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Chromatographic and Spectroscopic Method for Determination of Glycerol in Sanitizer Used During COVID-19 Pandemic

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Abstract

The use of sanitizers in the COVID19 pandemic is very common. While using sanitizers in Nepal, we have found people having problems with skin irritation and rashes; hence it was felt to know the amount of glycerol used as an emollient. A validated UV visible spectrophotometric and Reverse-phase High-performance liquid chromatography (RP-HPLC) method was designed to determine the amount of glycerol in the locally available sanitizers. The glycerol in sanitizers showed variation in amount, ranging from 0.78 to 1.66 g and 0.75 to 1.62 g/100 mL by HPLC and UV, respectively. The sanitizer samples with less purity or failed to meet the specification limit should be withdrawn, and their use must be limited. Hence this method seems to be easy, reliable, and cost-effective for determining glycerol based on the chemical derivatization technique.

Keywords: COVID-19, Sanitizers, Glycerol, 2, 4 Dinitrophenyl hydrazine, Color complex, UV Visible spectroscopy, RP-HPLC.

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. Coronaviruses, single-stranded RNA viruses, range about 120 nm in diameter. They are extremely diverse because of their susceptibility to mutation and recombination [1]. The COVID-19 virus is spreading from China's Wuhan city of Hubei Province (its origin) to the other countries of the world [2]. The COVID-19 cases are increasing day by day in Nepal. Till date, 23,60,557 cases are confirmed by RT-PCR (Reverse transcriptase – Polymerase chain reaction) method with 2,75,806 recovered and 3,101 deaths [3]. As per the Government, the cases may increase in the coming days if the government's decision of social distancing and using proper health precautions are not followed. World Health Organization (WHO) has developed two formulations that can be locally prepared during the COVID-19 pandemic. One formulation contains ethyl alcohol, glycerol and hydrogen peroxide in the strength of 80% (v/v), 1.45% (v/v), 0.125% (v/v), respectively and the other contains isopropanol 75% (v/v), glycerol and hydrogen peroxide in the strength of 80% (v/v) [4]. The addition of glycerol in both formulations serves as an emollient, an agent that protects the hand skin from dryness and other skin

related diseases, on multiple uses [5]. This is essential because of the incidence of dermatitis in healthcare workers' hands compliance with hand sanitation procedures [6, 7]. People have reported dermatitis and white stain on the skin due to the use of few brands of sanitizers. Hence, it was an urge to know the glycerol contained in those sanitizers to be aware people regarding the brands of sanitizers that don't follow the WHO guidelines during their manufacturing.
Glycerol is the 1, 2, 3-propanetriol. It is a polar, viscous, clear liquid at room temperature, soluble in water and polar solvents and insoluble in hydrocarbons and other non-polar media [8]. Glycerol is traditionally used in soaps, cosmetics, personal care products, pharmaceuticals, and food products. Other applications such as supplements for animal food, fermentation of biogas, and formulation of fluids for enhanced oil recovery [9, 10]. For pharmaceutical formulations, glycerin is mostly used as an excipient, and variation in its amount may alter the value of such formulations [11].

A few chemical methods have been established to determine the amount of glycerol [12, 13]. Other techniques reported include UV spectroscopy methods [14, 15], HPLC [16], capillary gas chromatography [17], GC [18], and GC-MS [19]. This paper deals with the development of a sensitive colorimetric and chromatographic method for the determination of glycerol in sanitizers. The proposed method was applied successfully to determine glycerol in the sanitizers. Validation of the developed method was performed according to the International Conference on Harmonization (ICH) guidelines [20, 21].

Materials and Methods

Chemicals and Equipment

UV-1800 Shimadzu Double beam UV-Visual spectrophotometer with 10 mm quartz cuvette was used to record the absorbance. The chemicals used were of analytical grade and were prepared freshly during analysis. The sanitizer samples were purchased from the local market of Nepal.

