RESEARCH ARTICLE Am. J. PharmTech Res. 2015; 5(1) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

A Stability Indicating ultra Performance Liquid Chromatography Method for Simultaneous Estimation of Verapamil Hydrochloride and Trandolapril in Combined Dosage Formulations

Vani Pusapati*[1], K. Kalyana Seela[2]
1. Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, India.
2. United States Pharmacopieal convention, Shameerpet, Hyderabad-500078.

ABSTRACT

An accurate, rapid, specific and stability indicating ultra performance liquid chromatographic method was developed and validated for the simultaneous estimation of Verapamil and Trandolapril in a combined pharmaceutical dosage form. The chromatographic separation was attained on Phenonemex Luna C_{18} (4.0 x 100 mm, 2.6μm) column by isocratic mode with the mobile phase components as 0.03M monobasic potassium phosphate buffer (pH6.5) and acetonitrile (70: 30v/v) at a flow rate of 1.0 mL/min and quantified at 210 nm. The average retention times for Trandolapril and Verapamil were 0.60 and 1.14 min, respectively. The UPLC method proposesoutstanding separation of two drugs with a good resolution of greater than 2.0 and tailing factor less than 2.0 with a run time of 3 minutes. The method shows linearity over the concentration range of 9-45 μg/mL for Verapamil and 0.1-0.5 μg/mL for Trandolapril with a correlation ≥ 0.999. The method is accurate with recoveries in the range of 98.0 -101.0% and precise with %RSD value lesser than2.0% for both the drugs. This method is very fast, cost saving, accurate and specific for the assay of commercially available tablets.

Keywords: Verapamil Hydrochloride (VER) and Trandolapril (TRD), Validation, Tablets, High performance liquid chromatography (HPLC) and Ultra performance liquid chromatography (UPLC)

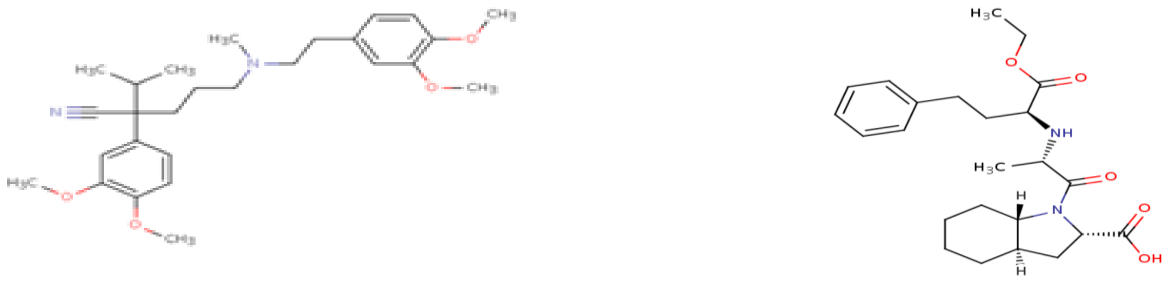
*Corresponding Author Email: vani_poosapati@yahoo.in

Received 27July 2014, Accepted13 August2014

Please cite this article as: Pusapati V *et al.*, A Stability Indicatingultra Performance Liquid Chromatography Method for Simultaneous Estimation of Verapamil Hydrochloride and Trandolapril in Combined Dosage Formulations. American Journal of PharmTech Research 2015.

INTRODUCTION

Verapamilis chemically 2-(3,4-dimethoxyphenyl)-5-{[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino}-2-(propan-2-yl)pentanenitrile[1]. It is a calcium channel blocker which is a class IV anti arrhythmia agent. Verapamil is an L-type calcium channel blocker which also possess anti arrhythmic activity. Verapamil reduces the voltage based calcium channels. Exclusively its effect on L-type calcium channels in the heart originates a diminution in ionotropy and chronotropy, thus decreases rate of heart beat and blood pressure. Effect of Verapamil in cluster headache problem can be attributed to its calcium-channel blocker property. Trandolapril is chemically (2S,3aR,7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydro-1H-indole-2-carboxylic acid[1]andbelongs to the class of ACE inhibitors. Generally it is metabolized to its Trandolaprilat in the liver which is a biologically active form. Trandolaprilat will inhibit ACE, the enzyme which converts angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a main part of the renin-angiotensin-aldosterone system. Trandolapril can be used to cure moderate hypertension, to recover endurance in patients with left ventricular dysfunction, as an adjunct healing for congestive heart breakdown, and to reduce the rate of advancement of the disease in hypertensive people with diabetes. The Chemical structures of Verapamil and Trandolaprilare given in Figure.1 (a) and (b).There are some spectroscopic, Thin layer chromatography[2]and chromatographic methodsreported for the quantitation of Verapamil[3,4]and Trandolapril[5,6]individually, and in combination[7,8]. Also some are reported in for biological fluids[9,10]. There are very few stability indicating chromatographic separation methods[11]wereavailable for the combination of trandolapril and Verapamil. But none of the stability indicating UPLC method is available for the estimation in combination pharmaceutical dosage form of Verapamil and Trandolapril. The present paperillustrates an easy, fast, precise, specific and accurate isocratic stability indicating UPLC method for the simultaneous determination of Verapamil and Trandolaprilin tablet dosage form.



