1. DRUG PROFILE

1.1. Trandolapril

Trandolapril [Figure 7.1] is a non-sulhydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to its biologically active diacid form, Trandolaprilat, in the liver [1]. Trandolaprilat inhibits ACE, the enzyme responsible for the conversion of angiotensin I (AT I) to angiotensin II (AT II). AT II regulates blood pressure and is a key component of the renin angiotensin-aldosterone system (RAAS) [2]. Trandolapril may be used to treat mild to moderate hypertension, to improve survival following myocardial infarction in clinically stable patients with left ventricular dysfunction, as an adjunct treatment for congestive heart failure, and to slow the rate of progression of renal disease in hypertensive individuals with diabetes mellitus and microalbuminuria or overt nephropathy [3-4].

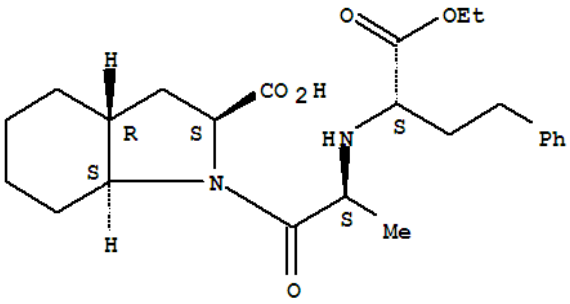

Figure 7.1: Molecular structure of Trandolapril

Molecular formula	:	$C_{24}H_{34}N_2O_5$
Molecular weight	:	430.54
Chemical name	:	(2S,3aR,7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydro-1*H*-indole-2-carboxylic acid

Solubility : Trandolapril is insoluble in water; soluble in methanol, dichloromethane or dimethyl sulfoxide.

Table 7.1: List of brand names of formulations of Trandolapril [5]

S. No	Brand name	Formulations	Available Strength	Address of Manufacturer
1	ZETPRIL	Tablet	2 mg 4 mg	Genix Pharmaceuticals Ltd

2. LITERATURE SURVEY

Several analytical methods have been reported for the determination of Trandolapril in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry [6], HPLC [7-11], HPTLC [12-13] and LC-MS [14-15] have been reported for the determination of Trandolapril in Pharmaceutical dosage forms.

Dubey et al [6] developed a simple, rapid and economical UV spectroscopic method for analysis of Trandolapril. Trandolapril shows maximum absorbance at a wavelength of 207.5 nm, which is used in this study. The method provides a linear response across a quantitation range of 5 μg/mL to 30 μg/mL in phosphate buffer pH 6.8. The method gave satisfactory results in terms of repeatability and intermediate precision. Also accuracy values were very good, the recovery being between 97.87 to 101.64%.The method was validated and proved to be robust and rugged. The results showed that this method can be used for rapid determination of Trandolapril.

Rambabu et al [7] developed a simple, sensitive and accurate reverse phase liquid chromatographic method to determine the amount of active pharmaceutical ingredient Trandolapril and applied for the analysis pharmaceutical formulation. Chromatographic determination is performed on a Hypersil gold C18 (100 x 4.6 mm., i.d; 5μm) with mobile phase consisting of 50% buffer and 50% acetonitrile v/v, and flow rate at 1.0 mL/min, keeping the column in thermostat at a constant temperature of 30^{o}C. The response of the instrument is recorded at maximum wavelength of absorption 215 nm. The standard curve is linear over a concentration range of 25.0-150 μg/mL (r^2=0.9999). The limit of detection and limit of quantification are calculated and found to be 1.149 and 3.832 μg/mL. The developed method is validated statistically. The low percent of relative standard deviation (0.297) and high percent of recovery of the drug (99.78-100.23%) indicate that the developed method is precise and accurate.

Gumieniczek et al [8] developed and validated an isocratic high performance liquid chromatographic procedure for the determination of Trandolapril and Verapamil in capsules. The drugs were analysed on a LiChrosorb RP18 column with a mobile phase composed of acetonitrile-methanol-phosphate buffer pH 2.7 (40:40:20 v/v/v) and UV detection at 220 nm. Peak height ratios were linearly related to amounts of the drugs in the range 4–20 μg/mL. The inter-day precision (CV) obtained for the standard solutions ranged from 0.40 to 2.18% for Trandolapril and from 0.35 to 2.57% for Verapamil. The inter-day coefficients of variation for replicate analyses in capsules ranged from 0.5 to 2.49% for Trandolapril and from 0.33 to 1.61% for Verapamil. The recovery of analytes after extraction from formulations using the described method was 99.94±1.69% and 98.13±1.20% (mean±SD) for Trandolapril and Verapamil, respectively.

