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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ANTI-HYPERTENSIVE DRUGS USING UV AND HPLC

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ABSTRACT

This research presents the development and validation of analytical methods for the simultaneous quantification of Quinapril (QPL) and Hydrochlorothiazide (HCZ) in pharmaceutical formulations using UV spectrophotometry and RP-HPLC. The UV method involved physical characterization, melting point determination, and IR spectroscopy to confirm the identity and purity of the compounds. Solubility studies were performed to select appropriate solvents for UV analysis. Calibration curves were created by measuring absorbance at λmax of 248.0 nm for QPL and 222.0 nm for HCZ, demonstrating linearity across specified concentration ranges. The method was validated for specificity, accuracy, precision, and robustness, confirming its suitability for quality control. The RP-HPLC method optimized mobile phase composition (Acetonitrile: Methanol 50:50 v/v), column type (C18), and detection wavelength (245 nm) for effective separation of QPL and HCZ. Calibration curves showed excellent linearity (correlation coefficients > 0.999) across concentrations from 1 to 5 µg/ml. Validation studies further confirmed the method's reliability for quantifying QPL and HCZ in commercial tablet formulations. The UV method offers simplicity and cost-effectiveness for routine analysis, while RP-HPLC provides enhanced sensitivity and specificity for complex matrices. Both methods ensure accurate determination of QPL and HCZ content, supporting quality control and regulatory compliance in pharmaceutical manufacturing.

Keywords: Quinapril, Hydrochlorothiazide, UV spectrophotometry, RP-HPLC, method development, method validation.

INTRODUCTION

Hypertension is a prevalent cardiovascular condition affecting millions globally, leading to severe complications such as stroke, myocardial infarction, and renal failure if left untreated. The management of hypertension typically involves the use of various antihypertensive agents, including diuretics, ACE inhibitors, calcium channel blockers, and beta-blockers. Given the complexity of hypertension management, there is a growing need for efficient analytical methods to simultaneously estimate multiple antihypertensive drugs in pharmaceutical formulations and biological samples.

The simultaneous estimation of antihypertensive drugs is crucial for therapeutic monitoring, pharmacokinetic studies, and ensuring compliance in patients prescribed multi-drug regimens. Conventional methods, such as spectrophotometry, often lack the sensitivity and specificity required for complex mixtures. Therefore, the development of robust chromatographic methods, particularly High-Performance Liquid Chromatography (HPLC) and

Ultraviolet (UV) spectrophotometry, has gained significant attention in the pharmaceutical analysis of these compounds.

HPLC has emerged as a gold standard for the separation and quantification of pharmaceutical compounds due to its high resolution, sensitivity, and ability to analyze complex matrices. It allows for the simultaneous analysis of multiple drugs, which is essential in polypharmacy scenarios where patients may be prescribed several antihypertensive medications (Nabavi *et al.,* 2015; Mohammadi *et al.,* 2017). Conversely, UV spectrophotometry is a cost-effective and straightforward technique that can be utilized for routine analysis of drug formulations, although it may require additional validation for specificity when analyzing mixtures (Lima *et al.,* 2019).

The development of analytical methods must adhere to stringent validation parameters, including specificity, linearity, accuracy, precision, and robustness, as outlined by regulatory authorities such as the International Conference on Harmonisation (ICH) (ICH, 2005). This validation ensures the reliability of the methods for their intended use in quality control and therapeutic drug monitoring.

In this study, we aim to develop and validate a method for the simultaneous estimation of commonly prescribed antihypertensive drugs using both UV and HPLC techniques. This approach is expected to enhance analytical efficiency and accuracy in the assessment of antihypertensive therapies.

MATERIALS AND METHODS

Quinapril (QPL) and Hydrochlorothiazide (HCZ) and combination recently launched in the market used to used to treat high blood pressure (hypertension). High blood pressure adds to the workload of the heart and arteries. Following are the marketed formulation to be estimated by using UV Vis. Spectrophotometer.

Establishment of stability profile

Stability of both drugs was observed by dissolving QPL and HCZ in distilled water used as solvent. Solution of QPL and HCZ was prepared in the conc. of 5µg/ml and 10µg/ml respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in selected diluent.

