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Quantitation of Methyl and Propyl Parabens in Cosmetics Product by HPLC

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Abstract:

Paraben are antimicrobial agents commonly used as preservative and flavoring agent. The high concentration of parabens causes cancer, genotoxicity and breast cancer. This experiment aimed to determine the concentration of methyl and propyl paraben in cosmetic products. Parabens, are permitted in cosmetics at acceptable concentrations of 0.8% (w/w) per total paraben and 0.4% (w/w) per individual paraben, according to the European Community (EEC) directive.

Seven different types of daily-use cosmetics were analyzed using high-performance liquid chromatography (HPLC). Methyl and propyl paraben, were extracted using sonication and methanol as the solvent. The separation was carried out using C_{18} (15 cm x 4.6 mm), composed of silane octadecyl bonded to porous (5 µm) silica and gradient elution employing potassium dihydrogen orthophosphate as the mobile phase. A UV-detector was used, and quantification was performed at 272nm using Max plot.

All the tested samples were found to contain between 0.01% and 0.18% methylparaben and between 0.001% and 0.12% propyl paraben, which are below the threshold limits set by European regulations. Thus, all analyzed samples complied with the safety standards for paraben content in cosmetics and safe for use.

Keywords: *Concentration, Cosmetics, Paraben, Preservative*

Introduction

Preservative are the substance that are usually added in the packaged foods, cosmetics, pharmaceuticals, personal hygiene items, and cosmetic products to prevent or inhabit the development of microorganism. Sodium salts of para hydroxy benzoic acid are known as parabens. The antimicrobial activity increases as the alkyl chain of the benzene ring ester group length longer (Mincea et al., 2009).

Propyl, methyl, butyl, and ethyl parabens are some of the commonly used parabens that are

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found in a variety of cosmetic products (Minnesota Department of Health, 2016). Other frequently used preservatives are chloromethyl oizotiazolinon (CMI), methyl isothiazolinone (MI), benzyl alcohol (BA) and methyl paraben (MP). Benzyl alcohol is frequently used in pharmaceuticals than in cosmetics as an antibacterial agent. The isothiazolinone group is used in a wide variety of industrial products, including emulsions, paints, cosmetics, resins, plasticisers, detergents, fibres and polishing products. Sodium benzoate (SB) is used to maintain freshness and inhibit the growth of yeast, mold and other bacteria. Potassium sorbate (PS) is used in the food, pharmaceutical and cosmetic industries. Additionally, it is especially effective as a food additive to prevent the growth of mold in product like fruit juice (Baranowska & Wojciechowska, 2013).

The importance of quantifying parabens both quantitatively and qualitatively has gained increased attention in recent years due to potential allergic reaction they cause. Moreover, these preservative do not change the physical properties of the final products such as colour, odour and test (Zhang et al., 2005). In this paper, the most widely used preservative methyl and propyl paraben concentration were determined using HPLC method. These compounds are commonly used because of its wide range of antibacterial activity.

These products can be used regularly or occasionally and often come to contact with the human skin, hair, lips, nails and scalp. According to European Union cosmetic product standards, allow preservatives containing 0.5% for salts and esters of benzoic acid(Council Directive 76/768/EEC, 1999). A European Economic Community Regulation (EEC) sets the maximum allowable concentration of parabens in cosmetics at 0.4% (w/w) for a single compound or 0.8% (w/w) for all the total parabens in the composition(Lee et al., 2006). It has been estimated that the average daily intake of parabens is approximately 76 mg, or 1.3 mg per kg of body weight. These include the following distribution of the outcomes, 1 mg from food, 50 mg from cosmetics, and 25 mg from medicines per day(Soni et al., 2001). Preservatives are used extensively in cosmetics, which create health hazards. Preservatives may be dangerous for consumers because most of them can result in allergic contact dermatitis. Several studies have also show that commonly used parabens exhibit oestrogenic effects in a various in vivo and vitro tests using animal models(Blair et al., 2000).

Product formulations comprising propyl, ethyl, methyl and butylparaben at concentration between 0.1% to 0.8% caused only slightly and temporary eye irritation in rabbits. In the study involve more than 100 human subjects their eyes repeatedly with aqueous solution of 0.1% to 0.3% of methyl paraben and none of them feel irritation reports of paraben awareness have been made (Report & Assessment, 1984).

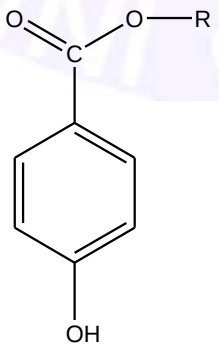

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Table 1: Structures of the parabens

Alkyl chain	Compound name	Abbreviation
$-CH_3$	Methyl paraben	MP
$-CH_2CH_3$	Ethyl paraben	EP
$-CH_2CH_2CH_3$	Propyl paraben	PP
$-CH_2CH_2CH_2CH_3$	Butyl paraben	BP

According to a review of the literature, numerous studies have been conducted on the analysis of parabens in biological fluids, food, cosmetic, and medicine. The analytical methods used include capillary electrophoresis (Memon et al., 2005; Liu et al., 2008), gas chromatography (Lin et al., 2000), HPLC (Lee et al., 2006) and HPLC coupled with chemiluminescence (Zhang et al., 2005).Wu et al., (2008)developed a Ultra-Performance Liquid Chromatography (UPLC) method that iseffective for and this simultaneously measuring 21 preservatives in cosmetic products.This method involves liquid-liquid extraction using ethyl acetate as solvent, and the concentration of methylparaben in pharmaceuticals and cosmetics was measured using spectrophotometry at a wavelength of 282 nm.