Glycerin Standard Solution

The glycerin primary stock was prepared of 10 g of glycerin standard in 100 mL water. This solution was diluted in order to obtain solutions of concentrations 2-10 mg/mL and analyzed.

Test Solution. It was prepared by diluting the sanitizer samples in water to attain a solution with the strength of 6 mg/mL of glycerol in water.

Method Development
Determination of solubility

The glycerol solubility was tested in various polar and non-polar solvents, and its solubility was found in polar solvents. Among them, water was selected as the solvent of solubility because of its availability, cost, and less hazards.

Selection of appropriate reagent

2,4-dinitrophenylhydrazine (DNPH) reagent was selected. The volume of the 2, 4 DNPH to be added was optimized.

Preparation of 2,4-dinitrophenylhydrazine reagent

A 3 g of 2, 4 DNPH was dissolved in 0.3 mL of concentrated sulphuric acid. 3 mL of ethanol was added to this slurry under continuous stirring at 25°C. The mixture was allowed to react for 10 min to achieve homogenization. The volume was then made up to 100 mL with distilled water.

Extraction of glycerol from the sanitizer

100 mL of sanitizer samples were taken and heated to 100°C. Ethanol has a boiling point of 78.5°C, whereas glycerol has a boiling point of 290°C [22]. On heating to 100°C, the alcohol in the sanitizer is evaporated, leaving behind only glycerol which is taken as the sample for analysis.

Oxidation of glycerol

This step was carried out based on Boyd et al. [23]. Each working standard and the sample were treated with 10 mM sodium periodate solution. During this step, glycerol oxidation occurs, and it gets converted to glyceraldehyde. The solution was shaken for 30 seconds.

Mechanism of colour production

The sample of sanitizer containing glycerol is first treated with sodium periodate. Sodium periodate reacts with free glycerol in the sample to generate formaldehyde [24]. The reaction between formaldehyde and DNPH produces the yellow complex known as diphenylhydrazone [25] (Fig. 1).

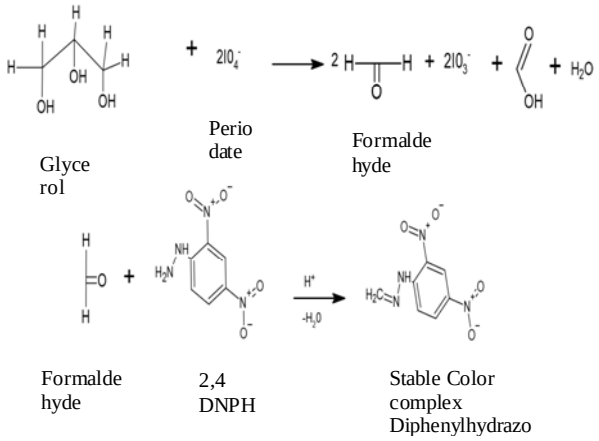

Figure 1. **Formation of color complex**

The absorbance of the colored complex was measured at 360 nm against a blank (Fig. 2).

