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Research Article

Analytical method development and validation for the estimation of quinapril and tolcapone using RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of Quinapril and Tolcapone 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) and 62.5- 187.5µg /ml for Tolcapone (r^2 =0.999) 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: Liquid Chromatography (LC), RSD Relative Standard Deviation, r^2 Correlation Coefficient.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis [2-7].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Quinapril and

Hydrochlorothiazide in pharmaceutical dosage form [8-15].

Plan of work

- Solubility determination of Quinapril and Hydrochlorothiazide various solvents and buffers.
- Determine the absorption maxima of the drug 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 [15-20].

METHODOLOGY

Mobile phase

A mixture of 40 volumes of mixed phosphate buffer (KH2PO4 +K2HPO4) 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.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug 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.

Preparation of standard stock solution of TOLCAPONE

50mg of TOLCAPONE 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.

RESULTS AND DISCUSSIONS

Solubility studies

These studies are carried out at 25 ^{0}C

Quinapril

Freely soluble in methanol,water and DMSO

Tolcapone

Slightly or very slightly soluble in water; sparingly soluble in alcohol; soluble in acetone; freely soluble in dimethylformamide; n-butylamine; and solutions of alkali hydroxides; insoluble in ether, chloroform, and dilute mineral acids.

Wavelength determination

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.

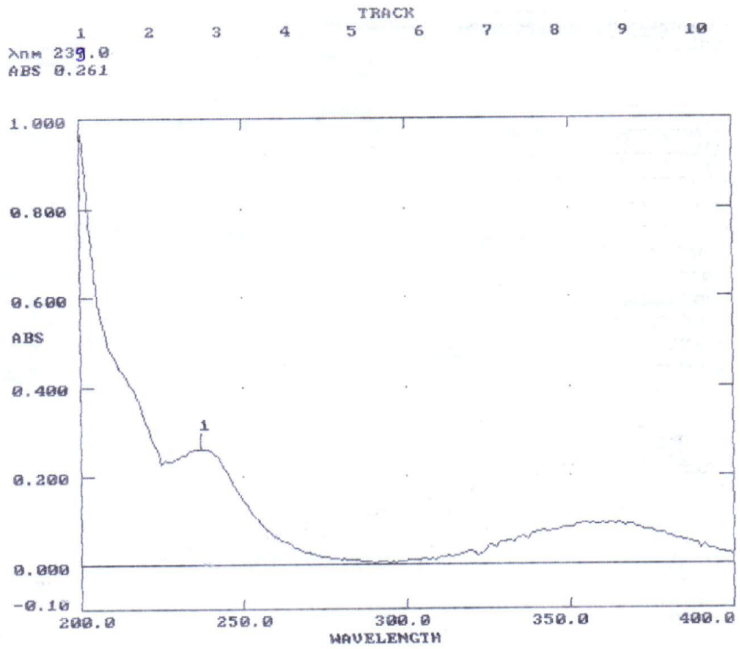
Preparation of standard stock solution of TOLCAPONE

50mg of TOLCAPONE 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.

Results

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. The resulting spectra are shown in the fig. no. 8.1 and the absorption curve shows characteristic absorption maxima at 239 nm for the combination.

Isobestic point of QUINAPRIL and TOLCAPONE



METHOD DEVELOPMENT OF QUINAPRIL and TOLCAPONE

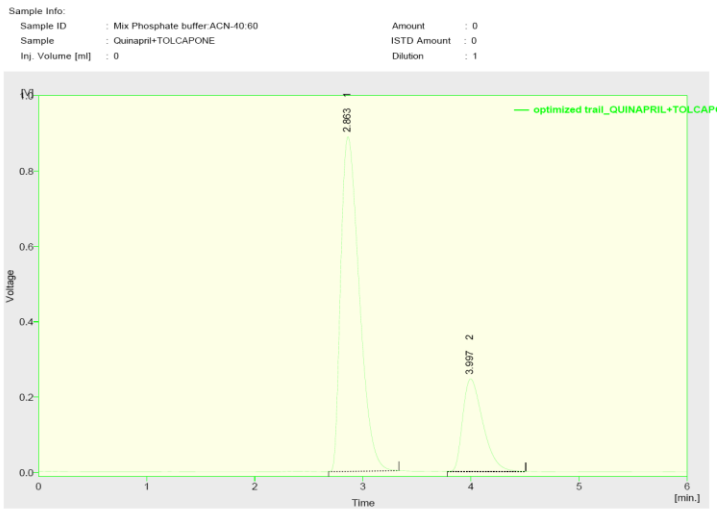
Trial- 5

Chromatographic conditions

Mobile phase : Mixed Phosphate buffer (KH^2PO4 +K2HPO4): Acetonitrile
Ratio40 : 60
Column : Zodiac, C18 (250×4.6× 5µ)
Wavelength : 239 nm
Flow rate : 1ml/min
.

Preparation of mixed standard solution

Weigh accurately 10mg of Quinapril and 12.5mg 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 10 µg/ml of Quinapril and 12.5µg/ml of Hydrochloro thaizide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



Result Table (Uncal - optimized trail_QUINAPRIL+TOLCAPONE)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.863	10576.379	887.838	76.9	78.4	0.19
2	3.997	3185.838	245.327	23.1	21.6	0.20
	Total	13762.217	1133.165	100.0	100.0	

Column Performance Table (From 50% - optimized trail_QUINAPRIL+TOLCAPONE)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.863	0.190	1.914	0.00	1258	25164	-
2	3.997	0.203	1.897	0.00	2140	42807	3.391

Observation

The peaks were good resolution and efficiency. Hence the method was optimized.

Table 8.3.8: Optimized chromatographic conditions

Mobile phase	Mixed Phosphate buffer (KH2PO4 +K2HPO4):Acetonitrile 40:60
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	239nm
Injection volume	20 µl
Run time	10 min
Retention time	About 2.86min for Quinapril and 3.99min for TOLCAPONE

ASSAY

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of Quinapril and 12.5mg of TOLCAPONE 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 TOLCAPONE is prepared by diluting 1ml of Quinapril and 1.25ml of TOLCAPONE to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5tablets (each tablet contains 10mg of Quinapril and 12.5mg of TOLCAPONE) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Quinapril (100µg/ml) and TOLCAPONE (125µg/ml) were prepared by dissolving weight equivalent to 10mg of Quinapril and 12.5mg of TOLCAPONE 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 TOLCAPONE was made by adding 1ml & 1.25ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Quinapril and TOLCAPONE 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 TOLCAPONE in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

