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ANALYTICAL PERFORMANCE AND VALIDATION OF HEAD SPACE – GAS CHROMATOGRAPHY – FLAME IONIZATION DETECTOR (HS-GC-FID) METHOD FOR ALCOHOL CONTENT AND EVALUATION OF EFFICIENCY AND POSSIBLE TOXICITY OF HAND SANITIZERS AT THE TIME OF PANDEMIC

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World Health Organization and Turkish National Health Authorities advice the public to clean their hands with an alcohol-based hand rub or to wash with soap and water. While the pandemic resulted in stockpiling and shortage of the products, but industry responded quickly, although concerns were raised about the safety and efficacy of these new products.

The alcohol content of hand sanitizer product samples that are available in Turkish market were evaluated with head space -gas chromatography- flame ionization detector (HS-GC-FID) to check and verify their safety for consumers amid the COVID-19 pandemic. The alcohol content of the hand sanitizers is in a range of 1.2 – 88.2% excluding methanol. The method indicated have satisfactory recovery, detection limits and standard deviations while the alcohol content of most of the products is below the advised limit for hand sanitizers; even some of them contain unwanted methanol impurities. The regulatory bodies should check these products more intensively.

INTRODUCTION

It has been reported that COVID-19 can persist on inanimate objects in a wide range of 2 to 8 h (paper and aluminium) to ≤5 days (plastic, ceramic, and others). Since the publication of this and other reports, the scientific community has debated the length of time SARS-CoV-2 can persist on inanimate objects, with some reporting that SARS-CoV-2 RNA persists on surfaces after >14 days.[1-4]

As survival times vary by surface types, guidelines for best disinfectant practices are vital, most notably for essential work locations including hospitals, grocery stores, and delivery centers that face higher traffic and risk rate than other locations. Hand hygiene is a widely accepted principle in the prevention of disease transmission because proper hand hygiene has a 24-31% likelihood of decreasing the spread of transmissible disease.[5,6]

Alcohol-based hand sanitizers work by penetrating the viral membrane to denature and coagulate proteins, disrupt cellular metabolism, and induce lysis of the viral particle.[7] In 2017, a study evaluated the virucidal activity of alcohol-based hand sanitizers against a variety of viral pathogens, including SARS-CoV. The study determined that ethanol-based and isopropyl alcohol-based hand sanitizers were effective disinfectants during the previous 2002 SARS-CoV outbreak.[8]

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Coronavirus pandemic has brought a shortage of hand sanitizers all around the World. Even because commercial products are hardly or no longer available due to the coronavirus disease (COVID-19) pandemic, alcohol-based hand rub formulations for hygienic and surgical hand treatment published by the World Health Organization (WHO) in 2009[9] for local production in developing countries are now being produced for everyone worldwide. Since the end of February 2020, pharmacies in Turkey have been producing the WHO-recommended formulations either for sale or as donations for personal use by the general population.[10] Use of hygienic hand preparations made with the original WHO-recommended formulations might be justifiable to prevent infection or transmission of pathogens outside patient care. The commercial counterparts, which are the preparations for hygienic hand antisepsis that are available outside the pharmacies, used in virtually in every shop nowadays in the pandemic, must meet the standards already established by WHO (see Table 1). Final product concentration suggested by WHO for household or local production is ethanol (80%, v/v), hydrogen peroxide (0.125%, v/v) and glycerine (1.45%, v/v) for formulation A and isopropyl alcohol (75%, v/v), hydrogen peroxide (0.125%, v/v) and glycerol (1.45%, v/v) for formulation B.[11]

According to the USA Centers for Disease Control and Prevention (CDC), best practices to slow the spread rely on good hand hygiene, including proper hand washing practices as well as the use of alcohol-based hand sanitizers. CDC only recommends hand rub products with greater than 60% ethanol or 70% isopropyl alcohol as active ingredients, but no other alternative.[12] Benzalkonium chloride, along with both ethanol and isopropyl alcohol, is deemed eligible by USA Food and Drug Administration (FDA) for use in the formulation of healthcare personnel hand rubs.[8] However, available evidence indicates that benzalkonium chloride has a less reliable activity against certain bacteria and viruses than either of the alcohols.