Preservatives are first applied to evaluate the quality of cosmetics. The study by Zareba et al., (2007) examined the effects of daily exposure to methyl paraben (MP) in dermatological formulations on human skin. It was found that methyl paraben accumulates in the stratum corneum (SC) of human skin and continued to exist in the skin even after daily application. This accumulation has been associated with effects on keratinocyte differentiation and ageing as well as on apoptosis, morphological changes, protein and gene expression and cell proliferation. Furthermore, the study assessed the impact of methyl paraben on epidermis differentiation in skin equivalents. The results showed that exposure to its accumulation and persistence in the skin, influencing both the aging process and differentiation of keratinocytes.

Methodology of the Study

The total methyl and propyl paraben were carried out according to the Mincea et al., (2009), Baranowska & Wojciechowska, (2013) and Nováková et al., (2004). The research work carried out in the following phases:

Collection of Samples

Sample of cosmetics (Ponds moisturizer, Pantene shampoo, Lotus facewash, Nivea body lotion, Monothrin and cetrimide lotion, Vaseline body lotion, Rose cleansing milk) were purchased from various retail shops from market.

Sample preparation

The extract were prepared by weighing approximately 1.5479 g of cosmetic using 32.5 mL of methanol. After 10 minutes of sonication, the solution volume was 50 mL using 17.5 mL of mobile phase. For five minutes, the sample extracts were vortexed. The extracts were passed through 0.22 µm Millipore membrane filters and placed into 2mL sample vials prior to injection for HPLC analysis.

Mobile phase

2.38 gm (Balance instrument were obtained from sartorius (Goettingen, Germany) of potassium dihydrogen orthophosphate and 350mL water (HPLC grade) was taken in a 1L

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volumetric Flask (Borosil, class A) and added 650mL methanol. Then, pH- 6.10 was measured (HANNA instruments Woonsocket RI, USA). After that solution was filter through filter assembly (nylon membrane filter 0.45µm). Then, solution was sonicated (PCi^{TM} ANALYTICS) for 10 min.

Stock solutions

Methyl paraben (Merck, Assay- 99.98%)

To prepare the initial stock solutions of methyl paraben (50mg/mL), about 25.5 mg of the methyl paraben were dissolved in 2.5mLof methanol which produced 50 mL of mobile phase. The stock solutions (5 mL) were further diluted in order to generate 50 mL of standard solutions using mobile phase.

Propyl paraben (Merck, Assay- 99.20%)

To prepare the initial stock solutions of propyl paraben (5mg/mL), measured amounts of the propyl paraben (approximately 12.5 mg) were dissolved in 2.5 mL of methanol to yield 50 mL of mobile phase. The stock solutions (1 mL) were further diluted in order to produce 50 mL of standard solutions using mobile phase.

For ten minutes, the solution was sonicated and then extracts were passed through 0.22µm Millipore membrane filter and place into 2mL sample vials before injection for HPLC.

HPLC instrument and conditions

Analyses were performed with a Shimadzu LC-2010 C system (Shimadzu, Japan) with built-in UV–visible detector. The employed HPLC system was consisting of a binary solvent manager, a sample manager with an integral column heater module, a solvent tray module and a UV- detector at wavelength 272nm. The analyte was determined using a stainless steel C_{18} (15cm×4.6mm), reversed phase column (shim-pack GIST C_{18}) packed with octadecylsilane bonded to porous silica (5µm). Lab solution software was used. The column temperature was maintained at 30 °C. The autosampler temperature was set to 25 °C. A mixture of 35mL of 0.68 percentage w/v solution of potassium dihydrogen orthophosphate (oualigens, thermo fisher scientific) and 65 mL of methanol (oualigens, HPLC grade, Thermo fisher scientific) was used as a mobile phase. The flow rate was 1.3 mL/min and injection volume was 10µl. A isocrate program was used starting with 100 % mobile phase. The column was saturated 30min by mobile phase. The mobile phases were filtered through a 0.22µm membrane filter. The UV signal was identified as the maximum plot within the range of 190-1100nm.

Result and Discussion

HPLC Optimization system

In this work, the mobile phase of potassium dihydrogen orthophosphate was found to be suitable for the detection and quantification of methyl and propyl paraben. Using aisocrate mobile phase composed of methanol and phosphate buffer (65:35, v/v), the retention time of methyl and propyl paraben was 2.208 min and 3.736min respectively. A chromatogram of methyl and propyl paraben with UV- detection at the max-plot obtained under these conditions. The compounds were monitored by measurement of the peak area of standard methyl and propyl paraben then the ratio of peak area was calculated.

