FORMULATION OF HYDROALCOHOLIC GEL WITH 80% ISOPROPYL ALCOHOL

A RESEARCH DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF PHARMACY IN INDUSTRIAL PHARMACY OF THE UNIVERSITY OF NAIROBI.

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This research dissertation has been submitted for review with our approval as University supervisors:

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DEDICATION

This research dissertation is devoted to God who has given me good health and opportunity to pursue my goals in education. I dedicate this research dissertation to my family whose honorable support and prayers were apparent through the entire course. Your sacrifices are truly treasured and lastly, I thank my classmate for the encouragement and motivation throughout this journey.

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ABBREVIATIONS AND ACRONYMS

AUC: Area under the curve

DARU: Drug Analysis and Research Unit

FDA: Food and Drug Administration (FDA)

GC: Gas chromatography

GMP: Good manufacturing practices

RPM: Rotation per minute

UoN: University of Nairobi

QbD: Quality by design

WHO: World Health Organization

DEFINITION OF TERMS

Disinfectant: A chemical agent designed to destroy or inactivate microorganisms.

Hydro alcoholic gel: A composition that includes water, alcohol, and other ingredients formulated to kill microbes.

Microbes: Microscopic organisms that are as unicellular, multicellular, or cell clusters and including bacteria, fungi, protozoa, algae, and viruses**.**

pH: A measure expressing the acidity or alkalinity of a formulation and ranges from 0 - 14

ABSTRACT

Background: Hydro alcoholic gels are used as hand disinfectants to kill microbes on the skin. Most of hydro alcoholic gels contain a WHO recommended alcohol concentration with a range of 60% to 95% which destroys the lipid that contains the cell membrane of the bacteria and fungi. Besides, alcohol denatures the proteins and enzymes inside bacteria and fungi. Alcohol also dissolves capsids of viruses.

Methodology: This study sought to develop a hydro alcoholic gel composed of carbopol 934 polymer, hydrogen peroxide, isopropyl alcohol, purified water or deionized water and glycerol using the direct addition method. To assess the quality of the formulated product analytical tests such as appearance, viscosity, pH, assay of isopropyl alcohol and stability were determined.

Results and discussion: A well calibrated equipment were used to determine the critical tests, the three formulated hydro-alcoholic gel which are clear colorless gels. Their pH varied from 5.8 to 6.4, their viscosity was tested using an SP 9 at the same RPM and same T^{O} and all the three sample were found to be in the standardized range of 800 – 45000 centipoise, using a chromatographic technique (GC) the assay was done and the three samples were presented as follows respectively 78%, 82% and 76%.

Conclusion: Therefore, in my view, it would appear that P3 can answer to this need; furthermore, it would be valuable to carry on the trials with the purpose to assess the optimum ratio of alcohol to moisturizers (with the least possible amount of alcohol) that provides the greatest antimicrobial activity.

CHAPTER ONE: INTRODUCTION

1.1Background to the research project

Hydro alcoholic gels are useful chemical compositions that people use to destroy microbes on the skin. Health experts have identified skin hygiene as one of the main mechanisms of preventing spread of infectious agents (Larson, 1999). Skin disinfection is a critical element of hygiene practice. The skin is the weightiest on its own body part in the body (Stephen et al., 2010). The epidermis, a tinny superficial layer, the dermis, a profuse innermost layer, and the hypodermis or sub-cutis, a fatty layer of subcutaneous tissue, are the three layers of the skin, each with a different thickness, strength, and function (Stephen et al., 2010). The epidermis is the skin's thin, resistant outer layer, which is without blood vessels and depend on on the dermis for feeding and blood flow. The dermis, on the other hand, is the skin's innermost supporting layer, comprised primarily of collagen and well provided with blood. The dermis houses the body's nerves, sensory receptors, blood arteries, and lymphatics (Stephen et al., 2010). The hypodermis, also called the subcutaneous layer, is a layer of adipose soft tissue that protects the skin from harm. It supports in temperature regulation and the preparation of fat for energy use as required (Stephen et al., 2010).

The skin protects the body from infection in a variety of ways. When it comes to attacking organisms, the skin is the first line of defense. It similarly supports to guard against water loss, pollutants, and other dangerous matters, as well as UV rays (Lachenmeier 2008). A few microorganisms are annihilated by unsaturated fats of the sebaceous discharges, and others, like the Gram negative bacilli, are to a huge sum obliterated when the liquid medium wherein they were put on the skin vanishes (Lachenmeier 2008). While the skin prevents microbes from incoming the body, it cannot protect the body from all disease-causing organisms. For instance,

the skin doesn't annihilate the steady bacterial populace of the skin which lives and expansions in the profundities and hair follicles just as by all accounts (Maiwald & Chan, 2014). Therefore, individuals must use effective disinfectants to protect against infection. The main properties prerequisite in an antiseptic for operation locations are (1) that it should be fast active against a wide range of microorganisms; (2) that it should destroy the organisms and not simply inhibit their growth; and (3) that it should not harm the skin or the fundamental tissues either by direct toxic action or by sensitization (Maiwald & Chan, 2014). Other properties, such as diffusion and temporary staining of the skin to express the disinfected area, may be desirable.

In the past, soaps and detergents were the most common tools for skin hygiene. However, the various negative outcomes that result from the use of soaps and detergents on the skin has created a need for effective yet affordable gels for skin hygiene (Larson, 1999). For example, some studies have shown that soaps raise the pH of the skin that causes skin damage and increased skin shedding (Pittet, 2001). Prolonged use of soaps to wash the skin raises pH levels to highs of 7.0-8.5 and they might remain high for several hours (Pittet, 2001).

Other studies have found that soaps and detergents destroy the skin by lowering the rate of lipid replenishment on the upper skin layer. For example, one study has concluded that the rate of lipid replenishment declines up to 20% one hour after washing the skin with soap (Shokrzadeh et al., 2009). A lowered lipid replenishment rate destroys the skin because the fatty acids have bacterial and fungicidal behavior that regulates the skin flora. Apart from skin damage, other factors lower the usefulness of soaps and detergents for skin hygiene (Shokrzadeh et al., 2009). Usually, the chosen skin hygiene tool must destroy all microbial skin flora from the hands including transient, resident, and infection flora.

Hydro alcoholic gels destroy resident and infection flora effectively without the threat of skin damage. Alcohol-based gels contain one or combinations of a highly concentrated alcohol (Ethanol and isopropyl alcohol), moisturizer/vehicle (glycerin) , polymers (base), pH stabilizers, thickening agents, colorants, fragrances agents and purified water (Lahouel et al., 2020). Hydro alcoholic concentrations evaporate rapidly and spread easily, their effectiveness depend on the concentration of alcohol which are another qualities that make them superior to soaps and detergents. Also, hydro alcoholic concentrations are bearable for the skin as long as they contain appropriate emollients. Numerous studies have found that hydro alcoholic concentrations destroy disease-causing organisms. For example, one study shows a positive correlation between reduction of microbial release and hand treatment (Lahouel et al., 2020).