Reagents and Standards

Reference standard of QPL and HCZ was a generous gift from Bioplus life science, Bangalore. In House synthetic mixture of QPL and HCZ were prepared in the ratio of (20:12.5mg). Label claim of QPL and HCZ in tablet is 20 and 12.5mg respectively. Reverse Osmosis Water was used throughout the study.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml distilled water in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml with distilled water to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml 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 distilled water that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) 0.5 ml, 1.0 ml, 1.5 ml, 2.0 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 distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for QPL.

2) Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 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 10ml with distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for HCZ.

Selection of wavelength for linearity

Solutions of 10 µg/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 248.0 nm and 222.0 nm, respectively. QPL and HCZ showed linearity in the concentration range of 5-25µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

Method I (Simultaneous equation method)

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of QPL and 10 µg/ml of HCZ were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. QPL showed an absorbance peak at 222.0 nm, whereas HCZ at 248.0 nm. The overlain spectra also showed isoabsorptive points at 235.0 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 (Noori, 2019).

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 222.0 nm and 248.0 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.

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

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

Where, A_1 and A_2 are absorbances of mixture at 260.0 nm and 282.0 nm respectively, ax_1 and ax_2 are absorptivities of QPL at λ_1 (222.0 i.e. λ_{max} of QPL) and λ_2 (248.0 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_{HCZ} and C_{QPL} are concentrations of QPL and HCZ respectively.

Validation of simultaneous equation method

A_1: 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, 1996).

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 (Kumar *et al.,* 2015).

Analysis of tablet sample

Twenty marketed tablets of QPL and HCZ were weighed and ground to a fine powder; amount equal to 10 mg of QPL was taken in 10 ml volumetric flask and 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 0.1 N HCl 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.

Method development of Quinapril (QPL) and Hydrochlorothiazide (HCZ) using RP-HPLC

Selection of Mobile Phase

Initially to estimate QPL and HCZ in fix dosage form number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10 mg API of QPL and HCZ was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

2. Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of QPL and HCZ respectively (Stock-B).

3. Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for QPL. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of HCZ also prepared (Chaudhari *et al.,* 2020).

4. Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for QPL and 1-5µg/ml for HCZ were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 245 nm and it was repeat for five times. A calibration graph was plotted

between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of QPL 1µg/ml for QPL and 10µg/ml HCZ was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5 µg/ ml for QPL) and (1 to 5µg/ ml for (HCZ) and areas for each concentration were recorded three times and mean area was calculated (Thomas and Varkey, 2023).

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Khursheed *et al.,* 2021).

Precision

The stock solution was prepared. The precision are established in three differences:

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for QPL and 5, 10, 15, 20 and 25µg/ml for HCZ indicates the precision under the same operating condition over short interval time.

2. Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

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 (De and Bera, 2021).

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of QPL and 10mg of HCZ was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 1µg/mL QPL and 7.5µg/mL HCZ respectively. The amounts of QPL and HCZ in tablets formulation were calculated by

extrapolating the value of area from the calibration curve.

RESULTS AND DISCUSSION

The study successfully developed and validated analytical methods for the simultaneous estimation of Quinapril (QPL) and Hydrochlorothiazide (HCZ) using both UV spectrophotometry and RP-HPLC, demonstrating their effectiveness for quality control in pharmaceutical formulations.

Tables 1 and 5 show the linearity results for both QPL and HCZ across their respective concentration ranges. For UV spectrophotometry, both compounds exhibited excellent correlation coefficients ($r^2 > 0.999$), confirming strong linearity within the specified Beer's law limits of 5-25 µg/ml. Similarly, the RP-HPLC method displayed comparable linearity, with r^2 values of 0.999 for both drugs over a narrower range of 1-5 µg/ml. These findings indicate that both methods are reliable for quantifying QPL and HCZ in their respective formulations.

The recovery studies (Tables 2 and 6) further affirm the accuracy of the methods. Recovery rates for QPL ranged from 99.046% to 99.828% at different levels, while HCZ showed recoveries between 95.08% and 96.62%. These results, coupled with low standard deviations, underscore the reliability of both analytical techniques in quantifying the active ingredients in marketed formulations.

