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DETERMINATION OF METHYLPARABEN IN PHARMACEUTICAL PRODUCTS IN TRNC-EVIDENCE BY HIGH PERFOMANCE LIQUID CHROMATOGRAPHY ANALYSIS.

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We certify that we have read the thesis submitted by Stephen Martor Johnson titled "..... **(in bold)"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Analytical Chemistry.

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DECLARATION

I hereby certify that all data in this document was gathered and presented in compliance with ethical standards and scholarly guidelines. I further affirm that I have properly cited and referenced all information and findings that are not unique to my work, as required by these rules and conduct.

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DAY/MONTH/YEAR

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I appreciate my beloved mother, relatives and friends who one way and the other contributed in this academic endeavor.

ABSTRACT

Determination of Methylparaben in Pharmaceutical products in TRNC-evidence by high performance liquid chromatography analysis.

Stephen Martor Johnson

Master of Science Thesis, Department of Analytical Chemistry,

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This study aims to quantitatively examine the methylparaben content of four distinct of Pharmaceutical products bought from few pharmacies in Turkey Republic of Northern Cyprus. The four samples were taken to the laboratory for analysis and direct dilution was done. The simultaneous determination of methylparaben in four samples in the Pharmaceuticals sample form has been established and confirmed using a straightforward, quick, and effective RP-HPLC method. Five minutes were allotted for the injection of the methylparaben standard in triplicate for ten different concentrations. MP was separated using an isocratic elution technique utilizing an ACE-C18.3mmIDX 12.5cm (um) column with a flow rate of 1.0 mL/min and a 258 nm detection wavelength. Methanol made up 60% while water made up 40% of the mobile phase. With a shorter run period, peak of MP in each sample was symmetrical and well-resolved. We can deduce that these concentrations are within acceptable ranges which are 0.4% of each paraben and 0.8% of all parabens combined in products sold in the European Union. Sample one limit of detection was 0.4 mg/L, two was 0.60 mg/L, and three was 0.81 mg/L while four was below detection range; meanwhile, their respective limits of quantification were 0.14 mg/L, 2.00 mg/L, and 2.69 mg/L. The method's linearity, accuracy, precision, LOD & LOQ and specificity were validated.

Keywords: Determination, Pharmaceuticals, HPLC, Toxicity, Evidence

ÖZET

KKTC'deki Farmasötik Ürünlerde Metilparaben'in yüksek performanslı sıvı kromatografi analizi ile belirlenmesi.

Stephen Martor Johnson
Yüksek Lisans Tezi, Analitik Kimya Anabilim Dalı,
Haziran 2023, 49 sayfa

Bu çalışma, Kuzey Kıbrıs Türkiye Cumhuriyeti'ndeki birkaç eczaneden satın alınan dört farklı Farmasötik ürünün metilparaben içeriğini niceliksel olarak incelemeyi amaçlamaktadır. Dört numune analiz için laboratuvara götürüldü ve doğrudan seyreltme yapıldı. Pharmaceuticals numune formundaki dört numunede metilparabenin eşzamanlı tespiti basit, hızlı ve etkili bir RP-HPLC yöntemi kullanılarak oluşturulmuş ve doğrulanmıştır. Metilparaben standardının on farklı konsantrasyon için üç kopya halinde enjeksiyonu için beş dakika ayrıldı. MP, 1,0 mL/dakika akış hızına ve 258 nm saptama dalga boyuna sahip bir ACE-C18.3mmIDX 12.5cm(um) kolonu kullanılarak izokratik elüsyon tekniği kullanılarak ayrıldı. Mobil fazın %60'ını metanol oluştururken %40'ını su oluşturuyordu. Daha kısa bir çalışma periyoduyla, her numunedeki MP'nin zirvesi simetrikti ve iyi çözümlenmişti. Bu konsantrasyonların Avrupa Birliği'nde satılan ürünlerde her bir paraben için %0,4 ve tüm parabenler için %0,8 oranında kabul edilebilir aralıklar içerisinde olduğu sonucunu çıkarabiliriz. Numunenin bir tespit limiti 0,4 mg/L, iki numune 0,60 mg/L ve üç numune 0,81 mg/L iken dördü tespit aralığının altındadır; bu arada, ilgili miktar sınırları 0,14 mg/L, 2,00 mg/L ve 2,69 mg/L idi. Yöntemin doğrusallığı, doğruluğu, kesinliği, LOD & LOQ'su ve özgüllüğü doğrulandı.

Anahtar Kelimeler : Tayin, Farmasötikler, HPLC, Toksisite, Kanıt

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LIST OF ABBREVIATIONS

TRNC	Turkey Republic of Northern Cyprus
HPLC	High Performance Liquid Chromatography
RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
p-HBA	P-hydrobenzoic Acid
DAD	Diode Array Detector
MP	Methylparaben
EP	Ethylparaben
PP	Propylparaben
BP	Butylparaben
EMA	European Medicine Agency
UV	Ultraviolet
ICH	International Conference on Harmonization
LOD	Limit of Detection
LOQ	Limit of Quantification
SD	Standard Deviation
RSD	Relative Standard Deviation
CLP	Clindamycin Phosphate
UA-CPME	Ultrasound -Assisted Cloud Point Microextration
BBD	Box-Berhken Design
ANOVA	Analysis of Variance
TRN	Tretinoin

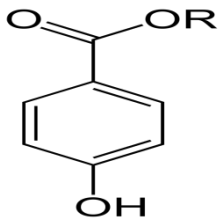
CHAPTER 1

INTRODUCTION

1.1 PARABENS

Parabens are aliphatic esters of p-hydroxybenzoic acid (pHBA), a preservative widely used in the cosmetics, pharmaceutical, and food sectors. Although they are a part of many pharmacological formulations, they are mostly found in topical treatments used in cosmetics. The most often used parabens are methyl, ethyl, propyl, and butyl. Due to their antibacterial efficiency, parabens were originally used in injections and ophthalmic treatments, but their use has subsequently been reduced to avoid potential irritations (Julia et al, 2020, p.17-32).

Each day, many people are exposed to packaged food, cosmetics, and pharmaceuticals. Para-hydroxybenzoic acid esters (Parabens) are present in several goods. Preservatives made synthetically called parabens are found in pharmaceutical preparations, food and drink, personal care goods, and medicine. The skin, gut, and urine both rapidly absorb and eliminate parabens (Rahul Tade, 2018). Their analysis is crucial for the assessment of their exposure due to official limits on the usage of these substances.



Parabens	R
Methylparaben	$-CH_3$
Ethylparaben	$-CH_2CH_3$
Propylparaben	$-CH_2CH_2CH_3$
Butylparaben	$-CH_2CH_2CH_2CH_3$

Fig. 1 Structures of Parabens

1.1.1 METHYLPARABEN

Preservative is substance that is frequently added to a variety of food and pharmaceutical formulations to extend the shelf life particularly Methylparaben, one of the most widely used preservatives in liquid medicinal formulations from microbial development. The health could be harmed if this preservative is used excessively. As a result, Methylparaben's minimum allowable quantities are regulated, and quantitative measurement of this preservative is crucial for the regular evaluation of pharmaceutical products (K. Supriya et al, 2020, p.13-18).

There are several extraction methods such as liquid-phase microextraction, dispersive liquid-liquid microextraction, homogeneous liquid-liquid microcroextraction, votex-assisted liquid-liquid microextraction, etc. Methylparaben is the paraben with the fewest carbon atoms, making it more polar than ethylparaben, propylparaben, and butylparaben.

The more polar molecules will elute on the column and show up first on the chromatogram in reversed-phase chromatography, where the stationary phase is nonpolar and the mobile phase is polar, typically water and polar organic solvent. This study served as the foundation for the development of an easy-to-use, low-cost, and high-performance liquid chromatographic method for the quantitative detection of methylparaben. Limit of detection and limit of quantification, specificity, accuracy, and considerable linearity have all been reported for pharmaceutics validation using RP-HPLC (Muhammad et al, 2023, p.52-59).