Hand hygiene is essential for reducing COVID-19 transmission, and this is one of the universal precaution simplemented,as many COVID-19 positive patients may be asymptomatic. Given the popularity of hand sanitizers and their importance in preventing the spread of COVID-19, the outbreak has triggered stockpiling of emergency, protection and cleaning supplies all around the world including the alcohol based hand hygiene products. To respond to the stockpiling and shortage, chemical industries and manufacturers reacted very fast and started to produce large amounts of products. Due to this high demand, most of them might not be regulated affectively, and sub-standard/falsified or even counterfeit products, which may contain low or unknown alcoholic content, may be available in market.

There are two key types of falsified alcohol-based hand sanitizers: 1) sanitizers that contain other unlisted alcohol content such as methanol; and 2) sanitizers with an alcohol content below 60%.

The typical unlisted alcohol is methanol that should not be used in hand sanitizers because it is highly toxic and can cause severe reactions when exposed to the skin, lungs or mouth.[18] Exposure to methanol can result in systemic toxicity and, in some cases, death with intentional, non-intentional or occupational or non-occupational exposures.[19-24] The substance's elevated intrinsic toxicity, its ready availability, and its widespread use make poisoning from undeclared methanol in hand sanitizers an important public health concern.[25] In acute poisoning, typically 12–24 h after exposure the main toxicity of methanol manifest as its metabolite formic acid has accumulated to toxic levels.[26] Severe metabolic acidosis, nausea, vomiting, headache, semi-coma, and ocular toxicity may be seen.[18] Unless timely antidotal therapy is given,[27] coma, seizures, death, permanent blindness, and permanent damage to the central nervous system have been reported with substantial exposures.[24] Inhalation and skin exposures have also been reported for subacute poisoning with workers.[27] There are few data on chronic toxicity while eye irritation and headache has been reported with chronic inhalation exposure to methanol with workers.[18]

Second, a hand sanitizer that contains less than 60% alcohol can be ineffective as a germicide and offers users no biocidal effect, leaving the public vulnerable to contracting and spreading COVID-19.

The safety of cheap priced and rushed production falsified alcohol-based sanitizers may jeopardize consumer health because of possible ineffectiveness or potential toxicity. In this study, we assessed the alcohol content of different types and price range of alcohol-based hand sanitizer samples that were purchased from various daily supermarkets and pharmacies in Turkey to check and verify their safety for consumers.

Table 1

Official recommendations for hand and/or surface hygiene

USA Centers for Disease Control and Prevention	Recommendations by March 2020; Minimum 60% ethanol or minimum 70% isopropanol[12]	Recommendations after March 2020; Hand sanitizers with 60% to 95% alcohol[13]
World Health Organization	Recommendations by March 2020; At least 60% alcohol-based hand sanitizers, or 70% for small objects[14]	Recommendations after May 2020; Proceed surface cleaning followed by 70%–90% ethanol[15]
Turkish / National Govermental & Non-govermental Organizations	WHO equivalent; alcohol based hand rubs recommended, for surfaces at least 70% alcohol recommended[16,17]	

MATERIALS AND METHODS

Experimental

Reagents and samples

All chemicals were of analytical gas chromatographic reagent grade. Standard solutions of ethanol (EtOH), isopropyl alcohol (IPA) (Merck, Darmstadt, Germany) and methanol (MeOH) (Sigma, Aldrich) were prepared with deionized water. Deionized water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). High purity helium, hydrogen, and dried air gases (for gas chromatography) were purchased from Hatgrup Industrial and Medical Gases Incorporated Company, Ankara, Turkey. Thirty commercial hand sanitizers were purchased from markets and pharmacies in Ankara, Turkey by May-June 2020. The samples were stored at ambient temperature before the analysis.

