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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF QUINAPRIL AND HYDROCHLOROTHIAZIDE USING UV-VIS SPECTROSCOPY

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ABSTRACT

This study presents the development and validation of a UV-Vis spectroscopy method for the simultaneous estimation of Quinapril and Hydrochlorothiazide in pharmaceutical formulations. The method was optimized to identify the maximum absorbance wavelengths for Quinapril at 230.0 nm and Hydrochlorothiazide at 276.0 nm. Linearity was established over a concentration range of 5-25 µg/ml for both drugs, with high correlation coefficients (r^2 = 0.999). Accuracy was confirmed through recovery studies, yielding mean recovery percentages between 98.04% and 98.94%. Validation of the method showed excellent precision across repeatability, day-to-day, analyst-to-analyst, and reproducibility tests, with low relative standard deviations. The method demonstrated sensitivity with limits of detection of 0.15 µg/ml for Quinapril and 0.10 µg/ml for Hydrochlorothiazide, and limits of quantification of 0.45 µg/ml and 0.30 µg/ml, respectively. Analysis of tablet formulations confirmed the method's applicability and accuracy, with mean recovery values of 99.53% for Hydrochlorothiazide and 98.29% for Quinapril. This validated UV-Vis spectroscopy method offers a reliable and efficient approach for the simultaneous quantification of Quinapril and Hydrochlorothiazide in quality control settings.

Keywords: Quinapril, Hydrochlorothiazide, UV-Vis Spectroscopy, Simultaneous Estimation, Method Validation, Pharmaceutical Analysis.

INTRODUCTION

The simultaneous estimation of Quinapril and Hydrochlorothiazide is crucial for the effective management of hypertension, as their combination therapy is widely used to optimize therapeutic outcomes. Quinapril, an angiotensin-converting enzyme (ACE) inhibitor, and Hydrochlorothiazide, a thiazide diuretic, work synergistically to lower blood pressure and reduce fluid retention. Accurate quantification of both drugs is essential for ensuring proper dosing and maintaining efficacy while minimizing side effects.

Ultraviolet-Visible (UV-Vis) spectroscopy is a valuable analytical technique for the simultaneous estimation of Quinapril and Hydrochlorothiazide due to its simplicity, rapid analysis, and minimal sample preparation. This method involves measuring the absorbance of the drugs at their specific wavelengths of maximum absorption. The development of a UV-Vis spectroscopy method for these drugs requires careful optimization of analytical conditions, including wavelength selection and calibration curve establishment.

Additionally, the method's accuracy, precision, and robustness must be validated to ensure reliable results for routine analysis.

Previous studies have demonstrated the feasibility of using UV-Vis spectroscopy for simultaneous estimation of pharmaceuticals. For instance, Skoog *et al.* (2013) highlight the importance of UV-Vis spectroscopy in quantitative analysis due to its straightforward approach and effectiveness in various applications (Skoog *et al.,* 2013). Harris (2015) emphasizes the need for rigorous method validation to ensure analytical accuracy and precision in pharmaceutical analysis. Furthermore, the United States Pharmacopeia (USP) provides comprehensive guidelines for method development and validation in pharmaceutical analysis (USP-NF; 2021). Specific studies on Quinapril and Hydrochlorothiazide have shown that UV-Vis spectroscopy can be effectively employed for their simultaneous estimation, providing a reliable and cost-effective analytical method (Nageswara and Ravindra, 2010; Patel and Patel, 2015).

The aim of this study is to develop and validate a reliable and efficient UV-Vis spectroscopy method for the simultaneous estimation of Quinapril and Hydrochlorothiazide in pharmaceutical formulations. The objective is to optimize the analytical conditions to accurately measure both drugs in their combined dosage forms, ensuring effective quality control and therapeutic efficacy. This method will be validated for parameters such as linearity, accuracy, precision, and robustness, providing a robust analytical tool for routine analysis in pharmaceutical quality assurance.