1.2 Quantitative Analysis of HPLC

A quantitative analysis involves many steps, which are briefly explained as follows: Recognize the subject matter you are analyzing.

Create a method for examining samples that include this chemical.

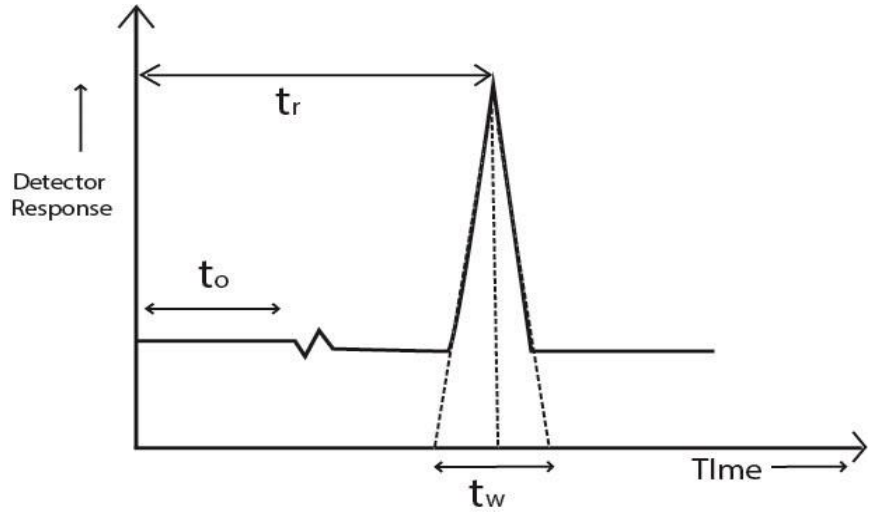
Analyzing a sample or samples (the Standard) that contain a known concentration or concentrations of the chemical is the process of "calibration," which aims to ascertain the response attributable to that concentration. It is possible to evaluate several of these samples with varied amounts when monitoring a wide concentration range in samples or when your detector has a non-linear response, also known as "multi-level calibration"—is necessary. To determine the reaction since the concentration is unsure, analyze the sample containing the chemical in the unknown quantity.

Compare the retention time of the unknown concentration to the response of the known

(Standard) concentration to determine the amount of the component present. For a fair comparison of the unknown sample response to the known standard, the data must be obtained and processed under same conditions.

1.2.1 Chromatogram

A chromatogram is the end result of a chromatography run. The data generated during the chromatography run is stored in a hardcopy or electronic file.



Retention time (tR) is the time needed for each component of the mixture after injection to reach the detector.

Dead time(tm) is the time taken for the mobile phase to pass through the column from injector to detector, also called void time.

1.2.2 Components of HPLC

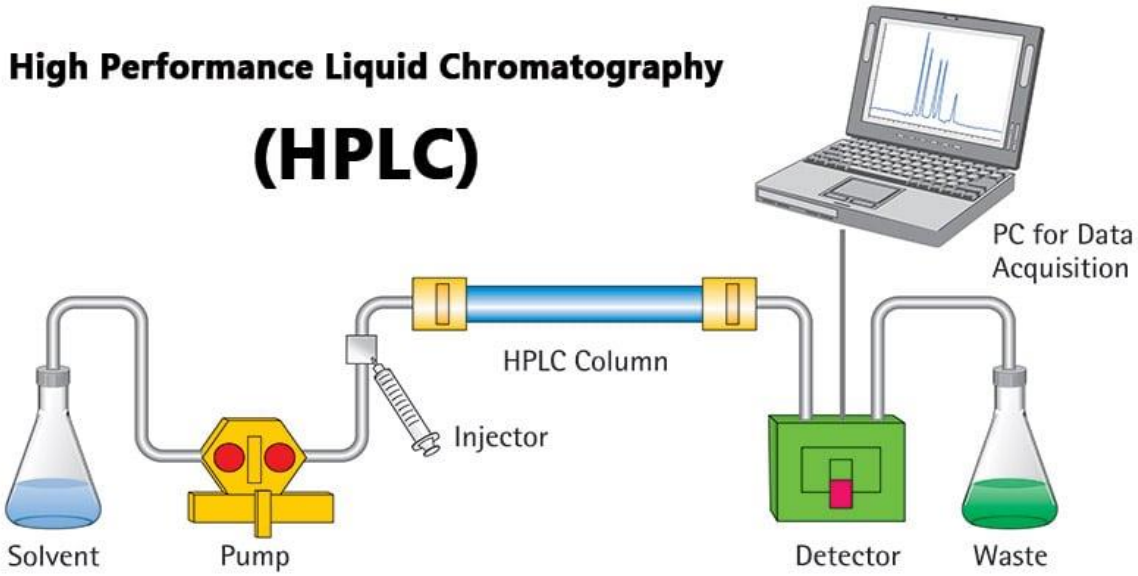


Fig.2: HPLC major Components

1.2.2.1 Reservoir/Solvent

The initial part of an HPLC system is the solvent reservoir, which stores the solvent required to transport the sample through the apparatus.

1.2.2.2 Pump

The column receives a precise flow of mobile phases with a predetermined composition from the HPLC pump, which is also referred to as a solvent delivery system.

1.2.2.3 Column

In the column, the sample separation procedure takes place. The components of the sample segregate in this case because the sample is sent through both the stationary and mobile phases at once.

1.2.2.4 Detector

After being gathered and processed via an HPLC column, the eluted mixture is analyzed for its components using an HPLC detector.

1.2.2.5 Waste

Volatile solvents, which must be appropriately disposed of, are routinely used in HPLC. Gravity or a pump can be used to remove waste from HPLC equipment. The waste from the device is drained using a small tube.

1.3 Statement of the Problem

Knowing whether a preservative like methylparaben is included in pharmaceutical products is essential for pharmacies and customers. This process would not be possible without modern analytical methods like HPLC, a piece of equipment utilized by the pharmaceutical sector for quality control. To lessen the disparity between the preservative concentrations that should be present in pharmaceutical products, the HPLC technique provides crucial information. This investigation was carried out to determine whether methylparaben was present in pharmaceutical items in the TRNC.

1.4 Purpose of the Study

To assess the methylparaben concentrations in four different samples of pharmaceutical products.

To determine if the methylparaben content in each sample is within the permissible limit.

To take into account how this can impact clients.

1.5 Research Questions

Where to obtain Standard for methylparaben?

What method should be selected to quantify methylparaben in the samples?

Will the concentration of methylparaben in each of the pharmaceuticals meets its recommended level?

What are the negative and positive impacts of methylparaben?

1.6 Significance of the Study

The importance of this research is not limited to the researcher only but also consumers of sample one, two, three and four in the TRNC. The quantification of methylparaben in these pharmaceuticals being in the recommended range will encourage more consumers to start using the products and less number of people would suffer from the sickness or complication the products treat.

1.7 Limitations

-Financial constraint made the researcher not to increase the number of samples to more than four. But does not affect the results of the research.

-The identification of specific articles for my study because past articles focused on methylparaben in combination with other preservatives or impurities.

-There was no enough previous practical knowledge on the operations of HPLC.

1.8 Definitions of Terms

Methylparaben is one of the most common parabens found in pharmaceutical products, cosmetics, etc. Chromatography is a separation technique with stationary and mobile phases. Lipophilicity refers to a chemical compound's capacity to dissolve in lipids, fats, and non-polar solvents. Otitis is the swelling in the ear.

Excipient (placebo): an inactive substance that serves as a medium for a drug or other active substance. Antimicrobials are drugs that treat or prevent illnesses in people, pets, and even plants. Antifungals are drugs that either eliminate or halt the development of the fungi that cause infections.

CHAPTER 2

Literature Review

Separation of methylparaben in pharmaceutical products is preferable with RP-HPLC especially in combinations with other active substances or preservatives. Because it has fewest carbon atoms among the parabens, methylparaben has highest polarity than ethyl, propyl and butylparabens. The more polar chemicals will elute on the column and appear first on the chromatogram in the case of reversed-phased chromatography because the column is nonpolar (V. Chornyi et al, 2020, p. 8).

