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Method development and validation for the estimation of Quinapril and Hydrochlorthiazide by RP-HPLC method

[1] Srikanth Choudary Pallothu*, [2] Sambana Srinivasa Rao and [3] G Ch D Nagalaskmi.

[1] Princeton College of Pharmacy, Korremula (V), Ghatkesar (M), Telangana, India.

[2] JJ College of pharmacy, Maheswaram, Telangana, India.

[3] NRS Government Ayurvedic College, Vijayawada, Andhra Pradesh, India.

* **Corresponding Author:** E-Mail: srikanthpallothu@gmail.com

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ABSTRACT

A Simple, specific and sensitive an isocratic Estimation by RP-HPLC analytical Method were developed and validated for the quantification QUINAPRIL AND HYDROCHLORTHIAZIDE. Quantification was achieved by by using the mobile phase Mixed Phosphate buffer (KH2PO4 +K2HPO4):Acetonitrile 40:60. Inertsil ODS,C-18,250×4.6mm ID, 5µm Particle size was used as stationary phase. The flow rate was 1.0ml/min. Measurements were made at a wavelength of 239nm. The average retention times for Quinapril and Hydrochlorothiazide was found to be 2.86 & 3.99min. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 50-150µg/ml & 62.5-187.5 µg/ml for Quinapril and Hydrochlorothiazide respectively. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Quinapril and Hydrochlorothiazide.

Keywords: Quinapril and Hydrochlorothiazide, RP-HPLC method, Inertsil ODS, Method development and Validation.

1. INTRODUCTION

Quinapril (Figure 1) is Angiotensin-Converting Enzyme Inhibitors, Antihypertensive Agent. There are two isoforms of ACE: the somatic isoform, which exists as a glycoprotein comprised of a single polypeptide chain of 1277; and the testicular isoform, which has a lower molecular mass and is thought to play a role in sperm maturation and binding of sperm to the oviduct epithelium. Somatic ACE has two functionally active domains, N and C, which arise from tandem gene duplication. Although the two domains have high sequence similarity, they play distinct physiological roles. The C-domain is predominantly involved in blood pressure regulation while the N-domain plays a role in hematopoietic stem cell differentiation and proliferation. ACE inhibitors bind to and inhibit the activity of both domains, but have much greater affinity for and inhibitory activity against the C-domain. Quinaprilat, the principle active metabolite of quinapril, competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII. Decreasing ATII levels in the body decreases blood pressure by inhibiting the pressure effects of ATII as described in the Pharmacology section above. Quinaprilat also causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.

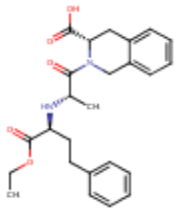

Figure - 1: Structure of Quinapril.

Antihypertensive Agents, Diuretics, Sodium Chloride Symporter Inhibitors. Hydrochlorothiazide (Figure 2), a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total

sodium reabsorption. Normally, the sodium-chloride symporter transports sodium and chloride from the lumen into the epithelial cell lining the distal convoluted tubule. The energy for this is provided by a sodium gradient established by sodium-potassium ATPases on the basolateral membrane. Once sodium has entered the cell, it is transported out into the basolateral interstitium via the sodium-potassium ATPase, causing an increase in the osmolarity of the interstitium, thereby establishing an osmotic gradient for water reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron. [1-8]

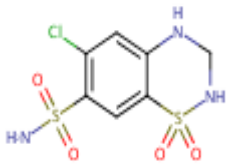

Figure - 2: Structure of Hydrochlorthiazide.

2. MATERIALS AND METHOD

Instruments The chromatographic technique performed on a Nicolet evolution 100 Liquid chromatography with Shimadzu(LC 20 AT VP) UV-visible detector and Spinchrom software , reversed phase Inertsil ODS 3V(250x4.6mm) 5µm as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

Pharmaceutically pure sample of Quinapril and Hydrochlorothiazide were obtained as gift samples from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drugs wer evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drugs were used without further purification.

HPLC-grade acetonitrile and OPA were from standard reagents pvt ltd. Potassium Phosphate dibasic (AR grade) sodium acetate AR Grade were from Merck.

2.1. Determination OF λ_{max} for Quinapril and Hydrochlorothiazide

2.1.1. Preparation of standard stock solution of Quinapril

50 mg of Quinapril was weighed and transferred in to 500ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10µg/mL of solution by diluting 1mL to 10mL with methanol.

2.1.2. Preparation of standard stock solution of Hydrochlorthaizide

50mg of Hydrochlorothaizide was weighed in to 500ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg/mL of solution by diluting 1ml to 10ml with methanol.