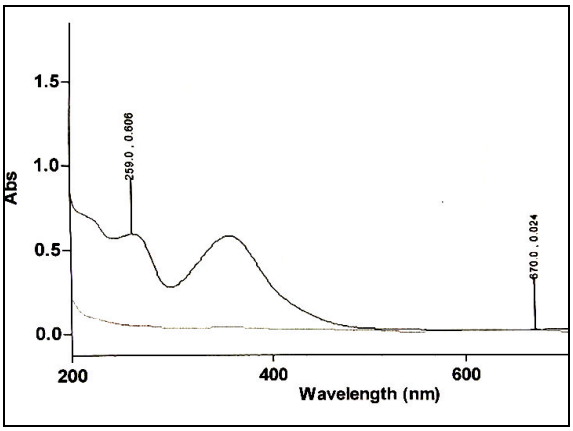

Figure 2. **Maximum absorbance of colored complex**

Optimized chromatographic conditions

Optimum conditions for chromatographic analysis of derivatized glycerol were performed on a reversed-phase C_{18} (250 mm × 4.6, 5 µm particle size) column and analyzed by Shimadzu LC 2010 system with Lab Solution software. The mobile phase comprised acetonitrile: water (40%: 60% v/v) and was pumped at 1.0 mL/min. A membrane filter of 0.45 µ was used to filter the mobile phase and all samples. The samples were monitored at 360 nm by a photodiode array detector (P-DAD). HPLC grade solvents were procured from Merck Pvt Ltd, India.

Sample Preparation for HPLC

Samples of sanitizers were extracted to obtain glycerol. The sample preparation for HPLC was as per the UV method, and analysis was carried out as per Koivusalmi et al., [26]. Finally, the sample was extracted with acetonitrile and analyzed by HPLC.

Preparation of calibration standards of Glycerol

Ten milligrams of glycerol was accurately weighed and dissolved in HPLC grade water, and successive dilutions were carried out using the mobile phase to achieve

the respective concentrations 10-50 µg/mL, in triplicate.

Results and Discussions

Effect of Reagent Concentration

The effects of concentration of the 2, 4 DNPH solution and sulphuric acid were studied on the related absorbance values. Different Volumes of 1–15 mL of 2, 4 DNPH (3%) were examined, and the optimum volume was selected as 10 mL (Table 1).

Table 1. **Volume optimization for reagent (2, 4 DNPH).**

S.N.	Volume of 2,4 DNPH (mL)	Absorbance
1	1	0.02
2	2	0.03
3	3	0.05
4	4	0.09
5	5	0.3
6	6	0.52
7	7	0.61
8	8	0.72
9	9	0.79
10	10	0.82
11	11	0.78
12	12	0.77
13	13	0.75
14	14	0.75
15	15	0.74

Effect of concentrated sulphuric acid

The different volumes of concentrated sulphuric acid were selected for the analysis, and 0.3 mL was selected as the optimized volume of acid for analysis, as it gave the maximum absorbance among the volumes selected, as shown in Table 2.

Table 2. **Volume optimization for concentrated sulphuric acid.**

S. No.	Volume of sulphuric acid (mL)	Absorbance
1	0.1	0.08
2	0.2	0.10
3	0.3	0.52
4	0.4	0.48
5	0.5	0.38
6	0.6	0.37
7	0.7	0.37
8	0.8	0.36
9	0.9	0.35

The optimized volumes of reagents are mixed with periodate treated glycerol to develop the color. The optimum time for the completion of the reaction between sample and 2, 4 DNPH was within a minute, and the color was stable for 24 hours. 10 mg/mL solution (the highest calibration curve concentration) was used to measure the absorbance of colored complex (Table 3).

Table 3. **Stability of colored complex at 10 mg/mL.**

S.N.	Time (hours)	Absorbance
1	2	0.55
2	4	0.54
3	6	0.55
4	8	0.55
5	10	0.55
6	12	0.56
7	14	0.55
8	16	0.56
9	18	0.55
10	20	0.55
11	22	0.54
12	24	0.46

Validation of UV Analytical method for Glycerol

The linearity complied with the regression plot in the 2–10 mg/mL concentration range with a regression correlation coefficient (R^2) of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.215 mg/mL and 0.652 mg/mL. The data for intra-day and inter-day precision studies were obtained for three different concentrations 4, 6 and 8 mg/mL in the linearity and % RSD were reported as less than 2. The accuracy of the developed method was established in terms of recovery study and was within the limit, as shown in Table 4.

