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ASSAY METHOD DEVELOPMENT AND VALIDATION OF QUINAPRIL USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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Abstract:

A simple, accurate, precise stability indicating HPLC method is developed for the determination of assay of quinapril. The chromatographic separation was achieved on a inertsil column,C18(150 X 4.6 ID) 5µm, It has a mobile phase consisting of a mixture of 40 volumes of mixed phosphate buffer and 60 volumes of acetonitrile. The detection wavelength is 239 nm. The method was validated for analytical parameters such as specificity, accuracy, precision, robustness and ruggedness as per ICH guidelines. The proposed method was found to be simple, accurate, precise, and robust and stability indicating HPLC method. Hence this method can be used for routine analysis.

Keywords: RP-HPLC, quinapril, method development, validation.

Introduction:

Quinapril is chemically known as (3S)-2-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. Its chemical formula and molar mass were $C_{25}H_{30}N_2O_5$ and 438.5161 g/mol[1]. Quinapril is a prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to quinaprilat (quinapril diacid) following oral administration[2,3]. Quinaprilat is a competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII)[4,5]. ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Quinapril may be used to treat essential hypertension and congestive heart failure[6]. The structure of quinapril[7] is given in the fig.1

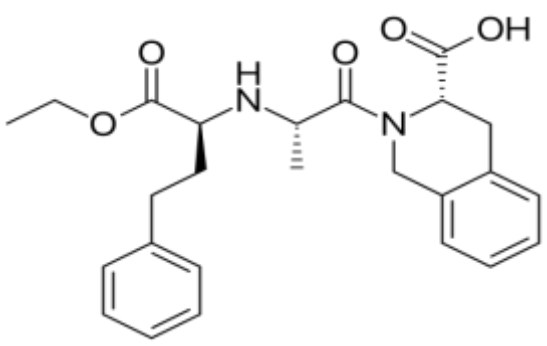

Fig 1: Structure of Quinapril

Mechanism of action:

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 pressor effects of ATII as described in the Pharmacology section above. Quinapril 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.

MATERIALS AND METHODS:

Chemicals and reagents:

The Samples of Quinapril were obtained as gift samples from Chandra labs, Hyd.

Table 1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSILcolumn,C18(150x4.6 ID) 5µm

Table 2: Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Preparation of Mobile Phase:

A mixture of 40 volumes of mixed phosphate buffer (KH_2PO4 +K_2HPO4) and 60 volumes of acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of Phosphate buffer :

Weigh 1.62 gms of KH_2PO_4 and 0.3 gms of K_2HPO_4 were weighed and dissolved in 550ml of water and volume was made up to 550ml with water. Adjust the pH to 6.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

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 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Validation parameters a) Specificity / Selectivity b) Accuracy c) Precision d) Linearity & Range e) Limit of Detection f) Limit of Quantitation g) Robustness h) Ruggedness i) System Suitability.

Results and Discussion:

Linearity and range

Preparation of mixed standard solution

10 mg of QUINAPRIL is weighed in 10ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Quinapril is 0.995 . The relationship between the concentration and area of Quinapril is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits. The results are given in the table 3 and shown in the fig 2 .

Table 3: Linearity of Quinapril

S.No.	Conc.(µg/ml)	Area
1	50	808.453
2	75	1164.555
3	100	1471.354
4	125	1944.375
5	150	2244.008

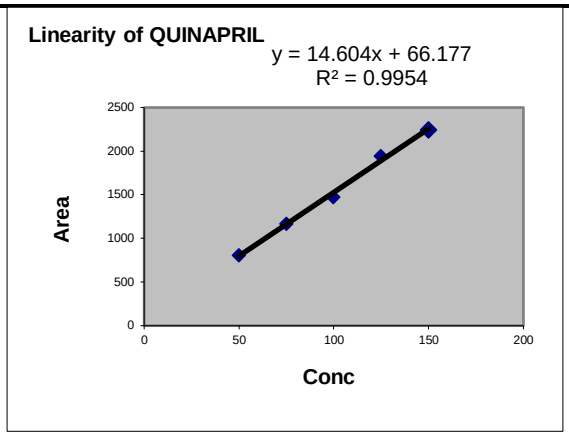

Fig.2: Linearity graph of Quinapril

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%. From the results the percentage mean recovery of Quinapril is 101.02%

. **Table 4** : 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				

Precision

Method precision

Prepared sample preparations of Quinapril as per test method and injected 6 times in to the column. Test results for Quinapril are showing that the %RSD of Assay results are within limits. The results are shown in the table 5.