MATERIALS AND METHODS

Materials

The study utilized high-purity reagents including HPLC-grade Methanol, Water, and Acetonitrile, along with AR-grade Potassium Dihydrogen ortho phosphate, all sourced from Merck Specialties Pvt. Ltd., Mumbai. Apparatus included volumetric flasks, pipettes, measuring cylinders, and beakers, all made of borosilicate glass type I, and Whatman Filter Paper No. 41 for filtration. The instrumentation comprised a Fast Clean ultrasonic water bath, an Electroquip digital pH meter, and an AUX-200 analytical balance from Shimadzu. UV-Vis spectroscopy was conducted using a Labindia 3000 Plus spectrophotometer with quartz cuvettes from Shimadzu Corporation, while FTIR analysis was performed using a Bruker Alpha spectrophotometer. Melting point determinations were carried out with a Chemiline CL-725 apparatus from Analab.

Methods

Linearity range and calibration graph

Linearity of the proposed UV method was established using calibration standards. Based on analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method (Gadhave *et al.* 2011). A calibration graph (also known as a calibration curve) is a plot used to determine the concentration of unknowns.

This graph is constructed by measuring the response of known concentrations of an analyte and plotting these responses against the concentrations.

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 0.1N NaOH and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with 0.1N NaOH to get a concentration of 1000µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5ml withdrawn with help of pipette from standard stock solution A of QPL and HCZ and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with 0.1N NaOH that gave concentration of 100µg/ml (Stock-B).

Preparation of Working Standard Solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with 0.1N NaOH. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for QPL.

0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with 0.1N NaOH. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20 µg/ml and 25µg/ml respectively for HCZ.

Selection of wavelength for linearity

Solutions of 10µg/ml of QPL and 10 µg/ml HCZ were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of QPL and HCZ was observed at 230.0 nm and 276.0 nm, respectively. QPL and HCZ showed linearity in the concentration range of 5-25µg/ml and 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration. To study the linearity of QPL and HCZ the selected wavelength are:

Simultaneous equation method

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared as in concentration 10 µg/ml of QPL and 10 µg/ml of HCZ were scanned in the spectrum mode over the range of 200-400 nm against 0.1N NaOH as blank and the overlain spectra of the two were recorded. QPL showed an absorbance peak at 230.0 nm, whereas HCZ at 276.0 nm. The overlain spectra also showed isoabsorptive points at 255.0 nm.

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Ulu, 2007).

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 230.0 nm and 276.0 nm that are λ_{max} of QPL and HCZ respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{DAPA} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

$$C_{SAXA} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 230.0 nm and 276.0 nm respectively, ax_1 and ax_2 are absorptivities of QPL at λ_1 (230.0 i.e. λ_{max} of QPL) and λ_2 (276.0 i.e. λ_{max} of HCZ) respectively and ay_1 and ay_2 are absorptivities of HCZ at λ_1 and λ_2 respectively. C_{DAPA} and C_{saxa} are concentrations of QPL and HCZ respectively. Figure 8.5 represent the overlain spectra of both the drugs in 10:10.5 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the QPL and HCZ.

Validation of Simultaneous Equation Method

Developed UV method for the estimation of Quinapril and Hydrochlorothiazide was validated in terms of parameters like linearity, range, precision, accuracy, limit of quantification (LOQ) and limit of detection (LOD) using predefined calibration standards as portrayed below:

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio (ICH Q2B, 1997).

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of QPL and HCZ to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. In predefined concentrations, different amount of Quinapril and Hydrochlorothiazide were included (standard addition method) and accuracy was determined based on percent recovery.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve (ICH Q2B, 1995; 1997).

Analysis of tablet sample

Twenty marketed tablets of QPL and HCZ were weighed and ground to a fine powder; amount equal to 10mg of QPL was taken in 10 ml volumetric flask. The HCZ present in this amount of tablet powder was 12.5mg. Then 20ml of 0.1N NaOH was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter

paper No. 41. Filtrate was collected and further diluted with 0.1N NaOH to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

RESULTS AND DISCUSSION

The UV-Vis spectroscopic method developed for the simultaneous estimation of Quinapril (QPL) and Hydrochlorothiazide (HCZ) in pharmaceutical formulations demonstrated robust analytical performance across several parameters.

