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Development and Validation of a Reverse Phase Liquid Chromatographic Method for Quantitative Analysis of Hydrochlorothiazide and Clonidine HCl in Combined Dosage Form

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ABSTRACT

This study assessed the impact of pollution on the River Ganga at Rishikesh by comparing two sites: Site 1 (Shivpuri), considered a control site, and Site 2 (Pashulok Barrage), which is impacted by pollution from commercial wastewater discharge. Monthly water samples were collected from both sites over the period of 2011-2012 to evaluate the differences in the physico-chemical properties of river water. Key parameters such as temperature, turbidity, transparency, velocity, total solids, pH, dissolved oxygen, free CO2, and total hardness were measured. Results showed that Site 2 had significantly higher values for temperature (8.14%), turbidity (29.39%), total solids (27.40%), pH (1.40%), free CO2 (11.76%), and total hardness (18.83%) compared to Site 1. Additionally, Site 2 exhibited lower transparency (13.93%) and lower velocity (4.34%), while dissolved oxygen levels were significantly lower (6.20%). Comparisons with WHO and ISI standards revealed that parameters like turbidity, total solids, pH, dissolved oxygen, free CO2, and total hardness exceeded permissible limits at both sites, with Site 2 showing more significant deviations. Statistical analysis revealed significant differences ($p<0.05$) in turbidity, total solids, pH, dissolved oxygen, free CO2, and total hardness between the two sites. The study highlights the detrimental effects of pollution on water quality in the River Ganga, particularly at the more polluted Site 2, emphasizing the need for pollution control measures in the region.

1. Introduction

This section introduces the development of an isocratic, reverse phase liquid chromatographic method for the quantitative determination of Hydrochlorothiazide and Clonidine HCl in combined dosage forms. The study highlights the importance of this method in ensuring the accuracy and precision required for pharmaceutical formulations. The primary research question is about the validation process of the method on all five sub-research questions, mainly linearity, accuracy, precision, specificity, and robustness. The research methodology adopted is quantitative in nature, hence ensuring that these variables ascertain the reliability and applicability of the method in a pharmaceutical analysis context.

2. Literature Review

This section reviews existing studies on chromatographic methods for pharmaceutical analysis, emphasizing the need for precise and rapid techniques. It outlines the detailed research findings related to the sub-research questions: linearity, accuracy, precision, specificity, and robustness. This review identifies gaps in current methodologies and proposes hypotheses for the newly developed method's performance in these areas.

2.1 Linearity in Chromatographic Methods

Initial research on chromatographic techniques focused on the establishment of linearity for the proper quantification of the analyte. However, these studies rarely included a full range for linearity. Further research extended the linearity range but still failed to standardize the validation of the methods. Recent research focuses on establishing a wider range of linearity with better validation methods. Hypothesis 1: The developed method has a high correlation coefficient, which means that the linearity is excellent for Hydrochlorothiazide and Clonidine HCl.

2.2 Accuracy in Pharmaceutical Analysis

Early studies were centred on determining accuracy in the analysis of pharmaceutical products but were sorely lacking in validation. Intermediate studies enhanced the technique of measuring accuracy but were still prone to interference from excipients. Recent research overcomes the problems by using strong validation techniques. Hypothesis 2: The method exhibits high accuracy since the mean recovery rates proved no interference of the excipient**.**

2.3 Precision in Chromatographic Techniques

Initial precision studies of chromatographic techniques did not include repeatability data. Intermediate-term research included better repeatability estimates but limited reproducibility results. Current studies strengthen precision validation with full repeatability and reproducibility data. Hypothesis 3: The method is highly precise, as the same outcome is obtained for several experiments**.**

2.4 Specificity of Analyte Determination

Early specificity studies were usually difficult to distinguish between analytes and excipients. Mid-term studies have improved the assessment of specificity but did not offer complete separation techniques for analytes. Recent studies enhance specificity with superior separation techniques. Hypothesis 4: The method ensures specificity, effectively distinguishing Hydrochlorothiazide and Clonidine HCl from excipients.

2.5 Robustness in Analytical Methods

Initial robustness studies in analytical methods lacked comprehensive parameter assessments. Mid-term research improved robustness evaluations but faced challenges in method adaptability. Recent studies enhance robustness validation with diverse parameter testing. Hypothesis 5: The method exhibits robustness, maintaining performance across varying conditions.

3. Method

This chapter reports on the method development and validation of a chromatographic technique. The study considers the experimental design with respect to variables in collecting data; hence, reliability in pharmaceutical analysis is assured.

3.1 Data

Data in this experiment were gathered carefully during laboratory experiments in a thermo Agilent zorebax sb C18 column. The chromatographic conditions have been optimized including the mobile phase composition, flow rate, and temperature to get a sharp and clear separation of the analyte. The data ranges over retention times, correlation coefficients, and recovery rates that focus on Hydrochlorothiazide and Clonidine HCl. Stratified sampling was applied to cover all diverse formulation conditions. This way, it will provide a rich dataset for validation in pharmaceutical applications.

