J Drug Dev Del 2019
Volume 2: 1

Journal of Drug Development and Delivery

To Develop New RP-HPLC Method for the Simultaneous Estimation of Quinapril and Tolcapone in Pharmaceutical Dosage Form

Syed Hafeez Ahmed[1*]
Khasim SM[2]
Sridhar Babu R[3]
Juweriya Shahnawaz[4]

Department of Pharmaceutical Analysis and Quality Assurance, Shadan College of Pharmacy, Hyderabad, India
Department of Pharmacology, Shadan College of Pharmacy, Hyderabad, India
Department of Pharmaceutics, Shadan College of Pharmacy, Hyderabad, India
Department of Pharmacy, Shadan College of Pharmacy, Hyderabad, India

Abstract

A simple and selective LC method is described for the determination of Quinapril and Tolcapone. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 55 volumes of Water, 45 volumes of Methanol with detection of 220 nm. Linearity was observed in the range 2.5-7.5 µg /ml for Quinapril (r^2 = 0.995) and 5-15 µg /ml for Tolcapone (r^2 = 0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

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.

Keywords: RP-HPLC, Quinapril, Tolcapone, Chromatographic separation.

Introduction

Quinapril

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. Quinaprilat is a competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). 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.

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

Article Information

Article Type: Research
Article Number: JDDD107
Received Date: 09 January, 2019
Accepted Date: 16 January, 2019
Published Date: 23 January, 2019

***Corresponding author:** Syed Hafeez Ahmed, Department of Pharmaceutical Analysis and Quality Assurance, Shadan College of Pharmacy, Hyderabad, India. Tel: +91-7702543154; Email: syed.hafeez30(at)gmail.com

Citation: Ahmed SH, Khasim SM, Babu RS, Shahnawaz J (2019) To Develop New RP-HPLC Method for the Simultaneous Estimation of Quinapril and Tolcapone in Pharmaceutical Dosage Form. J Drug Dev Del Vol: 2, Issu: 1 (01-06).

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. 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.

Tolcapone

Tolcapone is a drug that inhibits the enzyme catechol-O-methyl transferase (COMT). It is used in the treatment of Parkinson's disease as an adjunct to levodopa/carbidopa medication. It is a yellow, odorless, non-hygroscopic, crystalline compound. Tolcapone is associated with a risk of hepatotoxicity.

Mechanism of action: The precise mechanism of action of Tolcapone is unknown, but it is believed to be related to its ability to inhibit COMT and alter the plasma pharmacokinetics of levodopa, resulting in an increase in plasma levodopa concentrations. The inhibition of COMT also causes a reduction in circulating 3-OMD as a result of decreased peripheral metabolism of levodopa. This may lead to an increase distribution of levodopa into the CNS through the reduction of its competitive substrate, 3-OMD, for transport mechanisms. Sustained levodopa concentrations presumably result in more consistent dopaminergic stimulation, resulting in greater reduction in the manifestations of parkinsonian syndrome.

Aim and Objective

Aim

To develop new RP HPLC method for the simultaneous estimation of Quinapril and Tolcapone in pharmaceutical dosage form.

Objective

• Solubility determination of Quinapril and Tolcapone in various solvents and buffers.

• Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.

• Optimize the mobile phase and flow rates for proper resolution and retention times.

• Validate the developed method as per ICH guidelines.

Materials and Methods

Table 1 and 2

Mobile phase

A mixture of 55 volumes of Water and 45 volumes of methanol were prepared. The mobile phase was sonicated for 10 min to remove 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

10 mg of Quinapril was weighed and transferred in to 100ml 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.

Preparation of standard stock solution of Tolcapone

10 mg of TOLCAPONE was weighed in to 100ml 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. Were soluble it was used as solvent for **λ max** determination by UV-Visible Spectroscopy.