Nowadays, due to their high selectivity and ease of equipment accessibility, chromatographic methods are frequently employed to determine the presence of parabens in medicines. The preparation of complicated samples and the extraction of parabens are two issues that limit the implementation of simpler analytical procedures. Insufficient sample preparation prevents the approach from having good selectivity and accuracy (Slavica et al, 2022, p. 353-363).

Concerns about the potential for methylparaben to alter child endocrine function and result in reproductive toxicity are raised by its inclusion in infant medicines. In this study, an extraction and measurement method for methylparaben in baby medicines was established using a liquid-phase microextraction technique, RP-HPLC and a DAD detector (Nafiseh et al, 2021, p.1-8).

Antimicrobial activity increases with extending alkyl chain for the commonly used methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP). These factors all emphasize how crucial it is to maintain pharmaceutical products in good condition in order to stop microbial growth and drug degradation. Preservatives are active substances that prevent the growth of bacteria, fungi, and viruses. Oral administration of MP results in complete absorption, digestion, and formation of para-hydroxy benzoic acid and its metabolites, which are quickly removed through urination (Lakshmi et al, 2021).

Parabens are extensively used as preservatives in food, cosmetic, personal care, and pharmaceutical products to prevent the growth of undesirable microbes. However, because of their connection to estrogenic and carcinogenic dangers, parabens may be damaging to human health, claim a number of studies. The maximum permitted level of parabens in products sold in the European Union is 0.4% for each paraben alone and 0.8% for all parabens combined. To determine the presence of parabens in complex sample matrices, sample preparation techniques are required before chromatographic or hyphenated analysis. The process of clean-up and preconcentration makes it challenging to eliminate pollutants and enrich analytes because of the complex matrices used (Dandan Ge et al, 2020, p. 2120-2128).

The release specifications for a finished product should include identification and content determination tests for each antimicrobial preservative used in the formulation, according to the European Medicine Agency (EMA), which stated that the use of antimicrobial preservatives in a medical formulation requires special justification. Since methylparaben has antimicrobial properties and is one of the parabens in this category, identification and determination procedures are necessary. Due to its phenyl ring, this chemical may be detected by UV at exceedingly low concentrations. Due to the absence of an ionic functional group, it is characterized as lipophilic. Due to this lipophilicity, some accumulation in the fatty tissues of the body would be expected. Methylparaben dissolves at a rate of 0.25 (w/w) at 20 °C. In several organic solvents, such as ether, alcohol, and acetone, it is readily soluble.

According to the ICH recommendations, the proposed technique was validated, and it was found to meet all parameters for linearity, precision, accuracy, LOD&LOQ, and specificity. Using the described method, it was successfully determined that four commercial pharmaceutical samples contained the examined preservative (Zor & Donmez, 2018).

Due to the vast variety of textures and viscosities (such as liquid, solid, and semi-solid) of personal care products, extensive sample pretreatments are required prior to chromatographic testing. Using a rapid ultrasound-assisted extraction method, six parabens—methyl, ethyl, isopropyl, propyl, butyl, and benzyl—were simultaneously removed from a variety of complex-matrix cosmetic products (Lucas-Sanchez et al, 2022).

The most common and effective acne treatments are tretinoin and clindamycin phosphate. A reversed-phase HPLC stability-indicating technique for the simultaneous detection of tretinoin (TRN), imidazolidinyl urea (IU), methylparaben (MP), and clindamycin phosphate (CLP) has been developed and validated in this work. The bulk of the chromatographic parameters in the current study were enhanced to produce higher separation. Gradient elution with a C-18 (250 4.6 mm), 5 m column, a mobile phase made up of orthophosphoric acid (1 mL/L in water) and methanol, flowing at a rate of 1.0 mL/min, and UV detection at wavelengths of 200 nm and 353 nm. System appropriateness, precision, accuracy, specificity, robustness, linearity, range, detection limit, quantification limit, and reagent stability were among the criteria used to verify the novel method. According to the standards of the International Conference on Harmonization (ICH), all of the experimental parameters were certified to be within acceptable boundaries. Finding the contemporaneous concentrations of CLP, TRN, MP, and IU in pharmaceutical formulations was effective using the provided method. The chromatogram did not contain any interfering peaks according to the specified RP-HPLC procedure. The information suggests that pharmaceutical laboratories can simultaneously analyze CLP, TRN, and two preservatives, MP and IU, using the new RP-HPLC approach for both qualitative and quantitative evaluations. (Sarfraz et al, 2022, p.168).

Ultrasound-assisted cloud point microextraction (UA-CPME) was utilized to extract particular preservatives (p-HBA and its alkyl esters, methyl, ethyl, propyl, and butyl). Later, an HPLC approach was developed to concurrently detect them in samples from the pharmaceutical and cosmetic sectors. On a C18 column, the chromatograms of these substances were recorded utilizing a gradient elution technique, a number of solvent systems, various flow rates, and a diode-array detector (DAD) operating at a 254 nm wavelength. The conventional method's discovery of the analytical circumstances was optimized utilizing the Box-Behnken design (BBD). The impact of each factor was assessed for the UA-CPME and HPLC experiments using 3 and 4 design elements, respectively. For UA-CPME, the concentration, the quantity of Na2SO4, the extraction duration, the flow rate, column temperature, mobile phase 1, and mobile phase 2 ratios for HPLC analysis, as well as the values for the levels being studied, were collected. The experimental data was examined using regression analysis to determine the variables affecting resolution.

Analysis of variance (ANOVA) was employed to ensure the validity of the results. Using the ANOVA test, the results' repeatability was assessed. A model was created using the data that was gathered.

The new method was validated by looking at its linearity, reproducibility, accuracy, limit of quantification, and limit of detection. Methyl and propyl parabens were detected in the syrup sample using the established methodology (0.148% RSD value and 0.060% relative error) and 0.149% RSD value and 0.120% relative error, respectively. In a hand cream, ethyl paraben recovery values ranged from (99.17 to 99.41 percent), whereas values for methyl paraben ranged from 98.32 to 99.42 percent (Guray et al, 2022, p.1031-1040).

