# PRESERVATIVE PRECISION: A NOVEL UV METHOD FOR METHYL PARABEN QUANTIFICATION IN DRUG FORMULATIONS

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**Abstract.** A simple, precise, and sensitive UV spectrophotometric method was developed and validated for the quantitative determination of methyl paraben in pharmaceutical formulations. The method utilized methanol as a solvent, with methyl paraben exhibiting maximum absorption at 256 nm. The linearity range was established as 1-5 µg/mL, with a correlation coefficient of 0.999. Method validation was performed according to ICH guidelines, demonstrating acceptable precision, accuracy, and sensitivity. The limits of detection and quantification were determined to be 0.071 µg/mL and 0.2154 µg/mL, respectively. The validated method was successfully applied to analyze methyl paraben content in three commercial pharmaceutical products: Clindamycin phosphate gel USP, Ketoconazole cream 2% w/w, and Luliconazole cream. Results confirmed that the methyl paraben content in all tested products complied with their respective label claims and fell within the FDA-approved safety limit of 0.015-0.2% for pharmaceutical formulations. This method offers a rapid and reliable approach for routine quality control analysis of methyl paraben in pharmaceutical products, ensuring compliance with regulatory standards and product safety.
**Keywords**: *methyl paraben, UV spectrophotometry, pharmaceutical preservatives, method validation, quality control, FDA safety limits*

## Introduction

4-Hydroxybenzoate, commonly known as methyl paraben (methyl 4-hydroxybenzoate) (Ambarak, 2019), is one of the most widely used antibacterial agents, cosmetic preservatives, and flavouring agents (Soni et al., 2002). It appears as a white crystalline powder and is soluble in methanol, acetone, and ether, although it is only weakly soluble in chloroform (Leszczak and Tran-Minh, 1998; Wiberg, 1953). Industrial grades may vary slightly in colour, often appearing light grey or light tan. Upon exposure to air and light, methyl paraben can darken due to oxidation (Maeda et al., 1987). Methyl paraben is extensively used as a preservative in food, pharmaceuticals, and cosmetics (Garner et al., 2014). However, frequent use of products containing methyl paraben may have harmful effects on the body and has been associated with an increased risk of breast cancer and other health conditions (Darbre and Harvey, 2014). The concentration of naturally occurring parabens in plants is usually very low. For instance, while parabens can make up as much as 0.3% of a cosmetic product's formula (Soni et al., 2002), the naturally occurring level of methyl paraben in blueberries is less than 0.003% (Ambarak, 2019).

Parabens are widely utilized in various products due to their broad-spectrum antimicrobial activity, low toxicity, high stability, and non-volatility. Preservatives like


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parabens are essential for preventing food spoilage (Lincho et al., 2021). The U.S. Food and Drug Administration (FDA) have long allowed the use of methyl paraben in numerous products. Many commonly used parabens have been classified as Generally Recognized as Safe (GRAS) by the FDA since the early 1970s. This GRAS designation indicates that the substance is generally considered safe by qualified experts under the conditions of its intended use. The European Economic Community (EEC) has set the safety limit for methyl paraben in pharmaceutical products at 0.015-0.2% (Murjani et al., 2023). Methyl paraben is commonly found in personal care products such as hand creams, facial cleansers, moisturizers, and other pharmaceutical items (*Figure 1*) (Van Der Schyff et al., 2022). It acts as an antifungal agent and preservative in cosmetics and pharmaceuticals. Its ability to be easily absorbed through the skin, along with its generally non-irritating nature, makes it a popular choice for preserving formulations. However, concerns remain about the potential adverse effects of frequent paraben exposure (Hong and Chang, 2006). Some studies suggest that parabens, including methyl paraben, may cause rashes, urticaria, contact dermatitis, and even cancer. In males, parabens have been linked to decreased reproductive capacity, infertility, and skin conditions such as malignant melanoma and contact eczema (Mali et al., 2021; Fransway et al., 2019).

