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# DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METHYLPARABEN AND PROPYLPARABEN IN HERBAL CREAM FORMULATION

**Unmesh Keshwar*, Pranali Ingole, Anjali Charde, Sharad Dhurde and B. K. Shrikhande**

Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur, 441204, Maharashtra, India.

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***Corresponding Author**
**Unmesh Keshwar**
Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur, 441204, Maharashtra, India.

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## ABSTRACT

Preservatives play a crucial role in maintaining the microbiological stability and safety of cosmetic and herbal formulations. Methylparaben (MP) and propylparaben (PP) are widely used preservatives; however, their accurate and simultaneous determination in complex herbal matrices remains analytically challenging. The present study describes the development and validation of a simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of methylparaben and propylparaben in an herbal cream formulation. Chromatographic separation was achieved on a C18 column (250 × 4.6 mm, 5 µm) using a mobile phase consisting of potassium dihydrogen phosphate buffer, methanol, and triethylamine in the ratio of 347:650:3 (v/v/v), at a flow rate of 1.0 ml/min with UV detection at 272 nm. The retention times for methylparaben and propylparaben were found to be approximately 3.95 and 6.28 minutes, respectively, with good resolution and peak symmetry. The developed method was validated in accordance with ICH Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision, range, and robustness. The method showed excellent linearity with correlation coefficients greater than 0.999 for both analytes. Accuracy studies demonstrated recoveries in the range of 98–102%, while precision studies yielded %RSD values below 2%. The method was found to be robust against small deliberate variations in chromatographic conditions. The validated

RP-HPLC method is suitable for routine quality control analysis of methylparaben and propylparaben in herbal cream formulations.

**KEYWORDS:** Methylparaben, Propylparaben, herbal cream formulation, RP-HPLC.

## INTRODUCTION

Preservatives are indispensable components of cosmetic and personal-care formulations, safeguarding products from microbial contamination and ensuring stability throughout their intended shelf life. Among these, parabens particularly methylparaben (MP) and propylparaben (PP**)** remain the most widely used due to their broad-spectrum antimicrobial activity, chemical stability, and cost-effectiveness. Their inclusion is especially common in water-based formulations such as creams, lotions, shampoos, and other emulsions, where the risk of microbial growth is inherently high. Regulatory authorities, including the U.S. Food and Drug Administration (FDA), recognize methylparaben and propylparaben as safe within established permissible limits, restricting their concentrations to 0.4% for individual parabens and 0.8% for combinations to balance preservative efficacy and consumer safety.[1-2]

Methylparaben is the methyl ester of p-hydroxybenzoic acid and occurs naturally in some fruits. It functions as an antimicrobial and antifungal agent and is also used as a food and pharmaceutical preservative. Propylparaben, the propyl ester of p-hydroxybenzoic acid, demonstrates similar preservative properties and is frequently utilized in both cosmetics and foods.[3-4] Their extensive use, however, has prompted increasing scrutiny regarding potential health implications, particularly at higher concentrations or with prolonged exposure. The World Health Organization (WHO) has also emphasized the importance of monitoring the stability of preservatives throughout the shelf-life of herbal formulations.[5-9]

Consequently, accurate determination of MP and PP in cosmetic formulations especially in herbal creams, where complex plant matrices may interfere with analytical performance has become an important quality and safety requirement. Although several analytical procedures have been reported for the estimation of parabens in different cosmetic matrices, simultaneous determination of MP and PP in multicomponent herbal formulations often faces challenges related to chromatographic resolution, peak interference, and matrix complexity. High-Performance Liquid Chromatography (HPLC) remains the preferred analytical platform due to its sensitivity, precision, and suitability for routine quality control; however, method adaptability for herbal creams is limited in existing literature.[10-16]

In response to these analytical gaps, the present work reports the development and validation of a robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of methylparaben and propylparaben in an herbal cream formulation. The method has been validated following the International Council for Harmonization (ICH) Q2(R1) guidelines to ensure its reliability for routine quality control applications.[17]

## MATERIALS AND METHODS

### Instrumentation

Instrumentation details are mentioned in below Table 1.