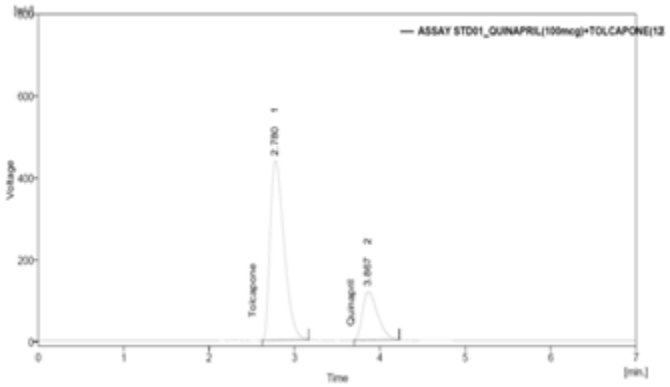


Fig: Chromatogram of Assay standard preparation-1

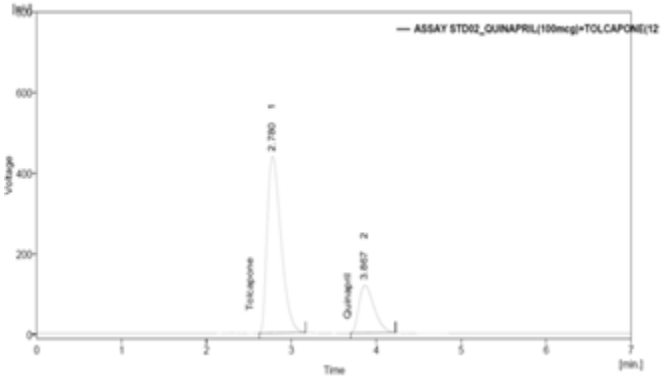


Fig: Chromatogram of Assay standard preparation-2

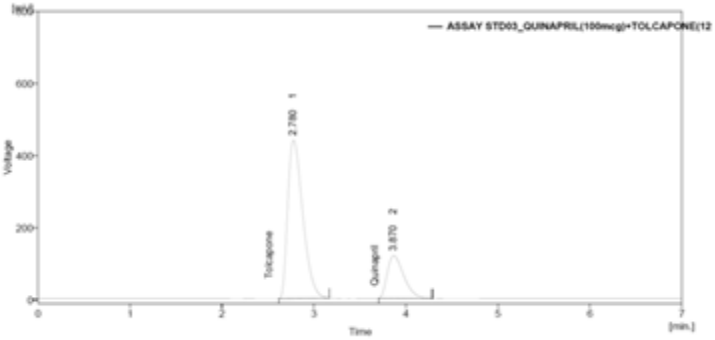


Fig: Chromatogram of Assay standard preparation-3

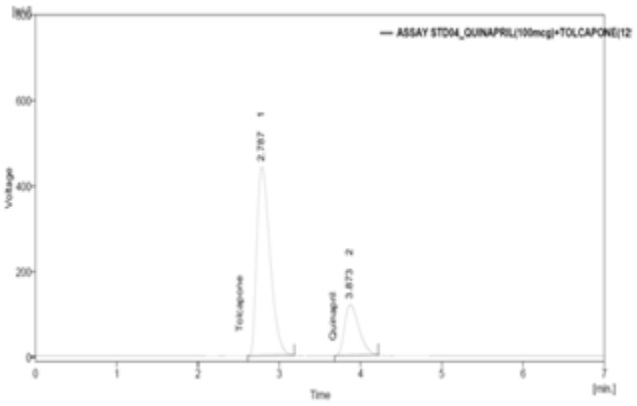


Fig: Chromatogram of Assay standard preparation-4

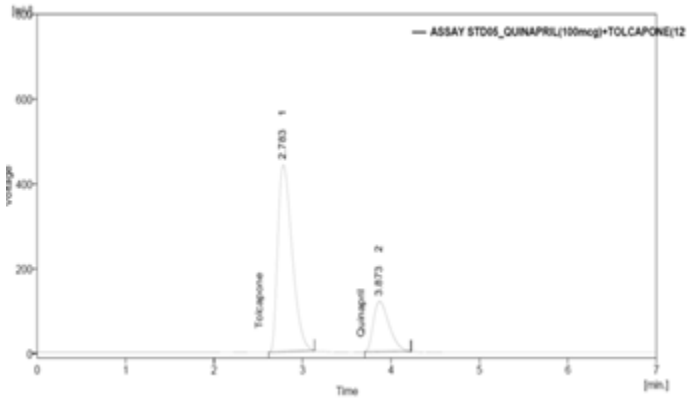


Fig: Chromatogram of Assay standard preparation-5

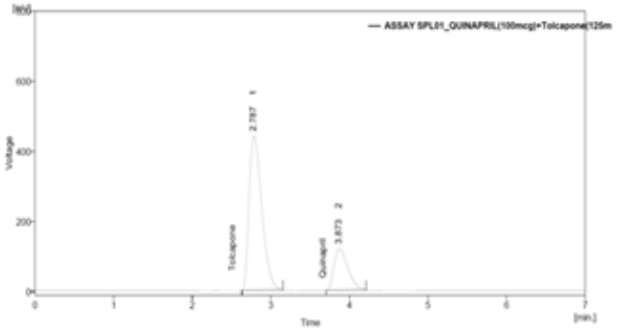


Fig: Chromatogram of Assay sample preparation-1

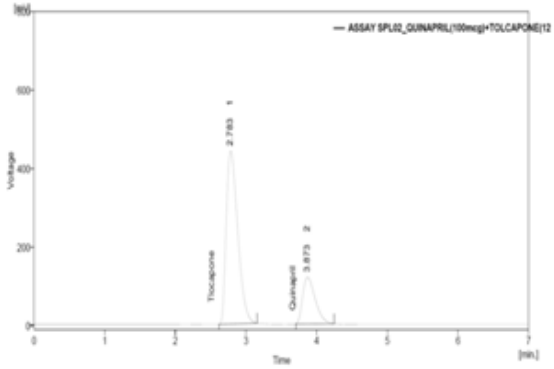


Fig: Chromatogram of Assay sample preparation-2

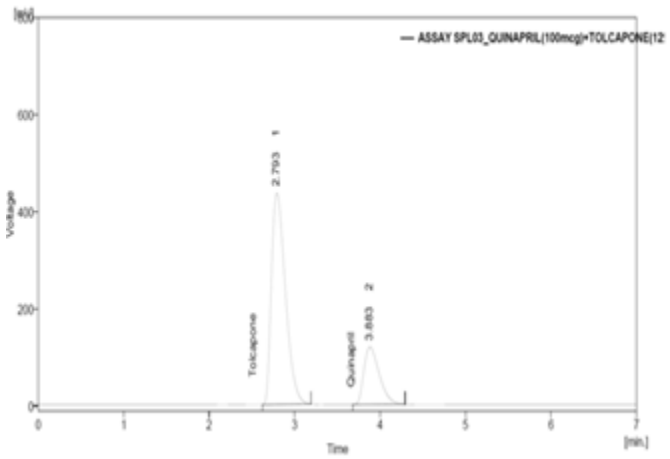


Fig: Chromatogram of Assay sample preparation-3

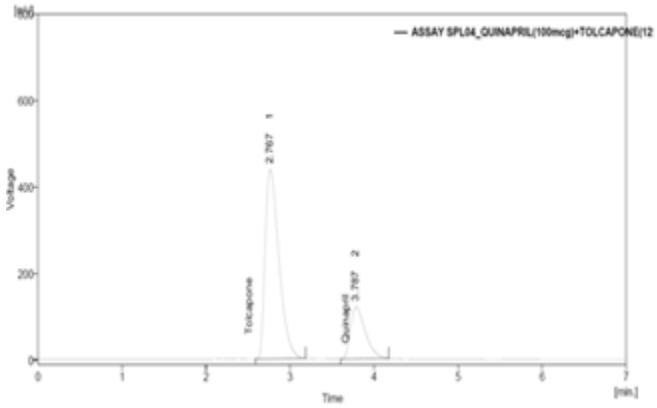


Fig: Chromatogram of Assay sample preparation-4

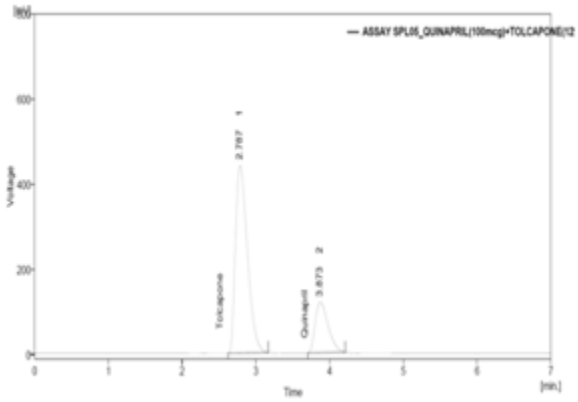


Fig: Chromatogram of Assay sample preparation-5

QUINAPRIL			TOLCAPONE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1467.356	1455.174	4976.555	4972.444
Injection-2	1467.356	1477.668	4976.555	4991.578
Injection-3	1485.812	1486.453	5000.73	4958.122
Injection-4	1470.195	1481.035	5001.709	5004.424
Injection-5	1468.436	1455.174	4973.886	4990.744

Average Area	1471.831	1471.101	4985.887	4983.462
Tablet average weight	60.25		60.25	
Standard weight	10		12.5	
Sample weight	60.25		60.25	
Label amount	10		12.5	
std. purity	99.8		99.8	
Amount found in mg	9.98		12.45	
Assay(%purity)	**99.75**		**99.75**	

Observation