Alcohol destroys the lipid that contains the cell membrane of the bacteria and fungi. At the same time, alcohol denatures the proteins and enzymes that are inside the bacteria and fungi. Consequently, hydro alcoholic gels kill bacteria and fungi instantly once they come into contact (Pramanik et al., 2018). The effectiveness of the prepared hydro alcoholic concentration depends on the concentration of alcohol. The Food and Drug Administration (FDA) of the United States says that effective hydro alcoholic gels contain ethanol at 60-90% or isopropanol at 70-90% of the prepared concentration (Pramanik et al., 2018). On the other hand, rheology modifiers thicken and stabilize the concentration. A combination of water, alcohol, rheology modifier, and a neutralizer creates thick clear gels to prevent saturated and make the most of contact of the alcohol with the skin to enhance effectiveness (Pramanik et al., 2018).

Hydro-alcoholic gels have also emerged as an effective intervention during the COVID-19 pandemic. The World Health Organization (WHO) acknowledged a pandemic in arrears to infection with Unadorned Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus on

11 March 2020 and the contamination spread crossways all nations and areas of the world (Berardi et al., 2020). The WHO, and public infectious prevention organizations, have constantly featured the remaining of hand cleanliness to diminish the spread of the infection. WHO rules notice maintaining hand cleanliness, by normal washing hands with cleanser and water for no less than 20 seconds especially in the wake of going to the rest room, ahead of time eating and subsequent to hacking, sniffling or cleaning out one's nose (Berardi et al., 2020). When soap and water are not accessible, the Food and Drug Administration (FDA) acclaims sanitizing of non-visibly soiled hands with a hydro-alcoholic gel or spray containing 80% v/v ethanol or 75% v/v isopropanol (Berardi et al., 2020).

Various antimicrobial composites have been used for hand sanitization and incorporate, among others, alcohols, chlorhexidine, hexachlorophene, benzalkonium chloride, triclosan and povidone-iodine (Singh et al., 2020). The alcohols, explicitly ethanol and isopropanol, are top generally utilized for skin sanitization because of their wide-running movement against microorganisms, infections and parasites. Liquor based gels have genuine antimicrobial effect through disbanding of the lipid film and denaturation of the protein prompting the obstruction of layer and hindrance of digestion (Singh et al., 2020). Cooperation of the infection with a liquor cause alteration in its layer ease. The existence of polar oxygen molecules break down the lipophilic associations among the non-polar buildups, and increment the inner proclivity of the layer for water, thus undermining and denaturing the protein structure. The antimicrobial system of liquor against encompassed infections is practically identical to that for microbes as both have a lipid-rich external film (Singh et al., 2020). Non-encompassed infections are generally more unaffected to this component because of the absence of a lipid layer.

This research dissertation conducted a study to prepare a hydro alcoholic gel to be used as a skin disinfectant. A hydro alcoholic gel is superior to soaps and detergents since it contains alcohol that destroys the membrane of the bacteria and fungi. A portion of the burdens of the liquor based gels is that rehashed utilization of it can cause skin disturbance, or dry out the skin. In the event that you have delicate skin, the impacts can be extremely awful. The drying out is brought about by liquor. The proposed study will develop a master formula of a hydro alcoholic hand disinfectant of standardized essential parameters like viscosity, pH, alcohol concentration and the appearance. Second, the study will define the physical and chemical properties of the developed hydro alcoholic gel.

1.2 Problem Statement

Excellent skin hygiene is a fundamental strategy to reduce microbial infections among human beings. Medical experts and professional bodies such as the World Health Organization advise people to wash their hands regularly to prevent the transmission of infections (Pramanik et al., 2018). The choice of disinfectant determines the effectiveness of infection-reduction for disease-causing organisms that spread through the hands. For a long time, people have used soaps and detergents to destroy disease-causing microbes from their hands (Pramanik et al., 2018). However, soaps and detergents are associated with numerous disadvantages including skin damage and reduced rate of lipid replenishment of the upper skin layer. Consequently, health professionals have experimented with numerous alternatives. Hydro alcoholic gels have emerged as the most popular and useful alternatives to soaps and detergents for skin hygiene. Hydro alcoholic gels contain alcohol that destroy the membrane of the bacteria and fungi an activity that destroys these disease-causing organisms instantly (Pramanik et al., 2018). This proposal aims to conduct a study to develop a hydro alcoholic gel that has 80% concentration of alcohol (isopropyl alcohol) to act as a hand disinfectant.

1.3 Purpose of the study

The objective of this research dissertation was to develop a hydro alcoholic gel that has 80% concentration of alcohol (isopropyl alcohol). Alcohol-based gels are superior to soaps and detergents because they do not damage the skin and they kill disease-causing microbes instantly. The study came up with a formula to prepare a hydro alcoholic gel that acts as a skin disinfectant. Second, the study determined the physical and chemical properties of the gel. Finally, the study validated the critical parameters of the developed hydro alcoholic gel with 80% isopropyl alcohol.

1.4 Objectives

The main goals of the research dissertation study were:

i. To develop hydro alcoholic gel with 80% vol. isopropyl alcohol.
ii. To determine the physical and chemical characteristics of hydro alcoholic gel with 80% vol. isopropyl alcohol
iii. To validate the critical parameters namely appearance, viscosity, pH and isopropyl alcohol content of the developed hydro alcoholic gel.

1.5 Research Questions

This research dissertation aimed to answer the following questions:

i. What is the formula used to develop hydro alcoholic gel with 80% concentration of alcohol?
ii. What are the physical and chemical characteristics of hydro alcoholic gels with 80% concentration of alcohol?
iii. What are the critical parameters such as viscosity, alcohol content, pH, and appearance of the developed hydro alcoholic gel?

1.6 Study significance

The findings of the study will provide useful insights that improve hygiene practices. Health professionals might use the findings of the study to improve hand hygiene practices and improve public health outcomes in the nation. Also, the government can use the findings of the study to enhance the development and use of hydro alcoholic gels to boost hand hygiene. Furthermore, the study will provide reference for researchers who want to conduct similar research in the future.

1.7 Delimitations

The study was conducted according to the research objectives. Specifically, the study will seek to develop a hydro alcoholic gel with 80% concentration of alcohol. Second, the study will describe the physical and chemical characteristics of the developed hydro alcoholic gel. The study will only present the physical and chemical characteristics of the gel develop in the current study. Finally, the proposed study will validate the varying parameters like viscosity, pH, alcohol content and appearance of the developed gel, Therefore, the proposed study will present the outcomes of only the gel developed in the study not any other gel.