(a) **(b)**

Figure. 1 Chemical structures of (a) Verapamil and (b) Trandolapril

MATERIALS AND METHOD

Ultra performance liquid chromatography equipped with auto Sampler and PDA detector separation module was used for the study with data acquisition EMPOWER software. The chromatographic separation was achieved on Phenonemex Luna C18 (4.0 x 100 mm, 2.6µm) column by isocratic elution mode with two mobile phase components with a combination of0.03M monobasic potassium phosphate buffer (pH 6.5) and acetonitrile in the ratio of 70:30. Mobile phase was operated at a flow rate of 1.0 mL/min and PDA detector was operated at 210 nm. The column temperature was maintained at ambient condition and an injection volume of 2µL was used.

General procedure

Preparation of pH 6.5 Buffer

0.03 M Potassium phosphate solution was prepared and adjusted to a pH of 6.5 with orthophosphoric acid. The solution was filtered through 0.45µ filter.

Preparation of Mobile phase

Mobile phase was prepared by the addition of 70 volumes of buffer and 30 volumes of acetonitrile. The mobile phase was subjected to ultra sonication for 5 minutes, filtered through 0.45µm membrane filter and degassed.

Preparation of Standard stock solutions

Prepared stock solutions of Trandolapril at 0.1mg/mL of and Verapamil at 0.9 mg/mL individually in the mobile phase.

Preparation of standard solution

Further dilutions were made to get a 0.3 µg/mL of Trandolapril & 27 µg/mL of Verapamil combined solution from the stock solution in the mobile phase. The solutions are injected and the chromatograms are shown in Figure 2A to Figure 2C.

Preparation of sample solution for the assay of Tablets

Twenty tablets of Trandolapril & Verapamil were weighed and their average weight was calculated. Accurately weighed and transferred an amount of Tablet powder equivalent to 0.1 mg and 9 mg of Trandolapril & Verapamil into a 100 mL volumetric flask, added 70mL of mobile phase, sonicated to dissolve and was made up to the mark with the mobile phase, filtered through 0.45 µm membrane filter. Further 3.0 ml of the above stock solution is taken in to a 10ml volumetric flask and dilute up to the mark with the mobile phase. The solution is injected in chromatographic system and the chromatogram is shown in Figure.3