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/mL solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.

2.1.3. Preparation of mobile phase

A mixture of 400volumes of Mixed Phosphate buffer pH 3.5 and 600 volumes of Acetonitrile was prepared. The mobile phase was sonicated for 10min to remove gases.

2.1.4. Preparation of mixed standard solution

Weigh accurately 10mg of Quinapril and 12.5mg of Hydrochlorothaizide in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 100 µg/mL of Quinapril and 125µg/ml of Hydrochlorothiazide is prepared by diluting 1ml of Quinapril and 1.25ml of Hydrochlorothiazide to 10mL with mobile phase. This solution is used for recording chromatogram.

2.1.5. Preparation of sample solution

5tablets (each tablet contains 10mg of Quinapril and 12.5mg of Hydrochlorothiazide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Quinapril (100µg/mL) and Hydrochlorothiazide (125µg/mL) were prepared by dissolving weight equivalent to 10mg of Quinapril and 12.5mg of Hydrochlorothiazide and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100 µg/mL of Quinapril and 125µg/mL of Hydrochlorothiazide was made by adding 1mL and 1.25 mLof stock solution to 10 ml of mobile phase.

Calculation

The amount of Quinapril and Hydrochlorothiazide present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS = Average peak area due to standard preparation.

AT = Peak area due to assay preparation

WS =Weight of Quinapril and Hydrochlorothiazide in mg.

WT = Weight of sample in assay preparation

DT = Dilution of assay preparation.

2.2. Method validation

2.2.1. Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents, plasma at retention time of drug substances.

2.2.2. Linearity and range

Preparation of standard stock solution

Weigh accurately 10 mg of Quinapril and 10mg of Hydrochlorothaizide in 10ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min. and dilute 100ml with mobile phase and further dilutions were given in the table No Calibration curve (Figure 3 and 4) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.2.3. Method precision

Prepared sample preparations of Quinapril and Hydrochlorothaizide as per test method are injected 6 times in to the column.

2.2.4. Accuracy:

The accuracy of the method was determined by calculating the recoveries of Quinapril and Hydrochlorothaizide Known amounts of standard solutions of Quinapril and Hydrochlorothaizide was added at 50% concentration to pre quantified sample solutions of Quinapril and Hydrochlorothaizide (50,100,125µg/mL)and Internal standard (50,100,150 µg/mL). The amount of Quinapril and 10mg of Hydrochlorothaizide recovered was estimated by using the following formulas.

$$\% \text{ Recovery} = \frac{\text{amount found}}{\text{Amount added}} \times 100$$

$$\text{Amount Found}(mcg/ml) = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard concentration}$$

2.5. Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ± 2nm and flow rate was varied ±0.2 mL/min.

2.6. Ruggedness

The ruggedness of the method was studied by analysing the sample and standard preparations by two analysts. The % RSD assay values between two analysts was calculated.

3. RESULTS AND DISCUSSION

In Analytical RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates for both Standard and Internal standard, and better cost effective and time saving method than the previously developed methods. The Maximum uv absorbance was found to be 239nm by scanning in UV region. The chromatographic method was optimized with mobile phase consisting of Phosphate buffer: acetonitrile (40:60) and Inertsil ODS,C-18,250×4.6mm ID, 5µm Particle size. All the validation parameters were studied at a the wavelength 239nm. Accuracy was determined by calculating the recovery and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Quinapril and Hydrochlorothaizide. The results obtained were in good agreement with the corresponding labelled amount (Table 1 and 2). The method was linear in the concentration range of 50-150µg/ml for Quinapril and 62.5-187.5µg/ml for Hydrochlorothaizide (Table 3 and 4). Precision, Robustness and ruggedness results were in acceptable range (<2%). As the Quinapril and Hydrochlorothaizide peaks were well separated, the method is more specific. Based on all validation[1-8] parameters the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Quinapril and Hydrochlorothaizide in formulation.

Table - 1: Recovery results for Quinapril.

