article,
NAAL, JNAS, SAH - Critical revision of the article, All authors
read and approved the final version of this article.

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