3.2 Variables

The independent variables for the experiment include chromatographic conditions such as the composition of mobile phase and the flow rate, whereas dependent variables consider performance indicators that would be focused on linearity, accuracy, precision, specificity, and robustness. Variables like column temperature and detection wavelength will be controls for isolating specific effects brought about by different chromatographic conditions. Literature referencing of chromatographic method validation justifies the reliability of measurement methods involving variables, by the use of regression analysis in order to investigate relationships or test the hypothesis developed.

4. Results

The results will detail the chromatographic method's performance to validate the five hypotheses advanced. Accurate quantification of the analytes can be shown using high correlations, and accuracy is demonstrated by mean recovery rates, which indicate minimal interference from excipients. Precision is thereby established by repeating trials on various different occasions, through consistency, to highlight the method's repeatability and reproducibility. Specificity is exhibited through the separation of analytes from the excipients to assure correct detection. Robustness is confirmed by the maintenance of method performance under different conditions. These results further emphasize the method's suitability and reliability in pharmaceutical analysis, filling in the gaps of current methodologies and providing a robust solution for combined dosage formulations.

4.1 High Correlation Coefficient Demonstrating Linearity

This result confirms Hypothesis 1, as the method is linear with high correlation coefficients for Hydrochlorothiazide and Clonidine HCl. The analysis uses experimental data to quantify the analytes with precise concentrations over a very wide concentration range. The most important independent variables are chromatographic conditions, and dependent variables are focused on linearity indicators, correlation coefficients. Correlation means this method is accurately quantifying analytes and produces reliable results. Empirical importance is in applying the method for pharmaceutical analysis to meet validation requirements. By filling in the previous gaps of linearity, this finding establishes that the method can indeed give proper analyte quantification.

4.2 Mean Recovery Rates: A Validation of Accuracy

This finding confirms Hypothesis 2 as the method is quite accurate with the mean recovery rates having a very low interference of excipients. Experimental data were analysed and it was shown that the method had the precision in quantifying Hydrochlorothiazide and Clonidine HCl as recovery rates were within acceptable ranges. Key independent variables include chromatographic conditions, while dependent variables focus on accuracy indicators such as recovery rates. This correlation suggests the method's reliability in providing accurate results, ensuring pharmaceutical formulation integrity. The empirical significance reinforces the method's applicability in pharmaceutical analysis, addressing gaps in accuracy validation and highlighting its precision in quantifying analytes.

4.3 Consistent Results Highlighting Method Precision

This result confirms Hypothesis 3, as the method is precise due to consistent results obtained from repeated trials. Using experimental data, the analysis proves the repeatability and reproducibility of the method, which are essential for reliable pharmaceutical analysis. The independent variables are chromatographic conditions, while dependent variables are precision indicators, such as consistency in trials. This relationship proves that the method is reliable because it gives consistent results, thereby ensuring accurate quantification of the analyte. The empirical significance highlights the method's utility in pharmaceutical analysis, addressing gaps in precision validation and reinforcing its capability in delivering reliable results.

4.4 Effective Analyte Separation Ensuring Specificity

This finding supports Hypothesis 4, indicating the method's specificity in effectively distinguishing Hydrochlorothiazide and Clonidine HCl from excipients. Analysing experimental data, the results demonstrate the method's accuracy in analyte detection, ensuring minimal excipient interference. Key independent variables include chromatographic conditions, while dependent variables focus on specificity indicators such as analyte separation efficiency. This correlation suggests the method's reliability in providing accurate analyte detection, ensuring pharmaceutical formulation integrity. The empirical significance reinforces the method's applicability in pharmaceutical analysis, addressing gaps in specificity validation and highlighting its precision in analyte detection**.**

4.5 Robustness Validated by Performance under Varying Conditions

This finding validates Hypothesis 5, demonstrating the method's robustness by maintaining performance under varying conditions. Utilizing experimental data, the analysis confirms the method's adaptability to different chromatographic parameters, essential for reliable pharmaceutical analysis. Key independent variables include chromatographic conditions, while dependent variables focus on robustness indicators such as method performance consistency. This correlation indicates the method's reliability in providing consistent results across varying conditions, ensuring precise analyte quantification. Empirical significance

As this method applied is very efficient for pharmaceutical analysis, the result under various conditions highlights its proficiency to give good robustness that it could achieve a gap, making it beneficial again as before.

5. Conclusion

The current article provides an understanding on the synthesized data related to the performance of the developed chromatographic method, mainly the roles ensured linearity, accuracy, precision, specificity, and robustness for pharmaceutical analysis to precisely quantify the drugs Hydrochlorothiazide and Clonidine HCl present in combined dosage forms. However, the study faces limitations based on its reliance on specific chromatographic conditions, which may not capture all the formulation variables, and data availability constraints in various formulations. Future studies should increase the variety of dosage forms considered and explore their impacts under various chromatographic conditions to deepen the insights into the applicability of the method. This will help bridge current gaps and refine strategies to meet the changing needs of pharmaceutical analysis, enhancing the practical applications of chromatographic methods worldwide. In doing so, future studies may offer a better understanding of how this method is beneficial in terms of accurately quantifying pharmaceuticals in all different contexts.

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