

# Analysis of Ethanolic Content and Detection of Methanolic Impurity in Marketed Hand Sanitizers Using Gas Chromatography

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## Abstract

Adulteration studies have a great impact on product development as we can analyze or terminate the impurity (adulterant) present in the formulation using different types of instruments used in quality processes. Impurities can be unintentional, which can come from active ingredients, excipients, solvents, and containers, or they may be intentionally added, like adulterants, to make products at a low cost using cheap materials, which alter the quality of the product and lead to various diseases. During COVID-19, the demand for sanitizers suddenly increased, so some companies started using adulterants like methanol instead of ethanol, which led to severe skin infections in people. The quality of sanitizer depends on the active and inactive ingredients as well as the impurities present. In this article, we have discussed the adulteration studies of some marketed ethanolic sanitizers containing methanol as an impurity using gas chromatography, which is a very sensitive instrument especially designed for volatile formulations. The results obtained for different branded sanitizers during analysis gave adequate details regarding purity and adulteration of the formulation.

**Keywords:** Gas Chromatography, Sanitizer, Adulteration, Impurity, Retention time.

## 1. Introduction

Adulteration of any unwanted entity leads to altering the quality of the formulation, which can cause serious or non-serious disease. The substitute used instead of the specified entity is called an adulterant. The main adulterant used in ethanolic sanitizers is methanol [1].

Sanitizer, also called hand rub, is the liquid, gel, or foam that is used to kill microorganisms on the hands. Alcohol-based sanitizers contain at least 70% alcohol, like ethanol or isopropyl alcohol. Care is the major concern in the case of alcohol-based sanitizers, as these are flammable. Non-alcoholic sanitizers typically contain benzalkonium chloride, but they are less effective than alcoholic sanitizers [8].

Sanitizers contain more than 70% alcohol (ethanol or isopropyl alcohol), humectants (glycerine and propylene glycol), emollients (isopropyl myristate), moisturizers (aloe vera and tocopherol acetate), emulsifiers, carbomers, and other ingredients like fragrance and FDA-approved colorants [9].

The analysis of methanolic adulteration in the sanitizers can be done by gas chromatography because of the volatile nature of the alcohols. We can do analysis by initiating sample preparation and then arranging all the required parameters to operate the instrument. There are two types of gas chromatography methods based on two principles [3].

**1.1 Gas Liquid Chromatography:** The principle of GLC is based on the partitioning of compounds between stationary liquids and mobile

**1.2 Gas solid chromatography:** The principle of GSC is based on adsorption, as chemicals are retained by their adsorption to the surface of the support. This support may be silica or alumina [10].

**1.3 Carrier gas:** The commonly used carrier gases are hydrogen, helium, nitrogen, and argon. The main purpose of carrier gas (the mobile phase) is to transport sample components through the column [10].

**1.4 Microsyringe:** A microsyringe is used to inject the sample through a rubber septum into a flash vaporizer chamber at the head of the column. The common volume range is between 0.5 L and 10 L [10].

**1.5 Liner:** The role of the liner in a GC system is to form a vessel into which the sample can be injected and heated. The liner converts the liquid sample into a vapor phase [10].

**1.6 Columns: Capillary columns** are gas chromatography (GC) columns that have the stationary phase coating their inner surfaces rather than being packed into the cavity.

**1.7 Packed column** (stainless steel or glass column): Short, thick columns made of glass or stainless-steel tubes, or packed columns, have been used since the early stages of gas chromatography.

**1.8 Detectors: Flame Ionization Detector**

The operation of the FID is based on the detection of ions formed during the combustion of organic compounds in a hydrogen flame [10].

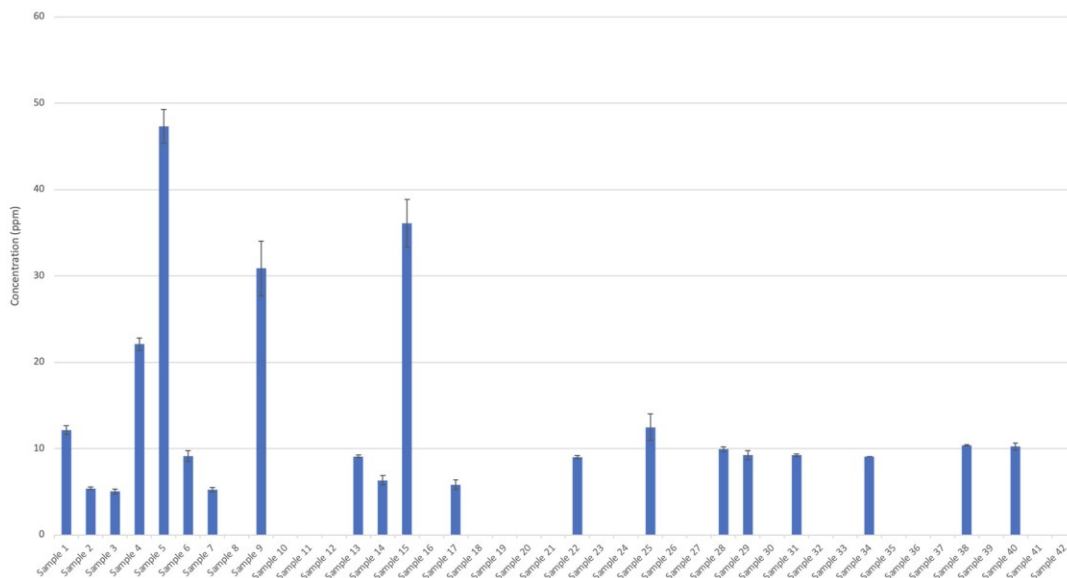
**1.9 Thermal Conductivity Detector**

This detector detects changes in the warm conductivity of the segment eluent and compares it to a reference stream of transporter gas.

**1.10 Retention time:** A component that adsorbs most unequivocally to the fixed stage will invest the most energy in the segment (will be held in the section for quite a while) and will, in this way, have the longest retention time (tR). It will emerge from the gas chromatograph last, and vice versa.