1.8 Limitations

The proposed study had several limitations. First, the study presented the findings of the hydro alcoholic gel that will be developed. As the formulated hydro alcoholic gel used different master formula Variations in the measurements of variables parameters such as the concentration of alcohol, pH, and viscosity might produce different results compared to other studies. Besides, the study is limited by a short study period that might not allow the researcher to carry out long term stability studies.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter analyses and discusses preceding studies that have studied the development of hydro alcoholic gels with high concentrations of alcohol. The chapter also reviews some of the preparation methods for hydro alcoholic gels. Furthermore, the chapter discusses some of the chemical and physical properties of hydro alcoholic gels.

The appearance of new microorganisms, bacterial or viral, have continually displayed smart experiences to general wellbeing wherever on the globe. One of these unsafe microorganisms is "serious intense respiratory disorder Covid 2" or SARS-CoV-2, all the more consistently recognized for starting Covid illness 2019 or COVID-19, which has been expressed an overall pandemic by the World Health Organization in walk 2020. Ever then its detection in December 2019 in Wuhan, close by over three million confirmed cases worldwide by April 2020 (1). With cases expanding dramatically all throughout the planet, it has caused groundbreaking burden on all highlights of mankind paying little mind to antagonistic segregation ways to deal with stop the spread of the infection. As of now, restorative plans to manage COVID-19 are just useful, making aversion expected at dropping transmission the best strategy.(Golin, Choi, & Ghahary, 2020a)

2.2 Preparation Methods for Hydro alcoholic Gels

The direct addition method begins with mixing all the components of the hydro alcoholic gel except the thickener in a water/ethanol mixture. Later, the thickener is added to the mixture and the solution is mixed thoroughly using mechanical or magnetic stirring (WHO, 2009). The direct addition method is preferred because it is in effect and inexpensive as long as an efficient mixing method is presented (WHO, 2009).

The reverse addition method is another popular method to prepare hydro alcoholic gels. In this method, the thickeners are pre-wetted by means of a water-miscible organic solvent (WHO, 2009). This method is the normal procedure in pharmaceutical companies where glycerol and propylene are the most commonly used wetting agents. A mortar and pestle are often used to conduct the wetting procedure (WHO, 2009). Next, a person mixes all the other components of the hydro alcoholic gels. Finally, the hydro alcoholic mixture is added to the water-miscible organic solvent (WHO, 2009).

The World Health Organization (WHO) has provided two formulations for the local production of hydro alcoholic gels (WHO, 2009). The two formulations are designed for the manufacture of a maximum of 50 liters per lot to guarantee security in production and storage (WHO, 2009).

2.3 Composition and mechanism of action of the hydro alcoholic gels

The hydro alcoholic gels can consist of ethanol, isopropyl liquor, n-propanol, or a blend of these, water, just as other excipients to work on the qualities. Plans involving alcohols somewhere in the range of 60% and 95% in volume are generally dominating and powerful. Humectants are held inside to forestall skin drying out and excipients support balance out the item just as expand the time wanted for the vanishing of liquor, subsequently expanding its biocidal action.(Golin, Choi, & Ghahary, 2020b)

N-propanol, is the greatest possible level of regularly utilized liquor compound in biocides.

It isn't notable with impressive sureness the exact instrument of liquor's antimicrobial action, however, it could be related film obliteration, and hindrance or disengagement of mRNA and protein union through consequences for ribosomes and RNA polymerase, or related with protein denaturation.

For action against microorganisms, it is ideal bactericidal viability is attained at fixations somewhere in the range of 60% and 95%.In detail, outright liquor, or liquor that is close to one percent water, is not as much of bactericidal than liquor among the in advance of referenced reach.

Water is therefore important in the protein denaturation procedure. Despite which procedure, if not numerous, are pretentious by alcohol, necessary metabolic pathways, membrane impairment and damage of cellular uprightness in the end take place (Ionidis et al., 2016).

The viral focal points of hydro alcoholic gels are generally the viral envelope, if existing, which is coming about due to have lipid envelopes, the protein capsid, which includes and guards the innate substantial and the genetic material itself. Taking into account that this huge number of constituents are essential for the viral life cycle and consequently hazardous for its capacity to spread to additional host, it will in general be expected that moving the development or limit of any of the recently referenced constituents will ordinarily convey the contamination incapable.

It is eminent that ethanol have a more broad and solid virucidal activity than propanol.

Thoroughly, critical level centralization of ethanol has revealed to be especially subsequently against incorporated contaminations and as needs be is dynamic against the typical of clinically appropriate diseases. It is fortifying to observe that development of acids to ethanol plans could assemble its ampleness against diseases that are extra impenetrable to ethanol in a manner of speaking. In any case the conceivable correspondence of ethanol and acridity, it stays understood that most hydro-alcoholic gels endure to be inadequate against non-incorporated contaminations (Kampf, 2018)

2.4 Benefits of hydro alcoholic gel compared to soap

Many hydro-alcoholic gels, containing of dissimilar constituents and methods of use, have been related. On the other hand, the Centers for Disease Control and Prevention (CDC) endorses

cleaning hands with soap and water on every occasion possible over hydro-alcoholic gels. The upside of hand cleaning branches from a few elements, like avoidance of a more extensive range of microbes and synthetic compounds, and disposal of bio-burden on dirtied hands. In 2016 precise audit arrangements the antiquated uncertainty about the utilization of hydro-alcoholic gels in food planning landscapes and suggests that hand washing with cleanser and water is more viable than elective hand sterilization approaches for end of soil and microorganisms from hands. On the other hand, we desired to precisely relate the effectiveness of hand soaps and hydro-alcoholic gel on their effectiveness incapacitating enveloped viruses.(Golin et al., 2020a)

2.5 Physicochemical Characteristics of Hydro alcoholic Gels

The effectiveness of hydro alcoholic gels depends on four major factors: nature of alcohol, concentration, extent applied, and time of contact. The common alcohols that are used in hydro alcoholic gels include isopropanol, ethanol, or n-propanol, or mixtures of these alcohols (Jing et al., 2020). While other antiseptics have acquired bacterial resistance, ethanol, isopropanol, or n-propanol do not have these weaknesses. At the same concentration, ethanol is more effective than propanols when they are used to kill bacteria. At the same time, skin tolerance is higher with ethanol related to isopropanol- or n-propanol-based gels (Jing et al., 2020). Therefore, many manufacturers prefer ethanol when making hydro alcoholic gels.

The Food and Drug Administration (FDA) of the United States says that hydro alcoholic gels with ethanol concentrations of between 60% and 95% (v/v) are effective and safe for use as disinfectants (Berardi et al., 2020).

Different researchers contend that the decision of definition, not the convergence of liquor, decides by the current exploration in the compositions, it is risky to most likely propose one methodology of hydro alcoholic gels movement over the other. What we can say, is that cleanser

and water is more prominent to hydro-alcoholic gel, and when hand washing is distant or badly arranged, a satisfactory volume of hydro-alcoholic gel is essential to guarantee extensive hand inclusion, and passive consent is risky for reasonable hand cleanliness. Also, in conclusion, with construing the virucidal information on infections of tantamount construction to SARS-CoV-2, this infection can be productively crippled by existing hand cleanliness produces, however inevitable exploration should endeavor to characterize this in an orderly fashion (Ionidis et al., 2016).