**Table 1: Instrumentation details.**

| Sr. No. | Instruments | Specification |
|---|---|---|
| 1 | HPLC instrument | Waters Arc HPLC |
| 2 | Detector | Photo diode Array Detector (2998 PDA) |
| 3 | wavelength range | 210 nm to 800 nm |
| 4 | Software | Empower |
| 5 | Balance | Mettler Toledo |
| 6 | pH meter | Toshnival |
| 7 | Sonicator | Tempo |

### Reagents and Materials

All chemicals used throughout this work were of analytical grade or HPLC grade purchased from Merck Chemicals, India. Water used for the analytical work is of Type 1 purified water. Reference standard Methylparaben and Propylparaben were purchased from Sigma Aldrich, India.

### Preparation of Standard-stock Solution for Methylparaben

The standard solution prepared by weighing 5.0 mg Methylparaben (MP) reference standard and then transferred to 10 ml volumetric flask and volume was adjusted with diluent (Methanol) to obtain concentration 500 µg/ml (500 ppm).

### Preparation of Standard-stock Solution for Propylparaben

The standard solution prepared by weighing 7.5 mg of Propylparaben (PP) reference standard and then transferred to 10 ml volumetric flask and volume was adjusted with diluent (Methanol) to obtain concentration 750 µg/ml (750 ppm).

**Preparation of Working-standard Solution**

From the standard stock solution 2 ml solution Methylparaben and 1 ml Propylparaben was pipette out and diluted with diluent (Methanol) up to 50 ml in volumetric flask to obtain concentration of 15 µg/ml (15 ppm) for PP and 20 µg/ml (20 ppm) for MP. This solution was used as working standard solution.

**Preparation of test solution (Herbal Cream Formulation)**

Accurately 1000 mg of cream sample was weighed in 100 ml of volumetric flask. About 80 ml of diluent (Methanol) was added and solution was sonicated on ultrasonic water bath for 15 min and warm on water bath for 10 min. The solution was allowed to cool at room temperature. Further, volume was made up to the mark with diluent (Methanol). The solution was filtered through 0.45 µ syringe filter and used as test solution.

**Preparation of Buffer Solution**

Buffer Solution was prepared by dissolving 6.8 g of Potassium dihydrogen phosphate in water (1000 ml).

**Mobile Phase Preparation**

Mobile phase was prepared by using Buffer solution, Methanol and Triethylamine in the ratio 347:650:3 v/v/v.

**Method Development**

Various mobile phases were tried to obtain the simultaneous elution of both the components PP and MP with good resolution and sharp peak. After various trials, the mobile phase containing Buffer solution, Methanol and Triethylamine in the ratio 347:650:3 v/v/v meets the criteria and finalised for the analysis. Quantification was carried out using a photodiode array detector at 272 nm.

**Chromatographic Conditions**

The details of optimized parameters of chromatographic conditions for estimation of PP and MP are mentioned in Table 2.

**Table 2: Optimized chromatographic conditions.**

| Sr. No. | Parameter | Optimized conditions |
|---|---|---|
| 1 | Mobile Phase | Buffer Solution, Methanol and Triethylamine |
| 2 | Ratio | (347:650:3 v/v/v) |
| 3 | Column | C18 (5 µm 4.6 x 250 mm) |
| 4 | Flow rate | 1.0 ml/min |
| 5 | Wavelength | 272 nm |
| 6 | Injection Volume | 20 µl |
| 7 | Column Temperature | 25°C |
| 8 | Sample Temperature | 25°C |

**Assay of Methylparaben and Propylparaben in Herbal Cream Formulation**

Herbal Cream Formulation was analysed for the assay of MP and PP as per the developed and optimised method. Sample and standard solution were injected under the same conditions. Sample solution was analysed in triplicate.

**Analytical Method Validation**

The developed RP-HPLC method was validated for the parameters like system suitability specificity, linearity, accuracy, range, precision, and robustness as per the International Conference on Harmonization (ICH) guidelines Q2 (R1). The details of the analytical method validation are as follows.

**System suitability**

System suitability was performed on standard MP and PP having concentration 5 ppm and 10 ppm respectively. Retention time, resolution, peak shape, theoretical plates, tailing factor and % RSD were ascertained for the suitability of the instrument for getting accurate and precise results.