Instrumentation and apparatus

All measurements were made with Gas Chromatography Flame Ionization Detector (GC-FID) (Agilent Technologies 7890A GC System, Santa Clara, US) equipped with Headspace (HS) Sampler (7697A, Agilent Technologies Inc, US), and DB-624 (6% cyanopropylphenyl and 94% dimethylpolysiloxane copolymer) capillary column (30 m x 0.32 mm ID x 1.8 µm film thickness, Agilent Technologies, US). Headspace vial (20 mL) and headspace crimp Aluminium caps, PTFE/Si septum (Agilent Technologies Inc., US) were used to prepare volatile standard and sample solutions.

Chromatographic conditions

Analysis of EtOH, IPA, and MeOH in hand sanitizer was performed on HS-GC-FID.[28] Helium at a constant flow rate of 1 mL/min was used as the column carrier gas. Hydrogen and dried air were used as flame gases. Separation was made by using Agilent J&W DB-624 capillary column. All the chromatographic and headspace sampler conditions are summarized in Table 2.

Preparation of calibration standard solutions

Working standard solutions of the analytes (EtOH, IPA, and MeOH) were prepared by the gravimetric-volumetric method. The procedure of the addition of analytes to water was used in order to prevent the evaporation of the analyte. Each accurately weighted standard was added to the volumetric flask and diluted to 10.0 mL with deionized water, the flask was closed immediately, and then vortexed. Further 2.0 mL of aliquot of standard was transferred to 20 mL of head space vial, and sealed it with Headspace crimp Aluminium caps, PTFE/Si septum immediately, then they were analyzed with HS-GC-FID. Calibration standards were prepared freshly. Six-point calibration curve was plotted against the percentage of standard EtOH, IPA, and MeOH concentrations *versus* peak areas (see Figure 1).

Table 2

Gas Chromatographic Conditions

GC-FID Conditions	
GC Column	Agilent J&W DB 624 (30 m × 0.32 mm, 1.8 µm)
Injector temperature	200 °C
Detector temperature	230 °C
Helium (carrier gas) flow rate	1.0 mL/min, constant flow mode
Hydrogen	30 mL/min
Dried air	400 mL/min
Split ratio	200:1
Oven program	40 °C hold for 5.0 min, increase at 5 °C/min ramp to 60°C and hold for 0.0 min, increase at 30 °C/min ramp to 210°C and hold for 4.0 min. Post run was 220 °C for 2 min
Run time	18.00 min
Sample injection	Headspace
Headspace sampler parameters	
Oven temperature	130 °C
Loop temperature	135 °C
Transfer line temperature	140 °C
Injection volume	1 mL
GC cycle time	19.00 min
HS vial equilibration time	2.00 min
Injection duration	0.06 min