Methyl paraben content

Formula=$\frac{AreaSP \times wt.STD \times 5 \times \times Assay of STD\%}{AreaSTD \times 50 \times 50 \times samplewt.} \times 100$ %

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Propyl paraben content

Formula=$\frac{Area\ SP \times wt.STD \times 1 \times \times Assay\ of\ STD\% \times (100-LOD)\%}{Area\ STD \times 50 \times 50 \times sample\ wt.}$×100 %

Chromatogram

Table 1-Standard Methyl paraben and propyl paraben retention time (RT), area and RSD%.

	RT	Area		RT	Area
Standard Methyl paraben Chromatogram 1	2.215	1283417	Standard propyl paraben Chromatogram 1	3.726	97665
Standard Methyl paraben Chromatogram 2	2.214	1282507	Standard Methyl paraben Chromatogram 2	3.726	98027
Average of standard		1282962	Average of standard		97846
RSD%		0.050	RSD%		0.262

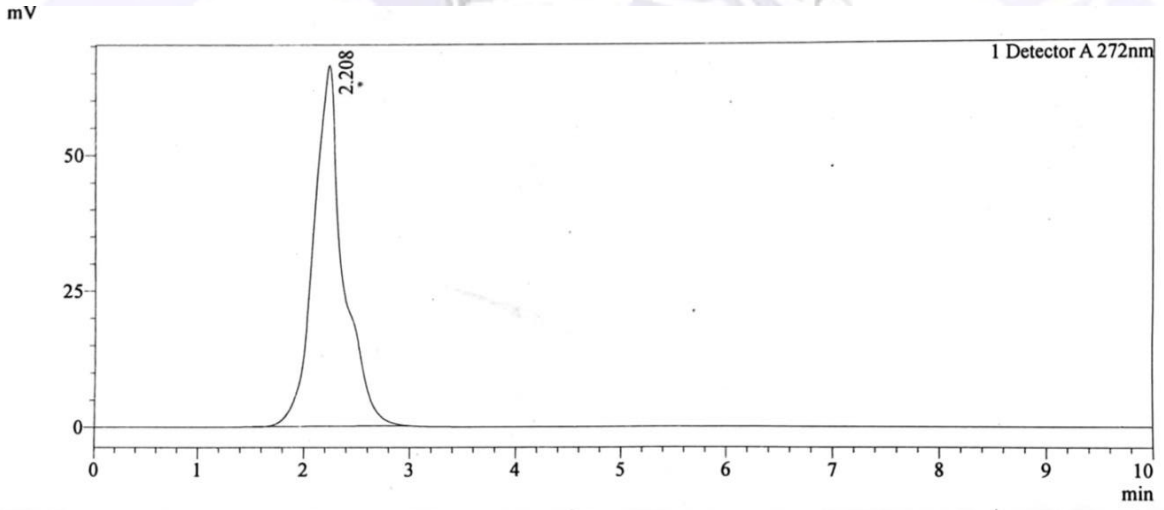

Figure 1: Standard methylparaben chromatogram with a UV detector at max-plot for isocratic elution