Recovery level	Accuracy Quinapril					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	
50%	50	828.835	835.010	50.70	101.40	101.02%
	50	838.098				
	50	838.098				
100%	100	1471.354	1495.917	101.67	101.67	
	100	1513.215				
	100	1503.181				
150%	150	2244.008	2245.461	150.10	100.06	
	150	2240.224				
	150	2252.152				

Table - 4: Recovery results for Hydrochlorothiazide

Recovery level	Accuracy Hydrochlorothiazide					Average % Recovery
	Amount taken(mcg/mL)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50	62.5	3473.134	3439.427	61.98	99.17	**99.55%**
	62.5	3598.073				
	62.5	3247.073				
100	125	5997.437	6111.610	124.54	99.63	
	125	6338.818				
	125	5998.575				
150	187.5	9428.872	9397.869	187.24	99.86	
	187.5	9338.19				
	187.5	9426.545				

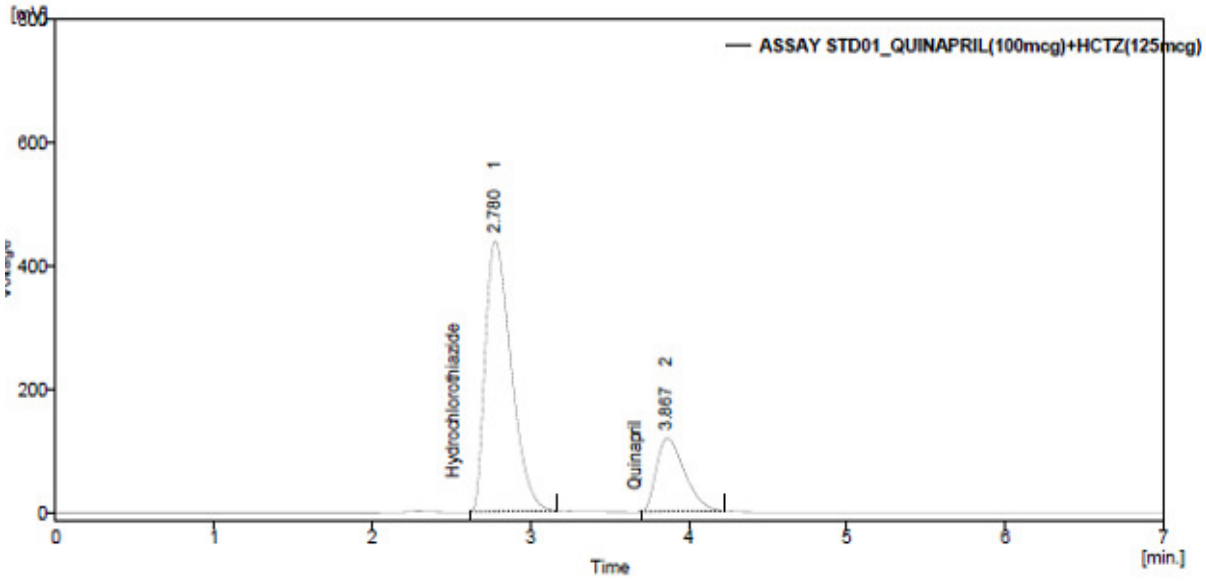

Figure - 3: Chromatogram of Standard.

.Table - 1: Linearity of Quinapril	
Conc (µg/mL)	**Area**
50	808.453
75	1164.555
100	1471.354
125	1844.375
150	2144.008

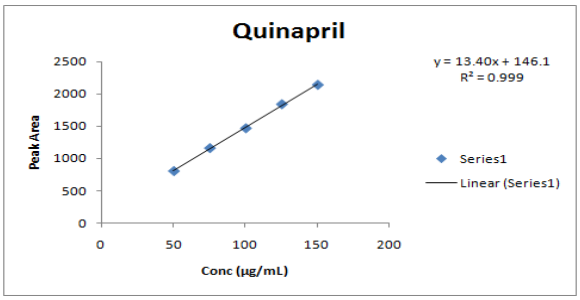

Figure - 3: Linearity graph of Quinapril.

Table - 2: Linearity of Hydrochlorothaizide	
Conc.(µg/ml)	**Area**
62.5	2774.562
93.75	3932.779
125	5007.437
156.25	6209.492
187.5	7328.872

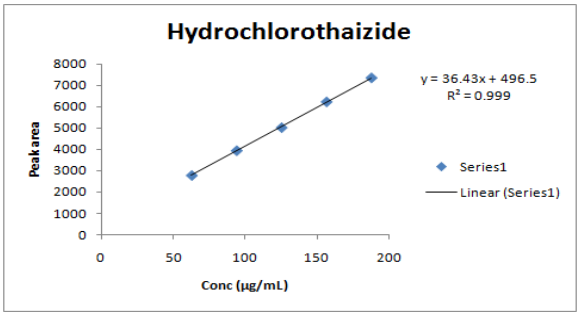

Figure - 3: Linearity graph of Hydrochlorothaizide

4. CONCLUSION

The proposed Isocratic Estimation by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Quinapril and Hydrochlorothiazide.. The method utilizes easily available and cheap solvent for analysis of Quinapril and Hydrochlorothiazide hence the method was also economic for estimation of Quinapril and Hydrochlorothiazide in formulation.

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