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Quantitative Estimation of Preservative Content in Herbal Skin Cream: Paraben and Niolone 950

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ABSTRACT

A new method for the determination of paraben by High-Performance Liquid Chromatography (HPLC) and determination of Neolone 950 by High-Performance Thin Layer Chromatography (HPTLC) was developed. Skin creams usually consist of many components, including preservatives. Consumer health and safety is the main reason for including preservatives as antimicrobial additives in skin cream formulations. Preservatives are often used as a multi-component mixture to increase the range of microorganisms against which the product is protected. Ingredients in cosmetic products are labelled in accordance with (European Union) EU legislation. Here we established a quantitative method to estimate the concentration of preservative in Herbal skin cream. Their methods were based on High-Performance Liquid Chromatography (HPLC) analysis and performed under different chromatographic conditions. The proposed method was successfully applied to the assay of methylparaben, propyl paraben and neolone 950 in cosmetic products with minimal sample preparation.

INTRODUCTION

The concept of beauty and cosmetics is as ancient as mankind and civilization. Indian herbs and its significance are popular worldwide. Cream is a semi-solid emulsion, which is a mixture of oil and water in presence of a suitable emulsifying agent [1]. Skin creams usually consist of many components, including preservatives. Consumer health and safety is the main reason for including preservatives as antimicrobial additives in skin cream formulations. Preservatives are often used as a multi-component mixture to increase the range of microorganisms against which the product is protected. However, preservatives are also important causes of allergic contact dermatitis [2]. Skin-care products are used daily by many people, correct and complete ingredient labelling is important, and the use of preservatives in skin products is strictly regulated. Ingredients in cosmetic products are labelled in accordance with EU legislation. Correct labelling means that people with sensitivities can be aware of any preservatives in a product formulation that could trigger an allergic reaction. Several studies of skin cream preservative content regarding correct ingredient labelling as well as maximum allowed concentration have been performed earlier. Many thousands of cosmetic, food and pharmaceutical products would deteriorate or lose their perfume, flavor and/or quality in a short time without any preservative.

Paraben As Preservative

To conserve the quality of food, pharmaceuticals and cosmetics for a relatively long time, different preservatives are added to them. Parabens (4-hydroxybenzoic acid esters), as oestrogenic chemicals, can protect against micro-organisms like bacteria and moulds **(Table 1)**.

Due to their broad antimicrobial spectra with relatively low toxicity, good stability and non-volatility, parabens are commonly used as preservatives to prevent alteration and degradation of cosmetics, pharmaceuticals and foods from microbial and fungal contamination and to protect the consumers [3].

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Table 1. Target Preservatives in the present study.

Preservative	Maximum allowed concentration % (m/m)
Parabens (esters of 4-hydroxy benzoic acid) **Methyl paraben** **Ethyl paraben** **Propyl paraben** **Isobutyl paraben** **Butyl paraben** **Benzyl paraben**	0.4% as 4-hydroxy benzoic acid for a paraben, 0.8% as 4-hydroxy benzoic acid for a mixture of parabens

Neolone 950 As Preservative

Neolone bactericides. These broad-spectrum bactericides are particularly suitable as replacements for formaldehyde donors in leave-on applications such as skin care formulations and sun care formulations, Zinc pyrithione-based antidandruff shampoos.

Neolone 950 preservative is effective at very low use levels, is compatible with existing fungicides like parabens and is more stable in difficult to preserve high pH formulations. And Neolone 950 is supported by extensive toxicology studies and global regulatory dossiers [4].

The active ingredient of Neolone preservative is an isothiazolinone identified by the Chemical Abstract and IUPAC system of nomenclatures as 2-Methyl-4-isothiazolin-3-one and 2-Methyl-3(2H) isothiazolinone. The recommended use level for Neolone 950 is 0.05%-0.1% (48-95 ppm of active ingredient) of Neolone [5,6] **(Table 2)**.

EXPERIMENTAL SECTION

Table 2. Formulation design for o/w cream 500 g.

Ingredients	Amount (g)	Ingredients	Amount (g)
Alovera powder ext	0.005	Rose Water	15
Herbal distillate	50	Neolone 950	0.5
Methyl paraben	1.03	Potassium hydroxide	3.5
Propyl paraben	0.51	DM water	350
Propylene glycol	2.5	Zinc Oxide (Lab)	1.5
Nicinamide	7.5	Tapioca Starch	20

Materials Use for Analysis

Acetic acid (HPLC grade) (MERCK)

Acetone (MERCK)

Methanol (MERCK)

Demineralized Water (Prepared in the laboratory)

Chloroform (MERCK)

Methyl Paraben (MERCK)

Propyl Paraben (MERCK)

Absolute Ethanol (MERCK)

Ethanol 95% (MERCK)

Acetonitrile (MERCK)

Neolone 950 (MERCK)

Instrument Used for Analysis

Camag HPTLC system with camag TLC scanner 4 with (i) TLC aluminium sheet Silica gel 60 F 254 plate (10/10 cm) and (ii) CamagLinomet 5 applicator (syringe size 100 µl).