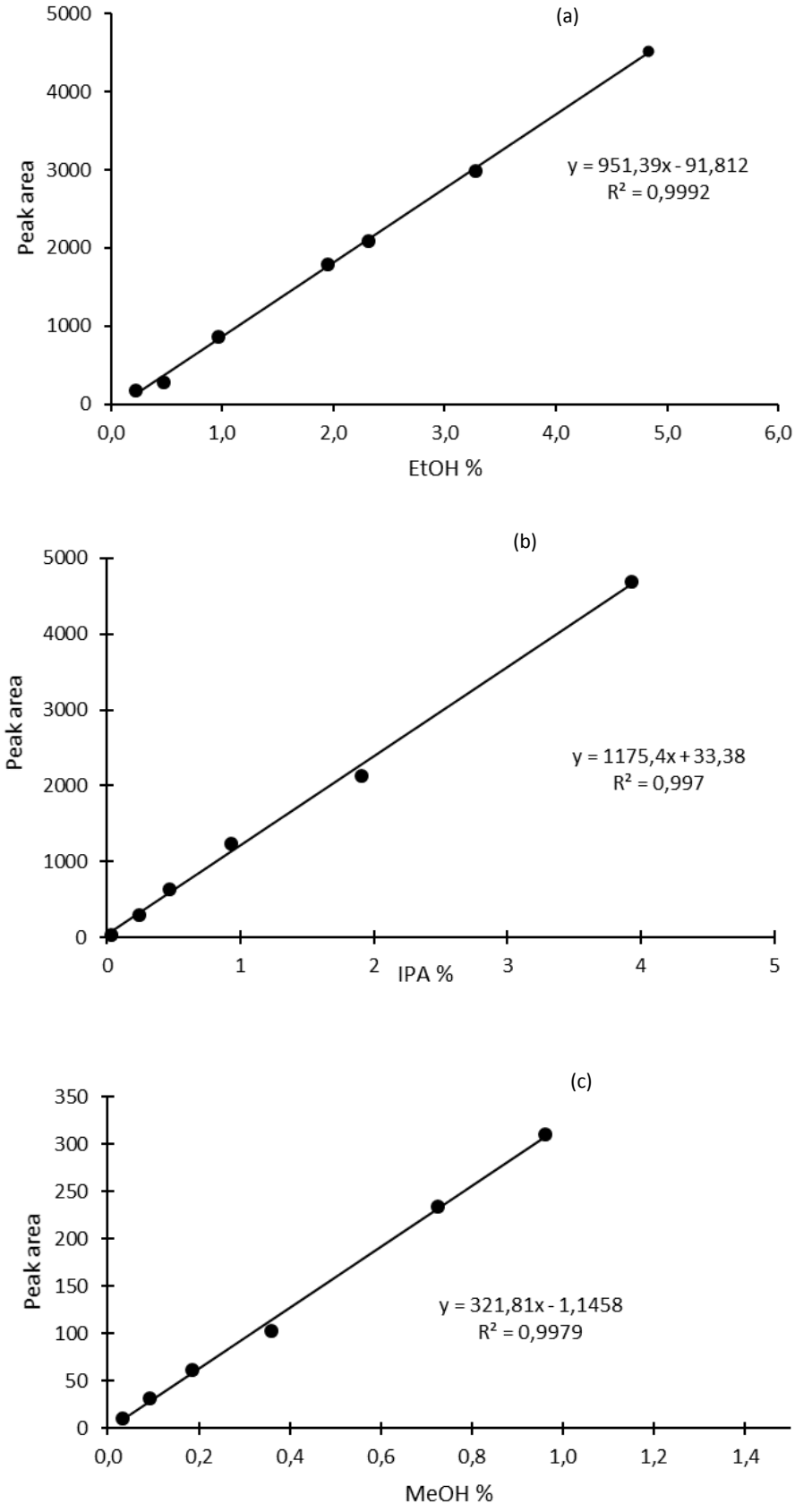

Fig. 1 – Linear calibration curve of standards (a) EtOH, (b) IPA, and (c) MeOH.

Table 3

Chromatographic data

Component	MeOH	EtOH	IPA
t_R, (min), (mean ± sd*)	$4.199 \pm 9.2\times10^{-3}$	5.644 ± 0.02	$6.845 \pm 5.2\times10^{-3}$
Minimum t_R, min	4.193	5.567	6.832
Maximum t_R, min	4.221	5.657	6.850
Resolution (R_S)	8 > 1.5	6 > 1.5	6 > 1.5
Selectivity (α)	1.3 > 1.0	1.2 > 1.0	1.2 > 1.0

*sd: standart deviation

Table 4

Quantitative characteristics of EtOH, IPA, and MeOH

Component	EtOH	IPA	MeOH
RSD % (n= 9)	5.4	5.1	4.6
LOD, %	0.014	1.12×10^{-3}	4.82×10^{-3}
LOQ, %	0.043	3.40×10^{-3}	0.015
Range, %	0.23 – 4.83	0.036 – 3.93	0.031 – 0.96
Linear regression equation*	y = 951.39x –91.812 (n= 7 point)	y = 1175.4x + 33.381 (n= 6 point)	y =321.81x – 1.1458 (n=6 point)
Determination coefficient (R^2)	0.9992	0.9970	0.9979

*y is the peak area, x is the % concentration

Table 5

Percentage recovery results

EtOH %	in Sample #n02	Spike	Detected	% Recovery
	0.88	0.46	1.32	98.5
	0.88	0.96	1.89	102.7
	0.88	1.91	2.75	98.5