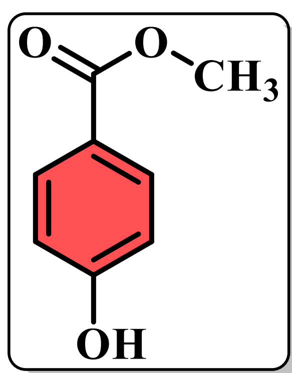


***Figure 1.*** *Methyl paraben structure.*

## Materials and Methods

A UV-Visible spectrophotometer (Shimadzu UV-1800) equipped with double-beam optics and two matched quartz cells with a 1 cm path length was used for all spectrophotometric measurements. The spectrophotometer was operated with the UV Probe software, which allowed for precise control of wavelength selection, data acquisition, and spectral analysis. This system is capable of high-resolution absorbance measurements across the ultraviolet and visible spectrum, providing accurate quantification of analytes in solution. For all mass measurements, an electronic analytical balance (Shimadzu BL-220H) was employed. The balance offers a high degree of precision, with a readability of 0.1 mg, ensuring the accurate weighing of reagents and samples required for experimental procedures. The balance was calibrated regularly to maintain accuracy, and all measurements were performed under controlled conditions to minimize environmental interference (*Figure 2*).


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***Figure 2.*** *UV-Visible spectrometer (Double beam) by Shimadzu used in the study.*

### *Reagents*

Methyl paraben was sourced from Hi Media Laboratories Pvt. Ltd. (Mumbai). Analytical grade methanol was obtained from S.D Fine-Chem Ltd. (Mumbai) and used as the solvent in all spectrophotometric analyses. Three pharmaceutical formulations containing methyl paraben were used in this study: Clindamycin phosphate gel USP [Clincitop, Universal Twin Labs (India)], which contains 0.114% w/w methyl paraben; Ketoconazole cream 2% w/w [Ketafung, East West Pharma (India)], containing 0.18% w/w methyl paraben; and Luliconazole cream [Lulifin, Sun Pharmaceutical Industries Ltd. (Mumbai)], with a methyl paraben concentration of 0.2% w/w. These formulations served as the test samples for method development and validation (*Figure 3*).

***Figure 3.*** *Commercial formulations used to analyze the concentration of methyl paraben (i) Clincitop, Universal Twin Labs (India), (ii) Ketafung, East West Pharma (India), (iii) Lulifin, Sun Pharmaceutical Industries Ltd. (India).*

### *Standard solution and sample*

A stock solution of methyl paraben was prepared by dissolving 10 mg of the reference standard in 5 mL of methanol, and then diluting the solution to a final volume of 10 mL in a volumetric flask using the same solvent. From this stock solution, 1 mL was further diluted with methanol in a 50 mL volumetric flask, and the volume was brought up to 50 mL. Working solutions with concentrations ranging from 1 to 5 µg/mL were prepared from this standard stock solution for subsequent analysis. The sample for

the study was accurately weighed, ensuring that the amount corresponded to the equivalent weight of the standard. The weighed sample was dispersed in a 10 mL volumetric flask, and the volume was adjusted with methanol to achieve a concentration of 1000 µg/mL. Subsequently, 1 mL of this solution was transferred to a 50 mL volumetric flask, and the volume was again adjusted with methanol to obtain a 20 µg/mL solution, which was used for further evaluation. The selected formulations for this study were Clindamycin phosphate gel USP (20 g), Ketoconazole Cream 2% w/w (30 g), and Luliconazole Cream (30 g). The calculated equivalent weights of these samples were 1.754 g, 4.918 g, and 1.5 g, respectively. Each weighed sample was dispersed in a 10 mL volumetric flask, and the volumes were made up with methanol to achieve a concentration of 1000 µg/mL. From each of these individual solutions, 1 mL was transferred into a 50 mL volumetric flask, and the volume was made up with methanol to yield a 20 µg/mL solution. These solutions were then used for further evaluation.

### *Validation*

The developed method was validated in accordance with the International Council for Harmonisation (ICH) guidelines (ICH Q2B) for the validation of analytical procedures. Key validation parameters included linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Validation is the process of generating documented evidence that provides a high level of assurance that a specific analytical method will consistently yield results meeting predetermined specifications and quality attributes.

## Results and Discussion

### *Linearity*

To evaluate linearity, standard calibration curves for methyl paraben were generated by plotting concentration against absorbance (*Table 1*). The method exhibited linearity within the concentration range of 1-5 µg/mL. The slope, intercept, and correlation coefficient ($R^2$) for methyl paraben were determined to be 0.3302, 0.0813, and 0.9997, respectively, indicating excellent linearity. The overlay spectrum of the standard methyl paraben is presented in *Figure 4*, and the corresponding calibration curve is shown in *Figure 5*.