Assay

Preparation of samples for assay

Preparation of mixed standard solution: Weigh accurately 10mg of Quinapril and 10 mg of Tolcapone in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 5 µg/ml of Quinapril and 10 µg/ml of Tolcapone is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation: 5 tablets (each tablet contains Tolcapone- mg, Quinapril- mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Tolcapone and Quinapril (µg/ml) were prepared by dissolving weight equivalent to 10 mg of Tolcapone and 10mg of Quinapril 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 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of Tolcapone and 5 µg/ml of

Table 1: Reagents used.

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Acetonitrile	HPLC Grade

Table 2: Drugs used.

QUINAPRIL AND TOLCAPONE drugs	Gift Samples obtained from Chandra labs, Hyd.
PFIZA (QUINAPRIL- 10mg & TOLCAPONE- 12.5**)** **Tablet dosage form**	Obtained from local pharmacy

Table 3: Assay results.

	QUINAPRIL		TOLCAPONE	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	1136.114	1120.050	2576.974	2541.448
Injection-2	1112.446	1121.051	2535.582	2551.500
Injection-3	1115.176	1123.043	2549.337	2545.160
Injection-4	1116.202	1118.821	2538.795	2551.600
Injection-5	1124.282	1112.446	2544.742	2535.582
Average Area	1120.844	1118.942	2549.086	2545.058
Standard deviation	3.615683		6.83985	
%RSD	0.323134		0.26875	
Assay(%purity)	99.83032		99.84198	

Quinaprilwas made by adding 1 ml of stock solution 1.5 to 10 ml of mobile phase.

Calculation: The amount of Tolcapone and Quinaprilpresent in the formulation by using the formula given below, and results shown in table 3.

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

Where,

AT = Peak area of sample preparation,

AS = Average Peak area of standard preparation,

WS = Weight of drug in mg,

DS & DT = Dilution of standard and sample preparation,

WT = Weight of Sample in Assay preparation,

P = Percentage purity of working standard,

LC = Label Claim of drug.

Observation

The amount of Quinapril and Tolcapone present in the taken dosage form was found to be 99.83% and 99.84% respectively.

Method validation

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. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity and potency of the drug substances and drug products.

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

Wavelength Optimization by UV– Spectroscopy

Figure 1

Method development and optimization of RP-HPLC method

Table 4

Method validation

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Tables 5 and 6

Table 4: Optimized chromatographic conditions.

Mobile phase	Methanol:Water
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	220
Injection volume	20 µl
Run time	6 min
Retention time	About 2.707 min for Quinapril and 3.953 min for Tolcapone.

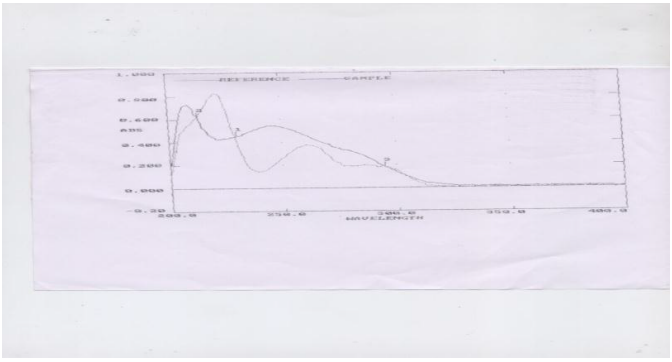

Figure 1: Wavelength optimization by UV– spectroscopy.

Table 5: Results for system suitability of Quinapril.

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.700	1136.114	2877	1.441
2	2.700	1112.446	2966	1.343
3	2.697	1115.176	2961	1.455
4	2.707	1116.202	2976	1.485
5	2.703	1124.282	2971	1.485
Mean	2.7014	1120.844	-	-
SD	0.003782	9.607	-	-
%RSD	0.139984	0.8574	-	-

Table 6: Results for system suitability of Tolcapone.