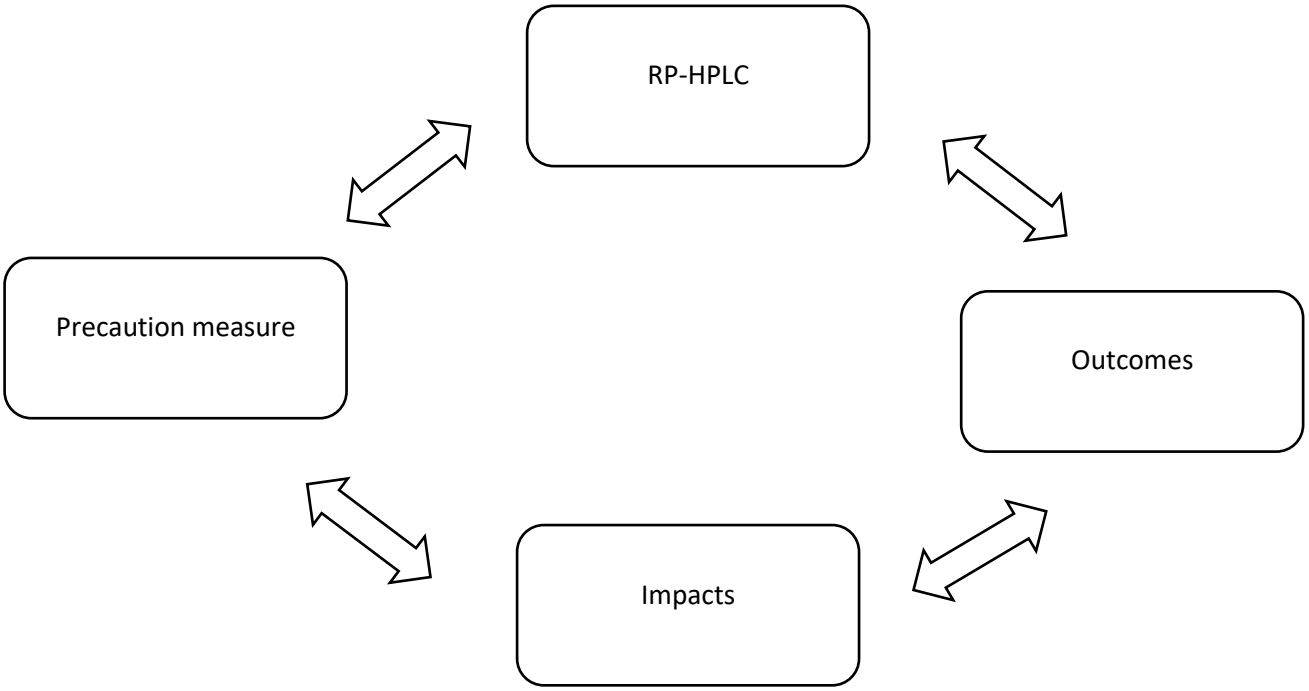
The innovative oral cephalosporin of the second generation, cefaclor, has proven to be the most effective treatment for acute otitis media in children. A novel RP-HPLC and RP-UPLC technique has been developed and validated for the simultaneous determination of CFC and MP in their powder for oral suspension dosage form and in their impurity cefaclor-delta-3-isomer. The chromatographic system is performed at ambient temperature using mobile phase composing of acetonitrile: methanol: 0.02M ammonium dihydrogen phosphate pH 4.7 ± 0.1 (25:10:65, v/v) on Agilent Eclipse XDB C18 column (250 mm X 4.6 mm, 5 µm particle size) at flow rate 1.0 mL/min, injection volume 20 µL for RP-HPLC and Waters CORTECS C18 column (50 mm × 4.6 mm, 2.7 µm particle size) at flow rate 0.3 mL/ min, injection volume 0.2 µL for RP-UPLC and UV detection at 265 nm. The approach is linear for all analytes in the concentration ranges of (70-700) g/mL for CFC and (10-200) g/mL for MP, with correlation values >0.999.According to ICH standards, the suggested strategy has been authorized. It is suitable for laboratory control of raw materials, bulk materials, and finished goods as a result (Hassouna & Mohamed, 2019).

A simple, precise, and exact RP-HPLC method is presented for the simultaneous quantification of methylparaben and propylparaben in pure form, pharmaceutical formulations, and environmental effluent samples. Chromatographic separation was carried out using methanol: water (adjusted to pH 4.8 with 0.1 N HCL) in the ratio of 45:55 v/v as the mobile phase on a supelco L7 reversed-phase column (25 cm 4.6 mm), 5 microns.

The flow rate was 1.0 mL/min, and the detecting wavelength was 254 nm. The two compounds were successfully resolved with retention durations of 5.34 min and 21.36 min for methyl paraben and propyl paraben, respectively. The linearities of propylparaben and methyl paraben were, respectively, in the 0.01-0.16 mg/mL and 0.01-0.12 mg/mL ranges (R^2 = 0.9991 and 0.998). These compounds can be estimated using the provided method in samples of environmental effluent and mixed dosage forms (Rahman, 2019).

According to several articles, parabens can be simultaneously separated in combination with other pharmaceutical products. The most common preservatives used by pharmaceutical companies or industries are methylparaben and propylbaraben .They are used in pharmaceuticals and other products due to their antimicrobial and antifungal activities but they cause allergic reaction and disrupt the endocrine system. There exists a gap between pharmaceutical products that are consumed with regulations due to parabens and those without parabens. Customers may avoid pharmaceuticals with parabens and go for those without parabens.

Theoretical Framework



RP-HPLC

Pharmacies and their consumers both benefit from being aware of whether or not pharmaceutical products contain preservative like methylparaben. One of the tools employed would not be possible without cutting-edge analytical methodologies. The pharmaceutical industry could not reasonably implement such a procedure to ensure product quality. The RP- HPLC method gives essential data for reducing the variation in the preservative concentrations that should be present in medicinal products. Although a DAD detector was utilized in this investigation, other RP-HPLC techniques using a variety of detectors are also used to detect MP in pharmaceutical products.

OUTCOME

Methylparaben is a preservative included in various pharmaceutical items of interest to consumers, and this study quantitatively evaluated its presence in four samples of pharmaceuticals. Preservative concentrations differed between samples. The observed distinctions are likely the result of organizational and manufacturing factors.

POSITIVE AND NEGATIVE IMPACTS

As preservatives, antifungal, and antibacterial agents, parabens are frequently utilized in a variety of pharmaceutical products. Although scientists disagree on how parabens affect people, animals, and ecosystems, it is known that they can operate as endocrine disruptors and that certain studies have found them to be carcinogenic substances. Furthermore, the assessment of toxicological research on humans addressed the risks associated with their use (Lincho et al, 2021, p. 2307).

PRECAUTIONARY MEASURES

To create high-quality products with superior appearance, applicability, and stability, these chemical substances must be included in pharmaceuticals. However, the excessive use of these chemicals in the products has raised safety concerns because many of them have been linked to serious health problems. Drugs containing dangerous materials should not be used excessively, especially by youngsters and pregnant women. Pregnant women are advised by gynecologists not to use medications that contain dangerous substances. To prove that medications are safe, a legal framework must be put in place. To safeguard human health and reduce safety concerns, pharmaceutical businesses and industries need to be properly regulated and brought into compliance with the rules (Manthan et al, 2023).

In addition to having negative health effects, common synthetic preservatives like parabens can also lead to allergic contact dermatitis, a serious form of skin inflammation characterized by rashes, blisters, and burning skin (Seeham & Abdul-Jabbar, 2020).

Related Research

The type and quantity of the extraction solvent as well as the type and quantity of the dispersive solvent were both modified in this investigation to increase the analytes' capacity to be extracted (Tugce U et al, 2023).

RP-HPLC with UV detection techniques was utilized to isolate and quantify the examined medicine from methylparaben and propylparaben. The established method can be utilized for routine drug analysis, therapeutic drug monitoring, and bioequivalence investigations by analyzing plasma samples collected from blood banks (Ahmed S et al, 2023).

A quick and simple RP-HPLC method has been developed and validated for the determination of MP and PP in pharmaceutical formulations. In this study, MP and PP were assessed using an RP-HPLC method developed for the investigation of medical cream formulations. On an ACE C18 Column 121-2546 (250x4.6 mm) at 25 °C, a gradient elution process was used with the mobile phase, an ACN: Buffer (pH: 3.9) mixture. In 17 minutes, MP and PP were eluted. The specificity of the approach could be shown in the peak homogeneity data for parabens in the medicinal cream samples that were identified using photodiode array detectors in the cream sample chromatograms (Ozen & Nemutlu, 2023).

A simple, isocratic HPLC method was developed and validated for the separation, identification, and detection of methylparaben and propylparaben. The ideal chromatographic conditions were established on CN column with 0.15% triethylamine in 10 mM KH2PO4 aqueous solution (final pH 3.0 adjusted with H3PO4) and methanol in the ratio 70:30 (v/v), enabling the selective identification of analytes within 5 minutes. The method was successfully validated in accordance with the ICH standards acceptance criteria for selectivity, linearity, accuracy, precision, and robustness. Gentamicin (0.32-1.04 mg mL1), methylparaben (0.0072-0.0234 mg mL1), and propylparaben (0.0008-0.0026 mg mL^{-1}) were tested in the method's linearity ranges (Ivkovic et al, 2023).

In this study, it was shown that the best method for detecting the presence of parabens required just 15 mg of adsorbent and a total desorption and adsorption duration of 10 minutes. The recovery rates attained with this method range from 90.78 to 104.89% for water matrix and from 85.19 to 1083.5% for cosmetics. The HPLC approach had detection limits of 0.0387, 0.0322, 0.0299, and 0.0339 ug/mL for methyl, ethyl, propyl, and butyl parabens, respectively. A new vortex-assisted MIL-101(Cr) adsorption-based solid phase extraction method that is rapid, inexpensive, and environmentally friendly was developed before HPLC analysis. Using the suggested VA-SPE-HPLC approach, four different parabens were extracted and identified from water and cosmetic samples (Yengin et al, 2023, p. 1383-1393).