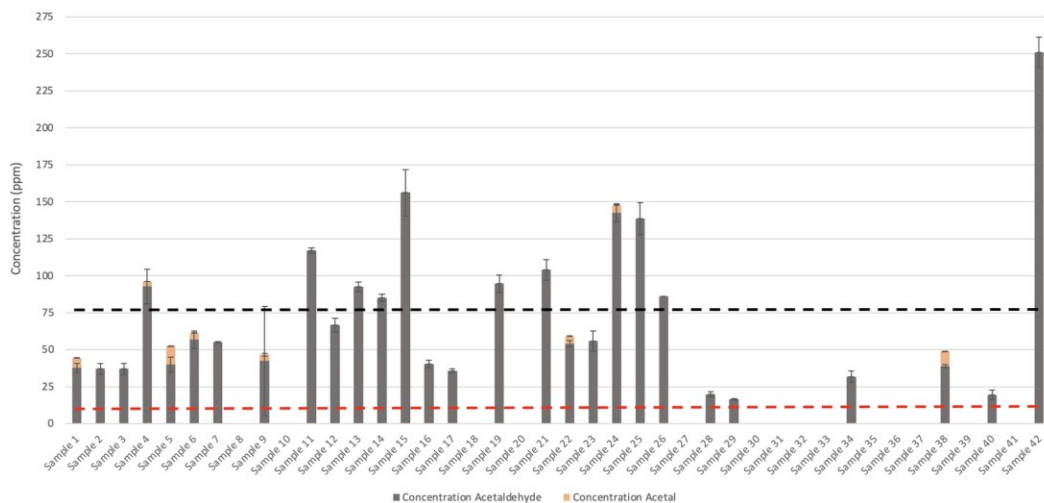
**2. Literature Review**

2.1 A group of scientists i.e., Timothy J. Tse et al., starts analysis of alcohol-based hand sanitizers using Agilent 7980 Gas Chromatography in USA [20]. The machine was equipped with flame ionization detector and the carrier gas used was Helium. Samples were injected using Agilent autosampler with the 10 µL micro syringe. Volatile components separated using Agilent DB-624 ultra-inert column (ID-0.32mm). The FID was operated at 250°C with flow rates of 400 mL min<sup>-1</sup> of air, 30 mL min<sup>-1</sup> of hydrogen. The temperature was increased from 40° C for 5 min to 225°C at 20°C min<sup>-1</sup> for 2.5 minutes. Helium was used as the carrier gas, with a flow rate of 6 mL min<sup>-1</sup>. The split injection of 40:1 was used at 140 °C and 1 µL of sample was injected. The syringe was washed continuously with Acetonitrile.



**Figure 1.** Methanol concentration in marketed Alcoholic sanitizers (Liquid samples 1-26, Gelled samples 27-42)

Methanol concentrations were below  $200 \mu\text{L L}^{-1}$  in accordance with USP criteria. Acetaldehyde peaks were also



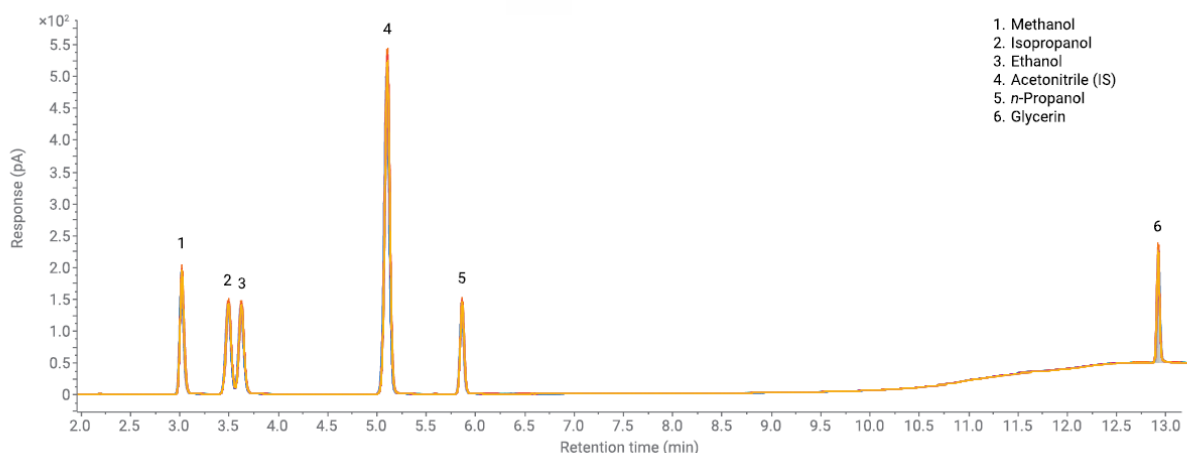
obtained in 28 samples. Both methanol and acetaldehyde were present in specified limits.

**Figure 2.** Acetaldehyde and acetal concentrations in marketed alcoholic sanitizers

2.2 Jie Zhang used the Agilent 8860 GC equipped with a Split inlet and an FID. Liquid sample injection was done using an Agilent 7693A automatic liquid sampler with a  $5 \mu\text{L}$  syringe [21]. The glycerine solution was prepared by weighing 58.4 mg of glycerine and diluting it in distilled water to 1 mL. The calculated volume concentration for glycerine working solution was 4.6% (v/v).

The alcohol calibration standards were prepared by adding pure alcohols or its working solution to distilled water with a final volume at 1 mL. Ethanol, IPA, and glycerine are in the group 1 calibration solutions. The calibration range for ethanol and IPA are from 1 to 4% (v/v). Glycerine concentration is from 0.01 to 0.18% (v/v). Methanol and *n*-propanol (*n*-PA) were prepared in group 2 calibration solutions. The calibration range for methanol was from 1 to 4% (v/v), and 0.1 to 1.5% (v/v) for *n*-propanol. The internal standard (IS) acetonitrile was added to the calibration solutions at a concentration of 5% (v/v). The calibration solutions were prepared as shown in Table 6. The QC sample was prepared by diluting  $25 \mu\text{L}$  of ethanol,  $25 \mu\text{L}$  of IPA, and  $50 \mu\text{L}$  of acetonitrile to 1 mL in distilled water [21].

The hand sanitizer sample of  $50 \mu\text{L}$  was dispensed into a flask. Next,  $50 \mu\text{L}$  of acetonitrile were added as internal standard and the sample was diluted to 1 mL by distilled water for later analysis [21].