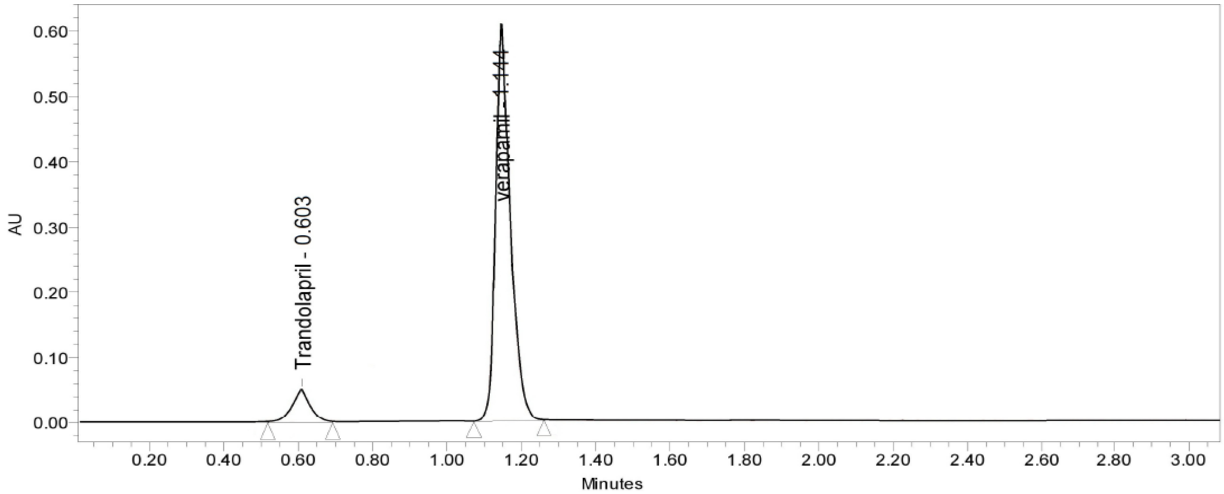


Figure. 2A Chromatogram of Verapamil and Trandolapril

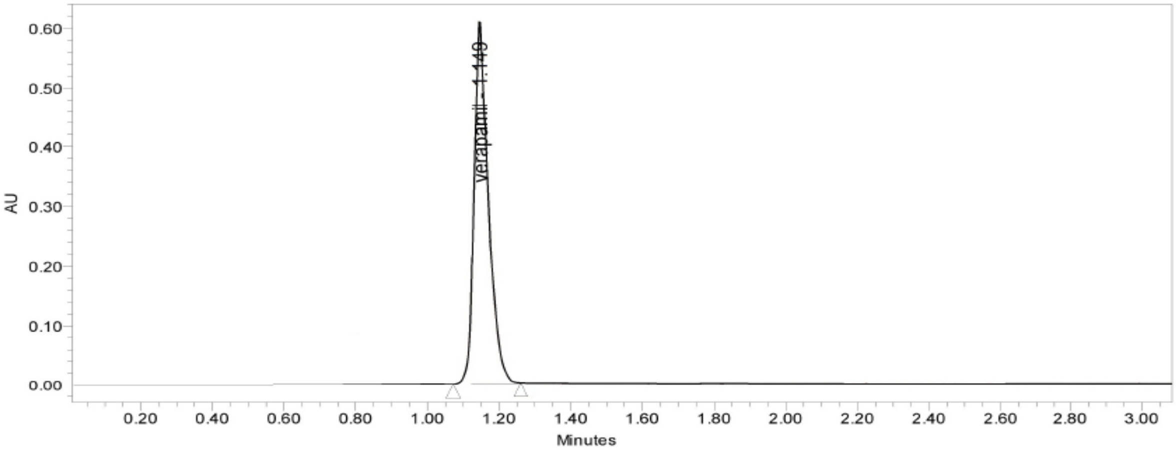


Figure. 2B Chromatogram of Verapamil

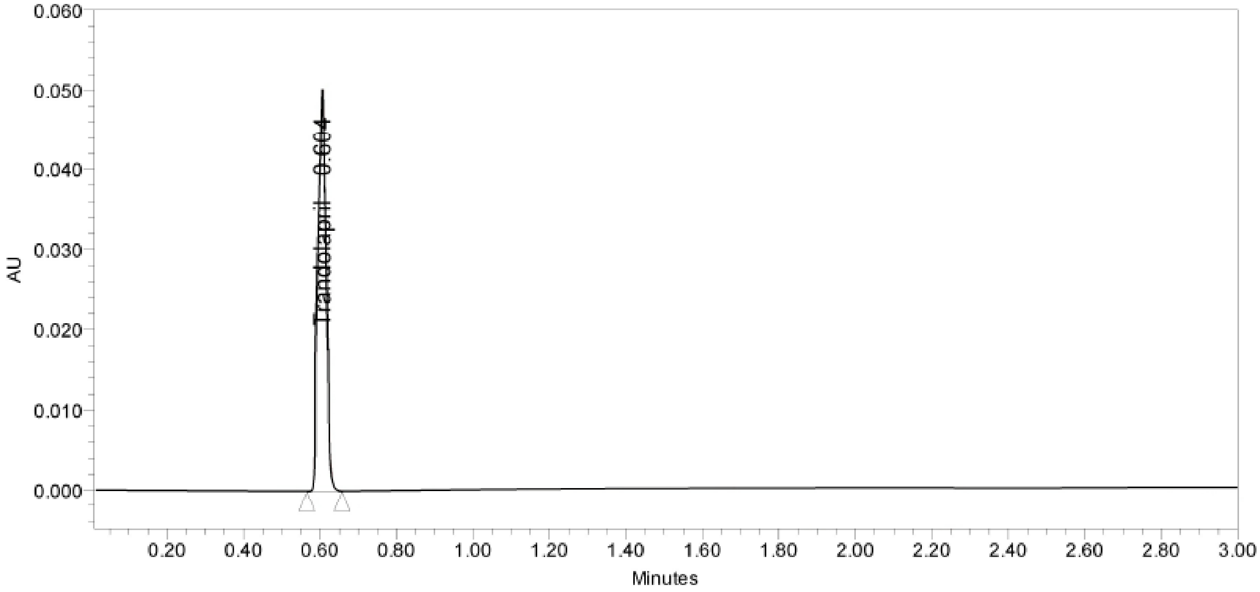


Figure. 2C Chromatogram of Trandolapril

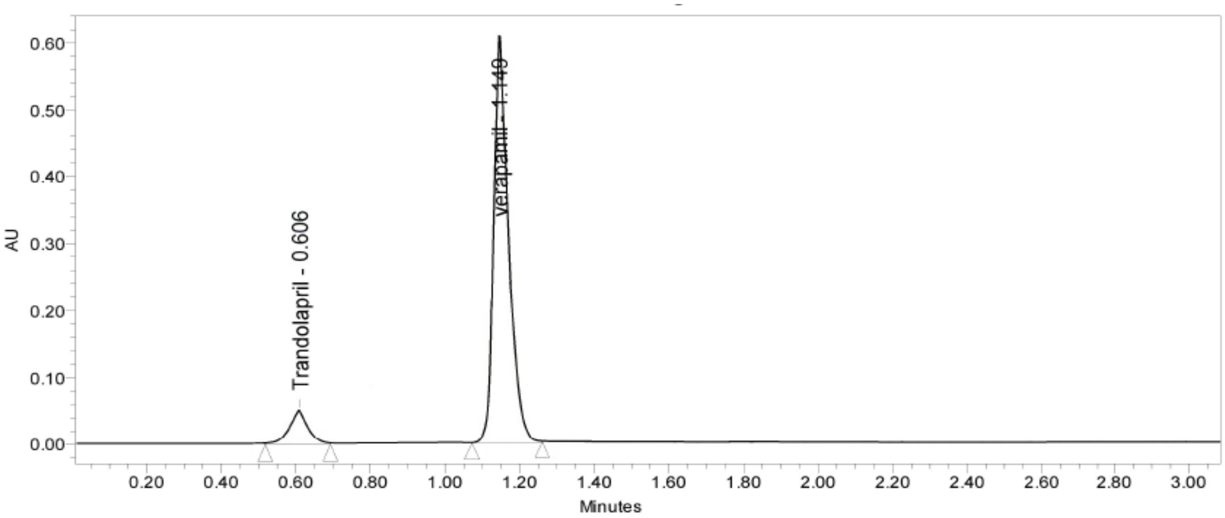


Figure. 3 Chromatogram of combined dosage form

Method Validation

System suitability

System suitability parameters were verified by injecting the standard solution 5 times into the UPLC and system suitability parameters were calculated.