Table 5: Results for Method precision of Quinapril

Quinapril		
S.No.	Rt	Area
1	3.827	1487.147
2	3.750	1496.768
3	3.750	1482.466
4	3.740	1448.567
5	3.827	1505.906
6	3.787	1463.248
avg	3.7802	1484.171
stdev	0.0397	21.855
%RSD	**1.05**	**1.47**

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like temperature and wavelength. System suitability parameters were compared with that of method precision.From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Table 6: Result of Robustness study

Parameter	**Quinapril**	
	Retention time(min)	**Tailing factor**
Flow		
0.8ml/min	4.670	1.902
1.0 ml/min	3.870	1.821
1.2ml/min	3.170	1.781
Wavelength		
237nm	3.740	1.838
239nm	3.870	1.821
241nm	3.750	1.861

Ruggedness

The ruggedness of the method was studied by the determining the analyst-to-analyst variation by performing the Assay by two different analysts**.** From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table 7: Results for Ruggedness

Quinapril	**%Assay**
Analyst 01	97.99
Analyst 02	98.37
%RSD	0.27

CONCLUSION:

A simple and selective HPLC method is developed for the determination of Quinapril tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a Mixed Phosphate buffer (KH2PO4 +K2HPO4): Acetonitrile 40:60, with detection of 239 nm. Linearity was observed in the range 50 - 150 µg /ml for Quinapril (r^2 =0.995)..

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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CONFLICT OF INTERESTS:

Declared None

REFERENCES:

1.Wishart DS. Jewison T. Guo AC, Wilson M and Knox C. et al The Human Metabolome Database. Nucleic Acids Res 2013; 1: 801-7
DOI: 10.1093/nar/gks1065

2.Espinoza S. Managó F. Leo D. et al Role of catechol-O-methyltransferase (COMT)- dependent processes in Parkinson's disease and L-DOPA treatment. CNS Neurol. Disord. Drug Targets 2012; 11(3): 251-63. DOI: 10.2174/187152712800672436

3.Goncalves D. Alves G.Soares-Da-Silva P. Falcao A et al Bioanalytical chromatographic methods for the determination of catechol-O-methyltransferase inhibitors in rodents and human samples: a review. Anal. Chim. Acta 2012; 710: 17-32. DOI: 10.1016/j.aca.2011.10.026

4.Bugamelli F. Marcheselli C. Barba E. Raggi MA. Et al Determination of L-dopa, carbidopa, 3-O-methyldopa and entacapone in human plasma by HPLC-ED. J Pharm Biomed Anal 2011; 54(3): 562-67. DOI: 10.1016/j.jpba.2010.09.042

5.Wright JM. Musini VM. Gill R.et al First-line drugs for hypertension. Cochrane Database Syst Rev 2018; 4: CD001841. doi: 10.1002/14651858.CD001841.pub3

6. Long CM, Tang K. Chokshi H .Fotaki N et al Surface dissolution UV imaging for investigation of dissolution of poorly soluble drugs and their amorphous formulation. AAPS Pharm Sci Tech 2019; 20(3): 113. doi: 10.1208/s12249-019-1317-z.

7.FDA Approved Drug Products: Quinapril Oral Tablet, www.accessdata.fda.gov/drugsatfda_docs/label/2017/019885s043lbl.pdf

8.Begum MH: Analytical method development and validation for the estimation of quinapril and tolcapone using RP-HPLC. Int J of Innovative Pharmaceutical Sciences and Research 2018; 6(01): 99-113. DOI: 10.13040/IJPSR.0975-8232.12(6).3375-80