Ultra-violate spectroscopy (Shimadzu model 1800).

High performance liquid chromatography with (i) Syringe filter (nylon, PVDF, 0.45 m porosity) (ii) HPLC detector diode array (190-950 nm) and (iii) Reversed phase analytical column (Nucleosil 5C18 or equivalent, stainless steel, 25 cm x 4.6 mm) with 20 L loop Micro syringe.

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Software Used

LC Solution (Shimadzu high performance liquid chromatography).

Win cats 'planar chromatography manager (software system).

UV probe 2.33.

Microsoft excels 2007.

Estimation Methyl Paraben, Propyl Paraben, in The O/W Skin Cream by HPLC

This method specifies a procedure for the determination of methyl 4-hydroxy benzoate (methyl paraben), propyl 4-hydroxybenzoate (Propyl Paraben), in cosmetic products.

The amounts of the preservatives determined by this method are expressed as percentage by mass.

Procedure for Preparing Standard

Stock solution contains the preservatives in ethanol/water 9:1 V/V. Weight 0.05 g of methyl Paraben/propyl Paraben and made the volume up to 100 by water. Transfer 0.5, 1, 2, 5, 10 and 20 ml stock solution (0.5 µg/µl) respectively into 50 ml volumetric flasks. To each flask and fill up to the line with ethanol-water (9:1 V/V) mixture. (Use a solvent mixture to fill up each flask made e.g. as follows: 270 ml absolute ethanol + 30 ml water.) Prepare these solutions fresh for every experiment [7,8].

Procedure for Sample Preparation

Weigh 1.0 g of cream into a 100 ml volumetric flask (20 ug/µl). Add 1.0 ml 2 mol sulphuric acid solution. Add 50.0 ml ethanol-water mixture (9:1 V/V; use a solvent mixture made in the following way: 90 ml absolute ethanol + 10 ml water). Shake the closed tube for at least one minute, to suspend the sample in the solvent mixture. Put the tube in a water bath kept at 60 ± 1°C. Leave for five minutes to facilitate the extraction of the parabens into the liquid phase. Immediately cool the tube in a stream of cold water and store the extract in the refrigerator for one hour. Filter the extract using a filter paper with 0.45 µm porosity. Transfer approximately 2 ml of the filtrate into a 5 ml volumetric flask. Store the extracts in the refrigerator and perform the HPLC determination within 24 hours [9].

Procedure for HPLC Separation

For HPLC separation, mix and filter the mobile phase (water-acetonitrile (50:50)). Adjust the flow rate of the mobile phase (1.5 ml min^{-1}) and the UV detector (290 nm).

The injected volume should be 20 µl of each solution (standard and sample). In all cases, record the chromatogram and measure the peak areas and peak heights. Calculate the amount of preservative present [10,11].

Estimation of Neolone 950 (2-Methyl-4-Isothiazolin-3-One (Mi)

This method is used for the determination of neolone 950 (2-methyl-4-isothiazolin-3-one (Mi) in cream. Estimation process used HPTLC (High Performance Thin Layer Chromatography). Chloroform: methanol (90:10) taken as Mobile phase and Stationary phase used was TLC Aluminium sheet Silica gel 60 F 254 plate (10/10 cm).

Preparation of Standard Curve

0.1 g of neolone 950 standard was weight accurately in a 100 ml volumetric flask (1 µg/µl) dissolved it by methanol: 0.4% Acetic acid (20:80) and make up to the mark with same solvent mixture (Stock solution is stable for 1 week, when stored at 4°C). Filter the solution by 0.45 µl, 1 µl, 2 µl, 3 µl, 4 µl above solution was injected (contain 1 µg, 2 µg, 3 µg, 4 µg) on TLC Aluminium sheet Silica gel 60 F 254 plate (10/10 cm) by using Camag Linomet 5 applicator (syringe size 100 µl).

After drying the TLC plate put into the mobile phase and run time is 10 min. Retention factor, peak height, peak area was measured by scanning the plate using camag TLC scanner 4 at 275 nm [12,13] **(Table 3)**.

Estimation of Neolone 950 in Sample

Weigh accurately 2.00 g sample in a 25 ml volumetric flask and add 20 ml methanol/0.4% acetic acid (20:80). Treat the sample by ultrasound for 10 min. Fill the measuring flask up to the mark and shake well. Filter the sample using a membrane filter (4.5). 10 ml above solution and condensed it 5 times, (2 ml). Apply the above solution in on TLC Aluminium sheet Silica gel 60 F 254 plate (10/10 cm) by using Camag Linomet 5 applicator (syringe size 100 µl). Concentration of sample is 400 ug/µl. After drying the TLC plate put into the mobile phase and run time is 10 min. Qualitative and quantitative estimation is done by observing Retention factor, peak height, pick area measured by scanning the plate using camag TLC scanner 4 at 275 nm.