IPA %	in Sample #n02	Spike	Detected	% Recovery
	0.51	0.37	0.89	101.1
	0.51	0.76	1.34	105.5
	0.51	1.50	1.96	97.5

MeOH %	in Sample #n24	Spike	Detected	% Recovery
	0.41	0.25	0.68	103.0
	0.41	0.46	0.85	97.7
	0.41	0.96	1.37	100.0

Preparation of hand sanitizer solutions

Sample solutions were prepared gravimetrically. The samples were added into water in the flask to prevent the evaporation of the analyte. Each accurately weighted sample was added to the volumetric flask and diluted to 10.0 mL with deionized water, the flask was closed immediately, and then vortexed. Further 2.0 mL of aliquot of sample was transferred to 20 mL of headspace vial, and sealed it with Headspace crimp Aluminium caps, PTFE/Si septum immediately, then they were analyzed with HS-GC-FID. Each sample was prepared in two replicates.

Method Validation

The method was validated according to standard procedures.[29,30]

Resolution and retention time

Variation in resolution (R_S) and retention time (t_R) was evaluated from the chromatograms generated in the precision test (see Table 3).

Precision

Precision was calculated from 9 replicate of sample #n03 determinations as RSD% (see Table 4).

Limits of Detection and Quantification

The limits of detection (LOD) for each analyte were defined as the detectable concentration yielding as S/N of 3 and the limit of quantification (LOQ) was defined as the detectable concentration yielding as S/N of 10 (see Table 4).

Linearity

Linear least squares regression calibration curves were constructed by plotting the peak area of the analyte versus the % concentrations of the working solutions (see Figure 1). Linear regression equations, determination coefficient and ranges are given in Table 4.

Accuracy

The accuracy of the method was evaluated by recovery experiment using standard addition technique. The percentage recoveries were determined by spiking three known quantities of the standards to the disinfectant sample and analyzed with HS-GC-FID. The results (recovery%) are given in Table 5.

Selectivity

Identification of the EtOH, IPA, and MeOH were performed based on the comparisons of their retention times (t_R) with pure standard under the same chromatographic conditions of the samples. The chromatogram of EtOH, IPA, and MeOH standard mixture is shown in Figure 2. It is known that some hand rub product solutions contain glycerol to soften the skin while this has not interfered with chromatograms. As shown in Figures 2 and 3, alcohols in the sample were detected in the presence of components without any need of purification process.