The amount of Quinapril and TOLCAPONE present in the taken dosage form was found to be 99.75% and 99.75% respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of Quinapril and 12.5mg of Tolcapone 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 Tolcapone is prepared by diluting 1ml of Quinapril and 1.25ml of Tolcapone to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5tablets (each tablet contains 10mg of Quinapril and 12.5mg of Tolcapone) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Quinapril (100µg/ml) and Tolcapone (125µg/ml) were prepared by dissolving weight equivalent to 10mg of Quinapril and 12.5mg of Tolcapone 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 Tolcapone was made by adding 1ml & 1.25ml of stock solution to 10 ml of mobile phase.

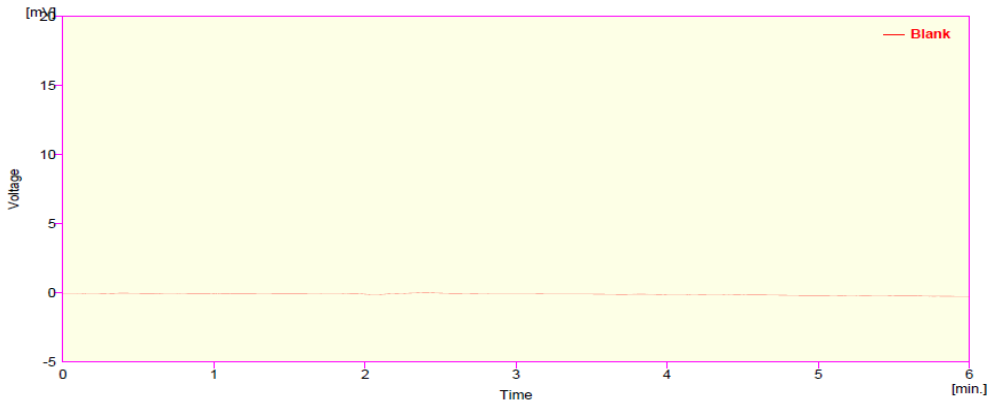
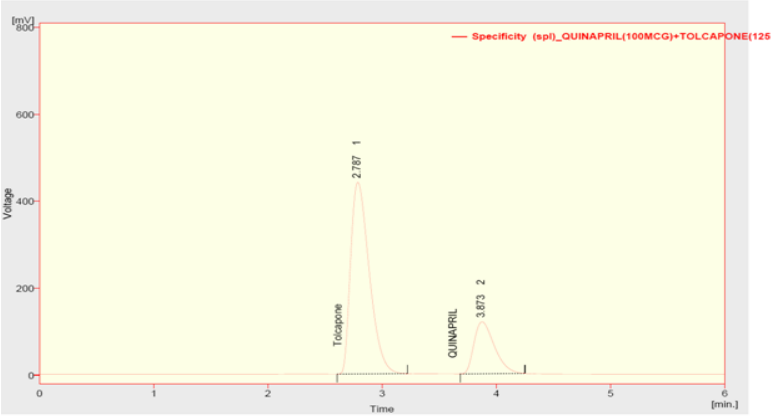
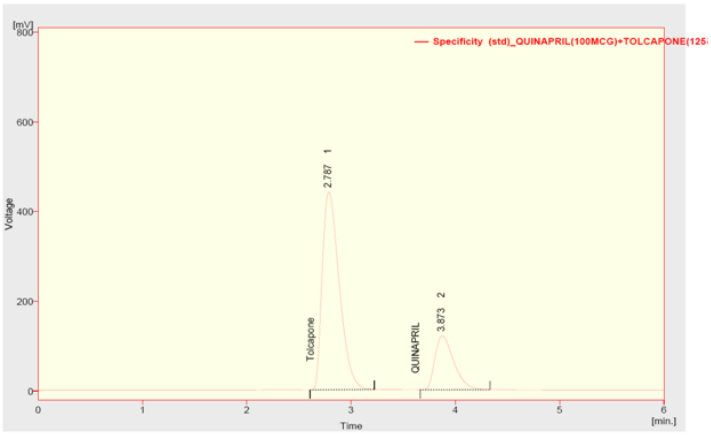


Fig: Blank chromatogram for specificity by using mobile phase



Chromatogram for specificity of QUINAPRIL & TOLCAPONE sample



Chromatogram for Specificity of QUINAPRIL & TOLCAPONE standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the QUINAPRIL & TOLCAPONE peaks.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 10 mg of QUINAPRIL and 10mg of TOLCAPONE in 10ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Table 9.3 .1: Linearity Preparations

Preparations	Volume from standard stock transferred in		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
	QUINAPRIL	TOLCAPONE		QUINAPRIL	TOLCAPONE
Preparation 1	0.5	0.625	10	50	62.5
Preparation 2	0.75	0.93	10	75	93.75
Preparation 3	1	1.25	10	100	125
Preparation 4	1.25	1.56	10	125	156.25
Preparation 5	1.5	1.87	10	150	187.5