RESULTS AND DISCUSSIONS

Estimation of Methyl Paraben in O/W Skin Cream

Methyl paraben in different concentration are scanned by HPLC detector diode array (190-950 nm) in reversed phase

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analytical column. λ_{max} is of methyl paraben was determined in 190 nm. The standard retention time of methyl paraben is 4.8 shown in **Figure 1**.

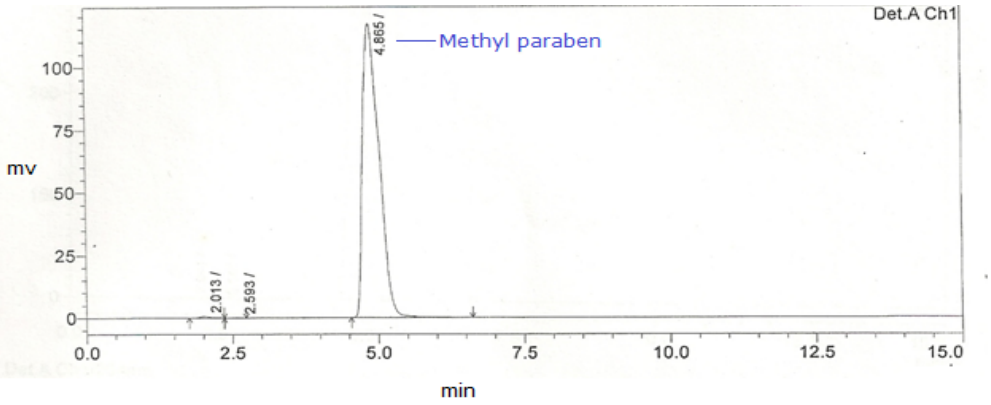

Figure 1. HPLC chromatogram of Standard Methyl Paraben.

Table 3. Standard curve for Methyl Paraben.

(According to Area)			
Track	**Retention time**	**Amount (µg)**	**Area**
1	4.884	0.20	1181329
2	4.865	0.4	2295050
3	4.850	1	5617441
4	4.839	2	11072487
5	4.828	4	22750992

Methylparaben is eluting out in this time. In different concentration methyl paraben shown in **Table 4** having same identical retention time. To determine the amount of an ingredient, present in the formulation at different concentration standard curve were calibrated shown in **Figures 2-5**.

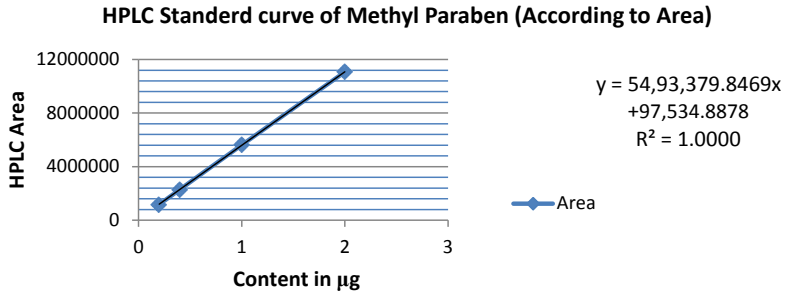

Figure 2. HPLC Standard curve of Methyl Paraben (According to Area). Regression *via* Area y = 54,93,379.8469 x + 97,534.8878.

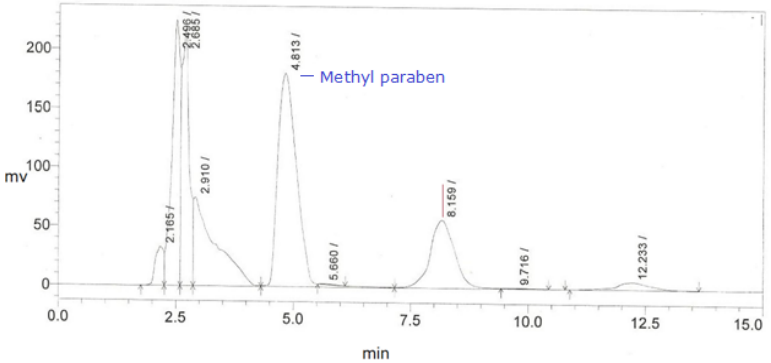

Figure 3. HPLC chromatogram of sample from batch 1. It shows the HPLC spectrum of sample (o/w skin cream). Where the identifying peak for methyl Paraben is come on retention time of 4.813.

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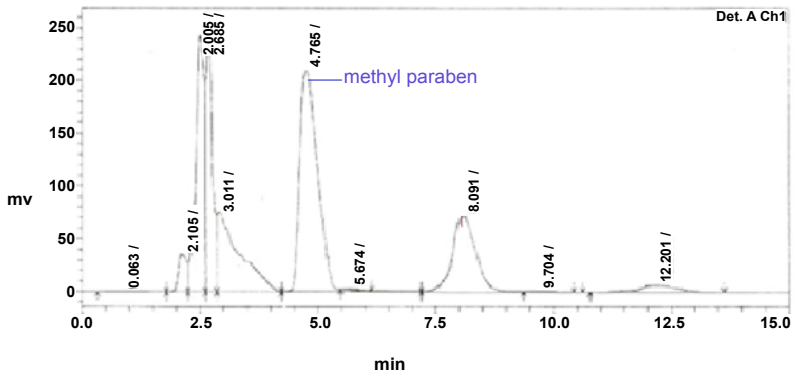

Figure 4. HPLC chromatogram of sample from batch 2. It shows the HPLC spectrum of sample (o/w skin cream). Where the identifying peak for methyl Paraben is come on retention time of 4.768.