The effectiveness of hydro alcoholic gels when used as disinfectants. Such scholars also believe that gels, liquids, and foam-based products are equally effective when the concentration of alcohol is within the 60-95% range (Berardi et al., 2020). Other studies indicate that increasing ethanol concentration from 80% to 95% (v/v) reduces the interaction time that is required to kill bacteria effectively (Jing et al., 2020).

Table 1: Chemical and Physical Properties of Hydro alcoholic Gels

No	Characteristic	Parameters
1.	Appearance/look	Clear/spotless and transparent/visible gel
2.	Color	Colorless
3.	pH	6-8
4.	Content of ethanol in mass %	60 – 95 %
5.	Viscosity	800 – 4500 Centipoises

CHAPTER THREE: EXPERIMENTAL

3.1 Introduction

This chapter discusses the preparation of a hydro alcoholic gel containing 80% concentration of alcohol for use as an antiseptic. The chapter includes the materials that were used to prepare the hydro alcoholic gel. Besides, the chapter details various chemical tests performed to determine the physical and chemical properties of the formulated hydro alcoholic gel.

3.2 Study design

This research study was done in the University of Nairobi Pharmaceutics Laboratory and some tests will be done in DARU, department of pharmaceutical chemistry at University of Nairobi, school of pharmacy.

In the development of this hydro alcoholic gel with 80% concentration of alcohol, the following ingredients, materials and tests will be done.

3.2.1 MATERIALS

1. (1% w/w) Carbopol polymer 934 (
2. Alcohol (isopropyl alcohol 99.5 %)
3. Glycerol 98%
4. Hydrogen peroxide 3%
5. Distilled water/ deionized water

3.2.2 Procedure

3.2.3 Experimental design

During the development of this hydro-alcoholic gel with 80% of alcohol concentration using the above mentioned ingredients, the researcher will prepare 3 different samples having different amount and ratios of ingredients for the purpose of observing the variations among the important parameters such as viscosity, pH ,color and appearance and alcohol content. Thereafter the researcher will choose the best sample with regards to set specifications and use its formula to prepare a formulation.

3.2.4 Recordings of the experiment

Number of samples	Ingredients	Quantity	Observations
Sample 1			
Sample 2			
Sample 3			

The prepared formulation will be also subjected to the same tests so as to confirm the varying parameters. And the formulation will be put in the primary packaging material (plastic bottle) for presentation.

3.2.5 Sample Preparation

The researcher will proceed with the following steps when preparing a hydro alcoholic gel:

1. The researcher will pour the alcohol (isopropyl alcohol) for the chosen formulation into a big plastic bottle.

2. The researcher will add H_2O_2 into the large bottle using the measuring cylinder.

3. Next, the researcher will add glycerol to the mixture by means of a measuring cylinder. Because the glycerol is very viscid and sticks to the walls of the measuring cylinder, the researcher can rinse the cylinder with sterilized purified or cooled boiled water and then dispensed in the large flask that contains the mixture.

4. The researcher will add carbopol polymer 934 in the mixture using a spatula

5. The researcher will top the large bottle up to the equivalent mark of the desired bulk using distilled or cold, boiled water.

6. The researcher will place a lid or a secure cap on the bottle directly after mixing to avoid evaporation.

7. The researcher will mix the solution by moderately shaking the mixture or by means of a wooden, plastic or stainless steel paddle.

8. The researcher will divide the mixture in a smaller containers (e.g. 50, 100 or 150 ml plastic container). The containers must be set aside in confinement for 72 hours. This consents time for any spores existent in the alcohol or the fresh or re-used container to be removed by H_2O_2.

3.3 Sample Analysis

3.3.1 Alcohol Content in the Hydro alcoholic Gel

The researcher will pour 100 ml of the hydro alcoholic gel in a beaker and use a hydrometer to determine the alcohol content in the gel. The researcher will record the concentration recorded using the hydrometer.

3.3.2 Physical and Chemical Properties of the Hydro alcoholic Gel

Color and Appearance

The researcher will observe and record the appearance and color of the hydro alcoholic gel.

pH

20 ml of the hydro alcoholic gel will be dispensed in a hygienic dry 25 milliliters beaker, next 13milliliters of purified water was additional in the beaker and agitated gently. It was chilled in a cold-water bed bath to 25^0C.pH electrode will be standardized with buffer solution then the electrode will be wrapped up in the sample and the pH value was read and recorded.

Viscosity

25 ml of the hydro alcoholic gel will be dispensed into a test tube and a viscometer will be used to measure the viscosity at a temperature of 20^0C.

Research Instruments to run tests

1. Viscometer
2. Hydrometer
3. pH meter
4. Gas chromatography

3.3.3 Data Analysis

The experiment will be done in the University of Nairobi Pharmaceutics Laboratory, some analytical tests will done from DARU at the University of Nairobi and the data will be collected and record in triplicate and in tabular form. The recorded data will be analyzed and recorded in tables.

CHAPTER FOUR: RESULTS AND DISCCISSION

4.1 Introduction

During formulation of this hydro-alcoholic gel 5 different ingredients were used at different ratios, 3 formulations of different concentrations of the ingredients were formulated with the purpose of selecting the best formulation among those 3 formulations. All of these experiment were done in the University of Nairobi pharmaceutics laboratory and in DARU which is also located at the University of Nairobi, school of pharmacy.

4.2 Ingredients and method

The following are the ingredients used and their concentrations:

I. Isopropyl alcohol 99.5%
II. Carbopol 934 0.5%
III. Glycerol 98%
IV. Hydrogen peroxide 3%
V. Boiled and cooled water.

First of all using the WHO recommended formula, 1 liter of a hydro-alcoholic hand sanitizer was made with the following ingredients:

I. Isopropyl alcohol 751.5 mls
II. Hydrogen peroxide 41.7 mls
III. Glycerol 14.5 mls
IV. Water (boiled and cooled) 192.3 mls

After preparing 1 liter of WHO recommended formula, then another formulation made of carbopol, boiled and cooled water and triethanolamane was prepare as follows;

Different quantity of carbopol was mixed in different quantity of boiled and cooled H2O, it was mixed at high speed until all the carbopol was dissolved , then different quantities of trietholamine were added for gelling purposes, then it was mixed well to form a very clear thick gel.

The WHO formulation was divided into 3 products with different volumes (100ml, 75ml and 50 ml). And in each volume of the WHO formulation, different ratios of the combination of carbopol, boiled and cooled water and trietholamine were added until desired consistency was achieved, it was mixed well and left it stand for the bubbles to settle.