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.947	2576.974	2476	1.500
2	3.937	2535.582	2554	1.477
3	3.933	2549.337	2550	1.512
4	3.953	2538.795	2576	1.477
5	3.947	2544.742	2567	1.512
Mean	3.9434	2549.086	-	-
SD	0.008173	16.46919	-	-
%RSD	0.207261	0.646082	-	-

Linearity

Tables 7,8 and 9

Figures 2 and 3

Observation: The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Quinapril and Tolcapone is 0.995 and 0.998.

Recovery

Tables 10 and 11

Observation: The percentage mean recovery of Quinapril and Tolcapone is 101.54% and 102.81% respectively.

Precision

Table 12

Observation: Test results for Tolcapone and Quinapril are showing that the %RSD of Assay results are within limits. The results were shown in table 12.

Limit of detection

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOD of Quinapril = 0.79 µg/ml

LOD of Tolcapone = Tolcapone

Observation: The LOD for this method was found to be 0.79 µg/ml Quinapril and 2.95 µg/ml for Tolcapone

Limit of quantification

$$LOD = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOQ of Quinapril = 0.98µg/ml

LOQ of Tolcapone = 3.79 µg/ml

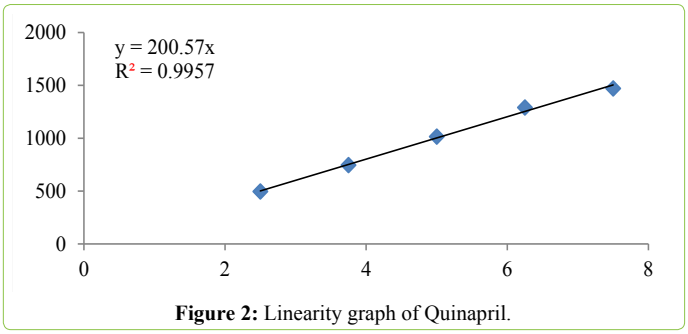

Figure 2: Linearity graph of Quinapril.

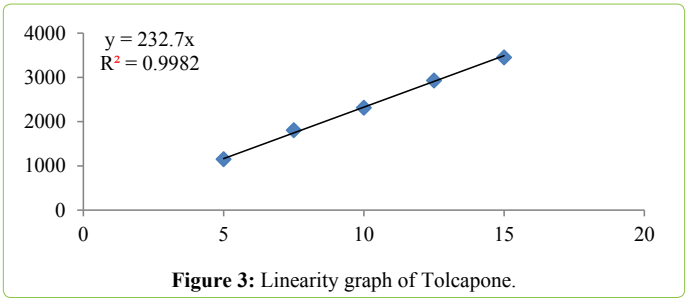

Figure 3: Linearity graph of Tolcapone.

Table 7: Linearity preparations.

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
			QUINAPRIL	TOLCAPONE
Preparation 1	0.75	10	2.5	5
Preparation 2	1.125	10	3.75	7.5
Preparation 3	1.5	10	5	10
Preparation 4	1.875	10	6.25	12.5
Preparation 5	2.25	10	7.5	1.5

Table 8: Linearity of Quinapril.

S.No.	Conc.(µg/ml)	Area
1	2.5	495.227
2	3.75	745.541
3	5	1015.117
4	6.25	1290.46
5	7.5	1470.799

Table 9: Linearity of Tolcapone.

S.No.	Conc.(µg/ml)	Area
1	5	1152.124
2	7.5	1807.304
3	10	2315.072
4	12.5	2929.514
5	15	3454.098

Table 10: Recovery results for Quinapril.

Recovery level	Accuracy Quinapril				Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	2.5	1147.472	1142.193	101.985	101.54
	2.5	1147.472			
	2.5	1131.636			
100%	5	1282.181	1287.862	103.48	
	5	1290.460			
	5	1290.945			
150%	7.5	1391.221	1388.523	99.18	
	7.5	1373.610			
	7.5	1400.738			

Table 11: Recovery results for Tolcapone.