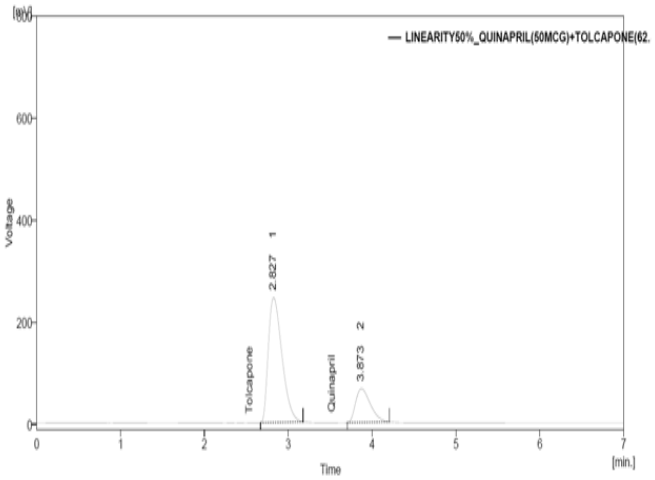


Fig: Chromatogram of Quinapril & Tolcapone preparation-1

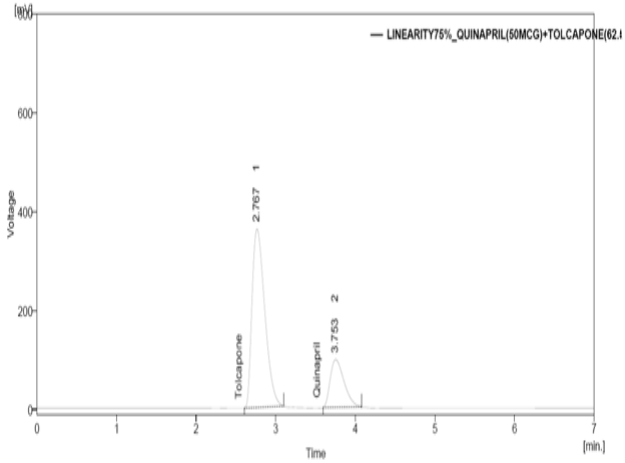


Fig: Chromatogram of Quinapril & Tolcapone preparation-2

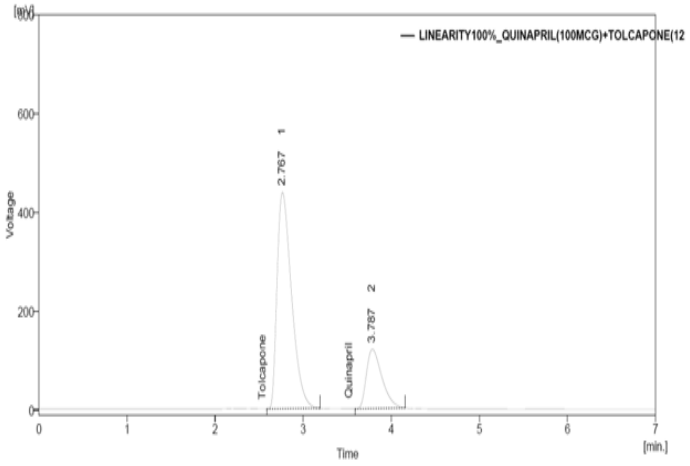


Fig: Chromatogram of Quinapril & Tolcapone preparation-3

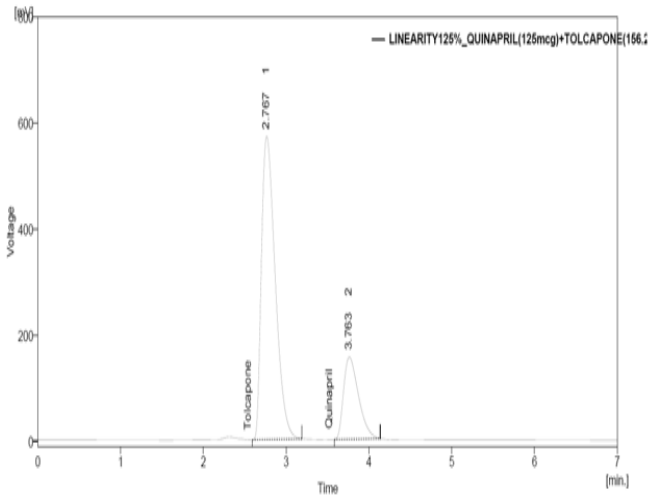


Fig: Chromatogram of Quinapril & Tolcapone preparation-4

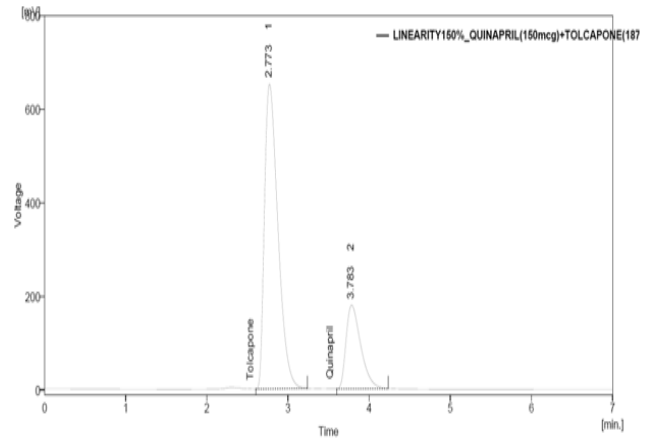


Fig: Chromatogram of Quinapril & Tolcapone preparation-5

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

Table 9.3.8: linearity of Tolcapone

S.No.	Conc.(µg/ml)	Area
1	62.5	2774.562
2	93.75	4032.779
3	125	5007.437
4	156.25	6609.492
5	187.5	7528.872

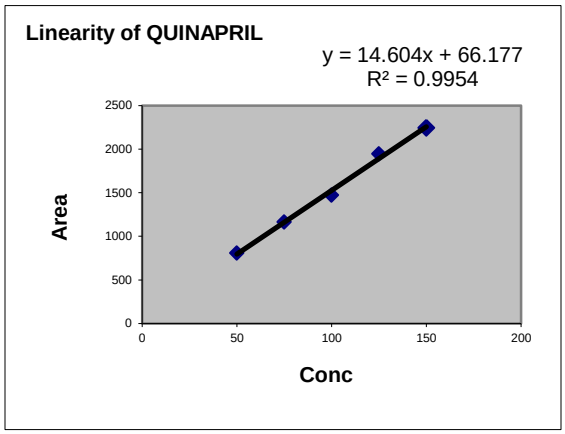


Fig. 9.3.9: Linearity graph of Quinapril

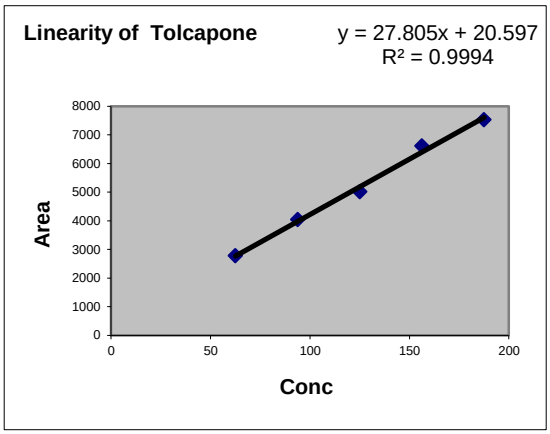


Fig. 9.3.9.1: Linearity graph of Tolcapone

Acceptance criteria

The relationship between the concentration of Quinapril and Tolcapone and area of Quinapril and Tolcapone should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Quinapril and Tolcapone is 0.995 and 0.999. The relationship between the concentration of Quinapril and Tolcapone and area of Quinapril and Tolcapone is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

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 for drug is shown in table. 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%.