***Table 1***. *Data for the linearity of Methyl paraben.*

| S.No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 1.00 | 0.251 |
| 2. | 2.00 | 0.406 |
| 3. | 3.00 | 0.578 |
| 4. | 4.00 | 0.737 |
| 5. | 5.00 | 0.911 |


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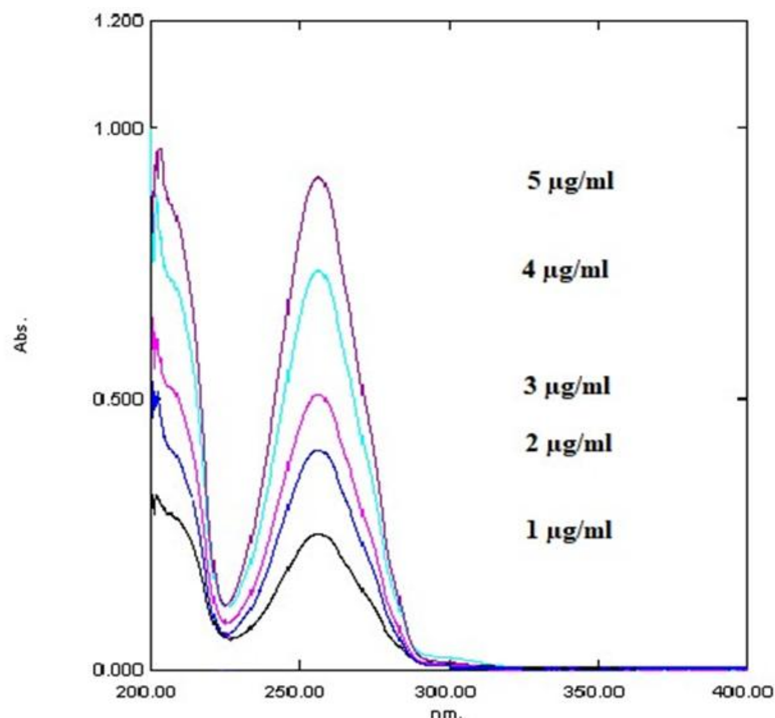


***Figure 4.*** *Overlay UV spectra of Methyl paraben showing linearity.*

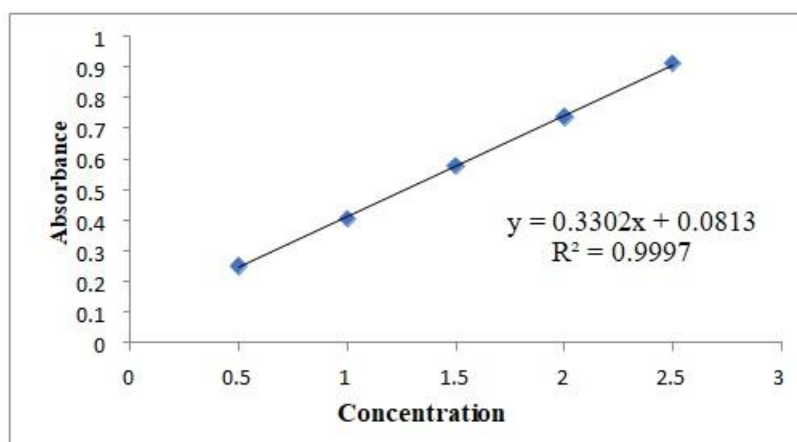


***Figure 5.*** *Calibration curve of Methyl paraben at 256 nm.*

### *Precision*

The precision of the method was assessed through repeatability (intra-day) and intermediate precision (inter-day) studies to evaluate the method's reliability. Precision was expressed as the percentage relative standard deviation (%RSD). The %RSD for intra-day and inter-day precision was found to be 1.432 and 1.435, respectively, both of which are within the acceptable limits, demonstrating the method's reproducibility.

### *Intra-day and inter-day precision*

Aliquots of 1 mL from the working standard solution of methyl paraben (20 µg/mL) were transferred into 10 mL volumetric flasks, and the volume was adjusted with methanol to achieve a concentration of 2 µg/mL. The absorbance of this solution was


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measured six times using a UV spectrophotometer. In the intra-day precision study, measurements were taken at different intervals throughout the same day, and the percentage relative standard deviation (%RSD) was calculated (*Table 2*). Similarly, for the inter-day precision study, aliquots of 1 mL from the working standard solution (20 µg/mL) were transferred into 10 mL volumetric flasks, and the volume was adjusted with methanol to obtain a 2 µg/mL solution. The absorbance was measured six times on different days, and the %RSD was calculated (*Table 2*).