Table 2: The summarized ratios of the three products formulated

The summarized ratios of the three products formulated

N/S	Product	Isopropyl alcohol	water	Hydrogen peroxide	trietholamine	glycerol	carbopol	Total volume
1	P1	115 ml	25 ml	5 ml	0.3 ml	3 ml	1.5 g	150 ml
2	P2	83 ml	10 ml	4 ml	0,2 ml	2 ml	1 g	100 ml
3	P3	60 ml	7 ml	3.5 ml	0.1 ml	2 ml	0.5 g	75 ml

The percentage expression of the ratios used to prepare the above three products

NS	Product	Isopropyl alcohol	water	Hydrogen peroxide	trietholamine	glycerol	carbopol	Total volume
1	P1	76%	18.3 %	3.1%	0.2%	2.4%	1%	100%
2	P2	78%	14%	4%	2%	1%	1 g	100%
3	P3	80%	9.3%	4.7%	1.3%	1%	0.7%	100%

4.3 pH

A well calibrated digital pH meter was used to test the three formulated hydro-alcoholic gel which are clear colorless gels. Their pH varied from 5.8 to 6.4.

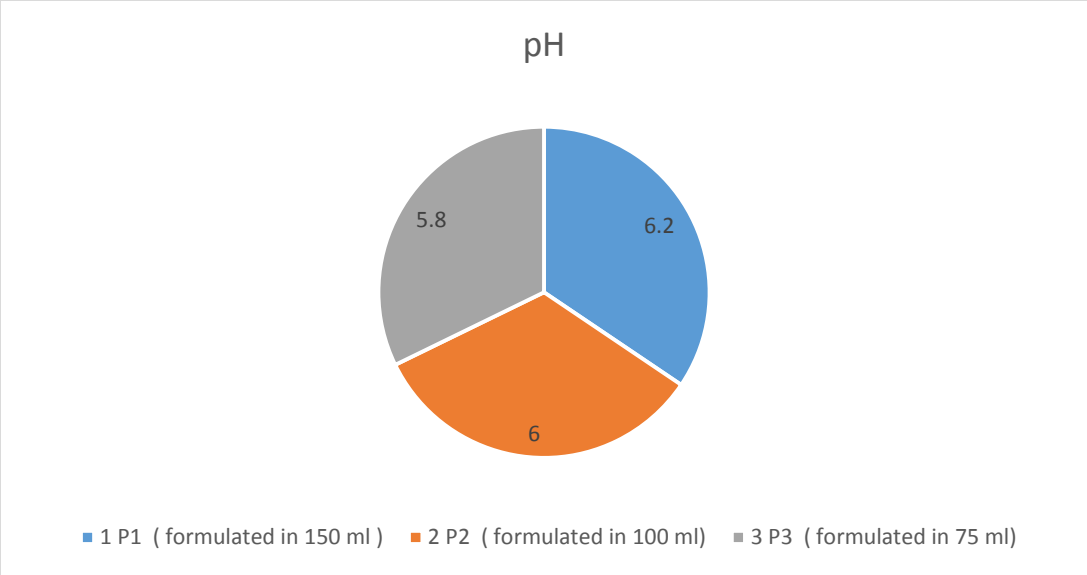


Figure1 shows the pH of the three formulated products

Figure1 shows the pH of the three formulated products

4.4 VISCOSITY

This is an important aspect to think through when preparing a hydro-alcoholic. This is to guarantee that the disinfectant in the hydro-alcoholic gel can cover as much of the superficial of the applied area before fade away with slight mess or remains.

The table below shows the readings for the three formulations

Table 3Table 3: readings for the three formulations

Table 3: readings for the three formulations

N/S	Product	Viscosity	Temperature	RPM	Percentage	Spindle size
1	P1	3705 CPs	20^0c	20	76%	SP-9
2	P2	4012 CPs	20^0c	20	81%	SP-9
3	P3	3926 CPs	20^0c	20	78%	SP-9

4.5 Stability Studies for a hydro-alcoholic gel optimization

4.5.1Evaluation of hydro-alcoholic Gels Stability After the formulation.

A sample was formulated representing each product, three samples of 100 ml were used so to assess the alcohol strength previously namely chemical and physical analysis at T_0 and later stability; accelerated and real-time storage stability study at temperatures of 20 ± 2 ◦C, 40 ± 2 ◦C for until 21 days. At the completion alcohol (isopropyl alcohol) strength by volume was measured. For the assessment of the organoleptic properties, a sensorial investigation was conducted, this is like an optical analysis of the "appearance", "color," and an olfactory analysis of "odor". For the pH studies, a digital pH meter was used, and the temperature was set at 20 ± 2 ◦C all through the test. The standards of the alcohol strength (concentration) by volume was measured using an alcoholmeter and recorded.

The Alcohol (isopropyl alcohol) concentration before the stability study (at T_O)

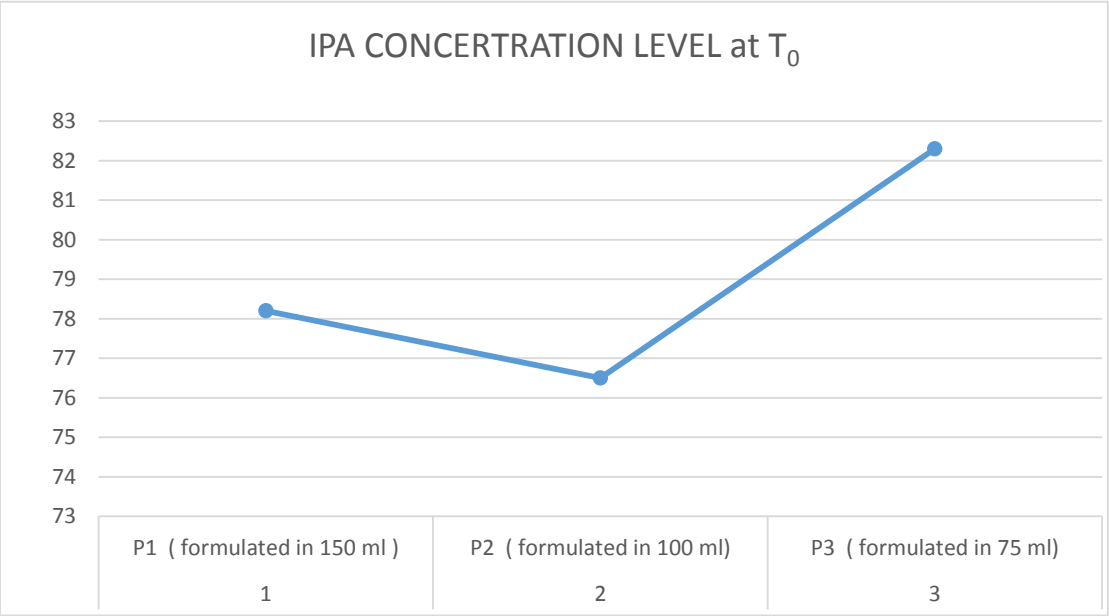


Figure 2: The Alcohol (isopropyl alcohol) concentration after 21 days of the stability study (at T_{21})