**Specificity**

The Specificity was proved by chromatographic comparison of blank, Placebo solution, standard solutions and the test solution. No interference in the peak observed from blank and placebo solution at the Retention time of Reference standard solutions confirms the specificity.

**Linearity**

Linearity for MP and PP standard solution was performed by evaluating minimum five concentrations. The slope, intercept and correlation coefficient should be reported by plotting the linearity graph of peak area against concentration of standards.

**Accuracy**

The accuracy was carried out by the standard addition technique by spiking the analyte into the matrix of the sample (placebo). Accuracy was assessed by minimum 9 determinations over a minimum of 3 concentration levels (3 Concentration / 3 replicate) covering the specified range by adding known amount of actives in placebo solution. These analysed samples and obtained results were compared with expected results.

**Range**

The range was derived from the linearity studies and accuracy. Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

**Precision**

Precision was evaluated in terms of system precision, method precision and intermediate precision. The system precision was determined by six measurements of mix standard solution of MP and PP containing each analyte at 100% of target concentration on the same day. The method precision includes repeatability and intermediate precision. They were determined by six measurements of sample solution containing each analyte at approximately 100 % of target concentration on the same day and on two different days, respectively. Overall RSD of 12 samples was calculated by taking precision and intermediate precision into consideration.

**Robustness**

The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. For assessment of the robustness of developed analytical method parameters like flow rate, detector wavelength and mobile phase composition were deliberately changed.

## RESULTS AND DISCUSSION

The RP-HPLC method was successfully developed for the simultaneous estimation of MP and PP having retention time 3.95 and 6.28 minutes respectively with good resolution and sharp peak.

**Assay Results**

Assay of MP and PP as per the developed and optimised method was performed in triplicate and results were incorporated in the Table 3.

**Table 3: Results of assay.**

| Sample ID | MP | | PP | |
|---|---|---|---|---|
| | Assay (%) | Mean | Assay (%) | Mean |
| Test Sample-1 | 0.2006 | 0.2007 | 0.1523 | 0.1536 |
| Test Sample-2 | 0.2017 | | 0.1583 | |
| Test Sample-3 | 0.1997 | | 0.1502 | |

**Validation Results**

This developed method was also validated as per the as per the International Conference on Harmonization (ICH) guidelines of which results are mentioned and discussed below.

**System suitability Assessment**

Standard Solution of MP and PP as per developed method were prepared and injected into HPLC system. Retention time, resolution, peak shape, theoretical plates, tailing factor and % RSD were evaluated and results were tabulated in the following Table 4.

**Table 4: Results of system suitability.**

| Sr. No. | Name of Analyte | Retention Time (RT) | Mean Peak Area | Theoretical Plates | Tailing Factor | % RSD | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | PP | 6.28 | 474992 | 2904 | 1.29 | 0.3 | 6.8 |
| 2 | MP | 3.95 | 733674 | 3199 | 1.28 | 0.2 | |

**Specificity Assessment**

Blank Solution, Placebo Solution, Standard Solution and Test Solution were injected and chromatograms were recorded. The Retention time was observed at 6.28 and 3.95 minutes for MP and PP respectively in Standard Solution. No peak interference was observed in blank and placebo at the retention time MP and PP. No peak purity flag was observed in Standard and Sample solution. The chromatograms are given below in "Fig. 1-4".