**Figure 3:** Gas chromatographic peaks of different volatile components [21].

Methanol and Isopropyl alcohol eluted before ethanol. The resolution of *n*-propanol to acetonitrile was 9 and the resolution of ethanol to acetonitrile was 12. The resolution and peak shape achieved on the 8860 GC. The resolution of ethanol and IPA was 1.31. It is not a baseline separation but usually does not impact the accurate quantitation of ethanol or IPA considering they do not co-exist in the same-hand sanitizer in most cases. Even if they are co-existent, the resolution of 1.31 is sufficient to give an accurate quantitation on the principal alcohol, which can be shown in the following analysis result on a hand sanitizer gel sample [21].

**Table 1.** The result summary of sanitizer sample [21].

| Gel 1         |          |             |            |             | Gel 2         |          |             |          |             |
|---------------|----------|-------------|------------|-------------|---------------|----------|-------------|----------|-------------|
| Injection No. | RT (min) | Quan (Vol%) | RT (min)   | Quan (Vol%) | Injection No. | RT (min) | Quan (Vol%) | RT (min) | Quan (Vol%) |
|               | Ethanol  |             | n-Propanol |             |               | IPA      |             | Ethanol  |             |
| 2             | 3.681    | 61.59       | 5.858      | 10.48       | 7             | 3.49     | 3.63        | 3.62     | 69.93       |
| 3             | 3.62     | 61.55       | 5.856      | 10.48       | 8             | 3.491    | 3.63        | 3.621    | 70.39       |
| 4             | 3.618    | 61.54       | 5.858      | 10.49       | 9             | 3.49     | 3.65        | 3.619    | 70.38       |
| 5             | 3.618    | 61.02       | 5.857      | 10.41       | 10            | 3.491    | 3.65        | 3.623    | 70.45       |
| 6             | 3.619    | 61.56       | 5.856      | 10.48       | 11            | 3.493    | 3.63        | 3.623    | 70.40       |
| 13            | 3.62     | 61.59       | 5.856      | 10.48       | 18            | 3.49     | 3.65        | 3.621    | 70.02       |
| 14            | 3.618    | 61.57       | 5.856      | 10.48       | 19            | 3.492    | 3.61        | 3.622    | 70.29       |
| 15            | 3.62     | 61.85       | 5.858      | 10.53       | 20            | 3.492    | 3.62        | 3.623    | 70.39       |
| 16            | 3.619    | 61.73       | 5.857      | 10.51       | 21            | 3.492    | 3.66        | 3.622    | 70.56       |
| 17            | 3.618    | 61.75       | 5.856      | 10.52       | 22            | 3.492    | 3.63        | 3.622    | 70.30       |
| 24            | 3.619    | 61.42       | 5.856      | 10.47       | 29            | 3.491    | 3.65        | 3.622    | 70.40       |
| 25            | 3.617    | 61.76       | 5.855      | 10.51       | 30            | 3.49     | 3.61        | 3.621    | 70.34       |
| 26            | 3.619    | 61.74       | 5.857      | 10.51       | 31            | 3.49     | 3.62        | 3.62     | 70.41       |
| 27            | 3.619    | 61.89       | 5.856      | 10.53       | 32            | 3.49     | 3.63        | 3.62     | 70.36       |
| 28            | 3.619    | 61.80       | 5.856      | 10.53       | 33            | 3.491    | 3.66        | 3.622    | 70.60       |
| 35            | 3.618    | 61.93       | 5.856      | 10.55       | 40            | 3.489    | 3.64        | 3.619    | 70.97       |
| 36            | 3.617    | 62.02       | 5.855      | 10.55       | 41            | 3.49     | 3.64        | 3.62     | 70.49       |

|      |       |       |       |       |    |       |       |       |       |
|------|-------|-------|-------|-------|----|-------|-------|-------|-------|
| 37   | 3.618 | 62.28 | 5.857 | 10.60 | 42 | 3.49  | 3.64  | 3.62  | 70.58 |
| 38   | 3.619 | 62.09 | 5.857 | 10.55 | 43 | 3.487 | 3.70  | 3.617 | 70.52 |
| 39   | 3.617 | 62.07 | 5.856 | 10.55 | 44 | 3.488 | 3.61  | 3.619 | 70.71 |
| Mean | 3.62  | 61.74 | 5.86  | 10.51 |    | 3.49  | 3.64  | 3.62  | 70.42 |
| RSD% | 0.03% | 0.45% | 0.02% | 0.38% |    | 0.04% | 0.55% | 0.04% | 0.31% |

The result summary for 20 injections of gel samples 1 and 2 is tabulated in Table 2. The concentration measured for the 40 injections of real hand sanitizer samples matched well with the labelled concentration range. The quantitation precision for ethanol and *n*-propanol in sample 1 was 0.45% and 0.38%, respectively, and the quantitation precision for IPA and ethanol in sample 2 was 0.55% and 0.31%. The RT repeatability for ethanol, IPA and *n*- propanol during 20 injections ranged from 0.02 to 0.04%, comparable to the RT RSD% of 0.01 to 0.03% generated in seven consecutive injections shown in Figure 2.

### RATIONALE OF STUDY

Public health is the major concern in recent past years due to SARS COV-19 as millions of people got serious respiratory infections and thousands of them can't survive due to low immunity. Research concluded that we could kill virus using Sanitizers and by maintaining distance of 2 meter. Before 2020 the use of sanitizers was limited due to which the demand of sanitizers increases but there was no sufficient production, so companies start adulteration of ethanolic sanitizers using methanol as adulterant due to which people got skin infections as methanol is toxic for human use. Food and Drug Administration give limits of methanol i.e., 0.063 Vol% but companies add more than specified quantity in Sanitizers. Methanol has low ability to kill viruses and is cheaper than ethanol. When a person got contact with methanol it absorbs through skin, lungs, stomach, etc and made formaldehyde which converts into formic acid leads to brain damage or death of the person. Methanol should not be used instead of Ethanol and isopropyl alcohol or more than specified quantity because this is injurious to human health and can lead to various skin infection or death in some cases.

Gas Chromatography is especially designed for volatile solvents in complex formulations and easily available in the laboratories, so we have selected the Gas Chromatographic method for detection of Ethanolic sanitizers and methanolic impurities.

### 3. EXPERIMENTAL MATERIALS AND METHOD:

#### 3.1 Equipments:

Gas chromatography system of Agilent Technologies 7890A, distillation apparatus, micropipette, round bottom flask, and microsyringe

#### 3.2 Chemicals used:

Methanol is 99.99% pure.

Ethanol is 99.9% pure.