Figure 2: The Alcohol (isopropyl alcohol) concentration after 21 days of the stability study (at T21)

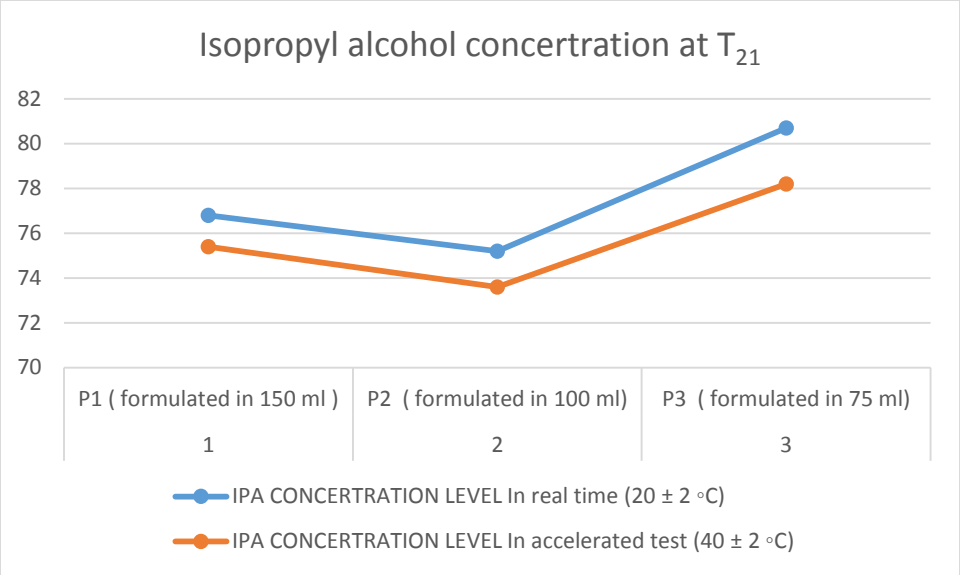


Figure3: comparison of isopropyl concertation before and after stability studies

Figure 1Figure3: comparison of isopropyl concertation before and after stability studies

4.6 TESTING THE ALCOHOL (ISOPROPYL ALCOHOL) CONCENTRATION IN THE FORMULATED HYDRO-ALCOHOLIC GEL.

A gas chromatography was used to produce chromatograms and use the area under the curve of the IPA and Acetonitrile to calculate the alcohol (isopropyl alcohol) concentration in percentage.

A mixture standard was prepared using IPA, water (deionized), Acetonitrile and then the sample to compare with the standard was also prepared and composed by the Acetonitrile 500 microliter, Water (deionized water) 200 microliter Sample(formulated hydro-alcoholic gel) 300 microliter.

The following are chromatograms of the three product that have been formulated with different formulation ratios.

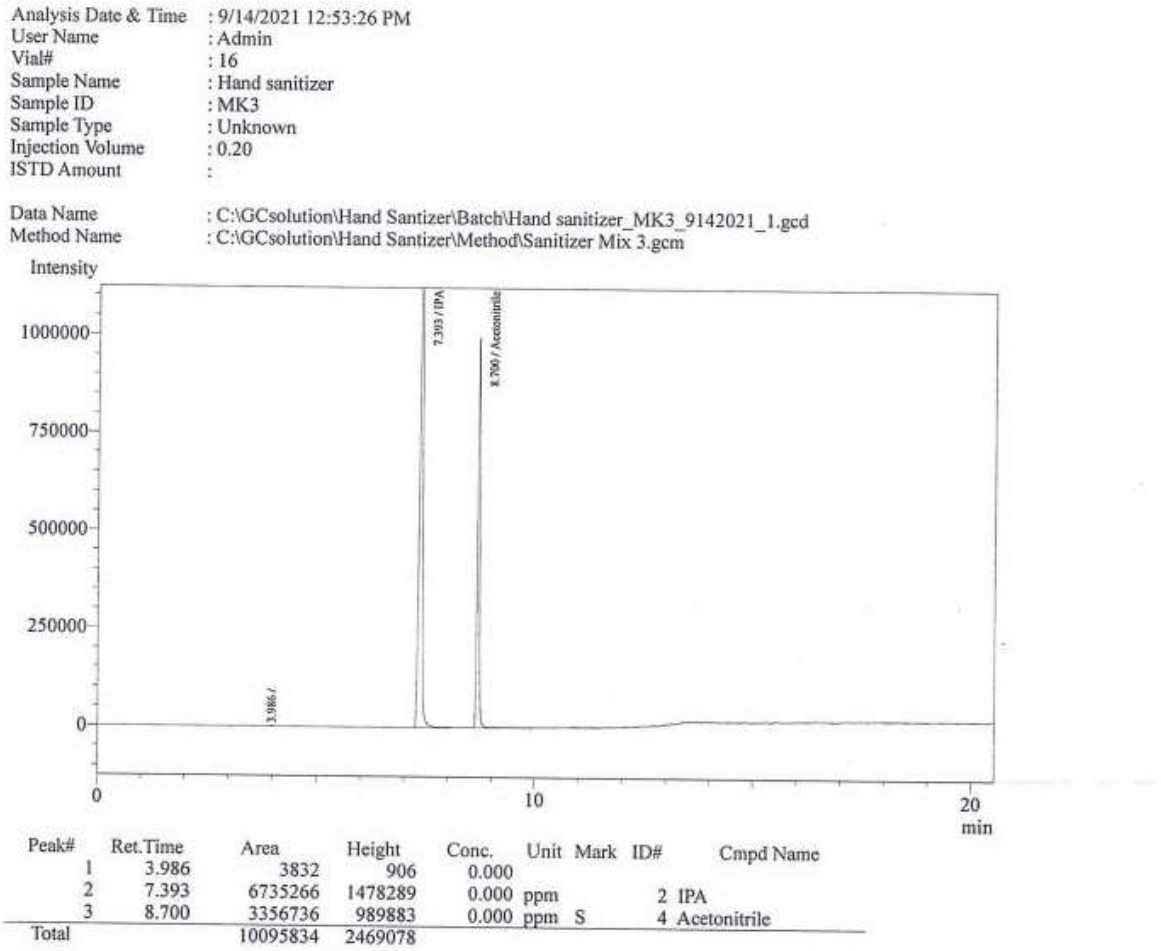
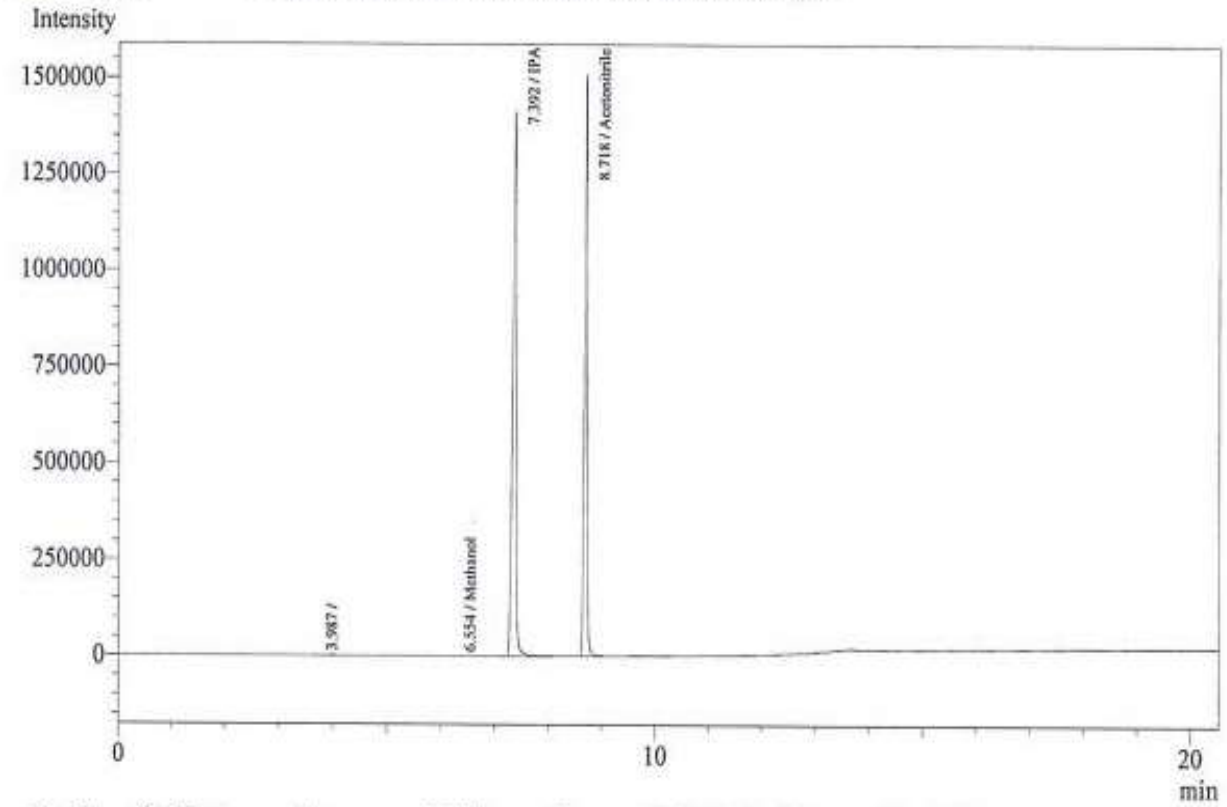