Table 4. **Linearity, LOD, LOQ.**

S. No.	Parameters	Values of Glycerol
1.	Concentration (mg/mL)	2-10
2.	Regression equation	y=0.056x+0.002
3.	Correlation coefficient (R^2)	0.999
4.	LOD (mg/mL)	0.215
5.	LOQ (mg/mL)	0.652
6.	Precision,(Intra and Interday)	<2.00
7.	Accuracy	98.47-100.69%

Method Optimization for RP-HPLC

The retention times of different derivatized glycerol were determined at first with isocratic conditions using (60:40% v/v) methanol: water separation was non-uniform and tailed, broad peaks were obtained with several trials. Using (40:60 % v/v) acetonitrile: water, a better resolution with a symmetrical peak was obtained. Hence selected as the optimized mobile phase for separation.

Chromatographic results

The elution profile of the glycerol in a standard solution and sanitizer samples (containing glycerol) were well separated. The retention time of glycerol was optimized at 6.15 min, where there was no interference from other ingredients present in a sanitizer sample (Fig. 3).

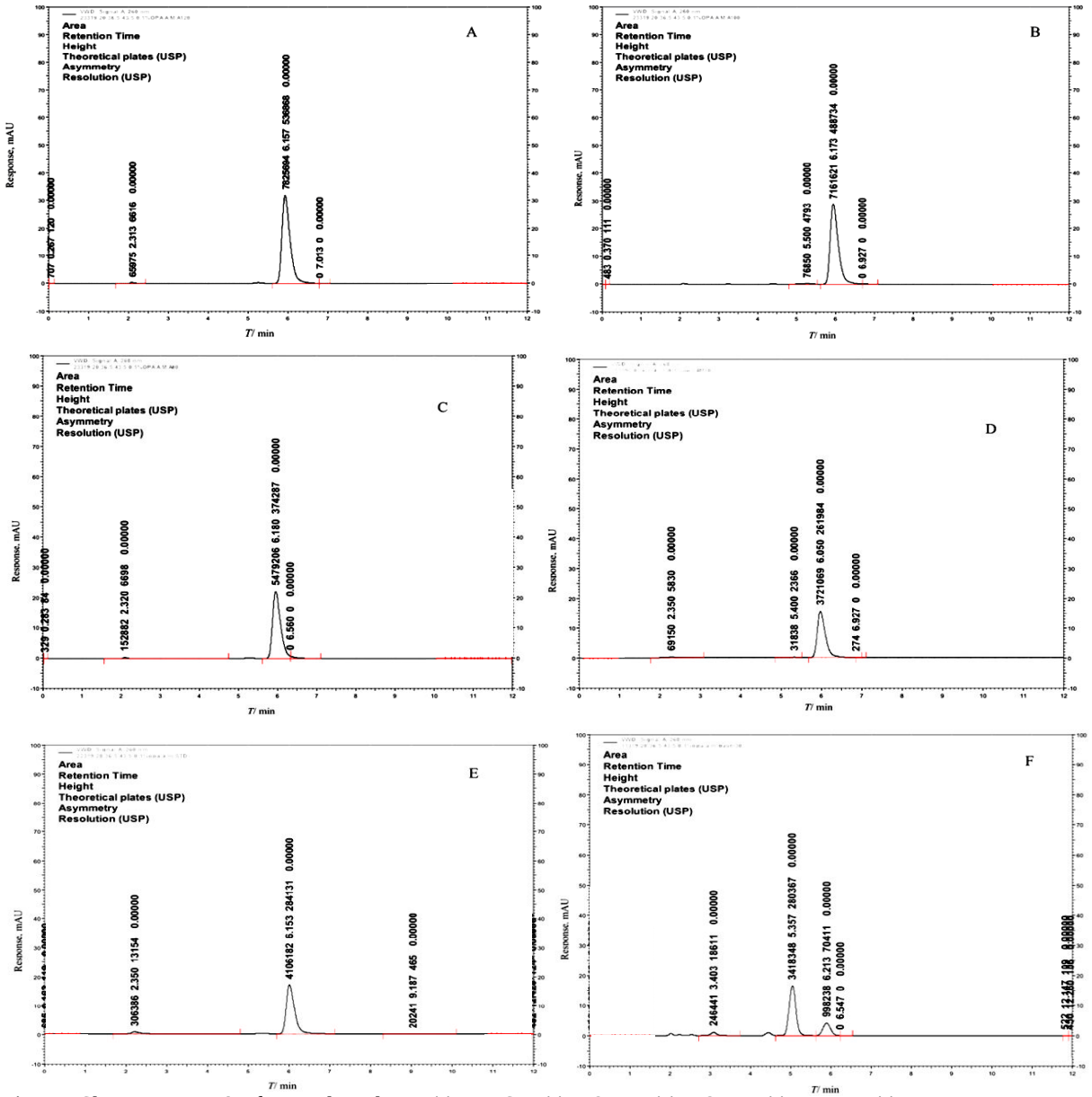

Figure 3. **Chromatograms; A reference glycerol, B sanitizer-1, C sanitizer-2, D sanitizer-3, E sanitizer-4, F sanitizer-5**

Validation of the HPLC Method

Using the external standard method, a glycerol calibration curve was constructed from 0-60 µg/mL. The calibration curves were found to be linear for the above-mentioned concentration range with R^2 = 0.999. All validation parameters are performed similarly to the UV method. The LOD and LOQ were 2.51 and 7.62 µg/mL, respectively. The precision of the method was performed based on Intraday and Interday and was found to be < 2.00%. The recovery of glycerol was 98.05-100.43%. All the details of validation are listed in Table 5.

***Table 5*. Calibration parameters, limit of detection, quantification for glycerol.**

S. No.	Parameter	Values of Glycerol
1.	Concentration (µg/mL)	0-60
