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# METHOD DEVELOPMENT AND VALIDATION STUDIES ON THE CHLORZOXAZONE USING UV SPECTROSCOPY

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## ABSTRACT

A simple, accurate, precise, and cost-effective UV spectrophotometric method was developed and validated for the quantitative estimation of Chlorzoxazone in pharmaceutical dosage forms. The method was based on measuring absorbance at the selected wavelength corresponding to the maximum absorption of Chlorzoxazone in the UV region. The method was validated according to International Council for Harmonisation (ICH) guidelines for analytical method validation. Specificity, linearity, precision, accuracy, and assay were evaluated. The method showed good specificity, with no significant interference from formulation excipients. A satisfactory linear relationship was observed between absorbance and concentration over the investigated concentration range. Precision studies demonstrated good repeatability and intermediate precision, as indicated by low percentage relative standard deviation (%RSD) values. Accuracy was confirmed through recovery studies using the standard addition method, with satisfactory recovery within acceptable limits. Forced degradation studies were performed under acidic, alkaline, and oxidative conditions to evaluate the degradation behavior of Chlorzoxazone. Significant degradation was observed under acidic conditions, whereas comparatively greater stability was observed under alkaline and oxidative conditions. The developed method provides a simple and economical approach for routine quantitative analysis and preliminary stability assessment of Chlorzoxazone in pharmaceutical formulations.

## 1. INTRODUCTION

Analytical chemistry plays a crucial role in pharmaceutical research and quality control, particularly in the development and validation of methods for drug estimation. Among various analytical techniques, UV-visible spectrophotometry is widely used due to its simplicity, cost-effectiveness, and reliability (10). The technique is based on the absorption of ultraviolet or visible radiation by molecules, resulting in electronic transitions from ground state to excited state. The intensity of absorbed radiation is directly proportional to the concentration of the analyte, as described by Beer–Lambert's law.

Chlorzoxazone is a centrally acting skeletal muscle relaxant commonly used in the management of musculoskeletal pain and associated conditions (1)(2). It belongs to the benzoxazolinone class and acts primarily by depressing spinal reflexes involved in muscle tone regulation. Due to its pharmacological importance and frequent use in combination formulations, accurate and reliable analytical methods are required for its estimation (4)(8)

Method validation is a critical step in analytical method development, ensuring that the method produces reliable and reproducible results. According to ICH guidelines, validation parameters include specificity, linearity, precision, accuracy, and robustness (10)(11)These parameters collectively ensure the suitability of the method for its intended purpose.

In addition to validation, forced degradation studies are essential for understanding the stability characteristics of pharmaceutical compounds. These studies involve subjecting the drug to various stress conditions such as acidic, alkaline, and oxidative environments to evaluate degradation pathways and establish stability-indicating methods (12),(13),(17)Such studies are important for ensuring drug safety, efficacy, and shelf-life (16)

The present study aims to develop a simple UV spectrophotometric method for chlorzoxazone, validate the method according to ICH guidelines, and evaluate the stability of the drug under different stress conditions.

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS:

Chlorzoxazone standard and marketed tablet formulations were used for the study. Ethanol and distilled water were employed as solvents, while hydrochloric acid, sodium hydroxide, and hydrogen peroxide were used for degradation studies. All chemicals were of analytical grade.

### 2.2 INSTRUMENTATION:

UV-VIS Spectrophotometer Shimadzu UV-1900i (Lab solutions software), Shimadzu AUX220 Weighing balance, Enertech Sonicator were used.

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**2.3 PREPARATION OF SOLUTIONS:**

The standard stock solution was prepared by dissolving an accurately weighed quantity of chlorzoxazone in ethanol to obtain a known concentration. Working standard solutions were prepared by appropriate dilution using ethanol and water in equal proportions.

The sample solution was prepared by accurately weighing and powdering tablet formulations, followed by dissolution in ethanol. The solution was filtered to remove insoluble excipients and further diluted to obtain the desired concentration suitable for analysis.