Durga Rao et al [9] developed a stability indicating HPLC method for the quantitative determination of Trandolapril in pharmaceutical dosage forms in the presence of degradation products. It involved a Xterra RP18 150 x 4.6 mm, 5 μm column. The separation was achieved on gradient method. The mobile phase A contains a mixture of pH 3.0, 10mM Na_2HPO_4 buffer: acetonitrile (65:35 v/v) and the mobile phase B contains a mixture of pH 3.0, 10mM Na_2HPO_4 buffer: acetonitrile (45:55 v/v). The flow rate was 1.2 mL/min and the detection wavelength was 210 nm. The retention time of Trandolapril is 5.7 min. The total runtime was 20 min within which drug and degradation products were separated. Trandolapril was subjected to different ICH prescribed stress conditions. Degradation was found to occur in hydrolytic and oxidative stress condition, while drug was stable to thermal and photolytic stress conditions. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The method developed was successfully applied to the determination of Trandolapril in pharmaceutical preparations. The developed RP-HPLC method was validated with respect to linearity, accuracy, precision and ruggedness.

Cendrowska et al [10] developed a HPLC method for the chiral separation of Trandolapril and octahydro-1*H*-indole-2-carboxylic acid. HPLC conditions for the identification of stereo isomers and stereochemical purity of the key intermediate in Trandolapril synthesis, octahydro-1*H*-indole-2-carboxylic acid, and final drug were elaborated. The chemical and stereochemical purity of synthetic Trandolapril was proved to be as high as 99.3-99.8%, on both non chiral and chiral RP-columns

Gumieniczek et al [11] developed a rapid, simple and accurate HPLC method for the determination of Trandolapril in capsules. Samples were chromatographed on a

LiChrosorb RP C-18 column and the mobile phase was acetonitrile: 0.067M phosphate buffer of PH 2.7 (7:3 v/v).The UV detection was at 220 nm and Benazepril was used as internal standard. Linearity was achieved in the range of 4-20 mg/mL. The method was validated and can successfully applied for the quantitative determination of Trandolapril in capsules.

Kowalczuk et al [12] developed simple, selective, precise and stability-indicating high-performance thin layer chromatographic method for analysis of both as the bulk drug and in a tablet formulation and validated. Aluminium foil TLC plates precoated with silica gel 60F(254) were used as stationary phase. Densitometry analysis was performed in absorbance mode at 220 nm. The method was validated for precision, recovery, and robustness. The drug was subjected to acid and alkaline hydrolysis, oxidation, and photochemical and thermal degradation and underwent degradation under all these conditions. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drug. It can be used for identification and quantitative analysis of in the bulk drug and in tablet formulations.

Sreekanth et al [13] developed a simple, precise, accurate and rapid high performance thin layer chromatographic method and completely validated for the estimation of Trandolapril in bulk and pharmaceutical dosage forms. Quantification of Trandolapril was carried out with percolated silica gel 60F(254) as stationary phase using mobile phase consisting of Chloroform: Methanol: Acetic acid (8:1.5:0.5 v/v/v) and scanned in Absorbance Reflectance mode at 212 nm using Camag TLC scanner 3 with WinCAT software. The R_f value of Trandolapril was found to be 0.54 (±0.03). The proposed method has permitted the quantification of Trandolapril over the linearity range

of 25-150 ng/spot and its percentage recovery was found to 99.7%. The intraday and inter day precision were found to be 1.26% and 1.4%, respectively. The limit of detection and the limit of quantification were found to be 18 ng/spot and 54 ng/spot, respectively. The proposed method can be successfully applied for the estimation of drug content of different marketed formulations simultaneously on a single plate and provides a faster and cost effective quality control tool for routine analysis of Trandolapril as bulk drug and in tablet dosage forms.

Nirogi et al [14] developed a sensitive high-performance liquid chromatography mass spectrometry (MS/MS) method was developed and validated for the simultaneous quantification of Trandolapril and its metabolite Trandolaprilat in human plasma using Ramipril as an internal standard. Following solid-phase extraction, the analytes were separated using an isocratic mobile phase on a reversed-phase column and analyzed by MS/MS in the multiple reaction monitoring mode using the respective $[M–H]^-$ ions, *m/z* 429/168 for Trandolapril, *m/z* 401/168 for Trandolaprilat and *m/z* 415/166 for the internal standard. The method exhibited a linear dynamic range of 20–10000 pg/mL for both Trandolapril and Trandolaprilat in human plasma. The lower limit of quantification was 20 pg/mL for both Trandolapril and its metabolite. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. A run time of 2.0 min for each sample made it possible to analyze more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies.