CHAPTER 3

3.1 Instrumentation

Agilent Technologies 1200 series HPLC with Diode Array Detector (DAD) detector, a solvent filtration system, and an electronic balance were used to determine the presence of methylparaben in pharmaceutical samples that were acquired locally from TRNC.

3.2 Reagents and Solutions

Pharmaceutical products, methanol & water (mobile phase), methyl-, ethyl-, propyl- and butylparaben (pure standards) and deionized water were used throughout. Methanol, MeOH (50%) and water, H_2O (50%) were used as dilution reagents.

3.3 Apparatus

Micropipettes, beakers, 10ml & 500ml graduated cylinders, from ISO LAB Germany, 10ml and 25ml volumetric flasks and vortex machine from Heidolph Reax.

3.4 Preparation of Standards and Samples

3.4.1 Stock and Standard solutions

10.0 mg of each paraben was weighed using electronic balance and separately transferred to 10ml volumetric flasks. 5.0ml of methanol was added to dissolve the solid and then complete the volume to the mark with DI water and each of these stock solutions contains 1000ppm ($1000mgL^{-1}$). 1.0ml of each stock solution was transferred into a 10ml volumetric flask to create a 100ppm intermediate stock solution (solution A), which contained the four parabens. The volume was then brought up to the mark with methanol. By pouring 200 ul of solution A into a 10 ml volumetric flask and filling the remaining volume to the mark with DI water (solution B) and create a 2.0ppm mixed standard solution containing the four parabens. The mixed standard solution was later vortex for 10 seconds.

3.4.2 Methylparaben standard for calibration curve

Methylparaben standard solutions containing 1.0, 5.0, 10.0, 20.0, 50.0, 100, 250, 500, 750, and 1000ppm in DI was prepared. 1000 ppm was transferred into an HPLC vial directly. 7500, 5000, 2500, 1000, 500, 200, 100, 50 and 10 uL were transferred in each 10 ml volumetric flask and the solutions were made up to the marks with DI water.

Note: 10 ml volumetric flask was used for all preparation of the 10 concentrations before transferring to HPLC vials and using dilution formula: $M_1V_1=M_2V_2$.

3.4.3 Sample Preparation

50 uL of each sample was transferred in four 10 ml volumetric flask each.

Mobile phase was prepared from 60:40% of methanol and water and placed in a 25 ml graduated cylinder. The solution was later transferred in each 10 ml volumetric flask, inverted/swirled four times and the solution was made up to the mark with DI water. The amount of methylparaben in four samples of Pharmaceutical products—namely, samples one, two, and three were quantified while sample four was below detection range. These samples were obtained from a selected few pharmacies in the Turkish Republic of Northern Cyprus. Using the same mobile phase, the researcher collected and prepared the samples, which were then filtered using four syringes and filters into four HPLC Vials. A triple injection was performed for each sample that was filtrated into vials and placed in an auto-sampler. The mobile phase was degassed and used to wash the isocratic column before injecting the sample. The proper HPLC method optimization procedures were taken. The peak area and methylparaben's retention time for each sample's chromatogram were observed after the sample's run time, and a calibration curve was made. Using the calibration curve, the researcher calculated the amount of methylparaben present in each sample.

The mobile phase was degassed and the column was watched with the mobile phase.

Forty two injections were done for five minutes each, which constitute 3 hours and 30 minutes.

CHAPTER 4

FINDINGS AND DISCUSSION

4.1 Optimization of HPLC

Table 1's column type was chosen since it produced reliable peaks for both my trial experiment and earlier experiments. The back pressure was above 210 bar with no reliable baseline at 0.8 ml min^{-1} flow rate. A decent baseline was produced and the back pressure decreased to 188 bar when the flow rate was adjusted to 1 ml min^{-1}. At 30 degrees Celsius, the baseline was unrealistic, whereas the baseline at 20 degrees Celsius was sufficient. 60% methanol and 40% water (mobile phase) provided a reliable baseline in earlier tests. The mobile phase was likewise employed in this study, and the chromatogram showed the desired peaks.

4.2 HPLC Operating Conditions

Table 1

Column	ACE-C18.3mm 10x12.5cm(um)
Mobile phase flow rate(mL min^{-1})	1.0
Column Temperature(degree C)	20
Detector/wavelength	DAD 258(BW4) Reference 360nm(100BW)
Injection volume(uL)	20
Mobile phase	Methanol:H2O 60:40(%v/v)

Table 2: Methylaraben concentration found in each sample

Each sample	Conc. of Methylparaben present(mg/L)	SD
Sample one	2066	1809+/-227.2
Sample two	1838	1809+/-227.2
Sample three	1524	1809+/-227.2
Sample four	Below detection range	Negative