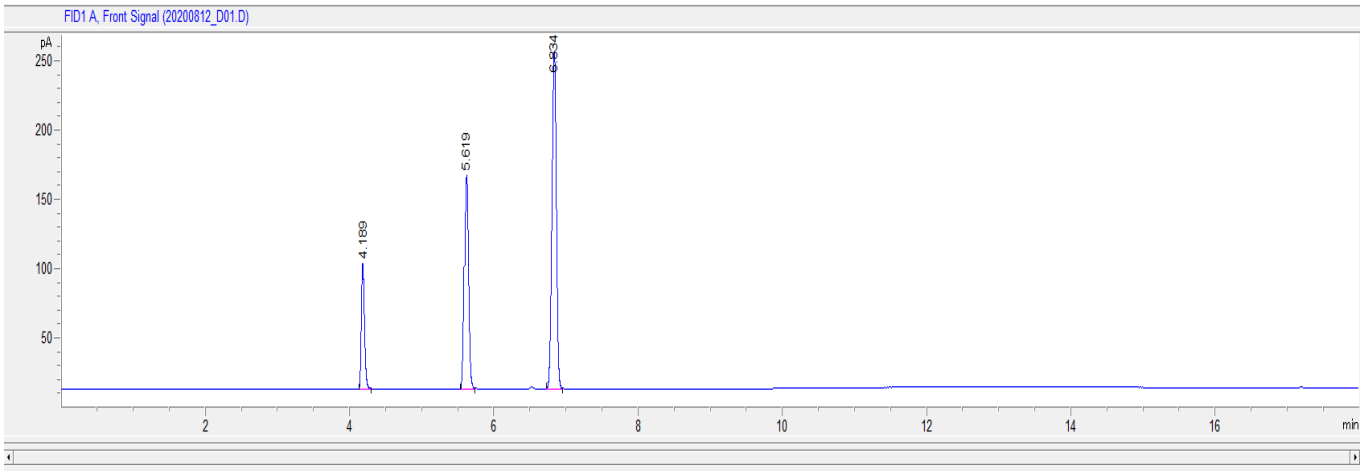

Fig. 2 – Chromatogram of standards (0.093% MeOH, 0.47% EtOH, and 0.47% IPA mixture) (t_R MeOH= 4.216 min, t_R EtOH=5.637 min,t_R IPA= 6.850 min).

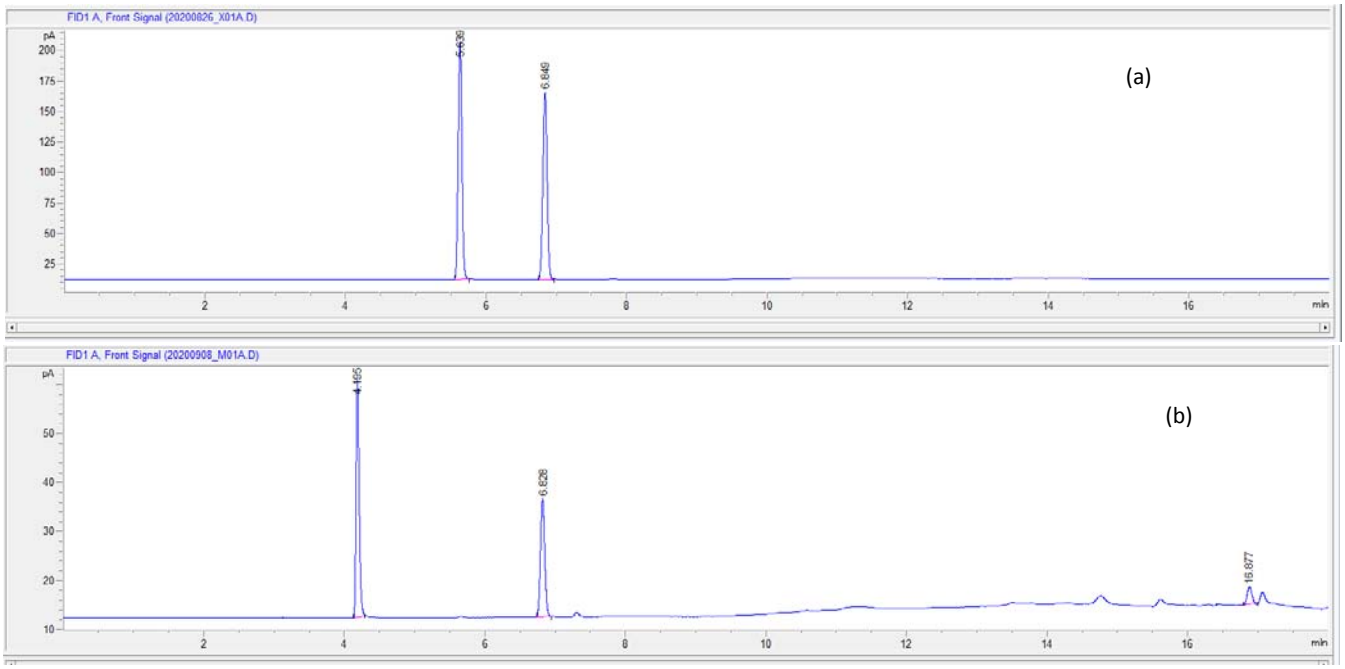

Fig. 3 – Chromatogram of (a) sample #n02 (t_REtOH= 5.639 min, t_R IPA= 6.849 min (b) sample #n24 (t_R MeOH= 4.195 min, t_RIPA= 6.828 min).