Acetone for washing glassware

#### 3.3 Sample 1: DXXXXL HAND SANITIZER

Active ingredients:

Alcohol IP (denatured ethanol) is equivalent to absolute alcohol. 72.4% v/v inactive ingredients

Water, PEG/PPG-17/6 Copolymer, Propylene Glycol, Acrylates/C10-30, Alkyl Acrylate Cross Polymer, and Tetrahydroxypropylethylenediamine

Mfd. 01/2022/Batch No. PXXXXX1 Exp. 12/2023/MRP. Rs. 25/Net Vol.: 50m

### 3.4 Sample 2: OXXXXXA AXXXXXA HAND SANITIZER

Active ingredients:

Ethyl Alcohol (70% v/v) Inactive Ingredients

Demineralized Water, Diethyl Phthalate, Glycerine, Phenoxyethanol, Aloe Vera Extract, Fragrance, and Color

Mfd. 05/2020/Batch No. SXXXXX0/Exp. 05/2023/MRP. Rs. 50/Net Vol.: 100ml

### 3.5 Sample 3: MXXXXXXS HAND SANITIZER

Active ingredients:

Ethyl Alcohol IP 70% v/v Inactive Ingredients

Glycerine IP 1.5% w/v and color: brilliant blue FCF

Mfd. 05/2021/ Lot No. AXXXXX1/ Exp. 04/2023/ MRP. Rs. 100/ Net Vol. 120ml

**3.6 Gas chromatographic method:** The samples of sanitizers of different brands were analyzed to determine the content of ethanol and methanolic adulteration in hand sanitizers using an Agilent GC equipped with a flame ionization detector from Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754 and nitrogen as a carrier gas. The methanol limit should not be more than 0.063 vol% (USFDA). The operating conditions of gas chromatography for the detection of ethanolic content and adulteration of methanol in sanitizers are mentioned in Table No. 1.

**3.7 Process of Distillation:** Distillation is the process of separating the components from the liquid or gel mixture by using selective boiling temperatures and condensation. The parts of the distillation assembly (condenser, still head, thermometer, round bottom flask, distillate, and still receiver) are assembled. A water bath was used to heat the liquid. The three samples of marketed ethanolic sanitizers are taken for analysis of the methanol and ethanol content in the samples. So, these samples were subjected to the process of distillation because, without distillation, the residual solvents present in the sample could damage the gas chromatography column. Each sample of 50 ml undergoes distillation to make it concentrate to 10 ml volume. This is done to clear any other residual solvent present that may alter the results of the gas chromatography analysis.

**Table 2.** GC-FID operating conditions for methanol and ethanol analysis in hand sanitizer using gas chromatography Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron -60 to 325/350C SN: USB474754H:

| Parameters             | Split Injection        |
|------------------------|------------------------|
| Injection Temperature: | 220°C                  |
| Injection Volume:      | 0.5 µL                 |
| Injection Liner:       | Split Liner            |
| Syringe Rinse:         | 5 rinses with DI water |

|                           |   |
|---------------------------|---|
| FID Detector Temperature: | 250°C   |
| H2 Flow Rate:             | 30 mL/min   |
| Air Flow Rate:            | 300mL/min   |
| Makeup Gas:               | Nitrogen  |
| Makeup Gas Flow Rate:     | 30 mL/min   |
| Carrier Gas:              | Nitrogen  |
| Carrier Gas Flow Rate:    | Constant flow: 30 mL/min  |
| Column:                   | Agilent Technologies, Inc.<br>HP-5, 30 m x 0.320 mm x 0.25 micron<br>-60 to 325/350C SN: USB474754H |
| Oven Temperature:         | 40 °C-220 °C  |
| Split Ratio:              | 50:1  |
| Temperature Program °C:   |   |
| Initial:                  | 40°C for 0 min  |
| Ramp 1:                   | 2°C/min to 70°C for 0 minutes   |
| Ramp 2:                   | 20°C/min to 220°C for 5 minutes   |
| Retention Time Order:     | Methanol, Ethanol   |
| Standard Sample           | Methanol And Ethanol  |

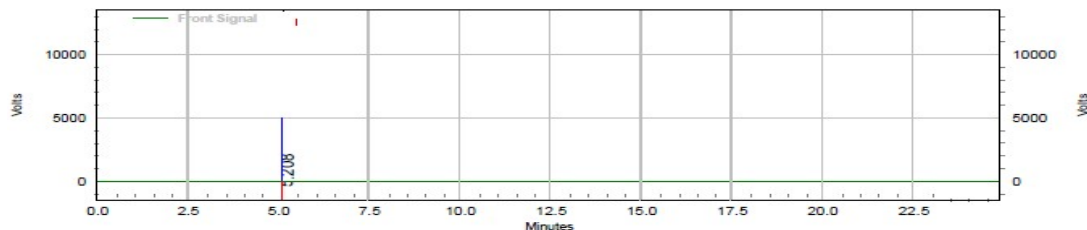
#### 4. RESULTS AND DISCUSSION:

The samples were analyzed for determination of ethanolic content and methanolic adulteration in hand sanitizers using Agilent gas chromatography equipped with a flame ionization detector at 250°C and an oven temperature of 40°C–220°C using Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754H column, nitrogen as carrier gas with a flow rate of 30 ml/min, hydrogen gas with a flow rate of 30 ml/min, and air with a flow rate of 300 ml/min. The injection temperature was set to 220°C, and the injection volume was 0.5 L. The process was operated with a constant flow of all the gases and sample injections. The split liner was used in the method that split the sample into 50:1. The microsyringe was used in the machine, which took samples for injection after five rinses with de-ionized water. Initial ramp temperature was 40°C for 0 minutes; ramp 1 temperature was 2°C/min to 70°C for 0 minutes; and ramp 2 temperature was 20°C/min to 220°C for 5 minutes.

After the setup of all the essential parameters of the GC machine, the samples were injected one by one into the GC machine to obtain the retention time and area under the curve of all the samples to quantify and qualify them. Three different samples from different brands of sanitizers were compared with the standard samples of methanol and ethanol to analyze the presence and amount of ethanol in the samples.