2.	Regression equation	y=123805x+10253
3.	Correlation coefficient(R^2)	0.999
4.	Precision	
	-Intraday	1.1
	-Interday	1.75
5.	LOD	2.516
6.	LOQ	7.624
7.	Accuracy	98.05-100.43%

Application of the UV and HPLC Method to Marketplace Sanitizer Samples

Glycerol contents in 5 different samples purchased in local markets were analyzed. All the sanitizers were produced in Nepal. The detailed results are listed in Table 6. Glycerol content in the sanitizer ranged from 1.64 to 1.83 g in 100/mL.

***Table 6*. Glycerol content of sanitizers.**

Samples[a]	Labelled claim (g)	Amount found (Mean±SD) HPLC	Amount found (Mean±SD) UV	Assay HPLC, %	Assay UV, %
Sanitizer-1	1.83[b]	1.66±0.016	1.62±0.016	91	88.52
Sanitizer-2	1.83[b]	1.26± 0.009	1.20± 0.030	69	65.57
Sanitizer-3	Unlabeled	0.86± 0.049	0.81± 0.16	47	44.26
Sanitizer-4	Unlabeled	0.96±0.124	0.89±0.012	52.47	48.63
Sanitizer-5	Unlabeled	0.786±0.024	0.75±0.020	43	40.98

[a]Sanitizer 1 to Sanitizer 5 are five alcoholic sanitizers from different companies, [b]Density of glycerol is 1.26 gm/cm^3; sanitizer-1 and 2 contained 1.45% v/v of glycerin, converting into mass yields 1.83 g of glycerin 100/mL

Conclusion

The use of sanitizer is exceeding day by day in the COVID-19 pandemic. The use of glycerol in several sanitizers as emollient showed the variation in the amount and the purity, ranging from 0.78 to 1.66 g and 0.75 to 1.62 g per 100 mL, by HPLC and UV, respectively. Sanitizers should have the required amount of glycerol recommended by the WHO, and those with less purity should be withdrawn and their uses must be limited. Due to the simple operation, the cost-effective reagent used, and without any sample pretreatment, with good precision, this method can use for routine analysis.

Conflict of Interest

The authors declare that there is no conflict of interest.

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