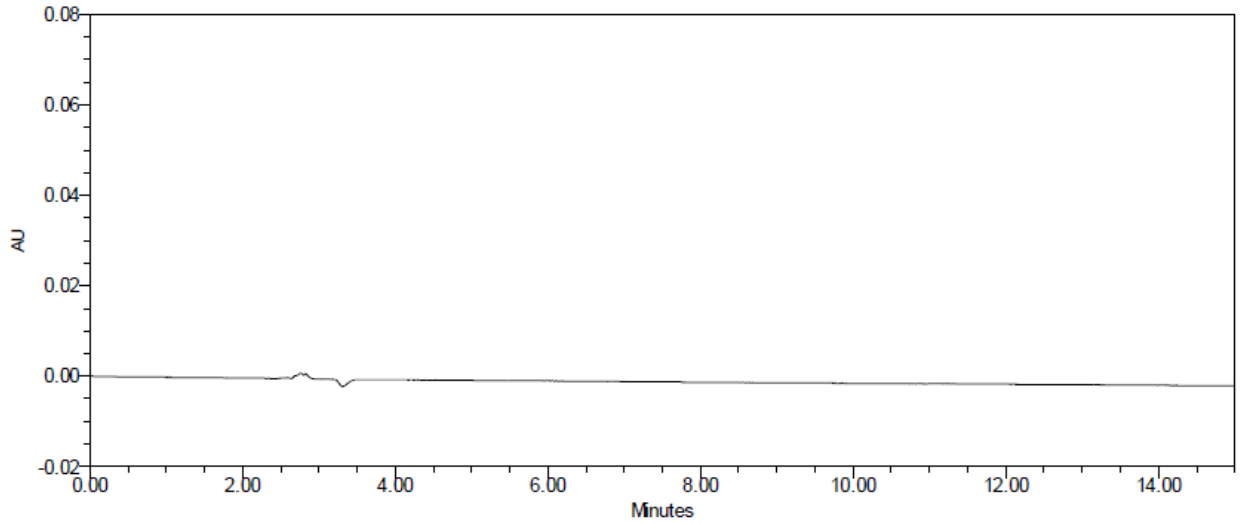


**Figure 1: Chromatogram of Blank Solution.**

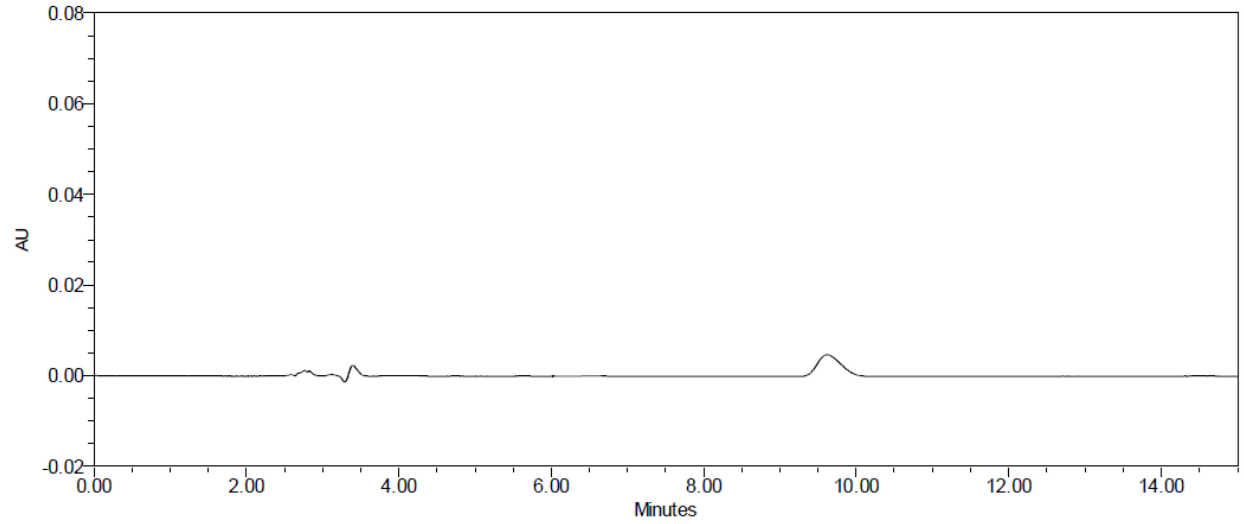


**Figure 2: Chromatogram of Placebo Solution.**

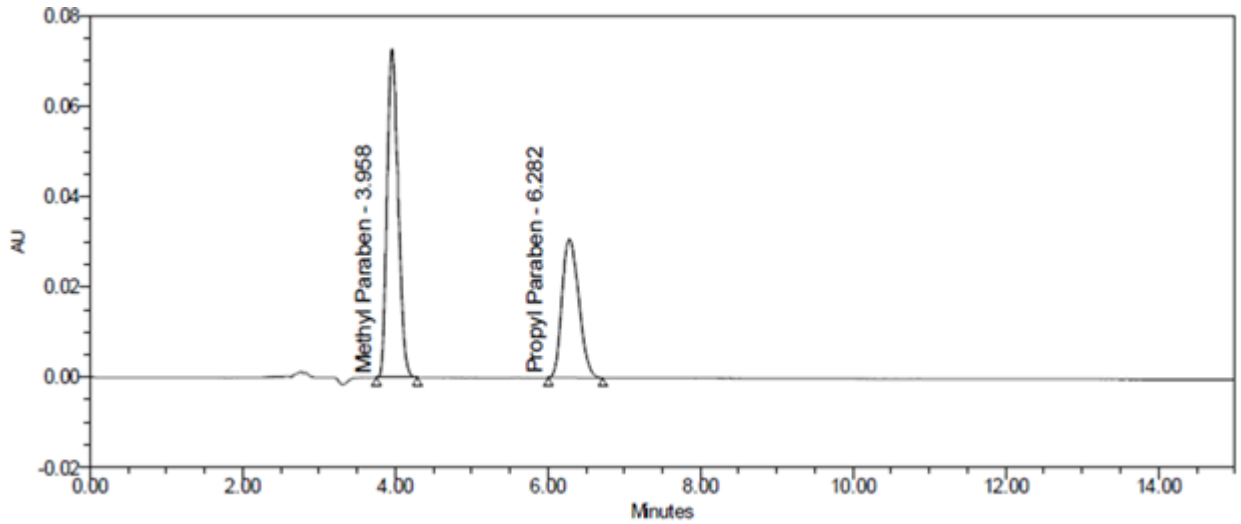


**Figure 3: Chromatogram of Standard Solution.**

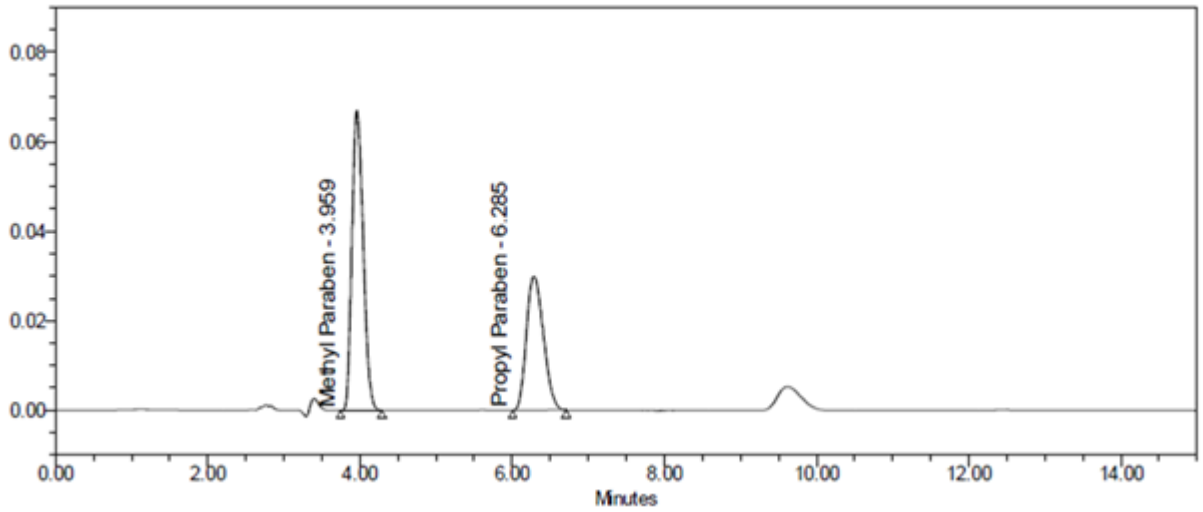


**Figure 4: Chromatogram of Sample solution.**

**Linearity Assessment**

The method gave linear response with concentrations of 10.05, 16.09, 20.1, 24.13 and 30.16 ppm for MP and 7.52, 12.04, 15.05, 18.06 and 22.57 ppm for PP. Linearity curve was obtained by plotting a graph of peak area vs. concentration. All data were calculated and given in Table 5 and "Fig. 5, 6".