**Area % Report**

Data File: D:\Enterprise\HHRC\Result\METHANOL STD D.rslt\M(Akash Sharma).dat  
 Method: D:\Enterprise\HHRC\Method\IPA\Ethanol.met  
 Acquired: 21-05-2022 10:14:29 (GMT +05:30)  
 Printed: 21-05-2022 10:45:07 (GMT +05:30)



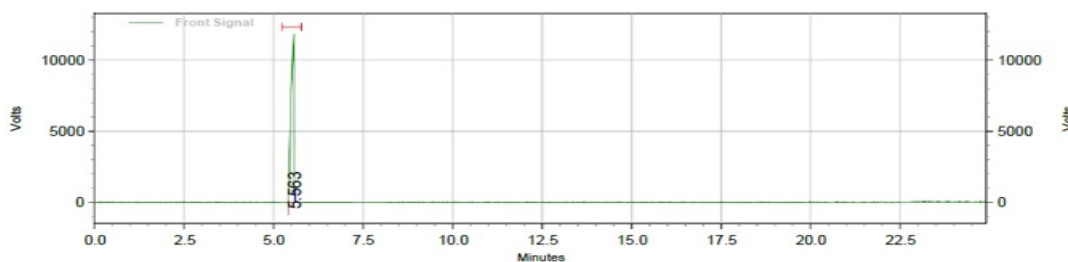
| Front Signal Results |           |        |          |          |          |
|----------------------|-----------|--------|----------|----------|----------|
| Retention Time       | Area      | Area % | Height   | Height % | Name     |
| 5.208                | 365206169 | 100.00 | 92220616 | 100.00   | METHANOL |
| <b>Totals</b>        |           |        |          |          |          |
|                      | 365206169 | 100.00 | 92220616 | 100.00   |          |

**Figure 4.** Gas chromatographic peak of standard sample of methanol

**4.1 Analysis of a standard sample of Methanol using gas chromatography**

**Discussion:** The sample of standard methanol was analyzed using an Agilent gas chromatography machine equipped with a flame ionization detector at 250°C and an oven temperature of 40°C–220°C, using Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754H column, nitrogen as carrier gas with a flow rate of 30 ml/min, hydrogen gas with a flow rate of 30 ml/min, and air with a flow rate of 300 ml/min. The injection temperature was set to 220°C, and the injection volume was 0.5 L. The split liner was used in the method that split the sample into 50:1. The microsyringe was used in the machine, which took samples for injection after five rinses with de-ionized water. Initial ramp temperature was 40°C for 0 minutes; ramp 1 temperature was 2°C/min to 70°C for 0 minutes; and ramp 2 temperature was 20°C/min to 220°C for 5 minutes. The sample was analyzed, and results showed that the retention time of the standard sample of methanol was 502.8 minutes. The peak area was 365206169, the peak height was 92220616, and the peak of retention time obtained is mentioned in figure no. 4.

**4.2 Analysis of DXXXXL hand sanitizer using gas chromatography**



| Front Signal Results |           |        |          |          |         |
|----------------------|-----------|--------|----------|----------|---------|
| Retention Time       | Area      | Area % | Height   | Height % | Name    |
| 5.563                | 539437695 | 100.00 | 90534630 | 100.00   | ETHANOL |
| <b>Totals</b>        |           |        |          |          |         |
|                      | 539437695 | 100.00 | 90534630 | 100.00   |         |

**Figure 5.** Gas chromatographic peak of ethanol

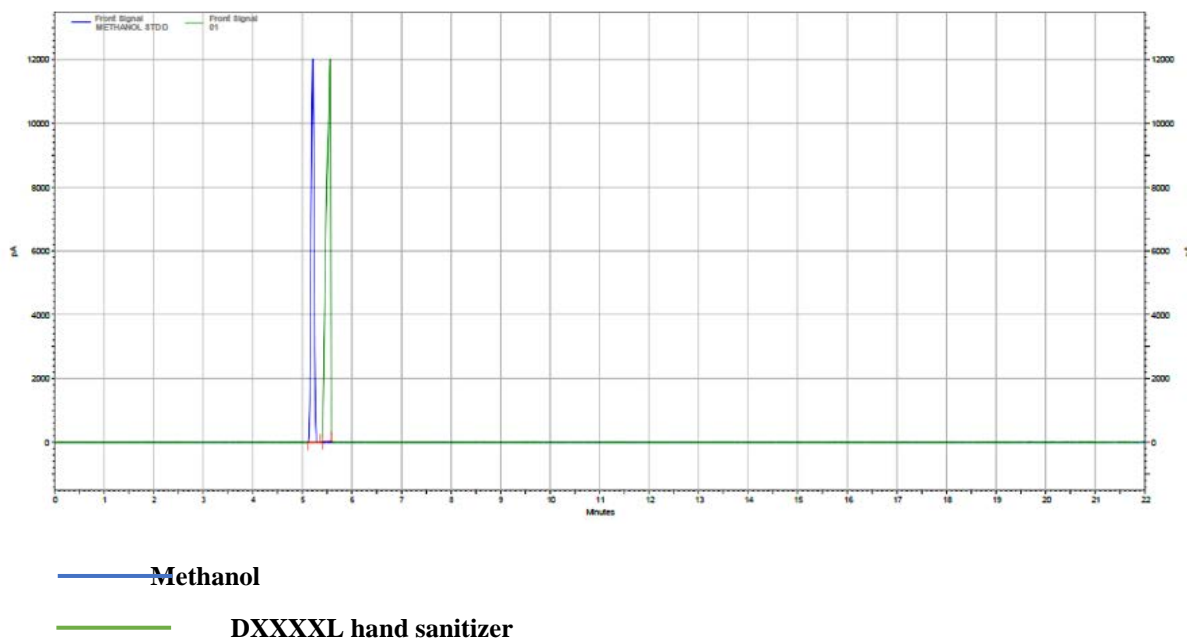
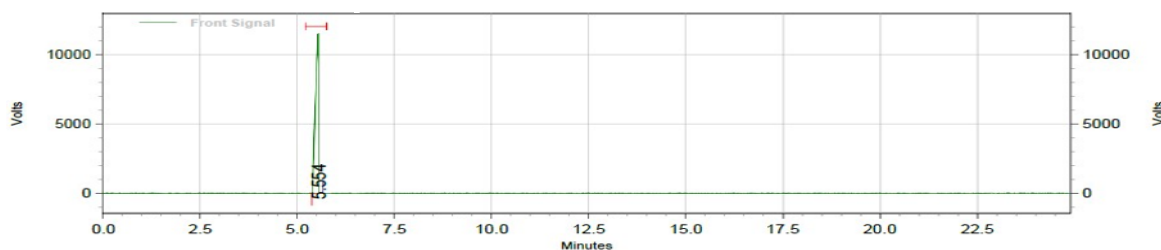