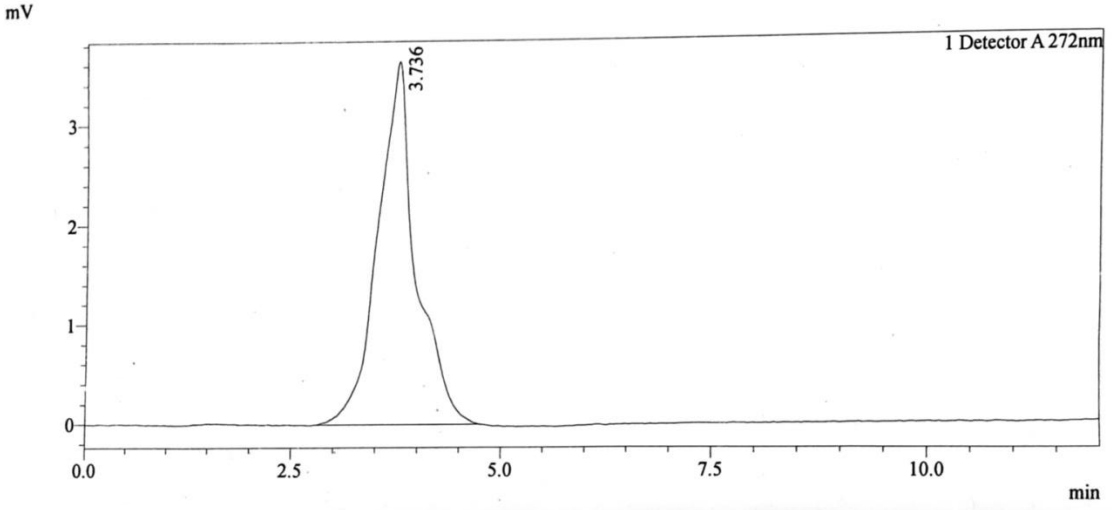

Figure 2: Standard propyl paraben chromatogram

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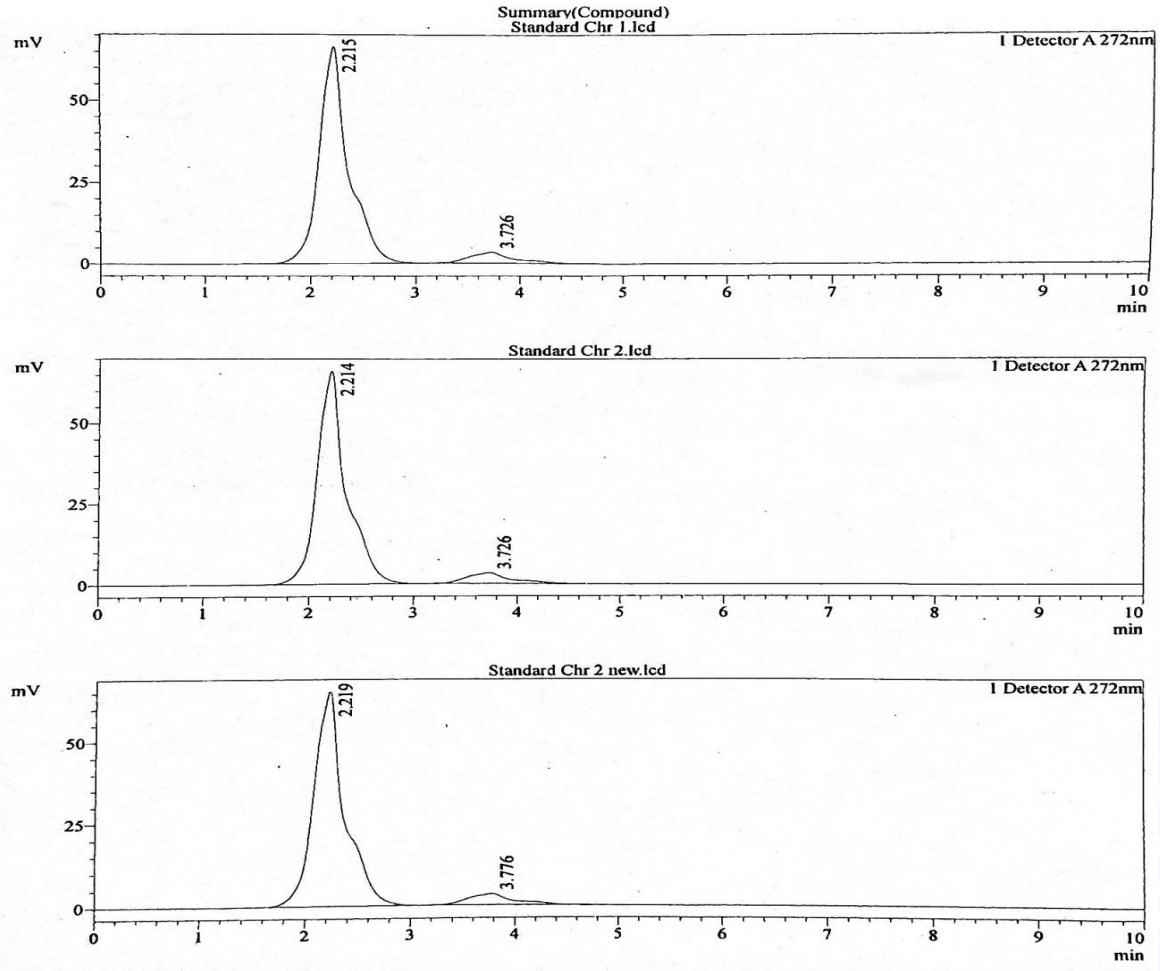

Figure 3: Summary standard (methyl and propyl paraben)

Table 2: Methyl paraben and propyl paraben concentration in seven cosmetics

Methyl paraben				
Cosmetics	Weight taken (g)	RT (Min.)	Area	Percentage of methyl paraben
Ponds moisturizer	0.0255 g	2.182	1379238	0.18%
Pentene shampoo	0.0129 g	Peak was not separated		
Lotus facewash	1.5538 g	2.226	77607	0.01%
Nivea body lotion	1.5485 g	2.236	1420122	0.18%
Monothrin and cetrimide lotion	1.5461 g	2.246	508842	0.07%
Vaseline body lotion	1.5161 g	2.239	1322344	0.17%
Rose cleansing milk	1.5117 g	2.224	1362658	0.16%