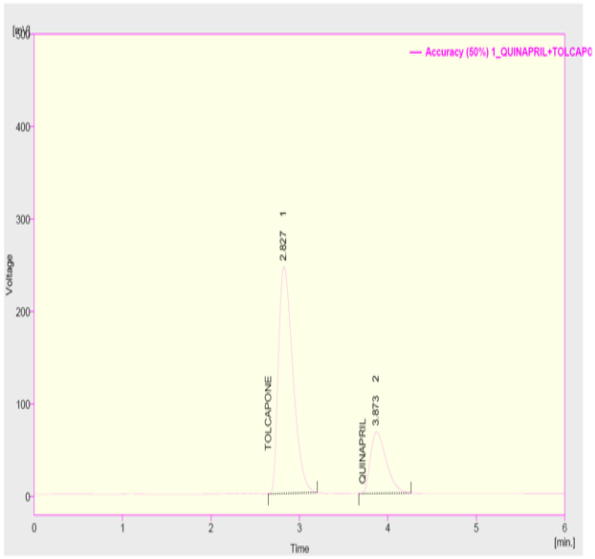


Fig: Chromatogram of 50% recovery (injection 1)

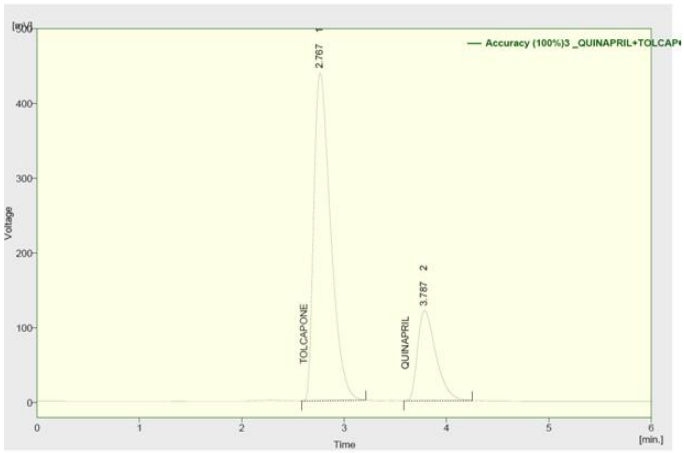


Fig: Chromatogram of 150% recovery (injection 2)

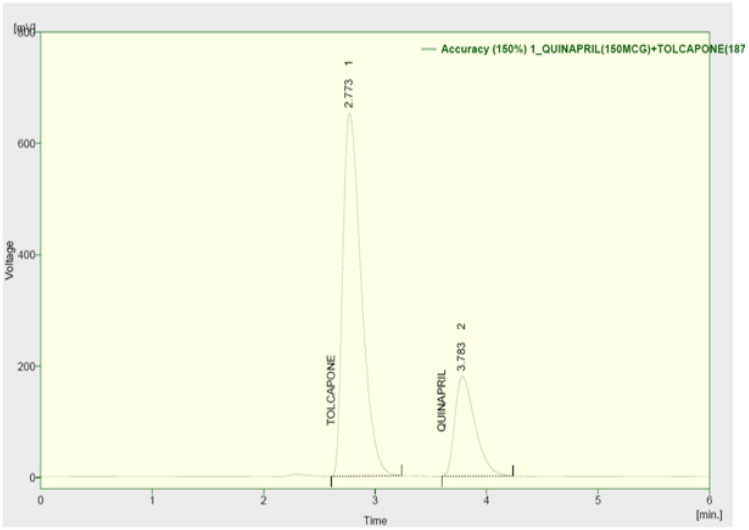


Fig: Chromatogram of 150% recovery (injection 3)

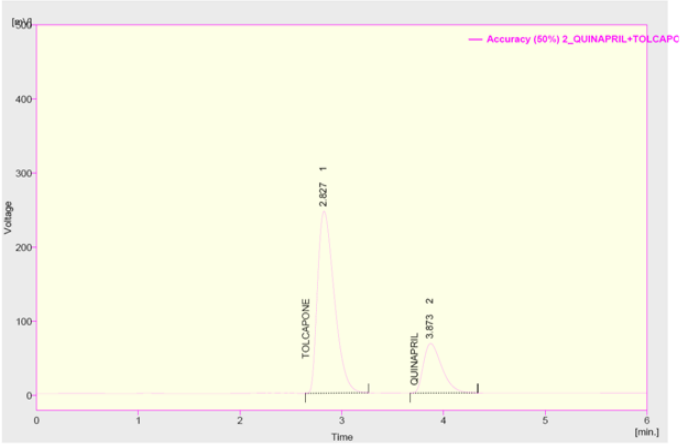


Fig: Chromatogram of 50% recovery (injection 1)

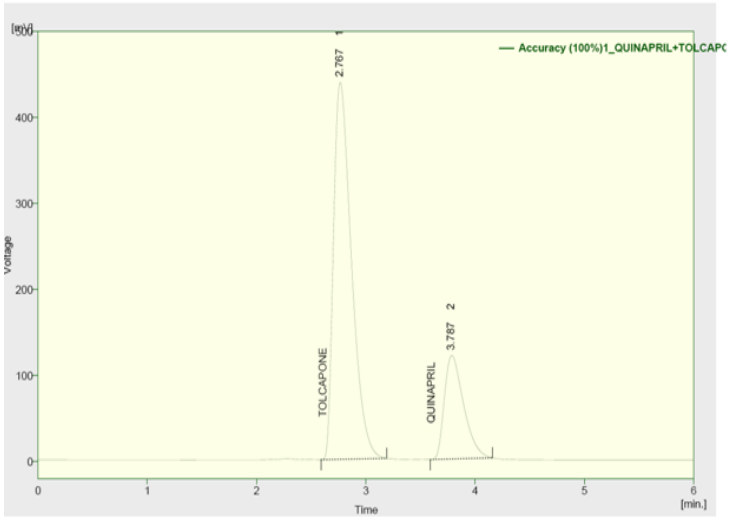


Fig: Chromatogram of 100% recovery (injection 2)