Specificity

Specificity of the method was shown by injecting blank, placebo, stress degraded solutions and mobile phase into the liquid chromatographic system and the chromatograms were integrated. The chromatograms were shown as Figure 4A to Figure 4F.

Method precision

Precision of the method was established by preparing the six individual sample solutions as per the test procedure mentioned in I.1and injecting them into the liquid chromatographic system. The %Assay values of the six determinations and the %RSD value for the assay values were determined.

Intermediate precision

Intermediate precision was verified by preparing the sample solutions six times on two different days by the two different columns and injecting them into the liquid chromatographic system. The %Assay values of the six determinations and the %RSD value for the assay values were determined.

Linearity

The Linearity was verified at 5 concentration levels for both the drugs. Standard stock solutions were prepared at 10 µg/mL of Trandolapril 90µg/mL of Verapamil. The solutions for linearity were prepared by transferring 0.1mL, 0.2mL, 0.3mL, 0.4mL and0.5mL of standard stock solutions of Trandolapril and 1.0mL, 2.0 mL, 3.0 mL, 4.0 mL and 5.0 mL of standard stock solutions of Verapamil into 10mL volumetric flask and diluted up to the mark with the mobile phase to get a concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 µg/mL of Trandolapril and 9, 18, 27, 36 and 45 µg/mL of Verapamil.