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Propyl paraben				
Cosmetics	Weight taken (g)	RT(Min.)	Area	Percentage of propyl paraben
Ponds moisturizer	0.0255 g	3.750	620688	0.100%
Pentene shampoo	0.0129 g	3.570	4610	0.001%
Lotus facewash	1.5538 g	3.835	19163	0.003%
Nivea body lotion	1.5485 g	3.873	662564	0.110%
Monothrin and cetrimide lotion	1.5461 g	3.895	51589	0.009%
Vaseline body lotion	1.5161 g	3.875	531310	0.091%
Rose cleansing milk	1.5117 g	3.861	726598	0.120%

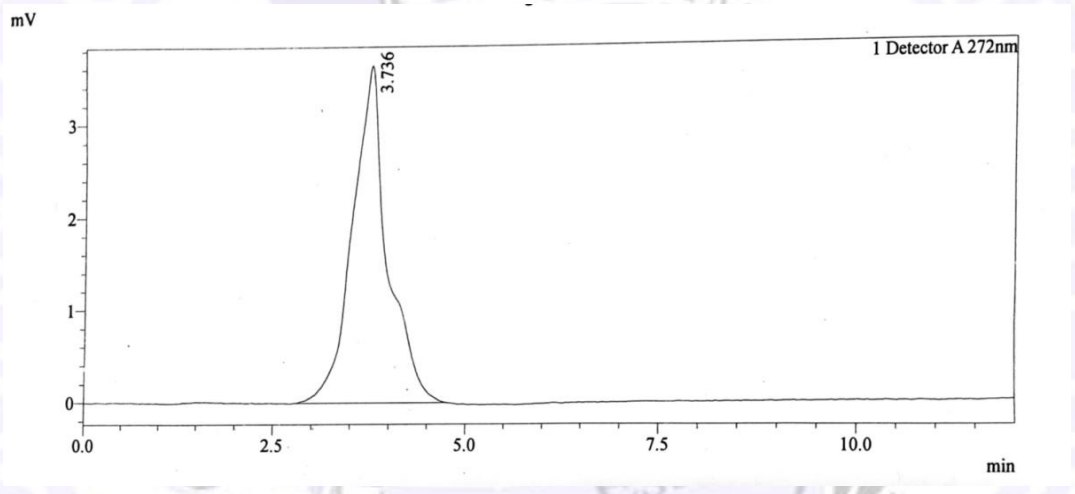

Figure 4: Chromatogram of pentene shampoo extract

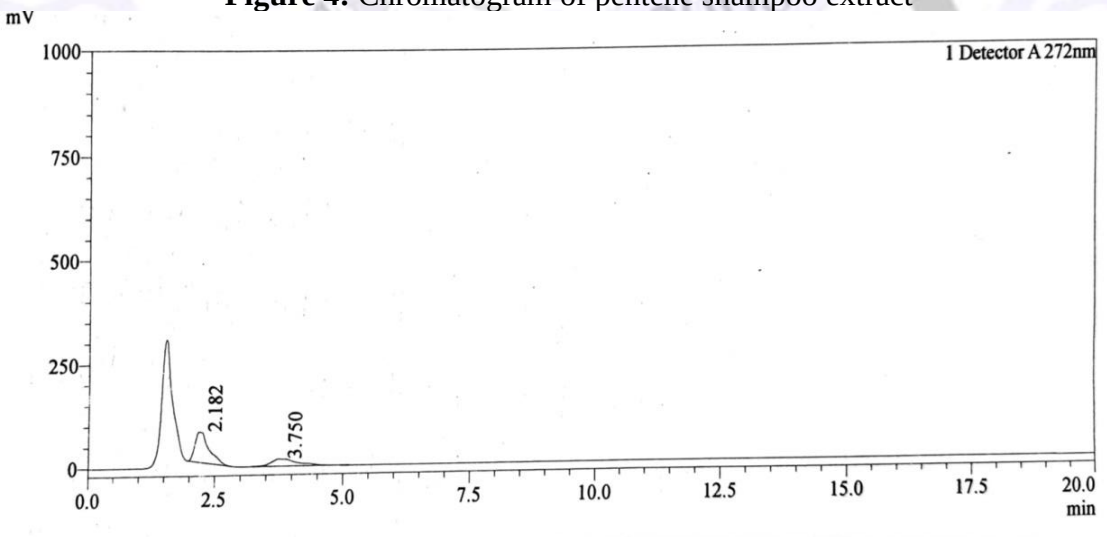

Figure 5: Chromatogram of Pond's moisturizer extract

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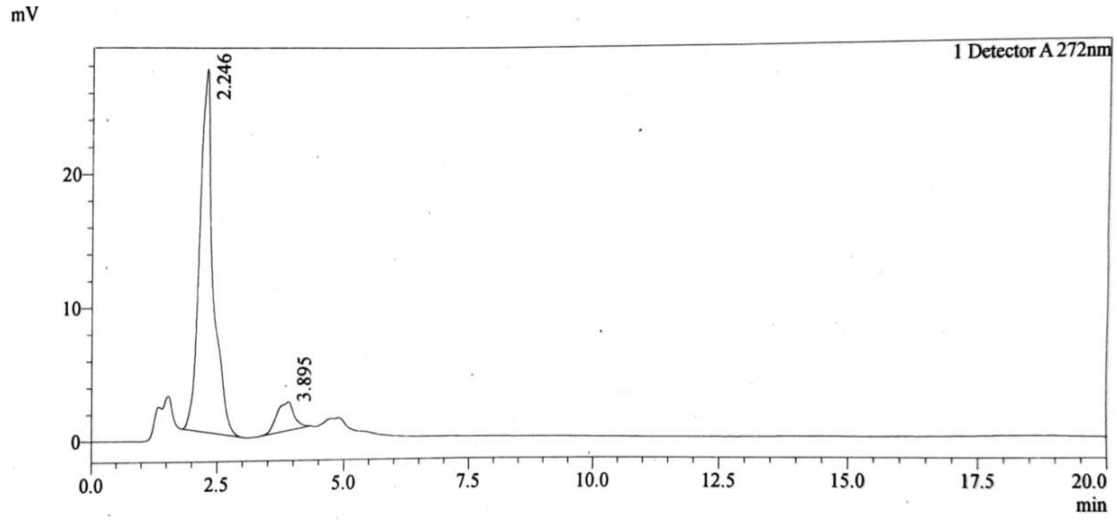