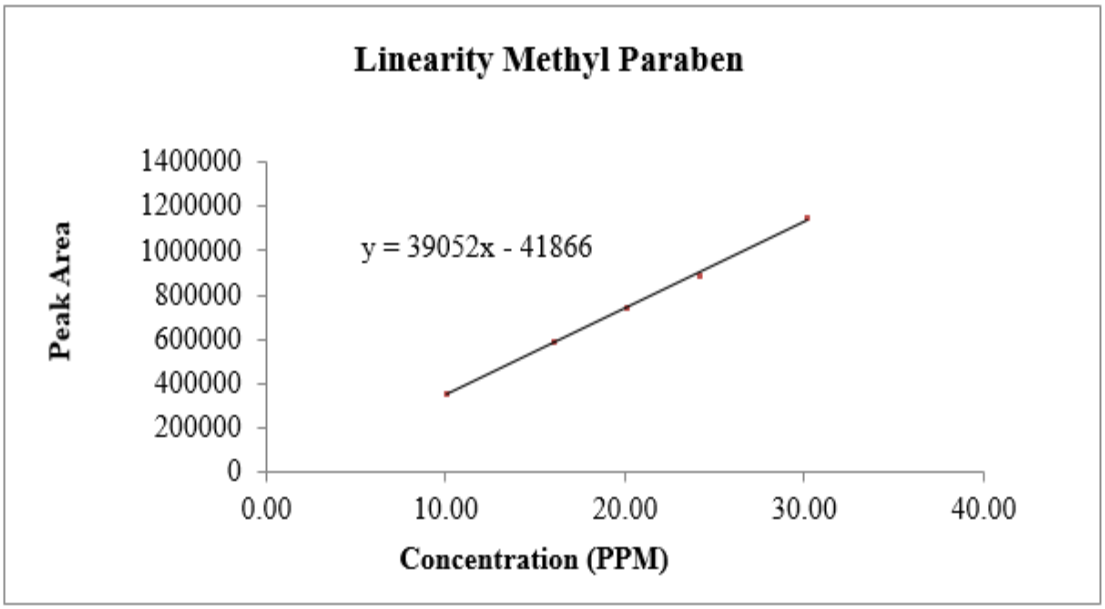


**Figure 5: Linearity Graph of Methylparaben.**

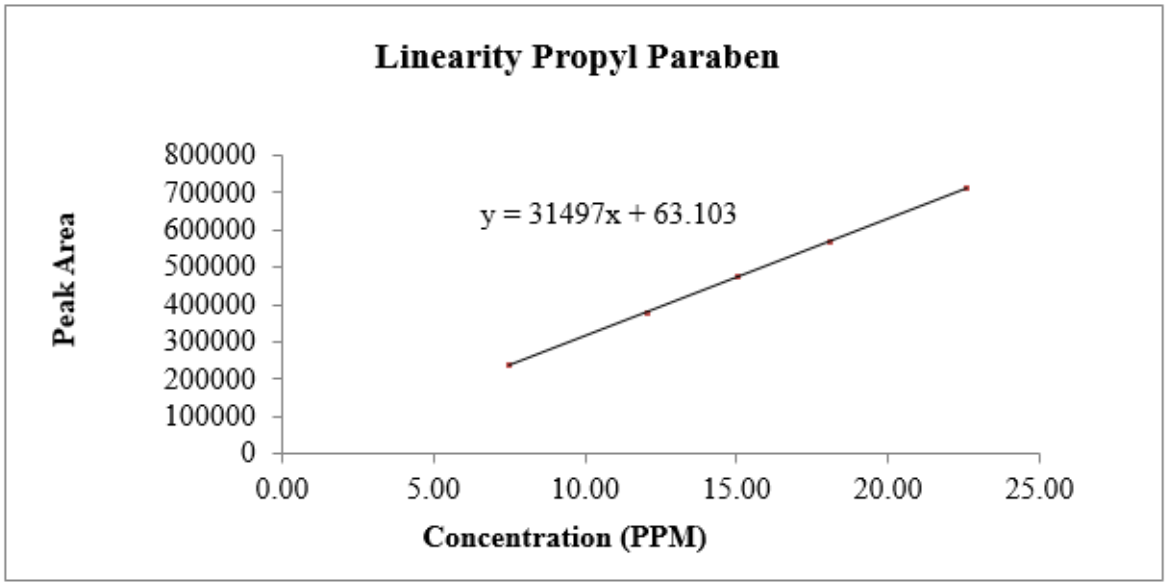


**Figure 6: Linearity Graph of Propylparaben.**

**Table 5: Results of linearity.**

| Sr. No. | Parameter | Results | |
|---|---|---|---|
| | | Methylparaben | Propylparaben |
| 1 | Linearity Range (µg/ml) | 10.05-30.16 | 7.52-22.57 |
| 2 | Correlation coefficient (R) | 0.999 | 1.000 |
| 3 | y-intercept | -41866.2 | 31497.3 |
| 4 | Slope | 39052.3 | 63.1 |

**Precision Assessment**

System precision was determined by performing the 6 replicates of standard MP and PP. The RSD of peak area was less than 2.0 % and performance of chromatographic system, represented by Retention time, number of theoretical plates and tailing factors were found well within the acceptable limits. The results are shown in Table 6.

In terms of method precision, the RSD of assay results for MP and PP in evaluation of repeatability, intermediate precision and overall RSD of 12 samples was less than 2.0 %, as shown in Table 7, 8. Therefore, the results showed that the method is precise.

**Table 6: Results of system precision parameter.**

| Sr. No. | Name of Standard | Retention time | Theoretical Plates | Tailing Factor | % RSD |
|---|---|---|---|---|---|
| 1 | MP | 3.95 | 3199 | 1.28 | 0.2 |
| 2 | PP | 6.28 | 2904 | 1.29 | 0.3 |

**Table 7: Results of method precision parameter.**

| Sr. No. | Name of Analyte | Mean Assay (%) | % RSD |