Figure 6. Overlay graph of methanol and ethanol peaks

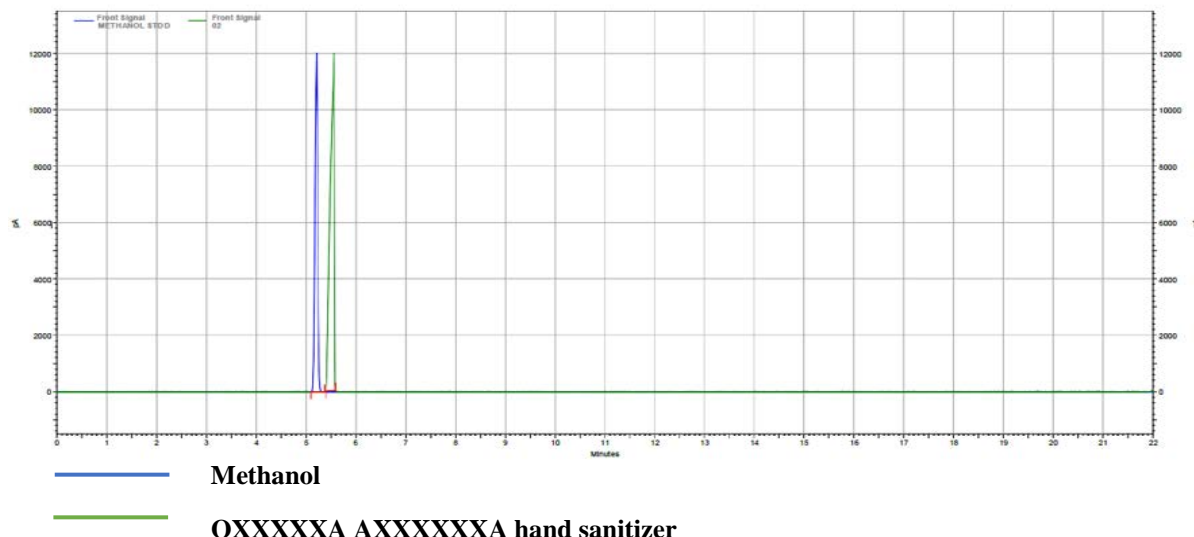
**Discussion:** The sample of DXXXXL hand sanitizer was analyzed for determination of ethanolic content and methanolic adulteration in hand sanitizers using Agilent gas chromatography equipped with a flame ionization detector at 250°C and an oven temperature of 40°C–220°C using Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754H column, nitrogen as carrier gas with a flow rate of 30 ml/min, hydrogen gas with a flow rate of 30 ml/min, and air with a flow rate of 300 ml/min. The injection temperature was set to 220°C, and the injection volume was 0.5 L. The process was operated with a constant flow of all the gases and sample injections. The split liner was used in the method that split the sample into 50:1. The microsyringe was used in the machine, which took samples for injection after five rinses with de-ionized water. Initial ramp temperature was 40°C for 0 minutes; ramp 1 temperature was 2°C/min to 70°C for 0 minutes; and ramp 2 temperature was 20°C/min to 220°C for 5 minutes. The sample was analyzed, and no trace of methanol was detected in the sample; however, a peak of ethanol was observed in the retention time graph. The retention time peak of ethanol is mentioned in figure 5, and the overlay peaks of the methanol standard and the ethanol of the sample are mentioned in figure 6. The retention time of ethanol was 5.563 minutes, the peak area of ethanol was 539437695, and the peak height was 90534630. The percentage purity of standard ethanol was 99.9%, and the peak area of standard ethanol was 725389511. So, the concentration of ethanol present in the sample was found to be 74.3%, but the label claim of DXXXXL hand sanitizer was 72.4%. The difference between the concentration of the test sample and the standard sample may be due to gelling agents and polymers present in the sample.

#### 4.3 Analysis of OXXXXXA AXXXX' XA hand sanitizer using gas chromatography



| Front Signal Results |           |        |          |          |         |
|----------------------|-----------|--------|----------|----------|---------|
| Retention Time       | Area      | Area % | Height   | Height % | Name    |
| 5.554                | 507772658 | 100.00 | 88686421 | 100.00   | ETHANOL |
| <b>Totals</b>        |           |        |          |          |         |
|                      | 507772658 | 100.00 | 88686421 | 100.00   |         |

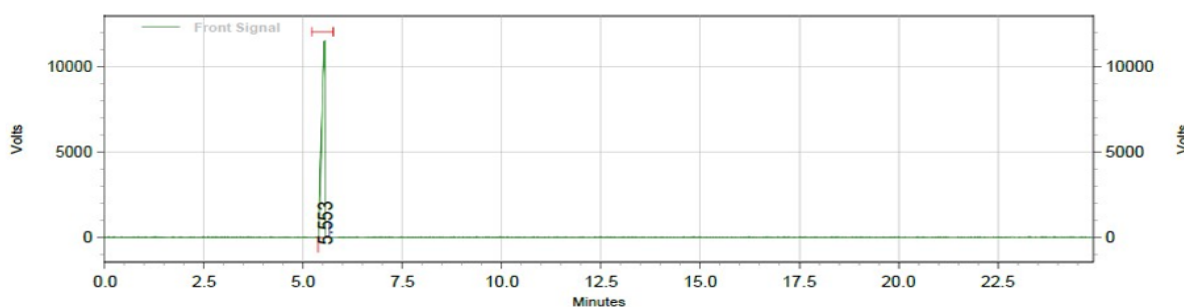
Figure 7. Gas chromatographic peak of ethanol



**Figure 8.** Overlay graph of methanol and ethanol peaks

**Discussion:** The sample of OXXXXXXA AXXXXXXA hand sanitizer was analyzed for determination of ethanolic content and methanolic adulteration in hand sanitizers using Agilent gas chromatography equipped with a flame ionization detector at 250°C and an oven temperature of 40°C–220°C using Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754H column, nitrogen as carrier gas with a flow rate of 30 ml/min, hydrogen gas with a flow rate of 30 ml/min, and air with a flow rate of 300 ml/min. The injection temperature was set to 220°C, and the injection volume was 0.5 L. The process was operated with a constant flow of all the gases and sample injections. The split liner was used in the method that split the sample into 50:1. The microsyringe was used in the machine, which took samples for injection after five rinses with de-ionized water. Initial ramp temperature was 40°C for 0 minutes; ramp 1 temperature was 2°C/min to 70°C for 0 minutes; and ramp 2 temperature was 20°C/min to 220°C for 5 minutes. The sample was analyzed, and no trace of methanol was detected in the sample; however, a peak of ethanol was observed in the retention time graph. The retention time peak of ethanol is mentioned in figure 7, and the overlay peaks of the methanol standard and the ethanol of the sample are mentioned in figure 8. The retention time of ethanol was 5.554 minutes, the peak area of ethanol was 507772658, and the peak height was 88686421. The percentage purity of standard ethanol was 99.9%, and the peak area of standard ethanol was 725389511. So, the concentration of ethanol present in the sample was found to be 70%, which was the same as the label claim of OXXXXXXA AXXXXXXA hand sanitizer, i.e., 70%.