Figure 6: chromatogram of monothrin permethrin extract

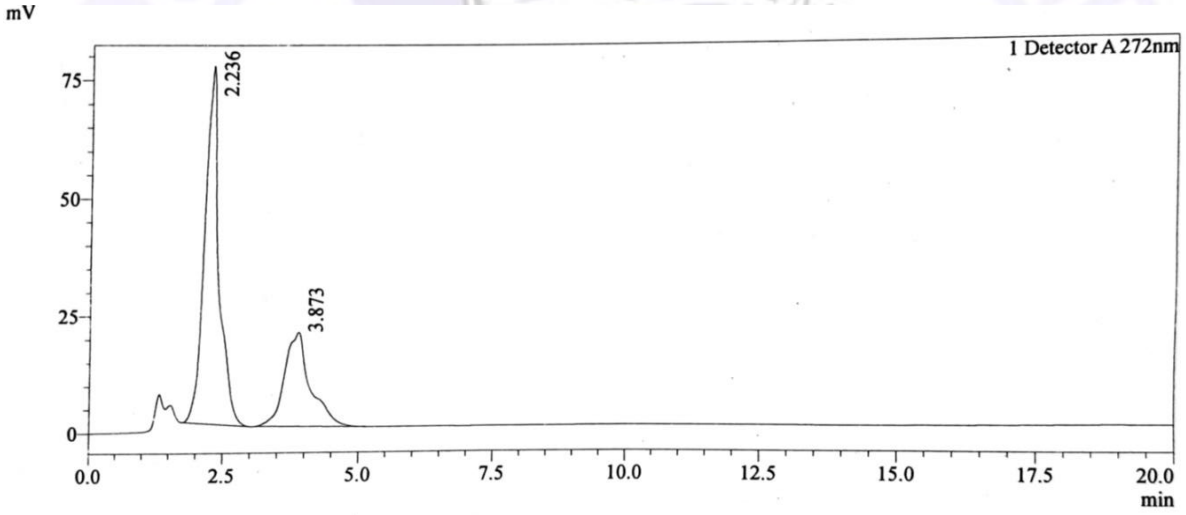

Figure 7: chromatogram of Nivea body lotion extract

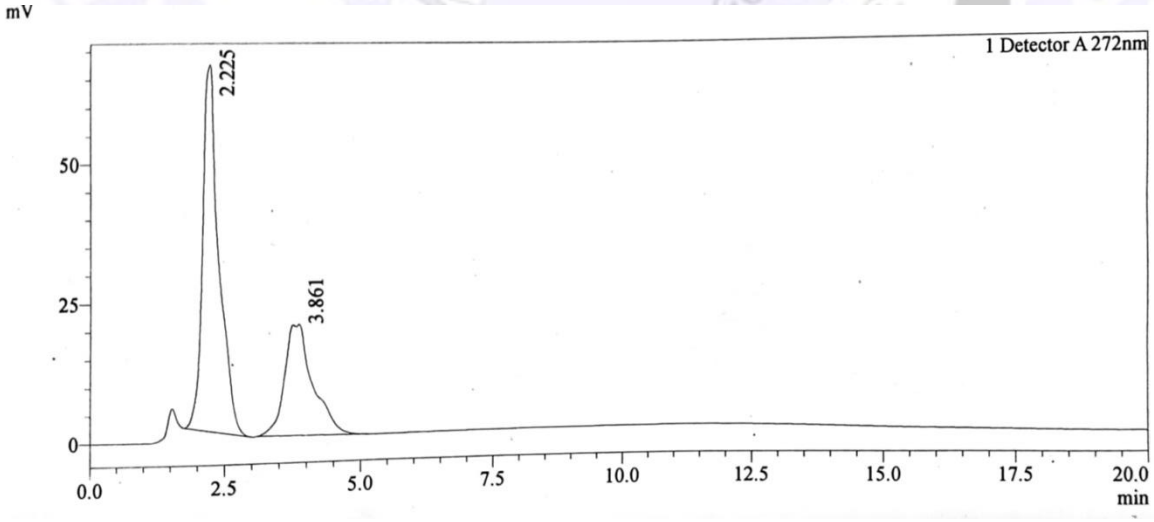

Figure 8: Chromatogram of Rose cleansing milk

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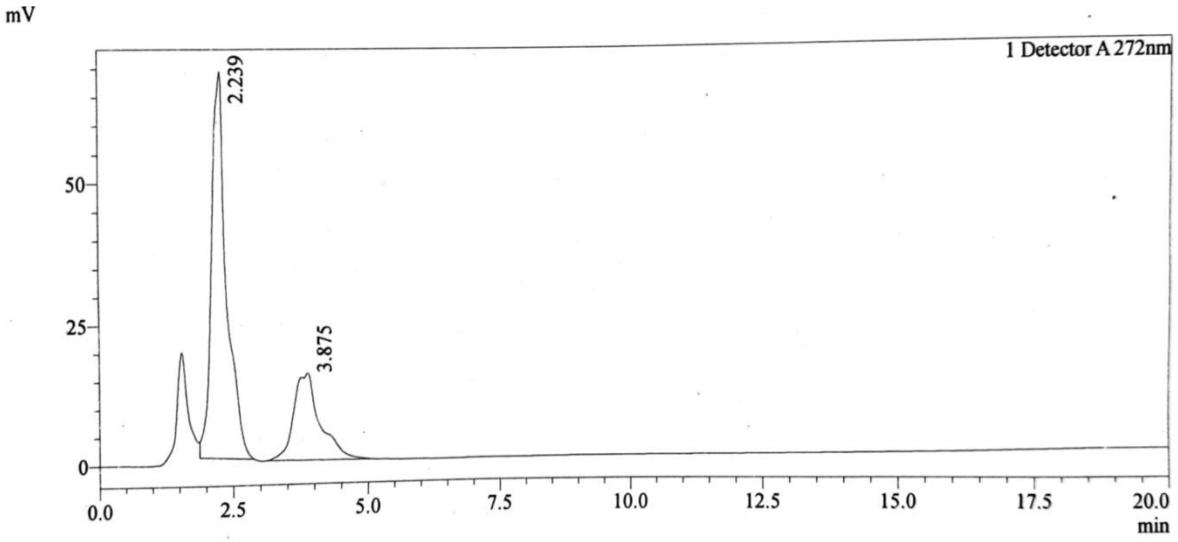