***Table 2**. Intra-day precision.*

| Concentration (µg/ml) | Intra-day precision Absorbance | Inter-day precision Absorbance |
|---|---|---|
| 2 | 0.418 | 0.410 |
| | 0.421 | 0.413 |
| | 0.425 | 0.415 |
| | 0.428 | 0.419 |
| | 0.435 | 0.422 |
| | 0.437 | 0.426 |
| Mean | 0.427 | 0.417 |
| SD | 0.0061 | 0.0059 |
| % RSD | 1.432 | 1.435 |

### *Accuracy study*

The recovery study was conducted using the standard addition technique, wherein a fixed quantity of the homogeneous formulation from each of the three selected samples was spiked with standard methyl paraben at levels of 80%, 100%, and 120%. The spiked samples were then reanalysed using the proposed method. The recovery results for methyl paraben ranged from 98% to 101% in sample 1, 98% to 102% in sample 2, and 98% to 100% in sample 3, indicating the accuracy of the method. The detailed recovery results are presented in *Table 3*, *Table 4* and *Table 5*.

***Table 3**. Accuracy data for Methyl paraben in Clindamycin phosphate gel USP.*

| Sample 1 | Level (%) | Amount present (µg/ml) | Amount added (µg/ml) | Amount recovered (mg) | % recovery |
|---|---|---|---|---|---|
| Clindamycin phosphate gel USP | 80 | 2 | 1.6 | 3.54 | 98.33 |
| | 100 | 2 | 2 | 4.01 | 100.25 |
| | 120 | 2 | 2.4 | 4.36 | 99.09 |
| | | | | Mean | 99.22 |
| | | | | SD | 0.9668 |
| | | | | % RSD | 0.974 |

***Table 4**. Accuracy data for Methyl paraben in Ketoconazole cream.*

| Sample 2 | Level (%) | Amount present (µg/ml) | Amount added (µg/ml) | Amount recovered (mg) | % recovery |
|---|---|---|---|---|---|
| Ketoconazole cream 2% w/w | 80 | 2 | 1.6 | 3.63 | 100.8 |
| | 100 | 2 | 2 | 4.05 | 101.25 |
| | 120 | 2 | 2.4 | 4.33 | 98.40 |
| | | | | Mean | 100.15 |
| | | | | SD | 1.5321 |
| | | | | % RSD | 1.529 |

***Table 5**. Accuracy data for Methyl paraben in Luliconazole cream.*

| Sample 3 | Level (%) | Amount present (µg/ml) | Amount added (µg/ml) | Amount recovered (mg) | % recovery |
|---|---|---|---|---|---|
| Luliconazole cream | 80 | 2 | 1.6 | 3.54 | 98.33 |
| | 100 | 2 | 2 | 3.95 | 98.75 |


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| | 120 | 2 | 2.4 | 4.37 | 99.31 |
|---|---|---|---|---|---|
| | | | | Mean | 98.796 |
| | | | | SD | 0.4915 |
| | | | | % RSD | 0.4975 |

## *LOD and LOQ*

The detection limit (DL) of an analytical procedure is defined as the lowest concentration of analyte in a sample that can be reliably detected, but not necessarily quantified with precision. This represents the minimum concentration at which the presence of the analyte can be distinguished from background noise or matrix effects (Eq. (1).

$$LOD = 3.3\ X\ \frac{Standard\ Deviation}{Slope} \quad \text{Eq. (1)}$$

The quantitation limit (QL), also known as the limit of quantification (LOQ), is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The QL is particularly important for quantitative assays measuring low levels of compounds in sample matrices, and is especially relevant for the determination of impurities and degradation products (Eq. (2).

$$LOQ = 10\ X\ \frac{Standard\ Deviation}{Slope} \quad \text{Eq. (2)}$$

In this study, the calculated detection limit and quantitation limit values were found to be 0.071 µg/mL and 0.2154 µg/mL, respectively. These results are presented in *Table 6*.