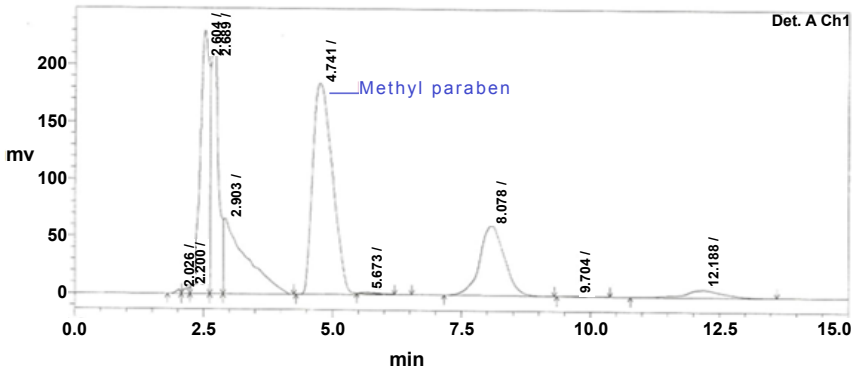

Figure 5. HPLC chromatogram of sample from batch 3. It shows the HPLC spectrum of sample (o/w skin cream) where the identifying peak for methyl Paraben is come on retention time of 4.741.

Table 4. Estimation of percentage of Methyl Paraben in sample (According to area). The percentage of methyl paraben present in 3 different batch are 0.225, 0.247 and 0.220, when calculated according to area.

Track	Batch	volume applied (µl)	Amount applied (µg)	Area	Ret time	Amount of Methyl Paraben (µg)	% of Methyl Paraben
1	1	20	400	5053259	4.813	0.9021	0.225
2	2	20	400	5533220	4.761	0.9894	0.247
3	3	20	400	4940002	4.741	0.8815	0.220

According to formulation 0.2% of Methyl Paraben is given in this o/w base cream. We analysed the percentage of Methyl Paraben in 3 batch of the cream. And the calculated amount of Methyl Paraben from HPLC analysis is shown in **Table 5**.

Table 5. Percentage of Methyl Paraben in Different Batch.

Batch No.	% of Methyl Paraben (according to Area)
1	0.225
2	0.247
3	0.220

The standard deviation between three batches is 0.0079 and average % of methyl paraben in three batches is 0.225, 0.247 and 0.220. From the above experiment percentage of Methyl Paraben in 3 batches is come in between 0.20-0.24.

Quantitative Estimation of Propyl Paraben in O/W Skin Cream (by HPLC)

Propyl paraben in different concentration are scanned by HPLC detector diode array (190-950 nm) in reversed phase analytical column. λ_{max} is of propyl paraben was determined in 190 nm. The standard retention time of propyl paraben is 8.6 shown in the **Figure 6** and **Table 6**. Propyl paraben is eluting out in this time. In different concentration propyl paraben shown same retention time [13,14].

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To determine the amount of active ingredient, present in the formulation at different concentration standard curve were calibrated by HPLC shown in the **Figures 7-16**.

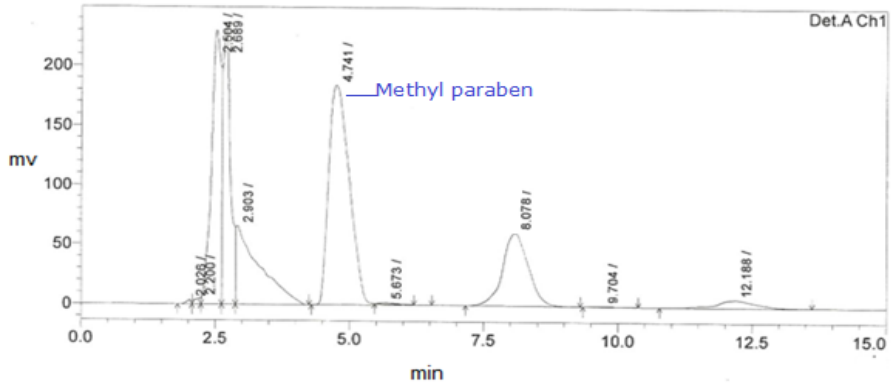

Figure 6. HPLC chromatogram for standard propyl paraben.

Table 6. Standard curve for Propyl paraben.