4.4 Figures of Chromatogram

Figure 3: Chromatogram of Paraben Standards

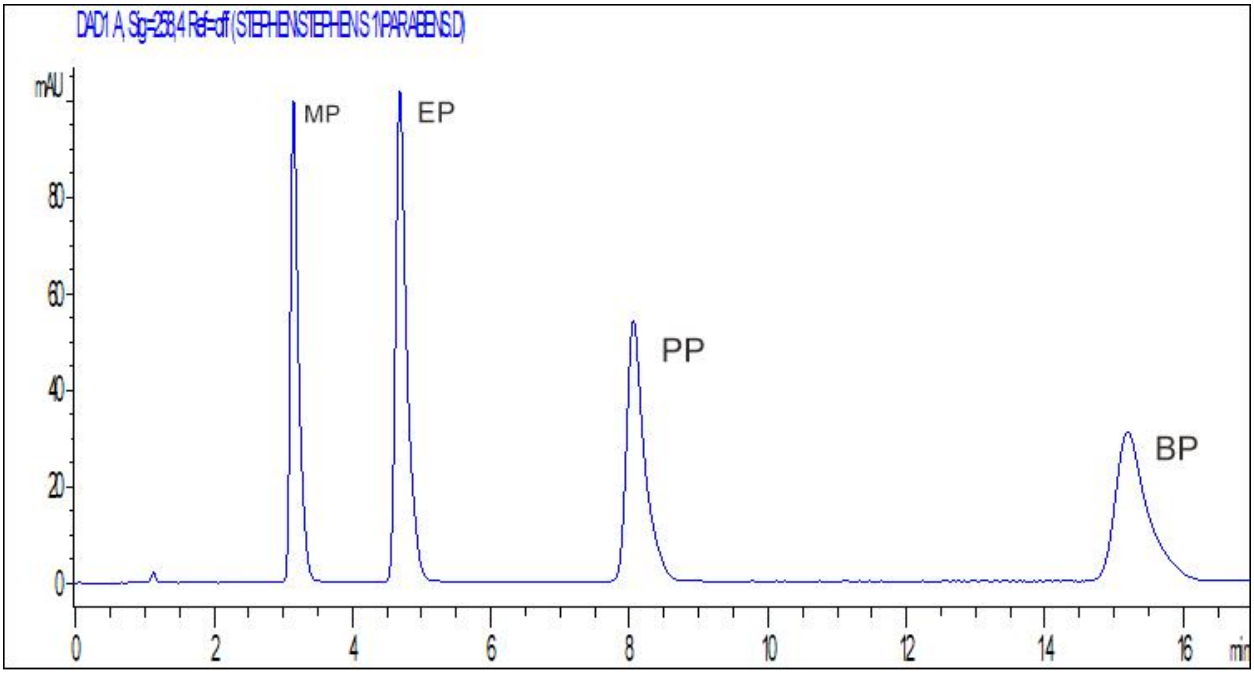


Figure 4: Chromatogram of sample 1

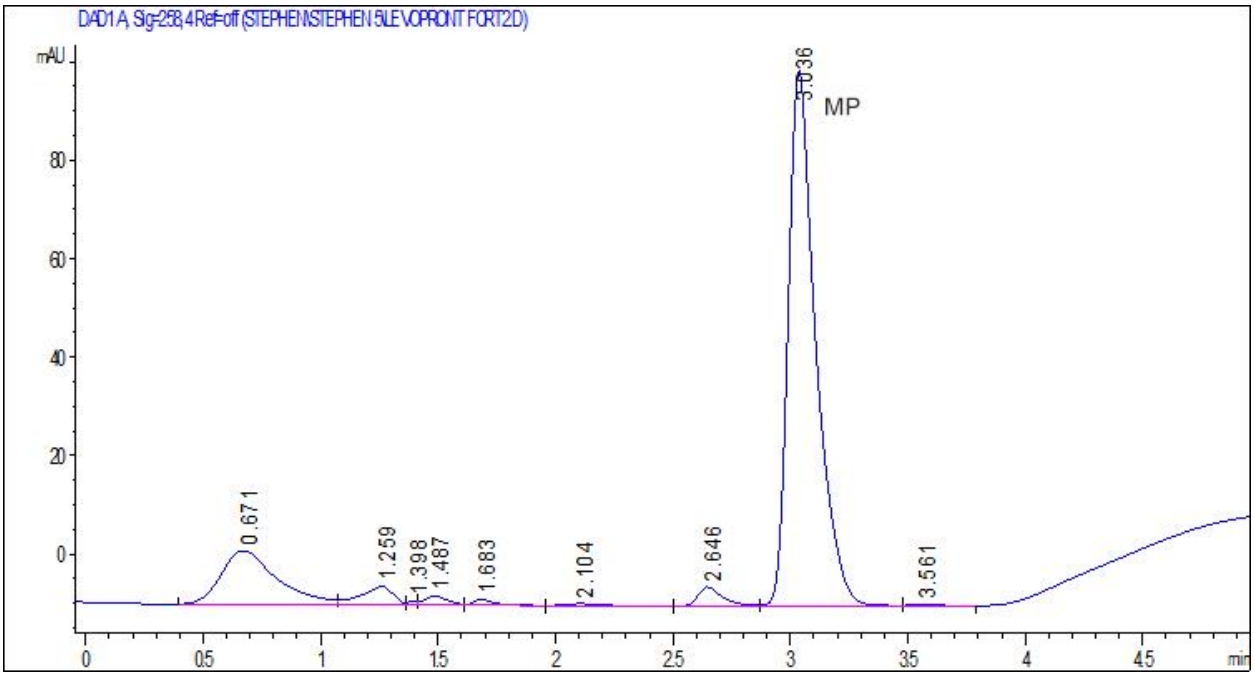


Figure 5: Chromatogram of sample 2

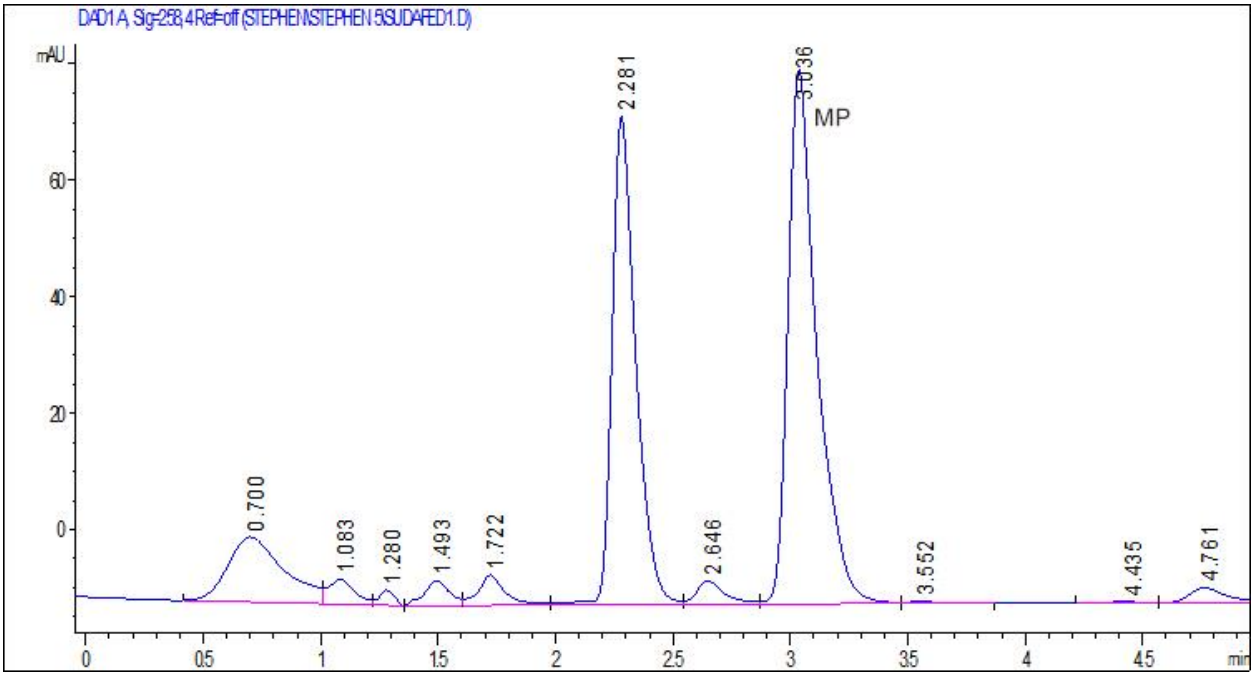


Figure 6: Chromatogram of sample 3

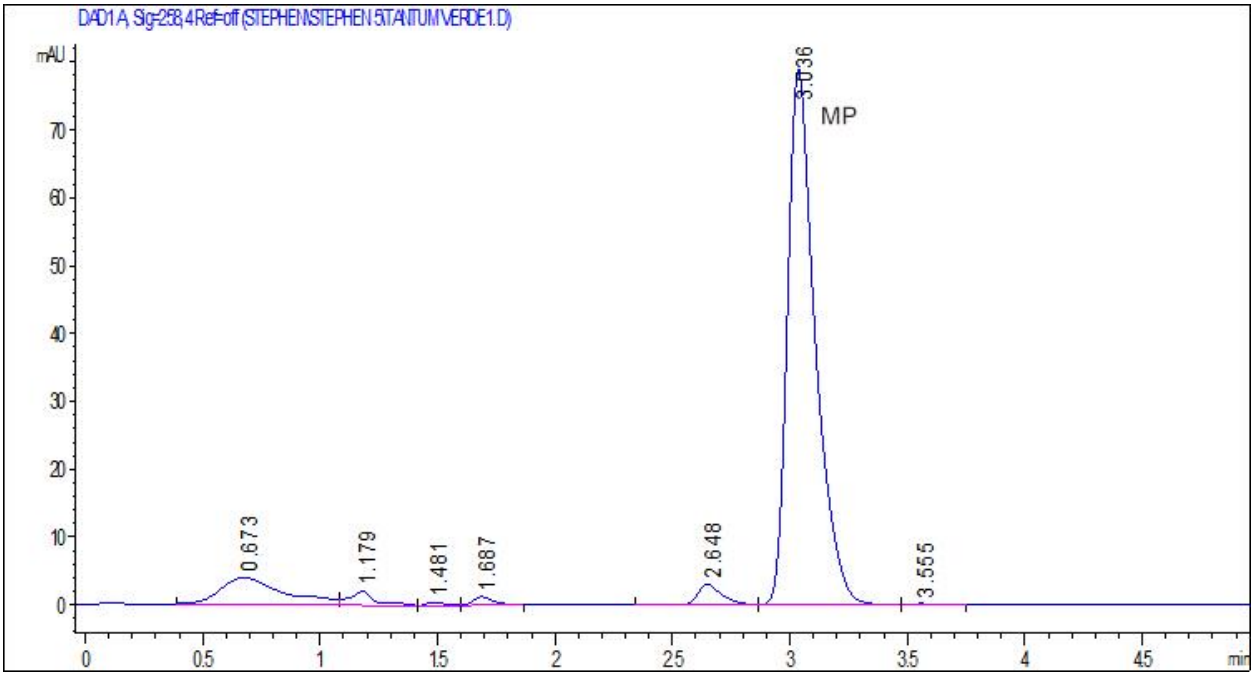
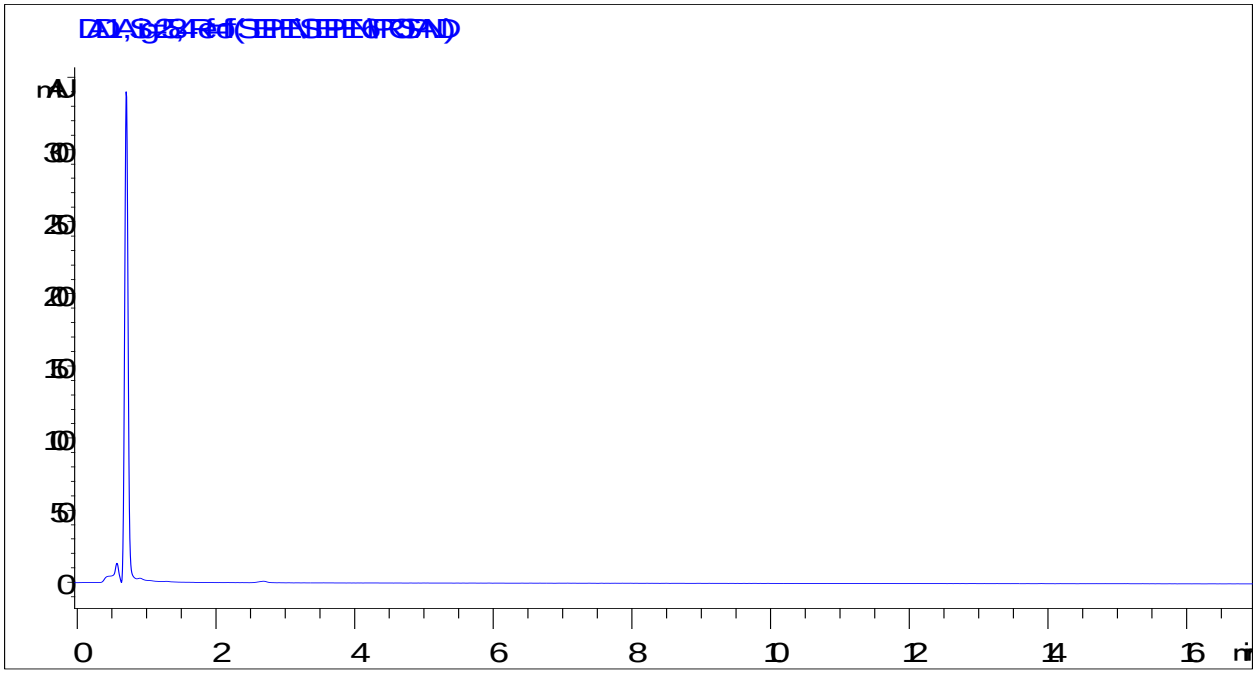


Figure 7: Chromatogram of sample 4

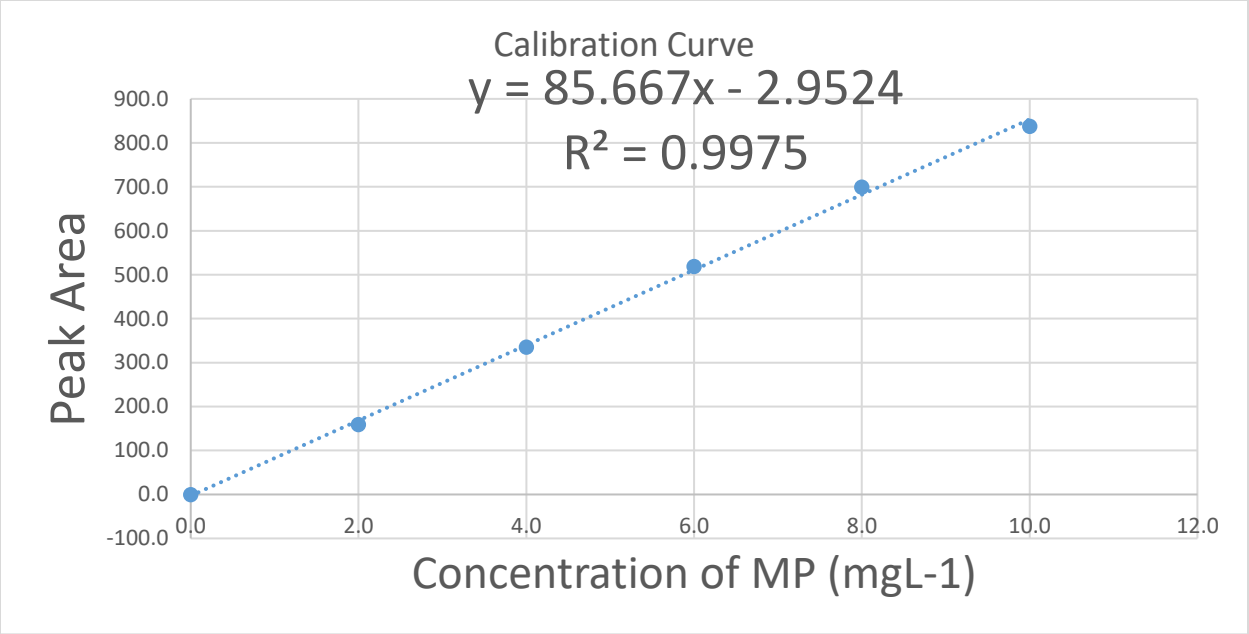


4.5 Method Validations

Linearity:

As can be seen in Fig.12 below, the study of ten standards concentrations of methylparaben was used to determine the system linearity.

Figure 8: Calibration Curve



Accuracy/recovery:

By comparing the experimental concentration of the solutions made for the linearity test to the nominal concentration, the accuracy of the procedure was examined. As illustrated in Table 3, a good recovery of methylparaben was seen.

Some validation parameters for Methylparaben

Table-3

Sample	Regression Equation	Correlation Coefficient	SD	Precision(%RSD)	$LOD^{mg/L}$	$LOQ^{mg/L}$
Sample one	Y=85.667x-2.9524	0.9975	1.011599	0.114681	0.04	0.14
Sample two	Y=85.667x-2.9524	0.9975	15.01111	1.913706	0.60	2.00
Sample three	Y=85.667x-2.9524	0.9975	20.1554	3.101307	0.81	2.69
Sample four	Y=85.667x-2.9524	0.9975	Negative	Negative	Negative	Negative

Method precision:

To ascertain the accuracy of the approach, four sample solutions in triplicate were examined on the same day. The method's usefulness for determining the presence of methylparaben in four distinct pharmaceutical products was demonstrated by the low RSD (10%), and the method's accuracy was presented in Table 4.

Table-4: Method precision

Sample	**Component**	**Peak area average**	**%RSD**
Sample one	Methylparaben	882.1	0.114681
Sample two	Methylparaben	784.4	1.913706
Sample three	Methylparaben	649.9	3.101307
Sample four	Methylparaben	Negative	Negative

LOD & LOQ

The calibration curve was used to determine the detection and quantification limits. The formulas 3*SD/S and 10*SD/S were used to determine the limits of detection and quantification based on the standard deviation of the intercept (SD =) and slope (S) of the calibration curve. Table 3 displays the concentrations at which detection and quantification limits were reached.

Specificity:

Methylparaben was found in three samples without any interference. Peaks had excellent clarity and specificity, and they were entirely separated. By contrasting the retention duration with that of the standard, the peaks of methylparaben in the samples were recognized, demonstrating the method's specificity.

CHAPTER 5

DISCUSSION

The concentrations of methylparaben in samples one, two and three are mentioned in table two while four was below detection range. Sample one has the greatest analyte concentration, followed by sample two, and sample three has the lowest concentration. These concentrations fall within permissible levels, as can be inferred.