***Table 6***. *Linearity data showing statistical data at the selected wavelength.*

| Wavelength (nm) | Regression Equation | $R^2$ | LOD (µg/ml) | LOQ (µg/ml) | % RSD |
|---|---|---|---|---|---|
| 256 | Y=0.330x+0.081 | 0.999 | 0.071 | 0.2154 | 1.648 |

The validated UV spectrophotometric method was applied to quantify methyl paraben in commercial pharmaceutical formulations, demonstrating its practical utility. A range of products containing methyl paraben as a preservative were analyzed to assess the method's versatility across different formulation matrices. Samples were prepared by extracting methyl paraben from each formulation and diluting appropriately. Each sample was analyzed in triplicate to ensure reproducibility. *Table 7* presents the comprehensive results, including mean methyl paraben content, standard deviations, labeled amounts, and percentage recovery for each analyzed formulation.

***Table 7***. *Estimation of Methyl paraben in selected pharmaceutical products.*

| Sample | Label claim of methyl paraben | Amount found | % Recovery | FDA approved safety limit of methyl paraben | Criteria range |
|---|---|---|---|---|---|
| Clindamycin phosphate gel USP | 0.114 % w/w | 0.103 % w/w | 90.35 | 0.015-0.2 % | Complies with the label claim and within the FDA limit |
| Ketoconazole cream | 0.18 % w/w | 0.163 % w/w | 90.55 | | Complies with the label claim and within the FDA limit |
| Luliconazole cream | 0.2 % w/w | 0.181%w/w | 90.85 | | Complies with the label claim and within the FDA limit |


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A validated UV spectroscopic method has been developed for the quantification of methyl paraben in pharmaceutical products. Methyl paraben exhibited maximum absorption at 256 nm using methanol as the solvent, which provided optimal solubility and peak resolution. The method demonstrated linearity in the UV absorbance range of 1-5 µg/mL. Validation was performed according to established guidelines, yielding a limit of detection (LOD) of 0.071 µg/mL, limit of quantification (LOQ) of 0.2154 µg/mL, and a correlation coefficient of 0.999. The U.S. Food and Drug Administration (FDA) permits the use of methylparaben in various pharmaceutical products within a safety range of 0.015-0.2% w/w. However, numerous studies have shown that excessive methyl paraben exposure beyond this safety limit can lead to adverse effects, including rashes, urticaria, contact dermatitis, and potential links to cancer and genotoxicity. In males, documented negative effects include decreased reproductive capacity, infertility, and skin disorders such as malignant melanoma and contact eczema. Our study's quantification results confirm that the selected pharmaceutical products (Clindamycin phosphate gel USP, Ketoconazole cream 2% w/w, and Luliconazole cream) comply with their respective label claims. The methyl paraben content in these products falls within the FDA-approved limit. The validation process demonstrates that the developed UV spectrophotometric method is accurate, precise, and sensitive. Moreover, this method is simple and time-efficient, requiring no complex extraction procedures.

## Conclusion

In this study, a novel UV spectrophotometric method was successfully developed and validated for the quantification of methyl paraben in pharmaceutical formulations. The method demonstrated excellent linearity within the concentration range of 1-5 µg/mL, with a correlation coefficient of 0.999. The method validation, performed in accordance with ICH guidelines, confirmed its accuracy, precision, sensitivity, and reproducibility. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.071µg/mL and 0.2154 µg/mL, respectively. The validated method was applied to three commercial pharmaceutical products-Clindamycin phosphate gel USP, Ketoconazole cream 2% w/w, and Luliconazole cream. The results confirmed that the methyl paraben content in these formulations complied with their respective label claims and fell within the FDA-approved safety limit of 0.015-0.2%. This ensures that the tested pharmaceutical products adhere to regulatory standards and are safe for consumer use. This UV spectrophotometric approach offers a rapid, cost-effective, and reliable method for routine quality control analysis of methyl paraben in pharmaceutical formulations. Unlike other complex chromatographic techniques, this method requires minimal sample preparation and utilizes methanol as a simple solvent, making it highly accessible for pharmaceutical quality control laboratories.

Despite the widespread use of methyl paraben as a preservative, concerns regarding its potential adverse health effects, including endocrine disruption and carcinogenicity, emphasize the need for stringent monitoring of its concentration in pharmaceutical products. The developed method provides an efficient tool for regulatory agencies and pharmaceutical manufacturers to ensure product safety and compliance with international guidelines. In conclusion, this validated UV spectrophotometric method is a valuable addition to the field of pharmaceutical analysis, offering a reliable approach for monitoring methyl paraben content in drug formulations. Future research could


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explore its application in broader pharmaceutical and cosmetic products to further enhance consumer safety.

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**Conflict of interest**

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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