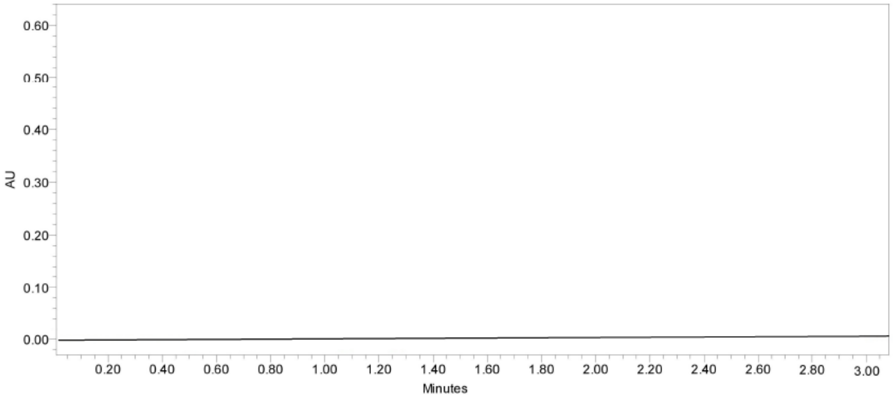


Figure. 4A Chromatogram of Blank

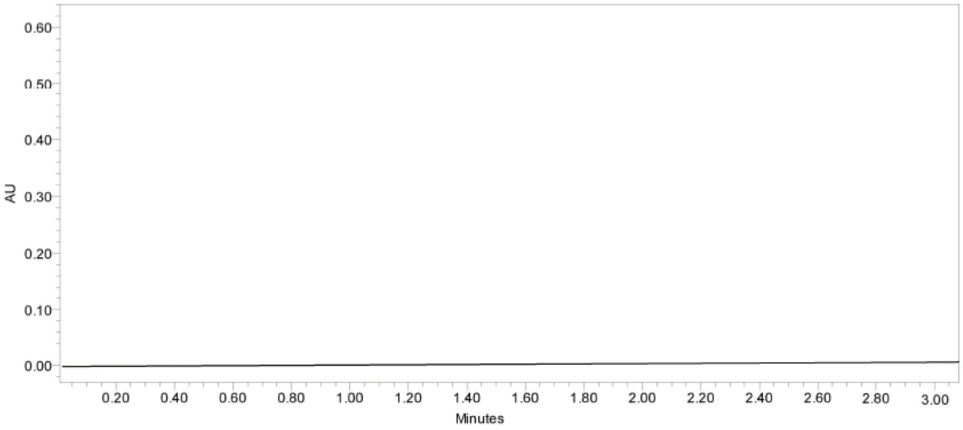


Figure. 4B Chromatogram of Placebo

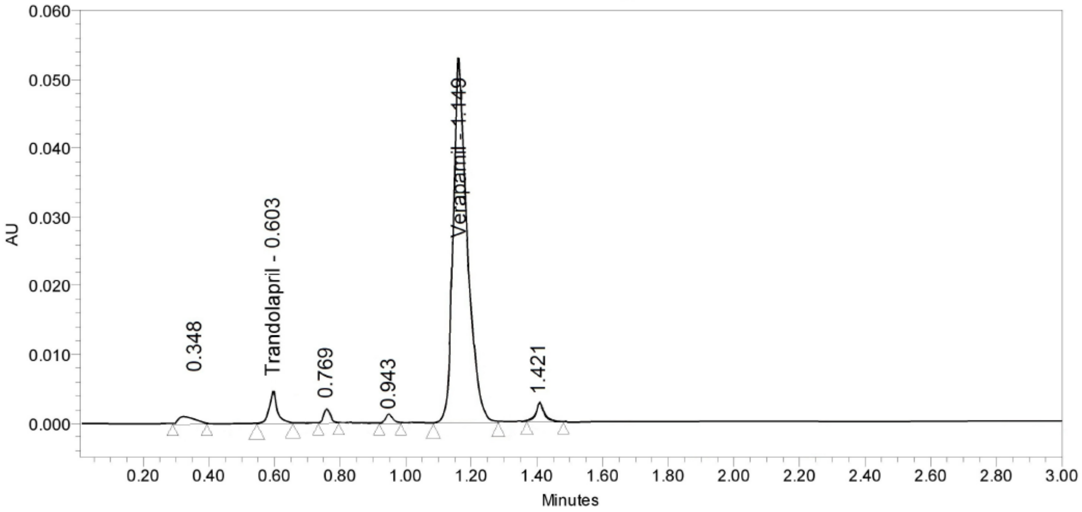


Figure. 4C Chromatogram of Acid Degradation

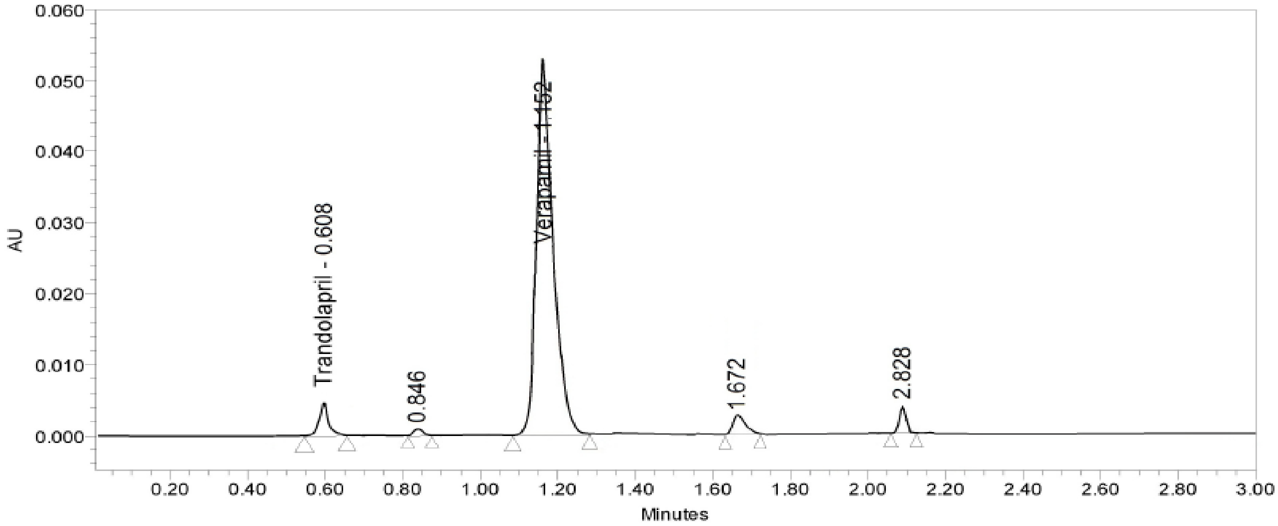


Figure. 4D Chromatogram of Base Degradation

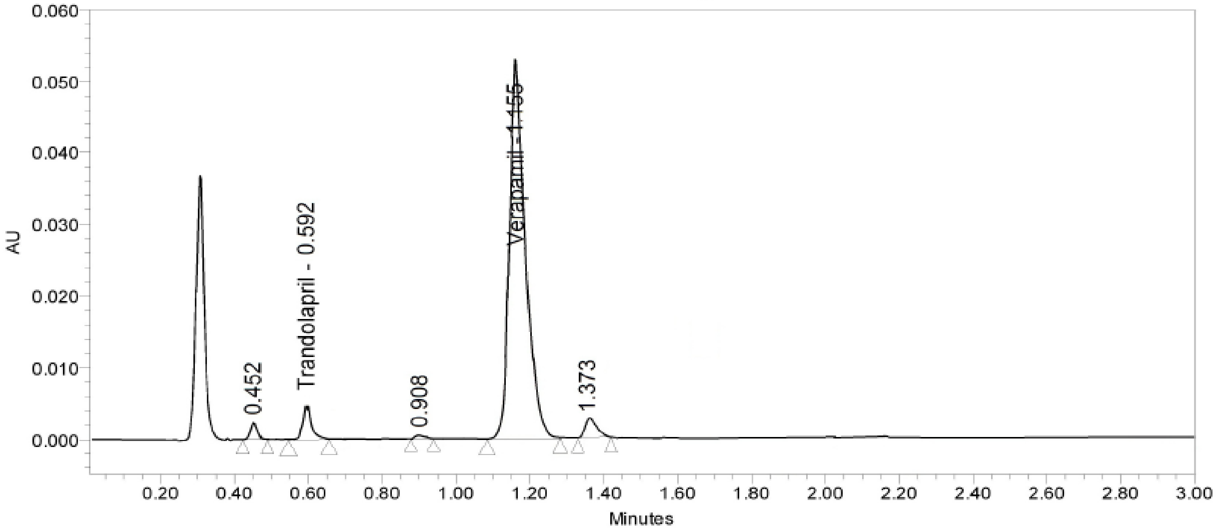


Figure. 4E Chromatogram of Oxidative Degradation

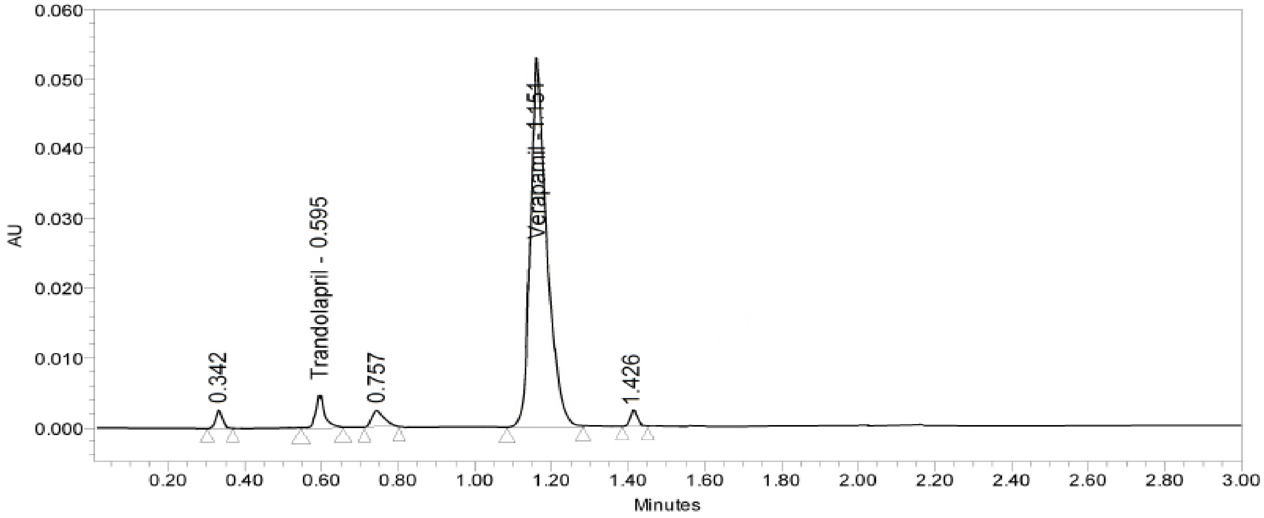


Figure. 4F Chromatogram of Thermal Degradation

Accuracy

The accuracy of the method was estimated in triplicate at three different concentration levels (50, 100, and 150%). The percentage recovery for each drug substance was calculated by preparing standard solution at the three concentration levels.

Robustness

The robustness of the proposed analytical procedure was calculated by altering in the method parameters like flow rate, column temperature and mobile phase composition. The system suitability parameters and the %Assay values were determined.

Limit of Detection and Limit of Quantitation

Trandolapril at 0.004 µg/ml and Verapamil at 0.02µg/ml were prepared in mobile phase for LOQ and Trandolapril at0.001 µg/ml and Verapamil at 0.003µg/ml were prepared in mobile phase for LOD based on the least concentration level in linearity. From these solutions LOD and LOQ were calculated.

Solution stability