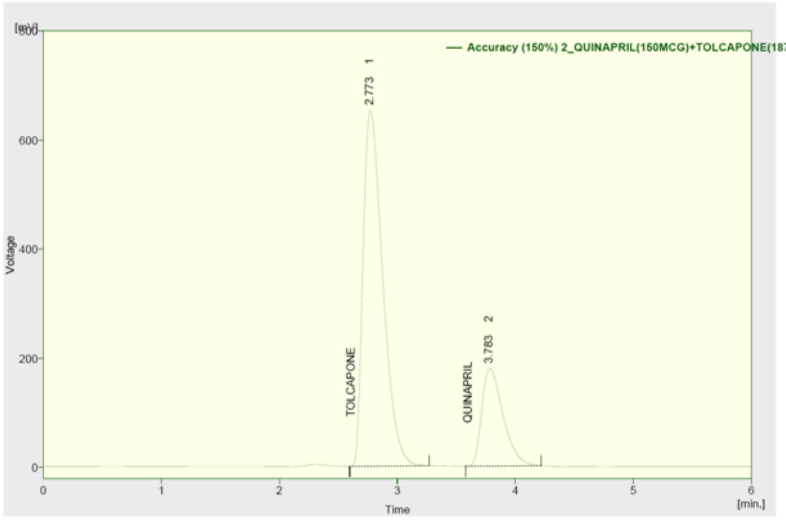


Fig: Chromatogram of 150% recovery (injection 3)

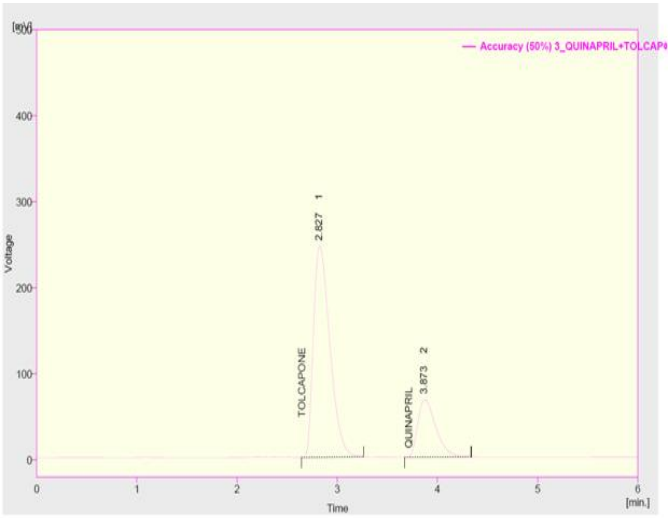


Fig: Chromatogram of 50% recovery (injection 1)

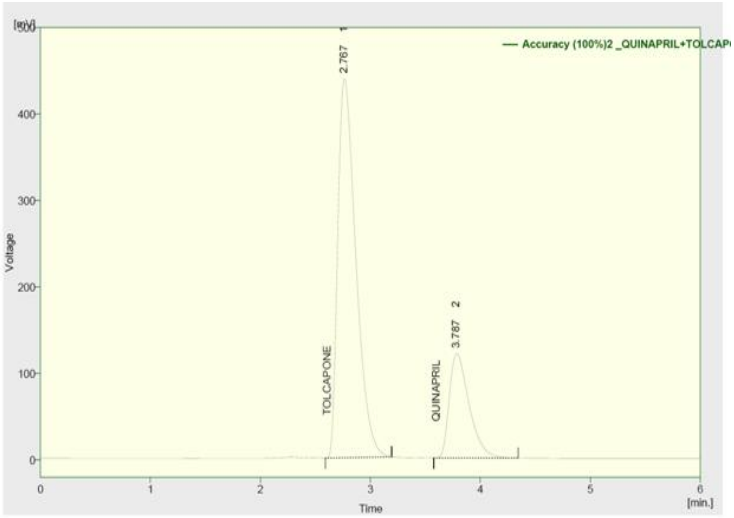


Fig: Chromatogram of 100% recovery (injection 2)

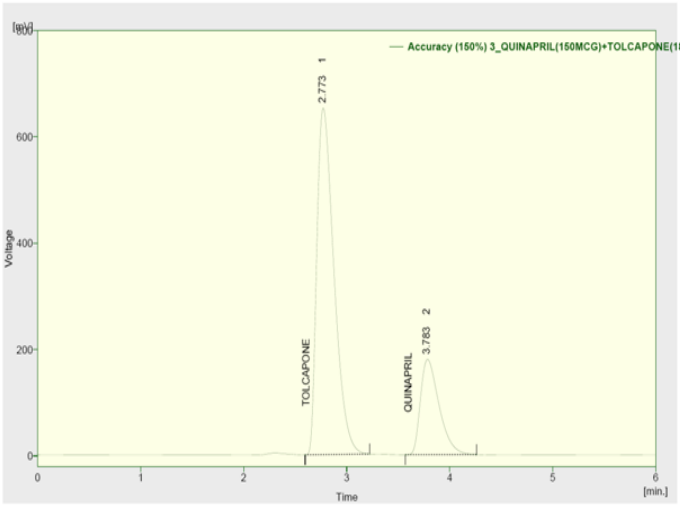


Fig: Chromatogram of 150% recovery (injection 3)

Recovery results for Quinapril

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

Table- 9.4.9.2: Recovery results for Tolcapone

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

Observation

The percentage mean recovery of Quinapril and Tolcapone is 101.02% and 99.55% respectively.

Precision

Method precision

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

Acceptance criteria

The % Relative standard deviation of Assay preparations of Quinapril and Tolcapone should be not more than 2.0%.