Figure 9: Chromatogram of Vaseline body lotion extract

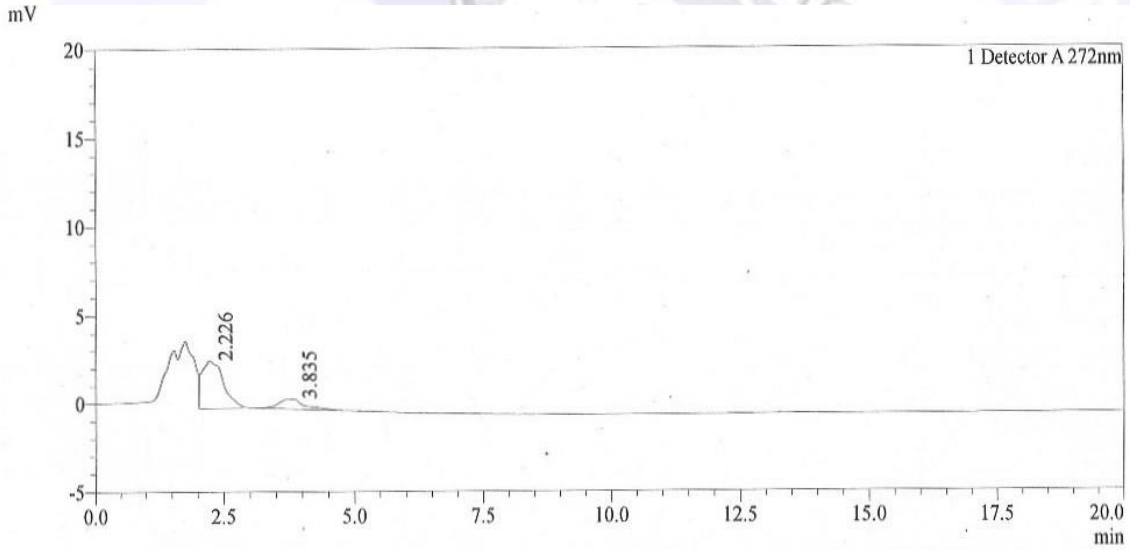

Figure 10: Chromatogram of lotus facewash extract

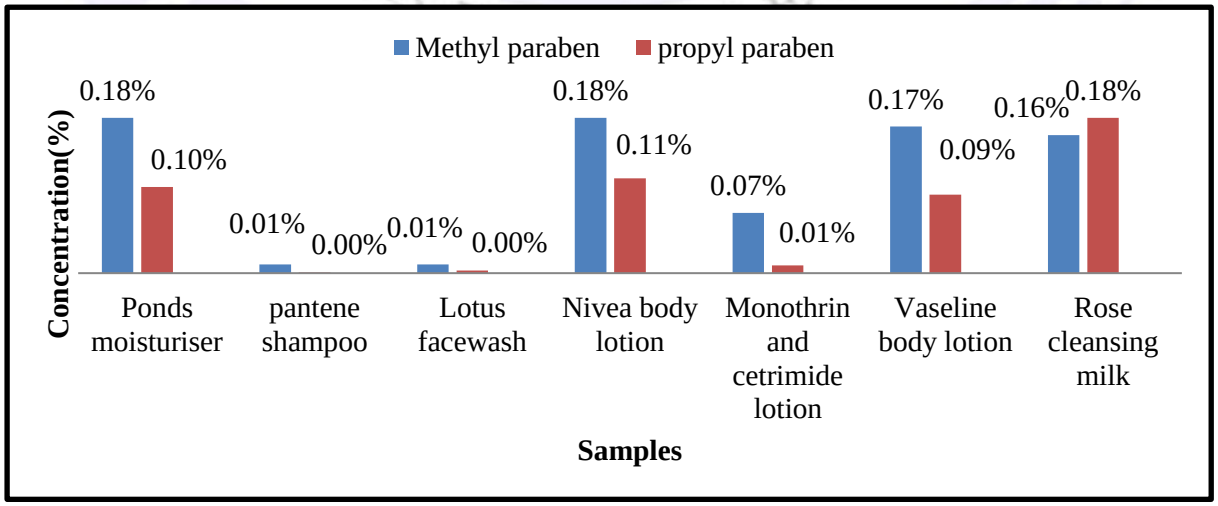

Figure 11: Percentage of methyl and propylparaben in different cosmetics

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In Nivea body lotion, the concentration of methylparaben was 0.18%, which exceeds the concentration of propylparaben by approximately 0.07%. Vaseline body lotion and Rose cleansing milk exhibited methyl paraben concentrations of 0.17% and 0.16%, respectively, both higher than corresponding propylparaben level. Conversely, Lotus facewash and Cetrimide lotion contained less than 0.1% of both preservatives. The concentration of methylparaben remained unchanged in both Pond's moisturizer and Nivea body lotion. Methylparaben was not detected in Pantene shampoo.

Propylparaben was present in Nivea body lotion and Rose cleansing milk at concentrations exceeding 0.1%. The concentration of propyl paraben were consistently lower, recorded at 0.11% in Nivea body lotion and 0.18% in Rose cleansing milk. Propylparaben was also detected in Pantene shampoo, Lotus facewash, Cetrimide lotion, and Vaseline body lotion at concentrations of 0.001%, 0.0032%, 0.009%, and 0.091%, respectively. Pond's moisturizer containing 0.10%.

In terms of overall preservative content, Nivea, Pond's, and Vaseline body lotions each contained more than 0.2% preservatives. Among the cosmetic products analyzed, Rose cleansing milk has the highest total preservative concentration at 0.34% among the cosmetics analyzed, while Lotus facewash and Pantene shampoo had the lowest concentration at 0.013%.