The stability of the solutions was determined by injecting blank, placebo, standard and sample solutions which were kept at room temperature for a particular period of time into liquid chromatographic system. %Assay of each drug was estimated at 3 hours, 6 hours, 12 hours, 14 hours, and 16hours.

Assay of the Tablets

Sample solutions were injected into the liquid chromatographic system and the %Assay was determined.

Forced Degradation Studies

The standard solution was prepared which contains1µg/mL of Trandolapril and 9µg/mL of Verapamil was exposed to stress conditions[12,13] like acid, base, Hydrolytic and thermal. To the 3ml of the standard solution, 3 ml of 0.1N Hydrochloric acid was added in a 10ml volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N sodium hydroxide and made up to 10ml with mobile phase. Filtered the solution with 0.45 µm syringe filters and injected in the chromatographic system. The base degraded solution also prepared by taking 3ml of standard solution, 3 ml of 0.1 N sodium hydroxide was added in a 10ml volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N Hydrochloric acid and made up to 10ml with mobile phase. Filtered the solution with 0.45 µm syringe filters and injected in the chromatographic system. The hydrolytic or oxidative degradation was carried out in 3% hydrogen peroxide at ambient temperature for 15minutes. The standards of

Trandolapril and Verapamil were taken in petridish and kept in hot air oven at 110°C for 6 hours as a part of thermal degradation condition.