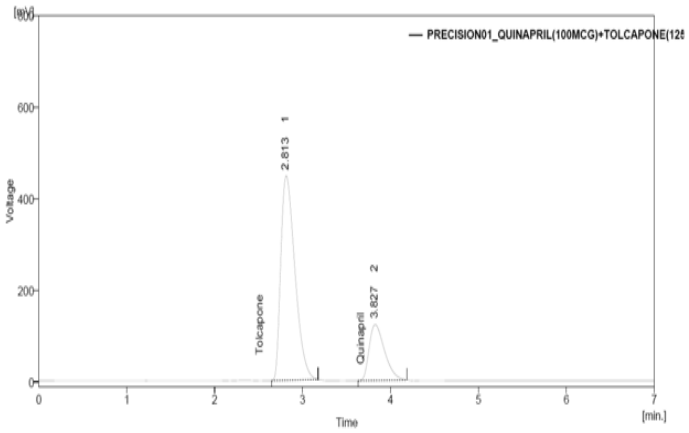


Fig: Chromatogram of precision injection 1

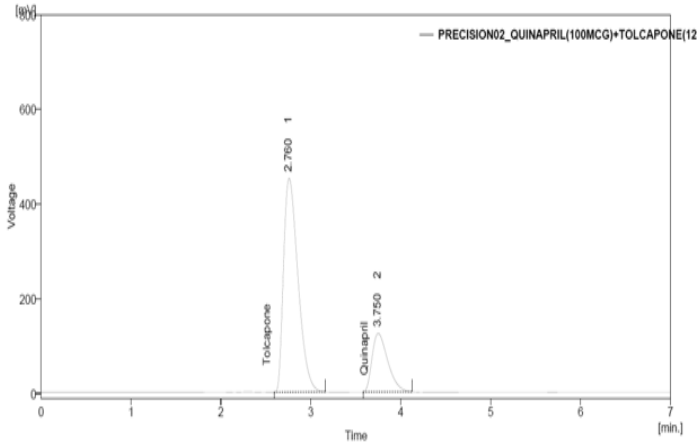


Fig: Chromatogram of precision injection 2

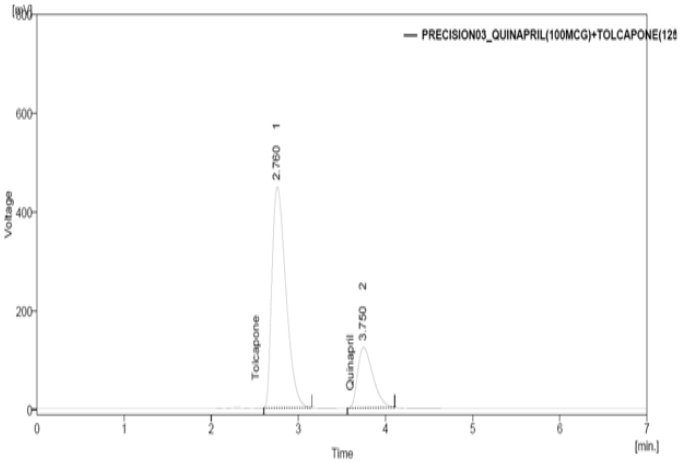


Fig: Chromatogram of precision injection 3

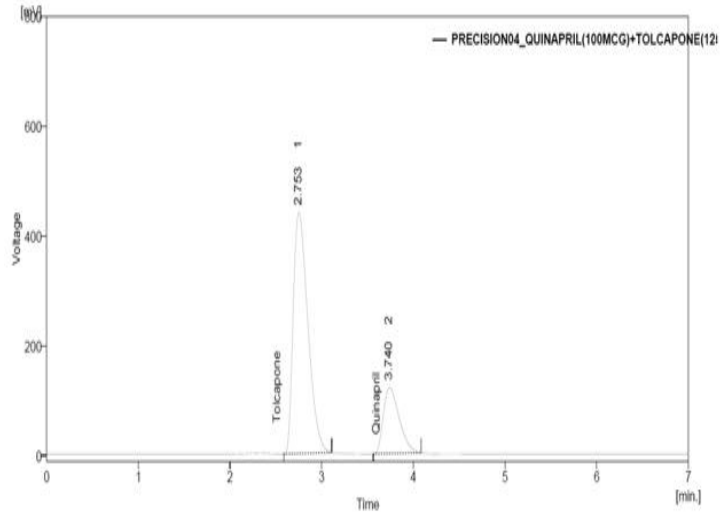


Fig: Chromatogram of precision injection 4

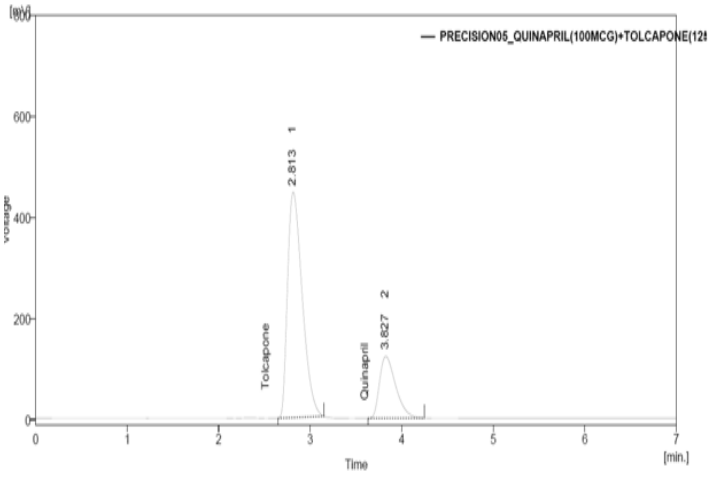


Fig: Chromatogram of precision injection 5

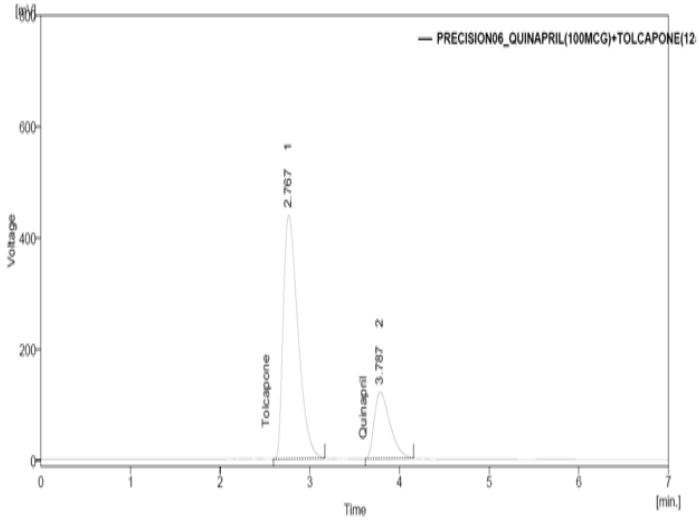


Fig: Chromatogram of precision injection 6

Quinapril			Tolcapone		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	3.827	1487.147	1	2.813	5005.745
2	3.750	1496.768	2	2.760	5044.357
3	3.750	1482.466	3	2.760	4993.823
4	3.740	1448.567	4	2.753	4890.628
5	3.827	1505.906	5	2.813	4976.613
6	3.787	1463.248	6	2.767	4997.266
avg	3.7802	1484.171	**avg**	2.778	4982.233
stdev	0.0397	21.855	**stdev**	0.028	56.942
%RSD	**1.05**	**1.47**	**%RSD**	**1.00**	**1.56**

Observation

Test results for Quinapril and Tolcapone are showing that the %RSD of Assay results are within limits.

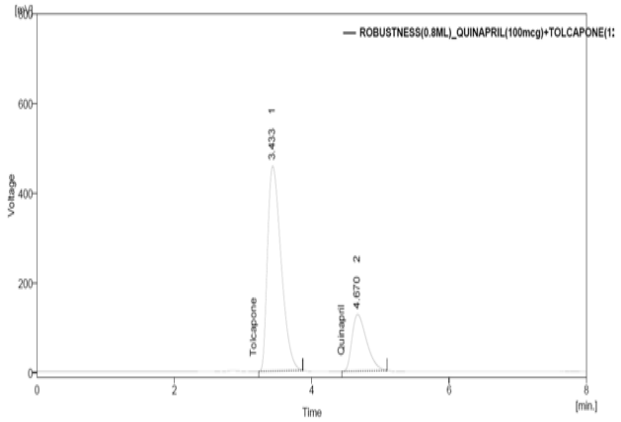
Robustness

Chromatographic conditions variation

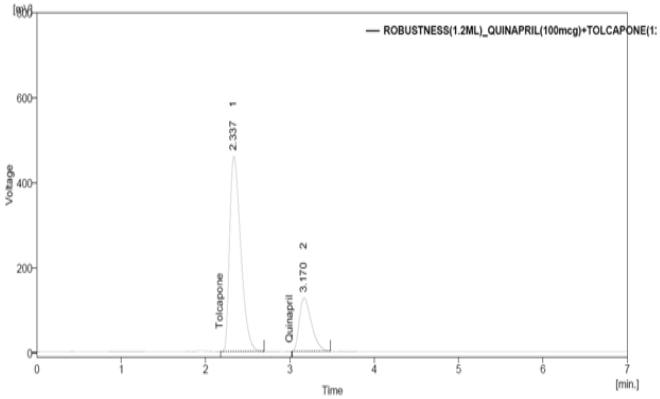
To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