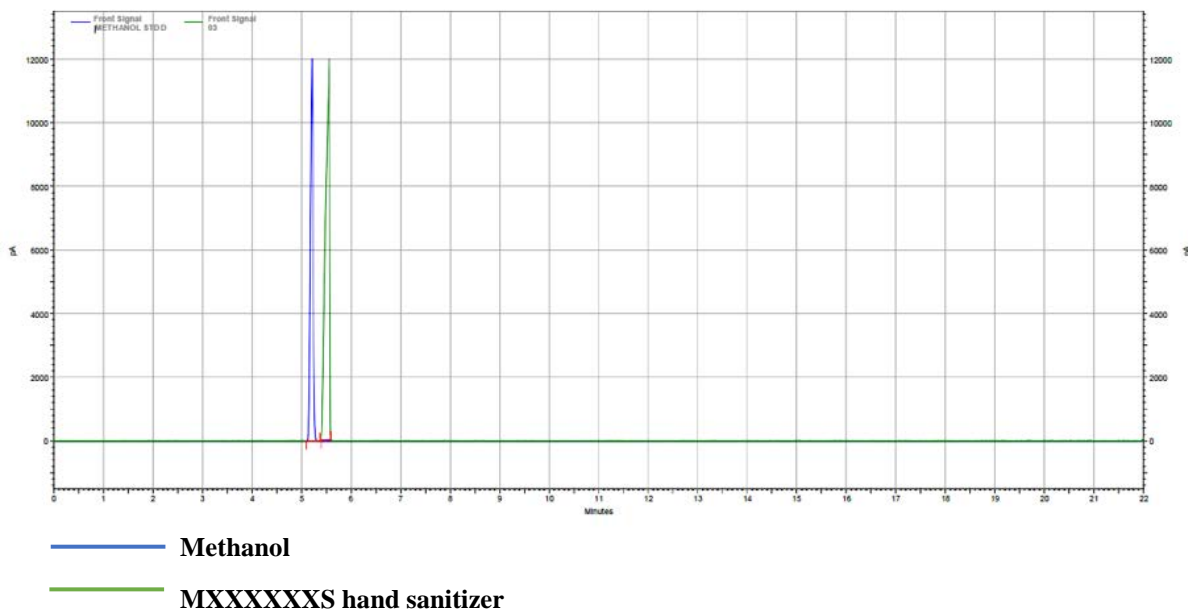
#### 4.4 Analysis of MXXXXXXS hand sanitizer using gas chromatography



**Front Signal Results**

| Retention Time | Area      | Area % | Height   | Height % | Name    |
|----------------|-----------|--------|----------|----------|---------|
| 5.553          | 517562457 | 100.00 | 89775729 | 100.00   | ETHANOL |
| <b>Totals</b>  | 517562457 | 100.00 | 89775729 | 100.00   |         |

**Figure 9.** Gas chromatographic peak of ethanol



**Figure 10.** Overlay graph of methanol and ethanol peaks

**Discussion:** The sample of MXXXXXXS instant hand sanitizer was analyzed for determination of ethanolic content and methanolic adulteration in hand sanitizers using Agilent gas chromatography equipped with a flame ionization detector at 250°C and an oven temperature of 40°C–220°C using Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754H column, nitrogen as carrier gas with a flow rate of 30 ml/min, hydrogen gas with a flow rate of 30 ml/min, and air with a flow rate of 300 ml/min. The injection temperature was set to 220°C, and the injection volume was 0.5 L. The process was operated with a constant flow of all the gases and sample injections. The split liner was used in the method that split the sample into 50:1. The microsyringe was used in the machine, which took samples for injection after five rinses with de-ionized water. Initial ramp temperature was 40°C for 0 minutes; ramp 1 temperature was 2°C/min to 70°C for 0 minutes; and ramp 2 temperature was 20°C/min to 220°C for 5 minutes.

The sample was analyzed, and no trace of methanol was detected in the sample; however, a peak of ethanol was observed in the retention time graph. The retention time peak of ethanol is mentioned in figure 9, and overlay peaks of the methanol standard and the ethanol of the sample are mentioned in figure 10. The retention time of ethanol was 5.553 minutes, the peak area of ethanol was 517562457, and the peak height was 89775729. The percentage purity of standard ethanol was 99.9%, and the peak area of standard ethanol was 725389511. So, the concentration of ethanol present in the sample was found to be 71.3%, which was close to the label claim of MXXXXXXS instant hand sanitizer, i.e., 70%.