(According to Area).			
Track	**Retention time**	**Amount (µg)**	**Area**
1	8.657	0.1	538446
2	8.649	0.2	1086943
3	8.643	0.4	2160759
4	8.625	1	5394568
5	8.608	2	10535781
6	8.594	4	21183637

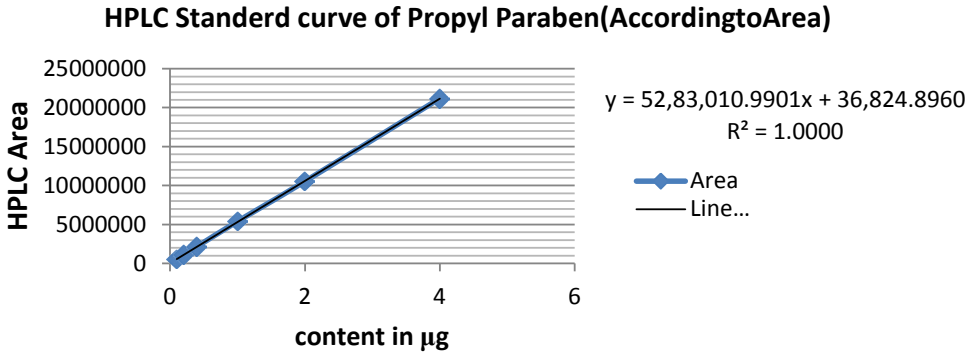

Figure 7. HPLC Standard curve of Propyl Paraben (According to Area). Regression *via* Area y = 52,83,010.9901x + 36,824.8960.

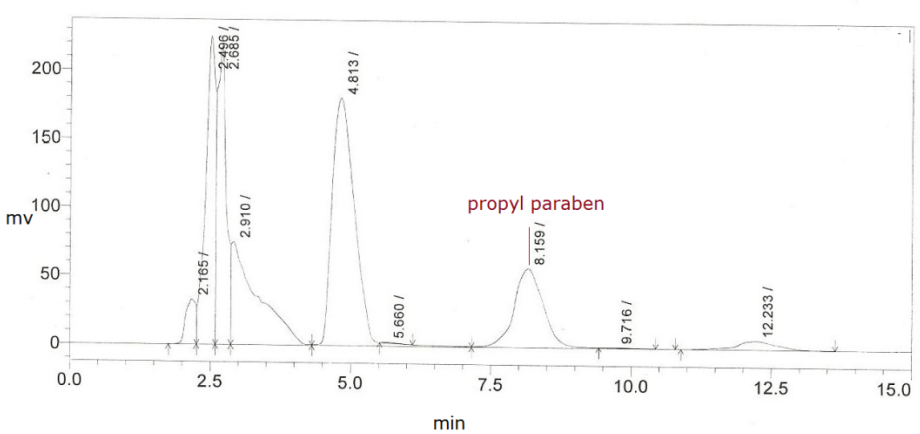

Figure 8. HPLC chromatogram of sample from batch 1. It shows the HPLC spectrum of sample (o/w skin cream) where the identifying peak for propyl Paraben is come on retention time of 8.159.

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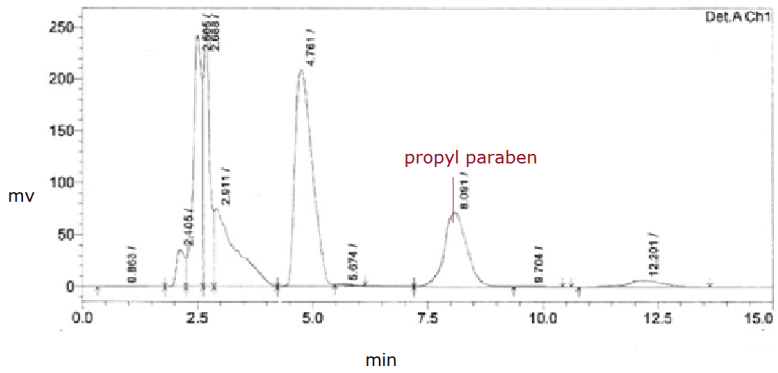

Figure 9. HPLC chromatogram of sample from batch 2. It shows the HPLC spectrum of sample (o/w skin cream) where the identifying peak for propyl Paraben is come on retention time of 8.091.

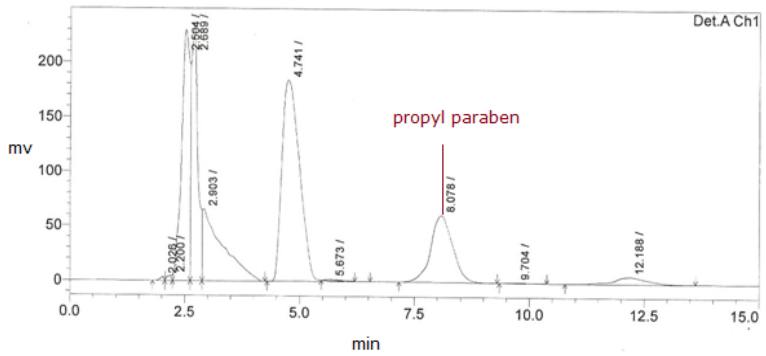

Figure 10. HPLC chromatogram of sample from batch 3. It shows the HPLC spectrum of sample (o/w skin cream) where the identifying peak for propyl Paraben is come on retention time of 8.078. Here the amount of propyl paraben in 3 different batch's sample are calculated by the regression equation of pure propyl paraben. Retention time of propyl paraben in sample is 8.1.