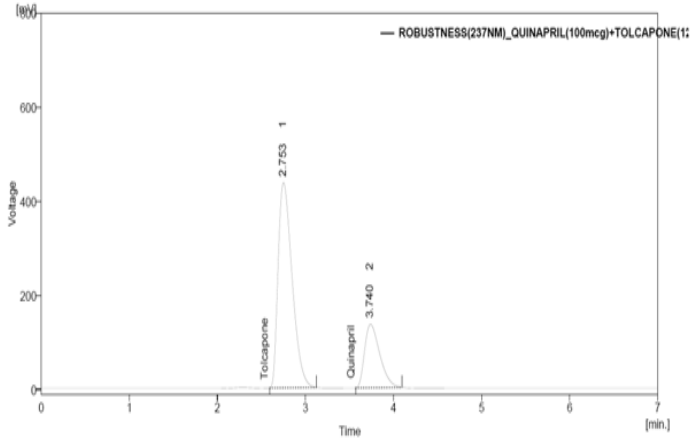
The system suitability should pass as per the test method at variable conditions.



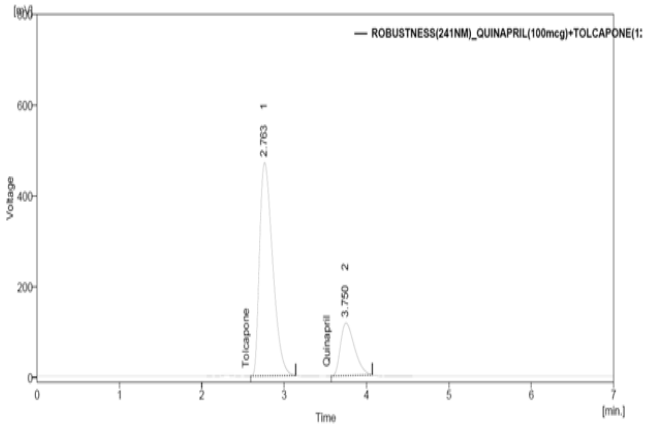
Chromatogram of Quinapril & Tolcapone Robustness (0.8 ml/min)



Chromatogram of Quinapril & Tolcapone Robustness (1.2 ml/min)



Chromatogram of Quinapril & Tolcapone Robustness (237 nm)



Chromatogram of Quinapril & Tolcapone Robustness (241 nm)

Result of Robustness study

Parameter	Quinapril		Tolcapone	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow				
0.8ml/min	4.670	1.902	3.433	1.902
1.0 ml/min	3.870	1.821	2.780	1.882
1.2ml/min	3.170	1.781	2.337	1.857
Wavelength				
237nm	3.740	1.838	2.753	1.824
239nm	3.870	1.821	2.780	1.882
241nm	3.750	1.861	2.763	1.909
				2.763

Observation

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

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

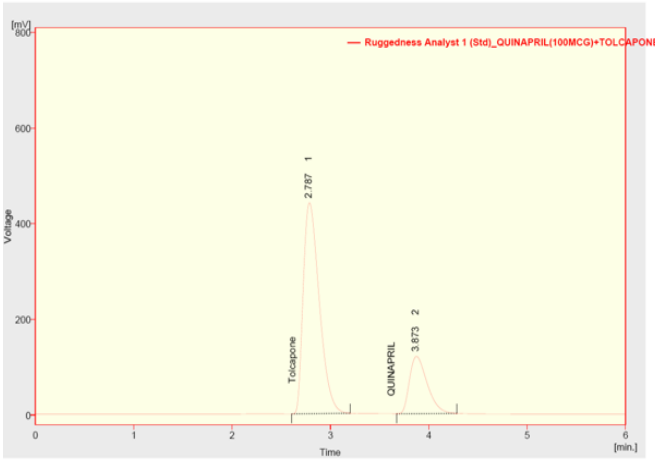


Fig: Chromatogram of Analyst 01 standard preparation

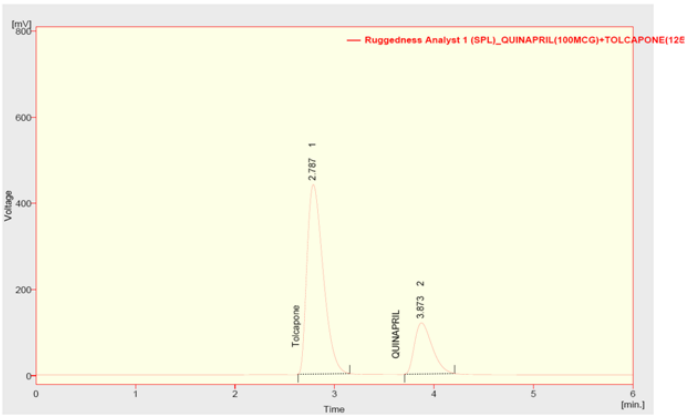


Fig: Chromatogram of Analyst 01 sample preparation

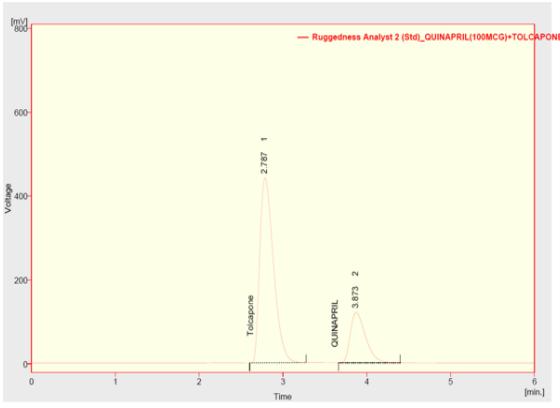


Fig: Chromatogram of Analyst 02 standard preparation

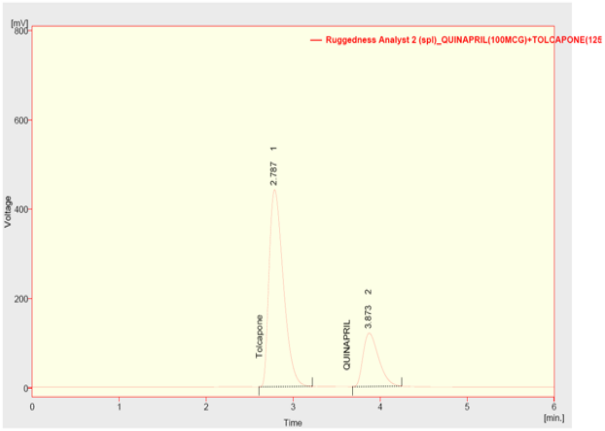


Fig: Chromatogram of Analyst 02 sample preparation

Results for Ruggedness

Quinapril	%Assay	Tolcapone	%Assay
Analyst 01	97.99	Analyst 01	99.96
Analyst 02	98.37	Analyst 02	97.59
%RSD	0.27	%RSD	1.69

OBSERVATION

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

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