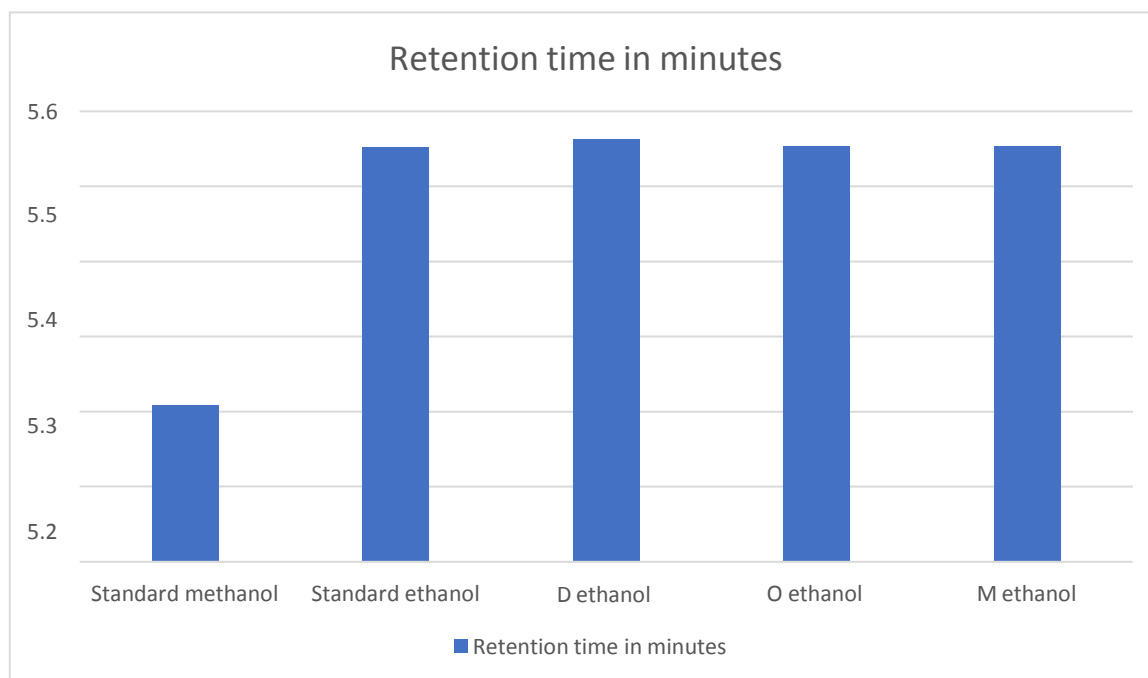
## 5. SUMMARY AND CONCLUSION

Impurity profiling is very essential in the pharmaceutical industry, as these impurities can be present in any form and come from any source. Nowadays, adulteration is present in everything, as each industry, whether it is pharmaceutical or cosmetic, uses adulterants to make products cheap without changing the selling price to gain commercial profits. So, we have selected some marketed ethanolic sanitizers and analyzed them with all their specifications to check whether they are safe for use or not. We used the gas chromatography technique, which is designed for volatile solvents. The instrument is equipped with a flame ionization detector from Agilent Technologies, Inc. HP-5, 30 m x 0.320 mm x 0.25 micron, -60 to 325/350C SN: USB474754 column and nitrogen as carrier gas. We analyze the impurities commonly used in sanitizers, such as methanol.

The samples of marketed sanitizers of different brands (DXXXXL hand sanitizer, OXXXXXA hand sanitizer, and MXXXXXXS hand sanitizer) were analyzed and compared with the standard samples of methanol and ethanol on gas chromatography to determine the content of ethanol and methanol in the marketed sanitizer samples. We concluded that there is no trace of methanol in any of the test samples, whereas the content of ethanol was found within the specified limits.

| Sr. No. | Samples                    | Retention tir min.) | Peak area | Height % |
|---------|----------------------------|---------------------|-----------|----------|
| 1       | Standard methanol          | 5.208               | 365206169 | 100%     |
| 2       | Standard ethanol           | 5.552               | 725389511 | 100%     |
| 3       | DXXXXL sanitizermethanol   | -                   | -         | -        |
| 4       | DXXXXL sanitizerethanol    | 5.563               | 539437695 | 100%     |
| 5       | OXXXXXA sanitizer methanol | -                   | -         | -        |
| 6       | OXXXXXA sanitizerethanol   | 5.554               | 507772658 | 100%     |
| 7       | MXXXXXXS sanitizermethanol | -                   | -         | -        |
| 8       | MXXXXXXS sanitizerethanol  | 5.553               | 517562457 | 100%     |

**Table 3.** Comparative data of the samples



**Figure 11.** Graph of Retention time of methanol standard and ethanol in all samples

We analyzed the 3 samples of different brands, i.e., DXXXXL hand sanitizer, OXXXXXA hand sanitizer, and MXXXXXXS hand sanitizer, and found that DXXXXL instant hand sanitizers contain 74.3% v/v ethanol, which is more than the label claim, i.e., 72.4% v/v. This is due to gelling agents and polymers present in the sample. OXXXXXA hand sanitizer contains 70% v/v ethanol, which is the same as the label claim, i.e., 70% v/v, and MXXXXXXS hand sanitizer contains 71.35% v/v ethanol, which is close to the label claim, i.e., 70% v/v.

The methanol was not found to be present in the test samples, and the ethanol content was found in close proximity to the label claim. So, we concluded that our samples of sanitizers are safe and effective for human use.

## 6. REFERENCES

1. World Health Organization. (2010). *Guide to local production: WHO-recommended handrub formulations* (No. WHO/IER/PSP/2010.5). World Health Organization.
2. Gold, N. A., Mirza, T. M., & Avva, U. (2018). Alcohol sanitizer. Government of Canada. Tse.
3. Driscoll, J. N. (1985). Review of photoionization detection in gas chromatography: the first decade. *Journal of chromatographic science*, 23(11), 488-492.
4. Meyers, C., Kass, R., Goldenberg, D., Milici, J., Alam, S., & Robison, R. (2021).
  - a. Ethanol and isopropanol inactivation of human coronavirus on hard surfaces. *Journal of Hospital Infection*, 107, 45-49.
5. T. J., Nelson, F. B., & Reaney, M. J. (2021). Analyses of commercially available alcohol-based hand rubs formulated with compliant and non-compliant ethanol. *International journal of environmental research and public health*, 18(7), 3766.
6. Devi, M. S. (2010). K. Satyanarayana. *Journal of Applied Geochemists*, 12(2), 294- 294.
7. Dimbat, M., Porter, P. E., & Stross, F. H. (1956). Apparatus Requirements for Quantitative Applications. *Analytical Chemistry*, 28(3), 290-297.
8. Ertl, P., Patiny, L., Sander, T., Rufener, C., & Zasso, M. (2015). Wikipedia Chemical Structure Explorer: substructure and similarity searching of molecules from Wikipedia. *Journal of cheminformatics*, 7(1), 1-7.
9. Güntner, A. T., Magro, L., van den Broek, J., & Pratsinis, S. E. (2021). Detecting methanol in hand sanitizers. *Iscience*, 24(2), 102050.
10. Hübschmann, H. J. (2015). *Handbook of GC-MS: fundamentals and applications*. JohnWiley & Sons.
11. hand rubs formulated with compliant and non-compliant ethanol. *International journal of environmental research and public health*, 18(7), 3766.
12. Alvarez-Bobadilla, G., Domínguez-Cherit, G., Acosta-Nava, V. M., Guizar-Rangel, M. T., Guido

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