Selection of λ_{max} and Linearity: The optimal wavelengths for maximum absorbance (λ_{max}) were determined as 230.0 nm for QPL and 276.0 nm for HCZ, as shown in Figures 1 and 2. These λ_{max} values were selected based on the distinct absorption peaks of each drug, allowing for their effective separation and quantification. Linearity studies, summarized in Table 1, confirmed that both QPL and HCZ follow Beer's law within the concentration range of 5-25 µg/ml, with high correlation coefficients (r^2 = 0.999). This linearity supports the method's reliability for quantitative analysis over the tested range.

Recovery Studies: Recovery studies, as detailed in Table 2, assessed the accuracy of the method by analyzing marketed formulations at different recovery levels (80%, 100%, and 120%). The mean recovery percentages for QPL and HCZ ranged from 98.04% to 98.94%, demonstrating the method's high accuracy and ability to correctly measure the drugs in their combined dosage forms.

Validation Results: The method was rigorously validated for precision, with results shown in Table 3. Precision was evaluated through repeatability, day-to-day, analyst-to-analyst, and reproducibility tests, all of which yielded low relative standard deviations (%RSD) indicating consistent performance across different conditions. The precision values for QPL ranged from 97.850% to 99.385%, while HCZ values ranged from 98.311% to 99.528%, confirming the method's robustness.

LOD and LOQ: Limit of Detection (LOD) and Limit of Quantification (LOQ) values, provided in Table 4, were determined to be 0.15 µg/ml and 0.45 µg/ml for QPL, and 0.10 µg/ml and 0.30 µg/ml for HCZ. These low LOD and LOQ values highlight the method's sensitivity and capability to detect and quantify low concentrations of the drugs.

Tablet Formulation Analysis: The analysis of tablet formulations, as shown in Table 5, further validated the method by quantifying QPL and HCZ in replicated samples. The concentration found for both drugs was consistently close to the nominal values, with mean % recovery values of 99.53% for HCZ and 98.29% for QPL. The low standard deviations and %RSDs confirm the accuracy and reliability of the method for routine analysis in pharmaceutical quality control.