According to formulation 0.1% of Propyl paraben is given in this o/w base cream. We analysed the percentage of Propyl paraben in 3 batch of the cream. And the calculated amount of Propyl paraben from HPLC analysis are shown in **Tables 7 & 8**.

Table 7. Estimation of % of Propyl paraben in sample (According to area).

Track	Batch	volume applied(µl)	Amount applied (µg)	Area	Ret time	Amount of Propyl paraben (µg)	% of Propyl paraben
1	1	20	400	2086488	8.159	0.3942	0.098
3	2	20	400	2435782	8.091	0.4603	0.115
4	3	20	400	2001240	8.078	0.3781	0.094

Table 8. Percentage of Propyl paraben in different batch.

Batch	% of Propyl paraben from Area
1	0.098
2	0.115
3	0.094

Standard deviation between the 3 batches is 0.011. From the above experiment percentage of Propyl paraben in 3 batches is in between 0.097-0.011, and the standard curve for Neolone shown in **Table 9.**

Quantitative Estimation of Neolone 950 in O/W Skin Cream (by HPTLC)

Table 9. Standard curve for Neolone 950.

(According to Area)				(According to Height)			
Track	**Rf**	**Amount (µg)**	**Area**	**Track**	**Rf**	**Amount (µg)**	**Height**
1	0.73	1	2462.25	1	0.73	1	97.04
2	0.72	2	3459.33	2	0.72	2	134.98
3	0.72	3	5614.21	3	0.72	3	207.11
4	0.72	4	6467.49	4	0.72	4	235.27

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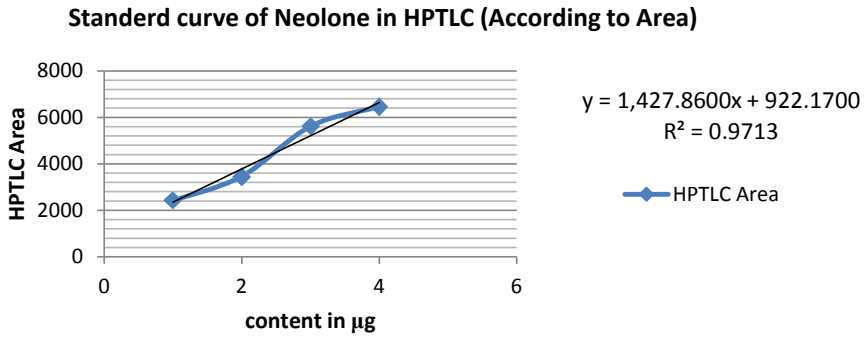

Figure 11. HPLC Standard curve of Neolone 950 (According to Area).

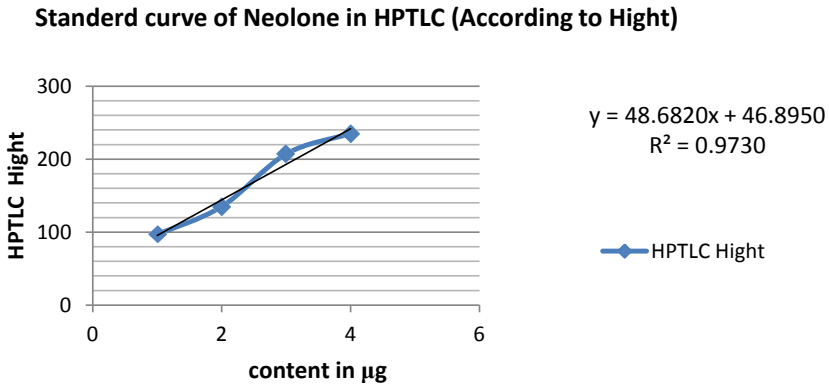

Figure 12. HPLC Standard curve of Neolone 950 (According to height). Regression *via* Area Y=958.2+1417*X Regression *via* Height =Y=958.2+48.68*X.

The retention factor (Rf) of sample and standard are estimated by spectrum scan, that is Rf of sample is 0.73 and Rf of standard is 0.72. It help to identify the presence of neolone 950.