Pistos et al [15] developed a liquid chromatographic tandem mass spectrometric method for the determination of Trandolapril in human plasma has been developed and fully validated. The samples were extracted using HLB Oasis solid-phase extraction cartridges. The chromatographic separation was performed on XTerra C8 MS column (150 × 4.6 mm i.d., 5 μm particle size) using a mobile phase consisting of acetic acid 20 mM and triethylamine 4.3 mM/acetonitrile (40:60 (v/v)), pumped isocratically at 0.35 mL/min. The analytes were detected using a micro triple quadrupole mass spectrometer with positive electrospray ionization in multiple reaction-monitoring (MRM) mode. Tandem mass spectrometric detection enabled the quantitation of Trandolapril down to 2.0 ng/ml. Calibration graphs were linear (*r* better than 0.996, *n* = 9) in the concentration ranges 2.0–750 ng/ml and the intra- and inter-day R.S.D. values were less than 3.83 and 3.86% for Trandolapril.

3. EXPERIMENTAL

3.1. Instrumentation

The author had attempted to develop and validate a liquid chromatographic method for determination of Trandolapril using an isocratic Waters HPLC system on a Xterra C18 column (150 mm x 4.6 mm, 5 µm). The instrument is equipped with a 2695 binary pump with inbuilt degasser, 2487 Dual absorbance detector and Rheodyne injector with 20 µL sample loop. A 20 µL Hamilton syringe was used for injecting the samples. Data was analysed using Waters Empower 2 software. A double-beam Elico SL-159 UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

3.2. Chemicals and Solvents

The reference sample of Trandolapril (API) was obtained from Glenmark Pharmaceutical Industries Limited, Mumbai, India. The branded formulation (tablets) (ZETPRIL tablets containing 4 mg of Trandolapril) was procured from the local market. HPLC grade acetonitrile and analytical grade potassium dihydrogen phosphate was obtained from Merck Specialities Pvt Ltd, Mumbai, India. Hydrochloric acid, sodium hydroxide, hydrogen peroxide and orthophosphoric acid of analytical grade were obtained from Merck Chemicals Ltd, Mumbai, India. Milli-Q water used throughout the experiment was dispensed through 0.22 µ filter of the Milli-Q water purification system from Millipore, Merck KGaA, Darmstadt, Germany.

3.3. The Phosphate buffer solution

Weigh about 7.0 grams of potassium dihydrogen phosphate and transfer to 1000 mL standard flask, add 400 mL of Milli-Q water mix and dilute to volume with Milli-Q water, sonicate for five minutes and cool to room temperature, measure the pH of above buffer solution and finally adjusted the pH to 3.0±0.05 with ortho phosphoric acid solution and filtered through 0.45 µ nylon filter.

3.4. The mobile phase

A mixture of potassium dihydrogen phosphate buffer pH 3.0 and acetonitrile in the ratio of 35:65 v/v was prepared and used as mobile phase.

3.5. The diluent

The potassium dihydrogen phosphate buffer pH 3.0 and acetonitrile mixture in the ratio of 35:65 v/v was used as diluent.

3.6. Preparation of standard solution of the drug

About 10 mg of Trandolapril was accurately weighed and transferred into a 100 mL clean dry volumetric flask containing 50 mL of diluent. The solution was sonicated for 5 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 100 µg/mL for Trandolapril (Stock solution). Further pipette 4 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the diluent.

3.7. Preparation of sample (tablet) solution

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of diluent. Then this mixture was filtered through 0.45 µ membrane filter. Further pipette 4 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the diluent.

4. METHOD DEVELOPMENT

For developing the method, a systematic study of the effect of various factors were undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and

choice of stationary and mobile phases. The following studies were conducted for this purpose.

4.1. Detection wavelength

The spectrum of diluted solution of the Trandolapril in diluent was recorded on UV spectrophotometer. The peak of maximum absorbance was observed. The spectra of Trandolapril showed that a balanced wavelength was found to be at 210 nm.

4.2. Choice of stationary phase

Preliminary development trials have performed with octadecyl columns and octyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded in Xterra C18 column.

4.3. Selection of the mobile phase

In order to get sharp peak and base line separation of the components, the author has carried out a number of experiments by varying the composition of various solvents and its flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a C18 stationary phase. A mixture of potassium dihydrogen phosphate buffer pH 3.0 and acetonitrile in the ratio of 35:65 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing.

4.4. Flow rate

Flow rates of the mobile phase were changed from 0.5-2.0 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 0.8 mL/min flow rate was ideal for the successful elution of the analyte.

4.5. Run time

No interference in blank and placebo solutions for the drug peak in the trail injections with a runtime of 5.0 min.

4.6. Optimized chromatographic conditions

Chromatographic conditions as optimized above were shown in Table 7.2. These optimized conditions were followed for the determination of Trandolapril in bulk samples and its combined tablet formulations. The chromatogram of standard and sample solutions of Trandolapril was shown in Figure 7.2 and Figure 7.3. The chromatograms of stability studies of Trandolapril were shown from in Figure 7.7 to Figure 7.10.