Figure4: a typical chromatogram of product number three named MK3 (P3)

Figure 2Figure4: a typical chromatogram of product number three named MK3 (P3)

Analysis Date & Time : 9/14/2021 12:24:44 PM
User Name : Admin
Vial# : 15
Sample Name : Hand sanitizer
Sample ID : MK2
Sample Type : Unknown
Injection Volume : 0.20
ISTD Amount :

Data Name : C:\GCsolution\Hand Santizer\Batch\Hand sanitizer_MK2_9142021_1.gcd
Method Name : C:\GCsolution\Hand Santizer\Method\Sanitizer Mix 3.gcm



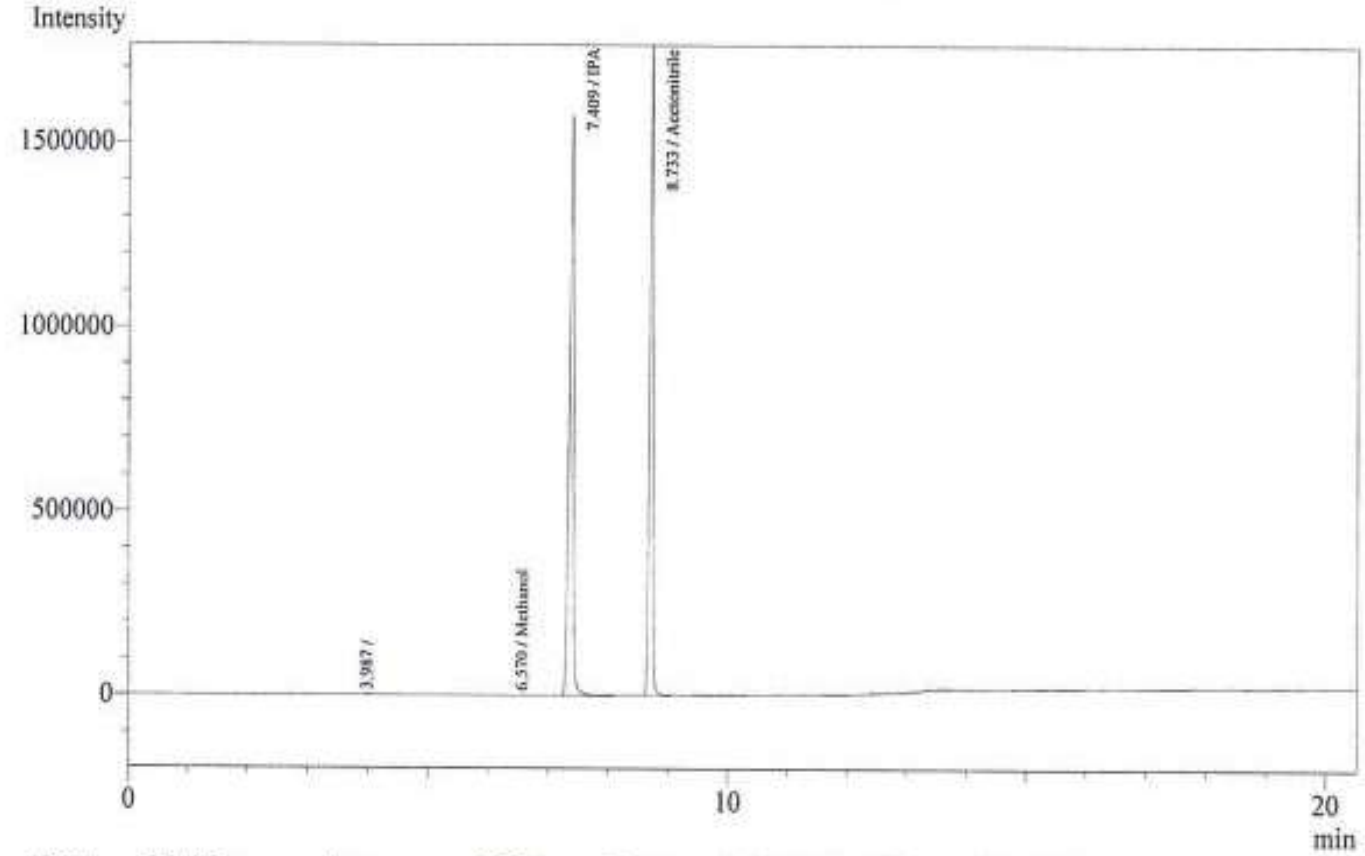
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	3.987	4416	1069	0.000				
2	6.554	1341	259	0.000	ppm		1	Methanol
3	7.392	6241965	1401532	0.000	ppm		2	IPA
4	8.718	5320660	1492950	0.000	ppm	S	4	Acetonitrile
Total		11568382	2895810					