RESULTS AND DISCUSSION

HPLC method development

The HPLC conditions were optimized with a view to develop a suitable LC method for the determination of Trandolapril and Verapamil in combined tablet dosage forms. Phenonemex Luna C_{18} (4.0 x 100 mm, 2.6µm) column was selected based on the polarity of the Trandolapril and Verapamil. The 0.03M pH6.5 mono basic potassium phosphate buffer was selected based on the pKa of Trandolapril(5.21) and Verapamil(9.04). Primarily various ratios of the buffer and acetonitrile were tried and the 30:70v/v/v of buffer: acetonitrile was found to be the optimized method. The flow rate of the mobile phase was adjusted to 1.0mL/min to get resolution greater than 2.0, tailing between 0.9-1.1and theoretical plates greater than 2000. The column temperature was kept at ambient.

VALIDATION

System suitability

The %RSD value for the peak areas and retention times for both the peaks were calculated and found to be less than 2%. The theoretical plate values were found to be greater than 2000 and the tailing factor was less than 2.0 for both the peaks. The chromatogram is shown in Figure .2and the results were tabulated in Table 1.

Table 1: System suitability Results standard solution of Trandolapril and Verapamil

System Suitability Results		
	RD	ER
USP Resolution	6.55	N/A
USP Tailing	1.5	1.3
USP Plate count	3595	5982
%RSD for the peak areas from replicate injections	0.79	0.42

Method precision and Intermediate precision

The % assay values for method precision and intermediate precision were found to be in between 98.0% - 102.0% for both the drugs. The %RSD value for the % assay values of both the method precision and intermediate precision was less than 2.0. The results were given in Table 2A and Table 2B.

Table 2A: Precision Results for the % Assay of Trandolapril and Verapamil

Precision	**TRD**	**VER**
Method Precision	100.18	98.59
% RSD	0.80	0.51

Table 2B: Intermediate Precision Results for % Assay Trandolapril and Verapamil

Intermediate Precision	TRD	VER
Method Precision	99.98	98.50
%RSD	0.37	0.28

Linearity

Calibration plots were constructed based on peak area versus concentration. The method was found to be linear over the concentration range of 0.1-0.5 µg/mL for Trandolapril and 9-45 µg/mL for verapamil with correlation co-efficient not less than 0.999for both the drugs. Linearity results were tabulated in the Table 3.

Table 3: Linearity Results for standard solution of Trandolapril and Verapamil

S.No	Linearity Level (%)	TRD		VER	
		Concentration (µg/mL)	Response	Concentration (µg/mL)	Response
1	20	0.1	30850	9	595621
2	40	0.2	65709	18	1098697
3	60	0.3	99642	27	1785681
4	80	0.4	131591	36	2356817
5	100	0.5	161180	45	2915842
Correlation Coefficient		0.999		0.999	

Accuracy

Accuracy of the method was determined at three different concentration levels by using recovery studies. The % recovery values were found to be in between 98.0% to 102.0%. The results were given in Table 4A and 4B.

Table 4A: Accuracy Results for standard solution of Trandolapril

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	48936	0.05	0.049	99.23%	99.79%
100%	98502	0.1	0.100	99.58%	
150%	148777	0.15	15.23	100.56%	

Table 4B: Accuracy Results for standard solution of Verapamil

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	859530	4.5	4.47	99.19%	99.28%
100%	1723279	9.0	8.96	99.43%	
150%	2579746	13.5	13.42	99.23%	

Robustness

The system suitability parameters and the percentage assay values for both the drugs were found to be within the limits by small, deliberate changes in the method parameters and the results were tabulated in Table 5A, 5B and5C.

Table 5A: Robustness (Flow rate) Results for Trandolapril and Verapamil

SR.No	Change in Flow Rate (ml/min)	System Suitability Results					
		TRD			VER		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	0.24	4029	1.3	100.12	5123	1.13	98.72
2	0.25 (Actual)	3594	1.5	100.18	5982	1.3	98.59
3	0.26	3664	1.4	100.05	5279	1.4	98.79

Table 5B: Robustness (Mobile phase composition) Results for Trandolapril and Verapamil

SR NO.	Change in Organic Composition in the Mobile phase	System Suitability Results					
		TRD			VER		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	10% Less	2618	1.5	100.12	4015	1.2	98.69
2	Actual	3594	1.5	100.18	5982	1.3	98.59
3	10% More	2505	1.5	100.08	2829	1.2	98.80

Table 5C: Robustness (Column temperature) Results for Trandolapril and Verapamil

SR NO.	Change in Column temperature (°C)	System Suitability Results					
		TRD			VER		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	23°	3514	1.4	100.08	5684	1.2	98.65
2	25°C (Actual)	3594	1.5	100.18	5982	1.3	98.59
3	27°	3619	1.3	100.13	5482	1.2	98.54

Limit of Detection and Limit of Quantitation

For calculating the limit of detection (LOD) and limit of quantitation (LOQ), the standard deviation and slope method was implemented. The LOD for Trandolapril and Verapamil was 0.001µg/mL and 0.003µg/mL with signal to noise ratio 3.0 and 3.0 respectively and the LOQ for Trandolapril and Verapamil was 0.004µg/mL and 0.02µg/mL with signal to noise ratio 9.96 and 9.98 respectively.