RESULTS

The peak area was used for the quantitative analysis and the samples were quantified by means of the linear regression equation of the calibration curves. The alcohol contents of disinfectants are listed in Table 6. The total amount of alcohol (ethanol and isopropyl alcohol) content of the products is in a range of 1.2–88.2% (weight/weight %) for each alcohol excluding methanol. Eleven of the 30 samples that were tested have alcohol content lower than 50% while two of the tested samples have methanol in their compositions.

Table 6

Results obtained from HS-GC-FID analysis of alcohol based disinfectants

Sample #	EtOH% (w/w) ± sd*	IPA% (w/w) ± sd*	MeOH% (w/w) ± sd*
n01	8.9 ± 0.3	15.0 ± 1.8	
n02	39.2 ± 2.3	24.7 ± 1.0	
n03	73.4 ± 0.5	14.8 ± 2.1	
n04	62.5 ± 0.5		
n05	53.2 ± 3.2		
n06	56.3 ± 1.5		
n07	56.6 ± 2.1		
n08	8.9 ± 0.1		
n09		5.1 ± 0.6	
n10	71.6 ± 3.0		
n11	71.6 ± 2.7		
n12	59.1 ± 3.4		
n13	67.0 ± 0.9		
n14	35.9 ± 2.6		
n15	67.3 ± 0.8	0.3 ± 0.03	
n16	75.0 ± 2.3	0.3 ± 0.02	
n17		5.7 ± 0.1	1.1 ± 0.02
n18	51.9 ± 2.7	2.5 ±0.03	
n19		63.5 ± 1.1	
n20		61.3 ± 2.4	
n21	63.9 ± 0.2		
n22		81.5 ± 0.6	
n23	78.5 ± 2.3		
n24		1.2 ± 0.1	10.1 ± 0.7
n25	47.6 ± 5.6		
n26	4.9 ± 0,05	0.4 ± 0.1	
n27	34.7 ± 0.8	1.1 ± 0.1	
n28	57.4 ± 1.9	2.8 ± 0.02	
n29	29.2 ± 0.5	1.3 ± 0.02	
n30	25.0 ± 1.4		

*sd: standart deviation (n, number of replicates= 2)

DISCUSSION

Alcohol-based hand rub is always preferred because of its greater effectiveness and better compliance rate, less time required for application, easy to use and easy to buy. Alcohol-based hand rub mostly contains ethanol, isopropyl alcohol, and their combinations are the most recommended hand hygiene products by many local and global health organizations to stop the spread of COVID-19.[10-12,16,17] However, when misused, or in case of accidental or deliberate ingestion of products or even an exposure to unexpected presence of methanol, these preparations can become toxic to humans and pose a significant public health risk. Methanol is very toxic, following oral, pulmonary and/or skin exposures.[18]Serious systemic toxicity including deaths may arise with intentional, non-intentional or occupational or non-occupational exposures.[19-24] In acute poisoning, typically 12–24 h after exposure the main toxicity of methanol manifest as its metabolite formic acid has accumulated to toxic levels.[26] Severe metabolic acidosis, nausea, vomiting, headache, semi-coma, and ocular toxicity may be seen.[18] Unless timely antidotal therapy is given,[27]coma, seizures, death, permanent blindness, and permanent damage to the central nervous system have been reported with substantial exposures.[24] Inhalation and skin exposures have also been reported for subacute poisoning with workers.[27] There are few data on chronic toxicity while eye irritation and headache has been reported with chronic inhalation exposure to methanol with workers.[18] Skin absorption from repeated exposures to methanol has also been reported to cause visual disturbances.[31] Even though infrequently reported, methanol as an "undeclared ingredient" in alcohol-based hand rub could be found in the market from time to time,[32-34] but the reasons behind for its use as if it is a contamination or a substitution for isopropyl alcohol or ethyl alcohol are not understood. It has to be clearly stated on their product labelling that hand rub should never be swallowed and the alcohol-based hand rub is for external use only. However, unintentional ingestion was reported for adults, adolescents and even for children.[35-37] In fact, consumption of illegally produced and also non-beverage alcohols is a major public health problem in many parts of the world.[38,39] When taken by mouth, if ever used as a substitute for isopropyl alcohol or ethanol, methanol in the hand rub will cause a much greater mortality and morbidity. Methanol has a lower lethal dose (~1.2 *versus* 1–4 and 3.6–6 mL/kg), and the poisoning often requires antidotal therapy (fomepizole or ethanol),[26] in addition to supporting therapy and critical care.[20] Methanol also causes severe metabolic acidosis and related target organ toxicities such as ocular toxicity.[18] Specific therapy[26] may be unintentionally delayed as the exposure to methanol may not be suspected at first. Our study shows that two of the tested samples contain methanol, which threaten the health of the consumer of these products.