Figure5: Atypical Chromatogram of the product Number two (P2)

Figure 3Figure5: Atypical Chromatogram of the product Number two (P2)

Analysis Date & Time : 9/14/2021 11:56:31 AM
User Name : Admin
Vial# : 14
Sample Name : Hand sanitizer
Sample ID : MK1
Sample Type : Unknown
Injection Volume : 0.20
ISTD Amount :

Data Name : C:\GCsolution\Hand Santizer\Batch\Hand sanitizer_MK1_9142021_1.gcd
Method Name : C:\GCsolution\Hand Santizer\Method\Sanitizer Mix 3.gcm



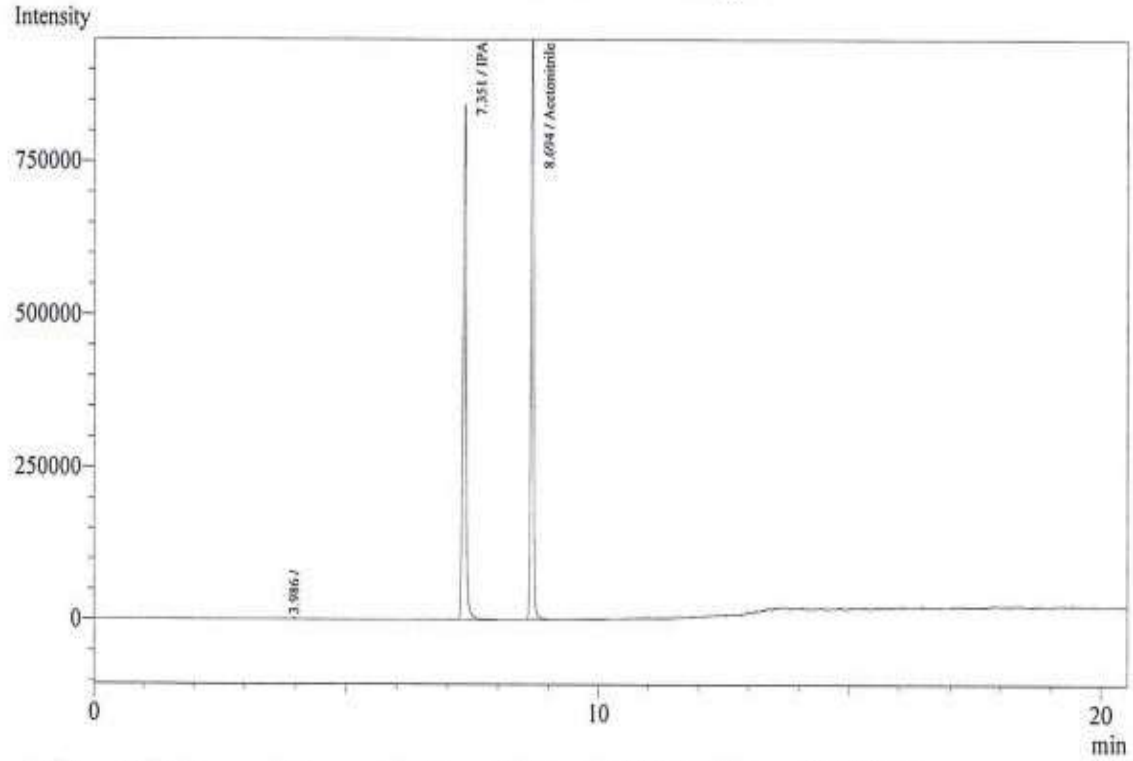
Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	3.987	4071	968	0.000				
2	6.570	1818	374	0.000	ppm		1	Methanol
3	7.409	7230744	1566538	0.000	ppm		2	IPA
4	8.733	6549323	1757566	0.000	ppm	S	4	Acetonitrile
Total		13785956	3325446					

Figure 6: A typical chromatogram of the product number one named MK1 (P1)

Figure 4 Figure 6: A typical chromatogram of the product number one named MK1 (P1)

Analysis Date & Time : 9/14/2021 1:57:35 PM
User Name : Admin
Vial# : 5
Sample Name : Hand sanitizer
Sample ID : Standard mixture
Sample Type : Unknown
Injection Volume : 0.20
ISTD Amount :

Data Name : C:\GCsolution\Hand Santizer\Batch\Hand sanitizer_Standard mixture_9142021_2.gcd
Method Name : C:\GCsolution\Hand Santizer\Method\Sanitizer Mix 3.gcm



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	3.986	3812	924	0.000				
2	7.351	3506438	839492	0.000	ppm		2	IPA
3	8.694	3722322	1097871	0.000	ppm	S	4	Acetonitrile
Total		7232572	1938287					

Figure7: A typical chromatogram of the standard mixture

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION

The major purpose of this study was conducted carefully and the specific objectives set were also achieved.

5.1 Discussion

In this study, Hydro-alcoholic gel shows many more advantages over other formulation like liquid or sprays formulation confines its use in arrears to the spillage and its striving to use in transportable dosage forms and may rise the alcohol evaporation level. Hydro-liquor gels contain either ethanol or isopropyl as the really dynamic fixing. The two structures additionally contain water, alongside glycerol or different humectants to help keep away from skin dryness.

In this manner after the solidness concentrate on done in just 21 days, it shows that liquor vanishes when open to the air. What's more, the hydro-alcoholic gels bottles are not impenetrable, so some liquor vanishing occur subsequent to removing the seal. Thus, the liquor convergence of liquor based details step by step diminishes after some time, diminishing the germ-free properties of the item.

5.2 Conclusion

Moreover, in a period when the use hydro-alcoholic gels are more recurrent, for case in point, amid healthcare personnel or all through a pandemic, it is crucial for find and pick items that have a commendable equilibrium concerning adequacy, wellbeing, and similarity with the skin, and that are appropriate to the clients.

Therefore, in my opinion, it would appear that P3 would answer back to this need; furthermore, it would be advantageous to carry on the trials with the intention to test the optimum ratio of alcohol to moisturizers (with the minimum possible amount of alcohol) that gives the greatest antimicrobial activity.

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