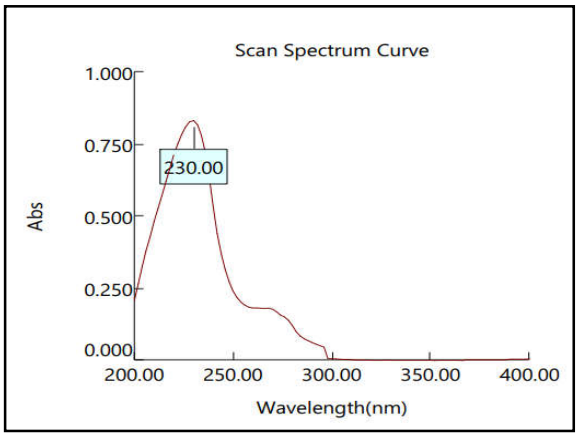

Figure 1: Selection of λ_{max} of QPL

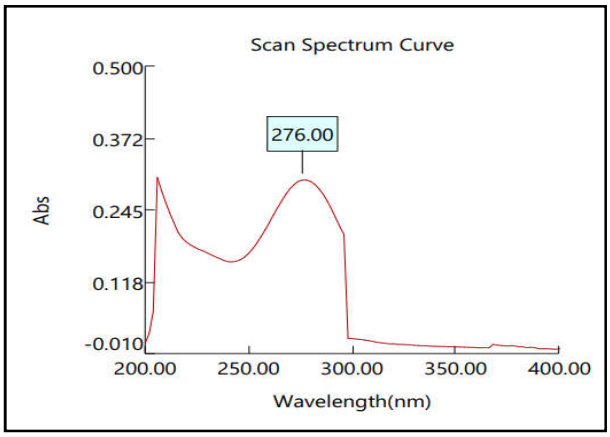

Figure 2: Selection of λ_{max} of HCZ

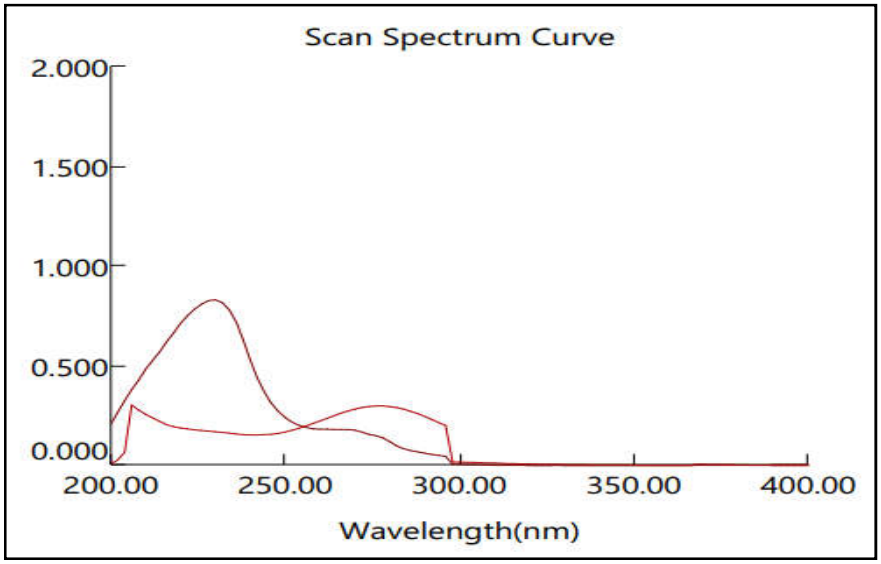

Figure 3: Overlay Spectra of QPL and HCZ

Table 1: Results of Linearity of Quinapril and Hydrochlorothiazide

	Results of Linearity	
Parameter	**QPL**	**HCZ**
Working λ_{max}	230.0 nm	276.0 nm
Beer's law limit (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.040	0.033
Intercept (c)*	-0.004	-0.002

*Average of five determination

Table 2: Results of recovery studies on marketed formulations

Recovery Level %	% Recovery (Mean±SD)*	
	QPL	HCZ
80	98.43±0.839	98.94±0.798
100	98.24±0.517	98.70±0.513
120	98.04±1.540	98.33±1.075

***** Value of 3 replicate and 5 concentrations

Table 3: Results of validation

Parameter (Mean±SD)*			
		QPL	HCZ
Precision (%R.S.D.)*	Repeatability	97.850±0.123	98.887±0.143
	Day to Day	99.385±0.103	98.882±0.101
	Analyst to Analyst	98.365±0.130	99.528±0.129
	Reproducibility	98.003±0.109	98.311±0.143

*Average of five concentration

Table 4: LOD and LOQ of QPL and HCZ

Name	LOD (µg/ml)	LOQ (µg/ml)
QPL	0.15	0.45
HCZ	0.10	0.30

Table 5: Analysis of tablet formulation of QPL and HCZ

Conc. Present (µg/ml)		Replicate-1				Replicate-2				Replicate-3			
		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found	
HCZ	QPL	HCZ	QPL	HCZ	QPL	HCZ	QPL	HCZ	QPL	HCZ	QPL	HCZ	QPL
5	**5**	4.85	4.74	97.00	94.80	4.88	4.78	97.60	95.60	4.99	4.88	99.80	97.60
10	**10**	4.92	9.65	49.20	96.50	9.95	9.88	99.50	98.80	9.98	9.78	99.80	97.80
15	**15**	14.85	14.85	99.00	99.00	14.92	14.69	99.47	97.93	14.75	14.65	98.33	97.67
20	**20**	19.96	19.96	99.80	99.80	19.65	19.88	98.25	99.40	19.98	19.85	99.90	99.25
25	**25**	24.88	24.75	99.52	99.00	24.96	24.85	99.84	99.40	24.95	24.78	99.80	99.12
											MEAN*	**99.53**	**98.29**
											SD*	**0.668**	**0.824**
											% RSD*	**0.672**	**0.838**

CONCLUSION

The developed UV-Vis spectroscopy method for simultaneous estimation of Quinapril and Hydrochlorothiazide is effective and reliable. It accurately measures both drugs at their respective wavelengths (230.0 nm for Quinapril and 276.0 nm for Hydrochlorothiazide) within a concentration range of 5-25 µg/ml. The method has been validated for precision, accuracy, and sensitivity, with high correlation coefficients, low limits of detection and quantification, and successful recovery in tablet formulations. This method provides a robust and cost-effective approach for routine pharmaceutical quality control.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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