Recovery level	Accuracy Tolcapone				Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	5	2581.774	2573.486	102.06	102.81
	5	2581.774			
	5	2556.911			
100%	10	2933.859	2948.693	105.45	
	10	2936.438			
	10	2975.781			
150%	15	3186.091	3175.224	100.94	
	15	3146.856			
	15	3192.726			

Table 12: Method precision results for Quinapril and Tolcapone.

Quinapril		
S.No.	Rt	Area
1	2.660	1109.066
2	2.667	1110.202
3	2.680	1113.271
4	2.683	1112.450
5	2.680	1108.599
6	2.690	1109.570
Avg	2.676667	1110.526
stdev	0.011057	0.1564
%RSD	0.412278	**0.3421**

Tolcapone		
S.No.	Rt	Area
1	3.890	2518.891
2	3.900	2515.559
3	3.917	2514.373
4	3.903	2512.866
5	3.913	2517.609
6	3.923	2519.468
avg	3.907667	2516.461
stdev	0.012193	0.4321
%RSD	0.311401	**0.2653**

Table 13: Robustness.

Parameter	Quinapril		Tolcapone	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.727	1.558	5.457	1.589
1.2 ml/min	2.127	1.464	3.113	1.421
Wavelength				
218nm	2.707	1.412	3.960	1.500
222nm	2.657	1.382	3.903	1.477

Table 14: Ruggedness.

Quinapril	%Assay	Tolcapone	%Assay
Analyst 01	99.516	**Analyst 01**	100.144
Anaylst 02	99.38	**Anaylst 02**	100.461

Observation: The LOQ for this method was found to be 0.98µg/ml for Quinapril and 3.79 µg/ml for Tolcapone.

Robustness

Table 13

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

Observation: From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table 14

Discussion

A simple and selective LC method is described for the determination of Quinapril and Tolcapone in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 55 volumes of water and 45 volumes of Methanol with detection of 220 nm. Linearity was observed in the range 2.5-7.5 µg/ml for Quinapril (r^2 = 0.995) and 5-15 µg /ml for Tolcapone (r^2 = 0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

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.

Conclusion

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Quinapril and Tolcapone was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

References

1. Douglas A, Skoog F, James H, Stanley RC (2007) Liquid Chromatography. In Instrumental Analysis. Cengage Learning India Pvt. Ltd, New Delhi.
2. Skoog, Holler, Crouch (2011) Liquid Chromatography. In Instrumental Analysis, Cengage Learning India, New Delhi.
3. Chatwal RG, Anand KS (2010) High Performance Liquid Chromatography. In Instrumental Methods Of Chemical Analysis. Himalaya Publishers, Mumbai.
4. Sharma BK (2005) High Performance Liquid Chromatography. In Instrumental Methods Of Chemical Analysis. Goel Publishers, Meerut.
5. Alfonso RG, Ara HDM, Glen RH, Thomas M, Nicholas GP, et al. (2000) Chromatography. In Remington: The Science and Practice of Pharmacy. Lippincott Williams & Wilkins, Philadelphia.
6. Manoj KS, Pramod KS, Sambhu CM, Preet KK, Nitin K, et al. (2011) A perspective review on method development and validation by HPLC. International Journal of Pharmaceutical Sciences 4: 1387–1413.
7. International Conference on Harmonization (1995) Q2A: Text on Validation of Analytical Procedures. Federal Register 60: 11260–11262.
8. International Conference on Harmonization (1997) Q2B: Validation of Analytical Procedures: Methodology; Availability. Federal Register 62: 27463–27467.
9. Michael Swartz E, Ira Krull S (2009) Analytical Method development. In Analytical Method Development and Validation. Marcel Dekker, Inc: New York.

Citation: Ahmed SH, Khasim SM, Babu RS, Shahnawaz J (2019) To Develop New RP-HPLC Method for the Simultaneous Estimation of Quinapril and Tolcapone in Pharmaceutical Dosage Form. J Drug Dev Del Vol: 2, Issu: 1 (01-06).