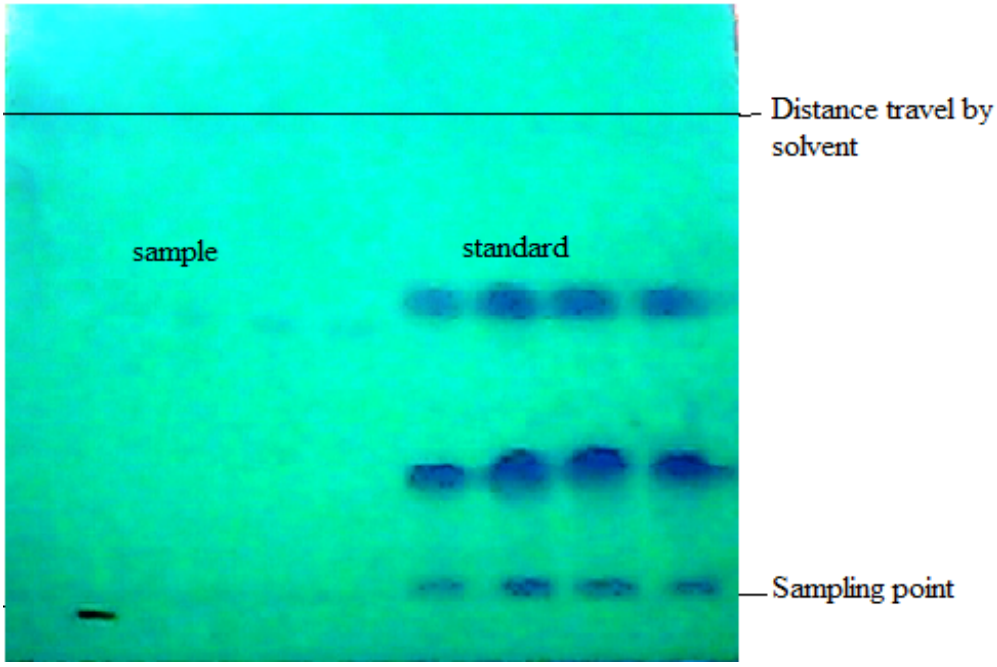

Figure 13. TLC plate of Neolone 950 (HPTLC). In this figure plate shown the standard and sample point for Neolone 950, With same Rf value. It helps to identify the presence of neolone 950 in sample.

Quantitative estimation of Neolone 950 in Sample

λ_{max} is of Neolone 950 was determined from UV Visible spectrophotometer by scanning through the entire range (190-1100). The λ_{max} was found to be 275 nm against the reagent blank. To determine the amount of an ingredient, present in the formulation at different concentration standard curve were calibrated. In this method area and height of the HPTLC spectrum curve was measured in different concentration [15].

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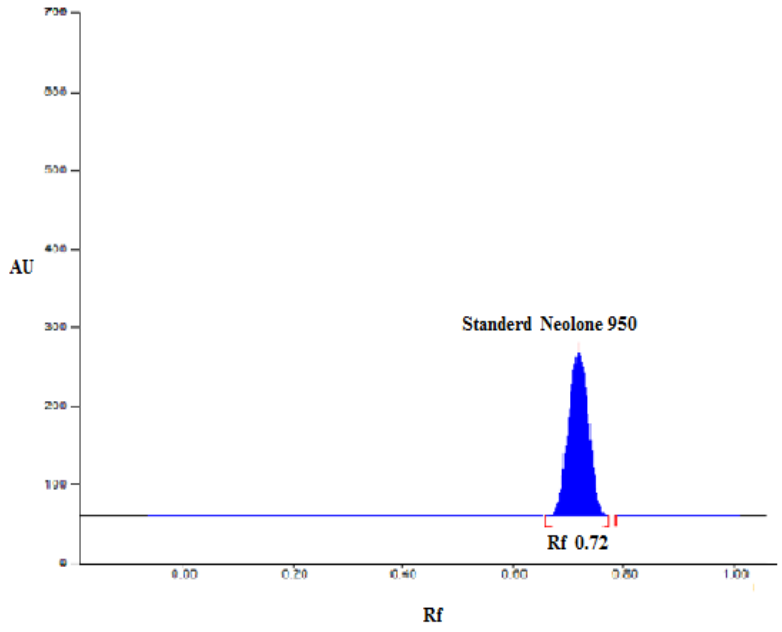

Figure 14. The chromatogram of standard Neolone 950.

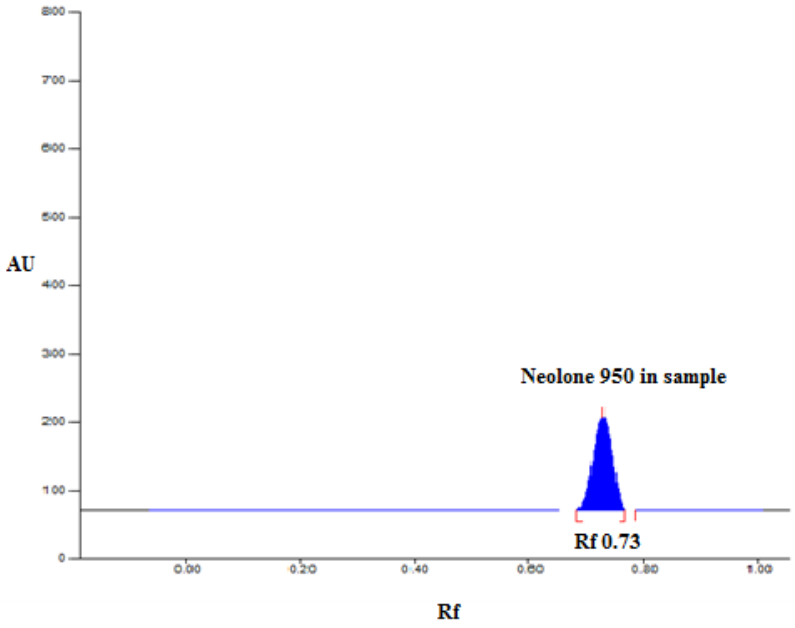

Figure 15. The chromatogram of Neolone 950 in sample.