There has been a great surge in demand for alcohol based hand disinfectant products in Turkey as a sanitization product in pandemic, leading to shortages in their supply. WHO and Turkish Pharmaceutical Federation recommended hand rub formulations with ethanol as an active component. These in-house-local productions are also formulized to produce final concentrations of ethanol 73.48% (w/w) or 80.0% (v/v).[10,11,40] These hand rub formulations have been recommended to be used both for hygienic hand antisepsis and for presurgical hand preparation by WHO, and the concentration can be expected to be changed at ± 5%. Our study shows that none of the tested products has over this amount of alcohol, and the alcohol based hand disinfectants should not be expected to be a routine hand antisepsis in most clinical situations. On the other hand, Kampf shows that ethanol has been effective against various enveloped viruses, with the minimum concentration of 42.6% (w/w), including the similar SARS coronavirus, MERS coronavirus.[41] The 42.6% (w/w) ethanol concentration can be assumed as 50% (v/v) approximately, while our study shows that, 10 of the tested products are under this effective concentration. Why the alcohol content is lower than the expected level is the main question that should be answered. It is mainly because of counterfeit and/or rushed production, as even some of the products seem to have methanol. While the boxing and the preservation of the alcohol even in the production phase until the end-product phase may also be the problem, as the alcohol easily vaporizes. In a pandemic period where production and deployment time is fast, this may seem like a weak link of the whole process, which can affect all products. The rest of the samples have an alcohol content above ~50% (v/v) and should be expected to be effective against novel coronavirus by helping to control the spread of the disease. During pandemic period, the production and distribution of possible toxic or too weak products starts to be a problem worldwide.

The weakness that can be interpreted to have very low concentration and also toxic ingredients such as methanol is a worldwide problem; by October 2020, FDA has already declared at least 14 hand sanitizers on their Do Not Use list or recall products that are already surged to the markets.[42]

The authors of this current study has before demonstrated that cologne products in Turkey, which are cosmetic products that can be used as alcohol based disinfectants because of their high alcohol content, have also some alcohol content problems at the time of pandemic.[43] In that study, three of the tested 19 colognes have ethyl alcohol content lower than 52% (w/w), as they should not be marketed as cologne at all, while one of the products has alcohol content that is noted to be ineffective against COVID-19. The previous study only investigated colognes, and the sample size was 19 while the current study has nearly 1.5 times the sample size. This can be interpreted as the larger the sample size, the more counterfeit/fake or below expected quality products can be detected. As mentioned before,[42] the hand sanitizers weakness and/or toxicity is a worldwide problem right now, wiser and rapid tests are needed for these products, while consumers should not rely on only one type of cleaning/disinfection solution.

CONCLUSIONS

The findings of this study show that approximately 1/3 of the alcohol-based hand sanitizers that are produced in a rush during the pandemic, available in daily markets, and affordable, have very low alcohol content with almost no effect against COVID-19. Careful selection of the raw material improves the quality and safety of these products; however, in our sample, two of the samples have toxic methanol content, which should not be used in any circumstances. At high demand times, falsified/counterfeit products may find a place in the shelves. However, as the surge and demand is balanced again, this undesirable situation will vanish as the regulatory bodies and control mechanisms can detect these counterfeit products more easily.

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