The development and confirmation of the simultaneous measurement of methylparaben in samples one, two, three, and four in pharmaceutical dosage form using a straightforward, quick, and effective RP-HPLC technique. All of the above pharmaceutical products were separated by isocratic elution using an ACE-C18.3mmIDX 12.5 cm (um) column with a flow rate of 1.0 mL/min and a detector wavelength of 258 nm. 40% methanol and 60% water made up the mobile phase. All of the peaks had shorter run times, better resolution (resolution was greater than 2.5 for any pair of components), and symmetry. Limits of quantification for samples one, two and three were 2.69 mg/L for quantification, 0.40 mg/L for detection, 0.60 mg/L for detection, and 0.81 mg/L for detection, respectively. While sample four was below detection range. The method's specificity, linearity, accuracy, and precision were all confirmed. The placebo and other excipients (the diluent) had no chromatographic interference at the retention time of the active peaks and their impurities.

CONCLUSION

The stability-indicating aspect of the approach is supported by the simple, selective, isocratic mode of the high-performance liquid chromatographic technology, which permits the selective measurement of methylparaben without interference from the blank, placebo, or any other degradants. The suggested approach is incredibly fast, precise, repeatable, linear, and accurate. The quality control for routine and commercial analyses can be successfully carried out using the method that is being provided. This information could be very helpful for assessing the quality of bulk samples and keeping track of them throughout stability testing.

RECOMMENDATIONS

Formal health education should be provided at all levels, with a focus on pharmaceutical product makers, pharmacies, drug stores, and end users of these goods on the advantages and disadvantages of methylparaben. Methylpaben prevents microbial growth in pharmaceutical products; however, it can also lead to allergic reactions in some people and have other harmful consequences for their health. Consumer health concerns will be reduced by using this preservative in pharmaceutical products at levels below those that are legal.

According to the findings, legislation should require public health education on the use of pharmaceuticals that contain methylparaben across the country. Leaders from a variety of sectors may also come out in favor of putting more emphasis on the creation of pharmaceutical products. Pharmacies and companies that use methylparaben at the permissible level are required to inform their clients about the possibility of adverse reactions when this preservative is used in higher concentrations during studies. The law, healthcare institutions, and pharmacists should all consider methylparaben's positive and negative impacts. They should also cover ways to lessen the risks that pharmaceutical products with preservatives in them pose to people's safety and health.

RECOMMENDATIONS FOR FUTURE STUDIES

This study looked at the methylparaben content of a few medicines that are widely used in the TRNC. The reason for the observed variances must be related to the company and the working environment. Methylparaben is a useful preservative for medicinal items even though it is only permitted in very small concentrations. Future research will primarily focus on expanding the sample size and examining additional preservatives, including ethyl, and propyl and butylparabens. In addition, research has been done to identify the many factors affecting clients inside the TRNC.

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Curriculum vitae

A dedicated professional Teacher with over seven (7) years of education and experience in teaching Chemistry, Biology and laboratory courses in both High School and College. Teaching laboratory rules on safety and handling of glass wares as well as busy concerts in Chemistry and Biology to elevate students' knowledge. Certificates' holder, a bachelor's degree holder, and a master's candidate in analytical chemistry.

Personal Data Curriculum Vitae

Name	:	Stephen Martor Johnson
Date of Birth	:	May 4, 1983
Place of Birth	:	Ganta City, Nimba County
Marital Status	:	Married with four (4) children
Nationality	:	Liberian
Cell #	:	0775-502-652/0886-725-457
Email	:	sjmartor@gmail.com

Education Background

.Bachelor of Science degree, General Chemistry 2009-2015

T.J. Faulner College of Science & Technology

Department of Chemistry, University of Liberia

High School Diploma 2003-2006

George Toe Washington Christian School System

Gompa City, Nimba County

TRAININGS

Certificate of Achievement 2019
Environmental Protection Agency (EPA)
Monrovia, Liberia

Certificate of Participation 2019
Helping Hands Network
Departments of Physics and Mathematics
University of Liberia

Certificate of Training 2018
Laboratotory Demonstration Skills
Ganta City, Nimba County

Certificate of Participation 2014
National Elections Commission (NEC)
Republic of Liberia

EMPLOYMENT HISTORY

Chemistry& Biology Laboratory Demonstrator, February-June, 2023
Notre Dame University College,
15TH Street Sinkor (Beach Side)
Monrovia, Liberia

Duties & Responsibilities

Submit a detailed Course Outline/Syllabus for the duration of the trimester before commencement of classes

Promote quality assurance strategies for learning and teaching

Ensure effective use of diverse and innovation teaching methodology

Employ effective classroom management techniques

Timely submission of Mid-trimester & Final Exams questions

Provide guidance for students

Ensure a smooth lecturer-student relationship

Keep appropriate records of classroom activities (eg. Attendance)

Chemistry Instructor & Chemistry Laboratory Demonstrator 2019-2020
Ganta United Methodist High School
Gompa City, Nimba County

Duties & Responsibilities

Prepare daily and weekly lesson plans

Ensure effective use of diverse and innovative teaching methodology

Employ effective classroom management techniques

Outline laboratory safety rules to students

Demonstrate practical for titration and food tests

Timely submission of periodic grade sheets and Final Exams report

Keep appropriate records of classroom activities

Perform any other duty as commensurate with my role and as instructed by the vice Principal for Instruction

Chemistry Instructor 2018-2019
Faith Academy High School
Gompa City, Nimba County

Duties & Responsibilities

Prepare daily and weekly lesson plans

Ensure effective use of diverse and innovative teaching methodology

Employ effective classroom management techniques

Outline laboratory safety rules to students

Demonstrate practical for titration

Timely submission of periodic grade sheets and Final Exams report

Keep appropriate records of classroom activities

Perform any other duty as commensurate with my role and as instructed by the vice Principal for Instruction

Chemistry Instructor, Chairman 2016-2020
Laboratory Demonstrator
Department of Science
St. Lawrence High School
Ganta City, Nimba County

Duties & Responsibilities

Prepare daily and weekly lesson plans

Inspect daily lesson plan from all Science Instructors

Ensure effective use of diverse and innovative teaching methodology

Employ effective classroom management techniques

Outline laboratory safety rules to students

Demonstrate practical for titration

Timely submission of periodic grade sheets and Final Exams report

Keep appropriate records of classroom activities

Perform any other duty as commensurate with my role and as instructed by the vice Principal for Instruction

Biology Instructor 2016-2017
Faith Academy High School
Ganta City, Nimba County

Duties & Responsibilities

Prepare daily and weekly lesson plans

Ensure effective use of diverse and innovative teaching methodology

Employ effective classroom management techniques

Outline laboratory safety rules to students

Demonstrate practical for food tests

Timely submission of periodic grade sheets and Final Exams report

Keep appropriate records of classroom activities

Perform any other duty as commensurate with my role and as instructed by the vice Principal for Instruction

Biology and Gen. Science Instructor 2015-2016
Nimba Rubber Incorporated (NRI)
Cocopa Rubber Plantation
Cocopa, Nimba County

Duties & Responsibilities

Prepare daily and weekly lesson plans

Ensure effective use of diverse and innovative teaching methodology

Employ effective classroom management techniques

Outline laboratory safety rules to students

Demonstrate practical for food tests

Timely submission of periodic grade sheets and Final Exams report

Keep appropriate records of classroom activities

Perform any other duty as commensurate with my role and as instructed by the vice Principal for Instruction

Election (s) Supervisor
Upper Nimba Sub-office
Sanniquellie City, Nimba County

Duties & Responsibilities

Train Presiding officers, Voter's card Identification officers, Ballot Paper Issuers, Inkers

and Queue Controllers

Deploy local Staffs and distribute materials before elections

Supervise local Staffs during elections

Collect election materials and report same to the Election Magistrate after the elections

Work with the Financial team from National Election Commission (NEC) to compensate the local Staffs

Appendices

Appendix A

Appendix X