|---|---|---|---|
| 1 | MP | 0.2088 | 1.4 |
| 2 | PP | 0.1566 | 0.7 |

**Table 8: Results of intermediate precision parameter.**

| Sr. No. | Name of Analyte | % RSD for Set-1 | % RSD for Set-2 | Overall % RSD |
|---|---|---|---|---|
| 1 | MP | 1.4 | 1.4 | 1.3 |
| 2 | PP | 0.7 | 1.1 | 0.9 |

**Accuracy Assessment**

The accuracy study was carried out by spiking known amount of standards into placebo solution at 50 %, 100 % and 150 % of working concentration, respectively. The overall recovery percent were calculated. The results of recovery studies gave the recovery rate from 98.3 % to 101.4 % for PP and 98.8 % to 100.9 % for MP at all three levels for all the two analytes. The details are mentioned in Table 9 and 10.

**Table 9: Results of accuracy study for methylparaben.**

| Recovery level | Spiked Conc. (µg/ml) | Mean Area | Recovered Conc. (µg/ml) | % Recovery |
|---|---|---|---|---|
| (50 %) Level_1_1 | 10.02 | 237016 | 10.07 | 100.5 |
| (50 %) Level_1_2 | 10.02 | 235015 | 9.98 | 99.6 |
| (50 %) Level_1_3 | 10.02 | 237456 | 10.09 | 100.7 |
| (100 %) Level_2_1 | 20.04 | 466035 | 19.80 | 98.8 |
| (100 %) Level_2_2 | 20.04 | 471424 | 20.03 | 100.0 |
| (100 %) Level_2_3 | 20.04 | 470011 | 19.97 | 99.7 |
| (150 %) Level_3_1 | 30.07 | 710953 | 30.20 | 100.4 |
| (150 %) Level_3_2 | 30.07 | 714421 | 30.35 | 100.9 |
| (150 %) Level_3_3 | 30.07 | 711531 | 30.23 | 100.5 |