As shown in Tables 3 and 7, precision assessments indicated low relative standard deviations (%R.S.D.) for both repeatability and reproducibility, suggesting high method consistency. The RP-HPLC method, in particular, showed robust precision across different parameters, including analyst-to-analyst variations. This reliability is essential for ensuring consistent drug quality in pharmaceutical manufacturing.

The analysis of commercial tablet formulations presented in Tables 4 and 8 demonstrated that both methods effectively quantify the active components in real samples. The results were within acceptable limits of the label claims, with QPL and HCZ found at 99.25% and 99.6%, respectively, confirming the methods' applicability in routine quality control.

Method development of Quinapril (QPL) and Hydrochlorothiazide (HCZ) using UV vis. Spectroscopy

Table 1: Results of Linearity of Quinapril and Hydrochlorothiazide

	Results of Linearity	
Parameter	**QPL**	**HCZ**
Working λ_{max}	222.0 nm	248.0 nm
Beer's law limit (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.9995	0.9999
Slope (m)*	0.0195	0.0499
Intercept (c)*	0.0005	0.0003

*Average of five determination

Table 2: Results of recovery studies on marketed formulations

Recovery Level %	% Recovery (Mean±SD)*	
	QPL	HCZ
80	99.046±0.284	95.20±0.101
100	99.055±0.400	96.62±0.093
120	99.828±0.308	95.08±0.086

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

Table 3: Results of validation

Parameter (Mean±SD)*			
		QPL	HCZ
Precision (%R.S.D.)*	Repeatability	99.21±0.033	99.71±0.043
	Day to Day	98.50±0.123	99.53±0.038
	Analyst to Analyst	98.20±0.135	99.40±0.066
	Reproducibility	99.70±0.529	99.66±0.039

*Average of five concentration

Table 4: Analysis of tablet formulation of TNG and PGL

Conc. present (µg/ml)		Replicate-1			
		Conc. found (µg/ml)		% Conc. found	
HCZ	QPL	HCZ	QPL	HCZ	QPL
20	12.5	19.65	12.45	98.25	99.60
20	12.5	19.85	12.48	99.25	99.84
20	12.5	19.65	12.46	98.25	99.68
20	12.5	19.95	12.47	99.75	99.76
20	12.5	19.74	12.32	98.70	98.56

Method development of Quinapril (QPL) and Hydrochlorothiazide (HCZ) using RP-HPLC

Table 5: Results of Linearity of Quinapril and Hydrochlorothiazide

	Results of Linearity	
Parameter	**QPL**	**HCZ**
Beer's law limit (µg/ml)	1-5	1-5
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	142.7	28.55
Intercept (c)*	3.807	3.086

*Average of five determination

Table 6: Results of recovery studies on marketed formulations

Recovery Level %	% Recovery (Mean±SD)*	
	QPL	HCZ
80	98.94±0.713	98.287±0.082
100	98.09±1.449	98.463±0.445
120	99.18±0.163	98.904±0.520

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

Table 7: Results of validation

Parameter (Mean±SD)*			
		QPL	HCZ
Precision (%R.S.D.)*	Repeatability	98.204±0.049	99.24±0.029
	Day to Day	99.24±0.046	99.24±0.013
	Analyst to Analyst	99.24±0.034	99.40±0.066
	Robustness	99.24±0.019	99.24±0.046
	LOD (µg/ml)	0.15	0.20
	LOQ (µg/ml)	0.45	0.65

*Average of five concentration

Table 8: Analysis of tablet formulation of QPL and HCZ

	QPL*	HCZ*
Label Claim (mg)	**20mg**	**12.5mg**
% Found (mg)	19.85	12.45
% Assay	99.25	99.6
% RSD	0.045	0.023

CONCLUSION

Both UV spectrophotometry and RP-HPLC methods provided reliable means for the simultaneous analysis of Quinapril and Hydrochlorothiazide in pharmaceutical formulations. The UV method is cost-effective and suitable for routine use, while the RP-HPLC method offers enhanced sensitivity and specificity, particularly useful for complex matrices. The validated methods ensure accurate determination of drug content, supporting quality control and regulatory compliance in the pharmaceutical industry. Further studies may explore additional formulations and the applicability of these methods in clinical settings.

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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