Similar study conducted by Zhang et al., (2005) on six different types of food samples including orange juice, pickle, soy sauce, vinegar and cola soda. Among all the tested samples, only soy sauce was found to contain 2.7 µg/g of methyl paraben and 2.5 µg/g of ethyl paraben, which are far below the 0.1% threshold. Likewise, Jung et al., (2015) studied the quantification of preservative in cosmetics for children, and found that 8 out of 10 samples contained methyl paraben in concentrarions ranging from 0.04 and 0.21% and propyl paraben from 0.02 to 0.09%. Muawana et al., (2017) found that 8 out of 10 body scrub samples tested positive for methyl paraben, with an average concentration of 0.05%, and 7 samples tested positive for propyl paraben, with an average concentration of 0.03%. The average total paraben content in the body scrub samples was 0.08%. Compared to the present study, all tested cosmetic samples were found to contain between 0.01% and 0.18% methyl paraben and 0.001% to 0.12% propyl paraben. The results of this study suggest that in all analyzed samples, the level of methyl and propyl paraben remained below 0.4 %, the upper limit imposed by European regulations.

Conclusion

The results of this study clearly demonstrate that parabens are commonly found in cosmetics, with nearly 99%. When compared to the European Community (EEC) directive, the study concludes that the levels of parabens in these products are below the permissible levels, ensuring their safety for daily use. However, it is essential that preservative tests be conducted regularly throughout the year to maintain the safe use of cosmetics, as contamination can occur frequently. To effectively reduce or eliminate cosmetic contamination, a reliable and routine monitoring system must be implemented.

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