Solution stability

The% assay of each drug substance was calculated at 3, 6, 12, 14, and 16 hours and the results were tabulated in Table 6.

Table 6: Solution stability Results for Trandolapril and Verapamil

SR NO.	Time (Hours)	System Suitability Results	
		TRD %Assay	VER %Assay
1	3	99.5	99.9
2	6	98.6	99.8
3	12	98.5	99.3
4	14	98.3	98.9
5	16	98.2	98.8

Forced degradation

The degradation peaks were well separated from Trandolapril and Verapamil peaks in all the degradation chromatograms. The % degradation is calculated for both the drugs and the results are summarized in Table 7.

Table 7: Degradation studies Results for standard solution of Trandolapril and Verapamil

Degradation Condition	TRD				VER			
	Area	% Assay	Purity angle	Purity Threshold	Area	% Assay	Purity angle	Purity Threshold
Standard	98433	NA	NA	NA	1729673	NA	NA	NA
Acid	90431	91.87	0.610	0.931	1561073	90.33	0.427	0.914
Base	90628	92.07	0.524	0.952	1547303	89.59	0.521	0.847
Oxidative	92301	95.17	0.615	0.941	1567958	90.89	0.356	0.756
Thermal	93679	93.77	0.607	0.925	1580005	91.47	0.384	0.745

CONCLUSION

The UPLC method demonstrates to be simple, accurate, Linear and precise. The total runtime was 3.0 minutes within which two drugs and their degradation products were well separated. The stability indicating method was validated and illustrates satisfactory analytical data for all the validation parameters performed. The proposed method is novel and challenging as the label claims have a large difference in concentrations and for which a UPLC method could be developed and validated successfully within a very short run time. The present stability indicating method can be adopted for simultaneous quantitative estimation of the drugs Trandolapril and Verapamil in presence of degradation products by the pharmaceutical industries.

REFERENCES

1. Merck Index - an encyclopedia of chemicals, drugs and biological, 13th edition, 7084.
2. Kowalczuk D, Simultaneous high-performance thin-layer chromatography densitometric assay of trandolapril and verapamil in the combination preparation, Journal of AOAC International 2005; 88, 1525-1529.
3. Nafisur Rahman, Nadeem Ahmad khan and Syed Najmul Hejaz Azmi, Optimized and Validated Spectrophotometric Methods for the Determination of Verapamil Hydrochloride in Drug Formulations, Science Asia 2005; 31, 341-348.
4. Satish Kumar K, Ravi Kumar K, Ravi Kumar B V V and Mathrusri M and Annapurna, Validated new spectrophotometric methods for the estimation of verapamil in pharmaceutical dosage forms, J Pharm Educ Res 2010; 1, 96-100.
5. Rambabu C, Ramu G, BikshamBabu A and VenkataRao S, Optimized and validated reverse

phase HPLC method for the determination of trandolapril in bulk and formulations, Rasayan J. Chem 2010; 4,777-782.

6. Sankar A S K, Alekhya M, SurajSythana, Ravichandiran V, Analytical method development and validation of trandolapril in tablets by RP-HPLC, International journal of frontiers in science and technology 2013; 1, 119-127.
7. Mehmet Gumustas, SenemSanli, NurullahSanli and Sibel A. Ozkan, Development and Validation of a Liquid Chromatographic Method for Concurrent Assay of Weakly Basic Drug Verapamil and Amphoteric Drug Trandolapril in Pharmaceutical Formulations, Journal of Food and Drug Analysis 2012; 20, 588-596.
8. Tejashwi E, Kiran Kumar S and Siddartha B, Simultaneous estimation and validation of trandolapril and verapamil in bulk and pharmaceutical dosage form by RP-HPLC Method, World journal of pharmacy and pharmaceutical sciences 2014; 3, 652-662.
9. VioletaIvanova, DragicaZendelovska, Marina Stefova, TrajceStafilov, HPLC method for determination of verapamil in human plasma after solid-phase extraction, Journal of Biochemical and Biophysical Methods 2008; 70, 1297-1303.
10. Scott M. Johnson &Shoukry K. Wahba Khalil, An HPLC Method for the Determination of Verapamil and Norverapamil in Human Plasma, Journal of Liquid Chromatography 2006; 10, 1187-1201.
11. Mahesh GorakhanathDeore and Sachin Shyamsundar Laddha, Development and validation of a stability indicating HPLC assay method for simultaneous determination of trandolapril and verapamil HCl in tablet dosage form, International journal of pharmaceutical development and research2014; 6, 138-147.
12. KapendraSahu, ChandraboseKarthikeyan, Narayana, Moorthy S H N and PiyushTrivedi, A Validated UPLC Method Used for the Determination of Trandolapril and its Degradation Products as per ICH Guidelines, Current Pharmaceutical Analysis 2011; 7, 182-188.
13. International Conference on Harmonization, “Stability Testing of New Drug Substances and Products Q1 A (R2),” International Conference on Harmonization, IF-PMA, Geneva, 2003.