**Table 10: Results of accuracy study for propylparaben.**

| Recovery level | Spiked Conc. (µg/ml) | Mean Area | Recovered Conc. (µg/ml) | % Recovery |
|---|---|---|---|---|
| (50 %) Level_1_1 | 7.54 | 237016 | 7.58 | 100.5 |
| (50 %) Level_1_2 | 7.54 | 239015 | 7.64 | 101.3 |
| (50 %) Level_1_3 | 7.54 | 231956 | 7.41 | 98.3 |
| (100 %) Level_2_1 | 15.08 | 467935 | 14.96 | 99.2 |
| (100 %) Level_2_2 | 15.08 | 471424 | 15.07 | 99.9 |
| (100 %) Level_2_3 | 15.08 | 476011 | 15.21 | 100.9 |
| (150 %) Level_3_1 | 22.62 | 710953 | 22.72 | 100.4 |
| (150 %) Level_3_2 | 22.62 | 717421 | 22.93 | 101.4 |
| (150 %) Level_3_3 | 22.62 | 698531 | 22.33 | 98.7 |

**Robustness Assessment**

The robustness of RP-HPLC method was evaluated by analyzing the influence of minor modifications in HPLC conditions by varying the parameters like flow Rate (±0.2 ml/min), pH of mobile phase buffer (±0.2) and mobile phase composition (±2 %). The method is found to be robust as there is no such variation (RSD less than 2.0 %) was found in the results after alteration of the evaluated parameters. The details are given in Table 11.

**Table 11: Results of Robustness.**

| Standard Solutions | Parameter condition | RT | Mean Area | SD | % RSD | Average % RSD |
|---|---|---|---|---|---|---|
| MP | **Flow Rate (± 0.2 ml/min)** | | | | | |
| | 0.8 ml/min | 5.39 | 0.8 ml/min | 3951.4687 | 0.8 ml/min | 0.7 |
| | 1.0 ml/min | 6.28 | 1.0 ml/min | 1325.5150 | 1.0 ml/min | |
| | 1.2 ml/min | 6.88 | 1.2 ml/min | 4270.2749 | 1.2 ml/min | |
| | **Change in Detector wavelength (± 2 nm)** | | | | | |
| | 270 nm | 6.10 | 270 nm | 297.3654 | 270 nm | 0.3 |
| | 272 nm | 6.28 | 272 nm | 1325.5150 | 272 nm | |
| | 274 nm | 6.41 | 274 nm | 2116.1731 | 274 nm | |
| | **Mobile phase composition (±2 %)** | | | | | |
| | 327:670:3 | 5.12 | 327:670:3 | 395.7225 | 327:670:3 | 0.2 |
| | 347:650:3 | 6.28 | 347:650:3 | 1325.5150 | 347:650:3 | |
| | 367:630:3 | 7.13 | 367:630:3 | 1461.2470 | 367:630:3 | |
| PP | **Flow Rate (± 0.2 ml/min)** | | | | | |
| | 0.8 ml/min | 3.23 | 0.9 ml/min | 3749.1416 | 0.9 ml/min | 0.3 |
| | 1.0 ml/min | 3.95 | 1.0 ml/min | 1567.7333 | 1.0 ml/min | |
| | 1.2 ml/min | 4.66 | 1.2 ml/min | 1813.0269 | 1.2 ml/min | |
| | **Change in Detector wavelength (± 2 nm)** | | | | | |
| | 270 nm | 3.69 | 270 nm | 332.9019 | 270 nm | 0.1 |
| | 272 nm | 3.95 | 272 nm | 1567.7333 | 272 nm | |
| | 274 nm | 4.01 | 274 nm | 1603.5823 | 274 nm | |
| | **Mobile phase composition (±2 %)** | | | | | |
| | 327:670:3 | 3.09 | 327:670:3 | 1683.5383 | 327:670:3 | 0.2 |
| | 347:650:3 | 3.95 | 347:650:3 | 1567.7333 | 347:650:3 | |
| | 367:630:3 | 4.91 | 367:630:3 | 387.7035 | 367:630:3 | |

## CONCLUSION

A simple, rapid, and reliable RP-HPLC method was successfully developed and validated for the simultaneous separation and quantification of methylparaben and Propylparaben in a herbal cream formulation. The developed method demonstrated good resolution between both preservatives, confirming its suitability for simultaneous analysis in a complex herbal matrix. Method validation was carried out in accordance with ICH guidelines, and the results established that the method is specific, precise, accurate, and robust across the tested

parameters. Owing to its simplicity, reproducibility, and consistent performance, the proposed RP-HPLC method is well suited for routine quality control analysis of methylparaben and Propylparaben in herbal cream formulations.

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