**2.4 SELECTION OF ANALYTICAL WAVELENGTH:**

The standard solution was scanned over the UV range to determine the wavelength of maximum absorbance. Chlorzoxazone showed a distinct absorption peak at 283 nm, which was selected as the analytical wavelength for further studies .

**2.5 METHOD VALIDATION:**

Method validation was performed according to ICH guidelines .

**SPECIFICITY:**

Specificity was evaluated by analyzing blank and placebo solutions. The absence of interference at the selected wavelength confirmed that the method specifically measures chlorzoxazone without influence from excipients.

**LINEARITY:**

Linearity was assessed by analyzing solutions at different concentration levels. A calibration curve was constructed, and the relationship between absorbance and concentration was found to be linear over the selected range.

**PRECISION:**

Precision was determined by repeatability studies. Multiple measurements of the same concentration were performed, and the results showed low variability, indicating high precision.

**ACCURACY:**

Accuracy was evaluated using recovery studies at different concentration levels. The percentage recovery values were found to be within acceptable limits, confirming the accuracy of the method.

**2.6 FORCED DEGRADATION STUDIES:**

Forced degradation studies were performed to evaluate the stability of chlorzoxazone under stress conditions. The drug was subjected to acidic, alkaline, and oxidative environments. Samples were analyzed at different time intervals to monitor degradation behavior .

## 3. RESULTS AND DISCUSSION

The developed UV spectrophotometric method for chlorzoxazone demonstrated excellent analytical performance across all validation parameters. The calibration curve showed a strong linear relationship between absorbance and concentration, confirming adherence to Beer–Lambert's law and indicating the suitability of the method for quantitative analysis.

The method exhibited high precision, as indicated by minimal variation between repeated measurements. The low %RSD values confirm the repeatability and reliability of the analytical procedure. Accuracy studies further supported the validity of the method, with recovery values falling within acceptable limits, demonstrating that the method is capable of producing results close to the true value.

Specificity is a critical parameter in analytical method validation, particularly for pharmaceutical formulations containing excipients. In the present study, specificity evaluation confirmed that there was no interference from blank or placebo at the selected wavelength of 283 nm. This indicates that the method is selective for chlorzoxazone and can be effectively applied for routine analysis in dosage forms.

The selection of 283 nm as the analytical wavelength provided optimal sensitivity and specificity for chlorzoxazone, which is consistent with previously reported spectrophotometric methods . The method validation results were found to be in compliance with ICH guidelines, confirming the reliability and reproducibility of the developed method .

Forced degradation studies were carried out to evaluate the stability-indicating capability of the method. Under acidic conditions, chlorzoxazone exhibited noticeable degradation, with a gradual decrease in absorbance over time. This suggests that the drug is susceptible to acid hydrolysis, which is an important consideration during formulation development and storage.

In contrast, the drug showed considerable stability under alkaline and oxidative conditions, with minimal changes in absorbance. This indicates that chlorzoxazone is relatively stable in these environments. The ability of the method to distinguish the drug from its degradation products confirms its stability-indicating nature.

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The results obtained from forced degradation studies are in agreement with previously reported findings on stability behavior and degradation pathways of pharmaceutical compounds . Similar analytical and stability-indicating methods have been reported for chlorzoxazone and related drugs using UV spectrophotometric and chromatographic techniques supporting the applicability and reliability of the present method.

Overall, the developed method is simple, precise, accurate, and specific, making it highly suitable for routine quality control analysis and stability studies of chlorzoxazone in pharmaceutical formulations.

## 4. CONCLUSION

A UV spectrophotometric method for the estimation of chlorzoxazone was successfully developed and validated. The method demonstrated excellent specificity, linearity, precision, and accuracy in accordance with ICH guidelines .

Forced degradation studies confirmed that chlorzoxazone is susceptible to acidic degradation while remaining stable under alkaline and oxidative conditions .

The method is simple, economical, and suitable for routine pharmaceutical analysis and stability studies.

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