The percentage of neolone in sample is measure by area and height of the HPTLC peak, which is estimated by spectrum scanning, was shown in **Table 10.**

Table 10. Estimation of % of Neolone 950 in sample (According to area). Average % of neolone in 1, 2 and 3 is respectively 0.11, 0.12 and 0.13, and their standard deviation are 0.014,0 and 0.014 according to area.

Batch	volume applied (µl)	Amount applied (µg)	Area	Rf	Amount of Neolone 950 (µg)	% Neolone 950	Std dev	Avg
1	3	1200	3379.05	0.72	1.708	0.12		
	5	2000	4560.19	0.72	2.542	0.10	0.0141	0.11
2	3	1200	3077.25	0.73	1.495	0.11		
	5	2000	4069.47	0.73	2.192	0.11	0	0.11
3	3	1200	2990.79	0.74	1.344	0.14		
	5	2000	4200.23	0.72	2.287	0.12	0.014	0.13

According to formulation 0.1% of Neolone 950 is given in this o/w base cream. We analysed the % of Neolone 950 in 3 batch of the cream.

And the calculated amount of Neolone 950 from HPTLC analysis is shown in **Tables 11 & 12.**

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Table 11. Estimation of % of Neolone 950 in sample (According to Height). Average % of neolone in 1, 2 and 3 is respectively 0.14, 0.13 and 0.13 and their standard deviation are 0.0141, 0.0070and 0. 012 according to height.

Track	Batch	volume applied (µl)	Amount applied (µg)	Height	Rf	Amount of Neolone 950 (µg)	% of Neolone 950	Std dev	Avg
1	29	3	1200	135.82	0.73	1.827	0.15		
2	29	5	2000	177.94	0.73	2.692	0.13	0.0141	0.14
3	30	3	1200	130.98	0.74	1.727	0.14		
4	30	5	2000	178.97	0.74	2.711	0.13	0.0070	0.13
5	31	3	1200	139.00	0.73	1.892	0.15		
6	31	5	2000	172.99	0.72	2.594	0.12	0.0212	0.13

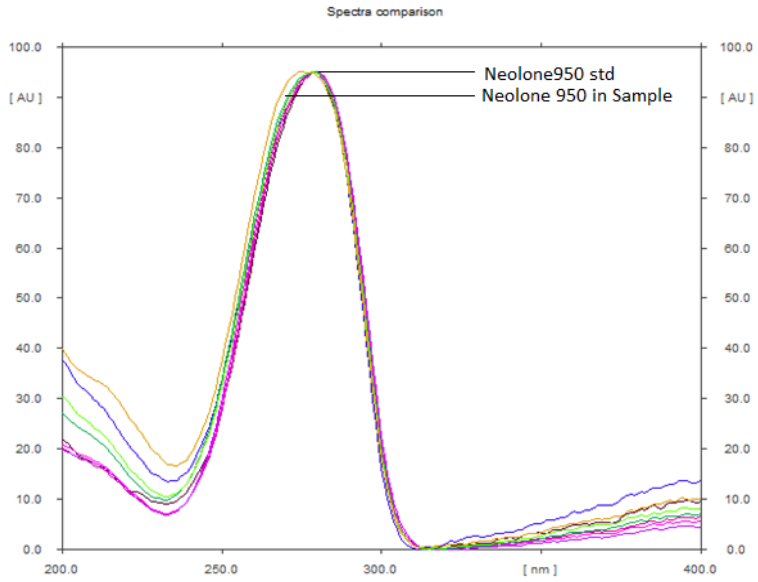

Figure 16. Spectrum scan of neolone 950. It shows the comparison between the standard and sample for spectrum matching of neolone 950 spectrum for sample and standard shows the super imposable image.

Table 12. Percentage of Neolone 950 in Each Batch. The average percentage of Neolone 950 in 3 batch are 0.12, 0.12 and 0.13. standard deviation within the batch are 0.012247, 0 and 0.01.standerd deviation between 3 batch is 0.005774 [16].

Batch	Percentage of Neolone 950 according to Area	Percentage of Neolone 950 according to Height	Std dev	Average	Std dev
1	0.11	0.14	0.012247	0.12	
2	0.11	0.13	0.01	0.12	
3	0.13	0.13	0	0.13	0.005774

CONCLUSIONS

In conclusion, the proposed method allows a rapid and sound quantification of methylparaben, propayl paraben and neolone 950 in cosmetic samples, being based on a simple and rapid sample preparation procedure and a very fast and reliable chromatographic separation. The method can be used to monitor the occurrence at trace level of this preservative in cosmetic.

CONFLICT OF INTEREST

The author does not have a commercial or other association that might pose a conflict of interest with any organization.

ACKNOWLEDGMENTS

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