Table 7.2: Optimized chromatographic conditions for the estimation of Trandolapril in tablet dosage form

Mobile phase	**:** Potassium dihydrogen phosphate buffer pH 3.0:acetonitrile, 35:65 v/v
Pump mode	**:** Isocratic
pH of Buffer	**:** 3.0±0.05
Diluent	**:** Potassium dihydrogen phosphate buffer:acetonitrile, 35:65 v/v
Column	**:** Symmetry C18 column, 150 mm x 4.6 mm, 5 µm
Column Temp	**:** Ambient
Wavelength	**:** 210 nm
Injection Volume	**:** 20 µL
Flow rate	**:** 0.8 mL/min
Run time	**:** 5 min
Typical t_R	**:-**
Trandolapril	**:** 2.732±0.5 min

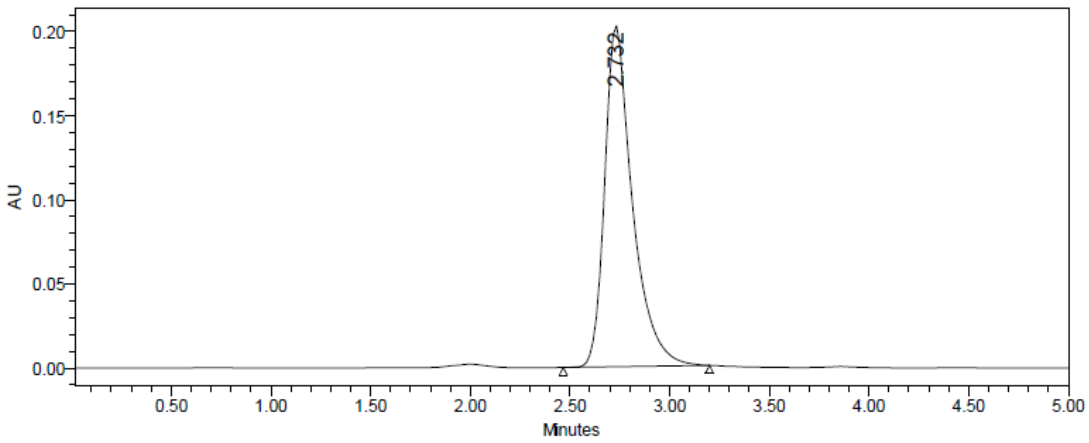

Figure 7.2: Chromatogram of standard solution of Trandolapril

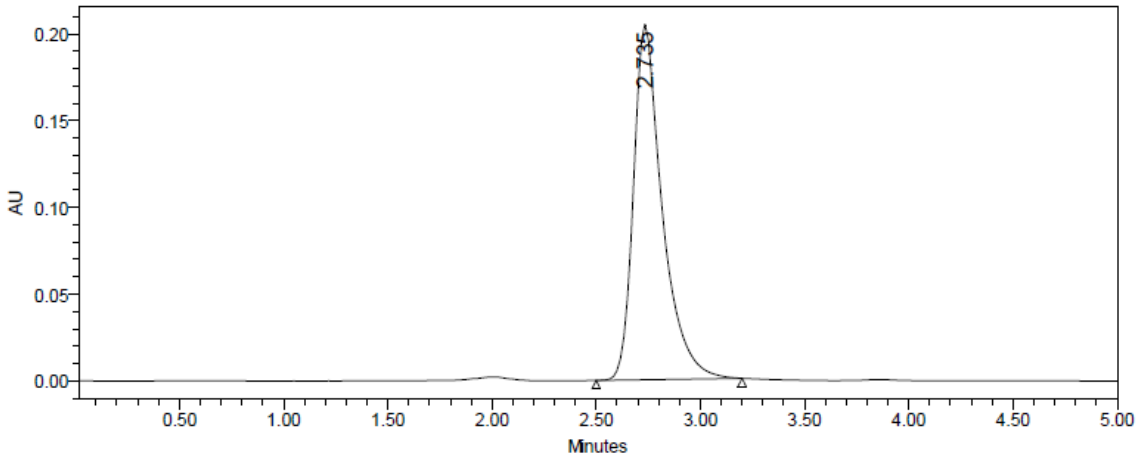

Figure 7.3: Chromatogram of sample solution of Trandolapril

5. VALIDATION OF THE PROPOSED METHOD

The proposed method was validated as per ICH [16-17] guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification and solution stability.

5.1. Specificity

A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in Table 7.3. The chromatograms of blank and placebo for Trandolapril was shown in Figure 7.4 and Figure 7.5.

Table 7.3: Specificity study

Name of solution	Retention time (min)
Blank	No peaks
Trandolapril	2.73

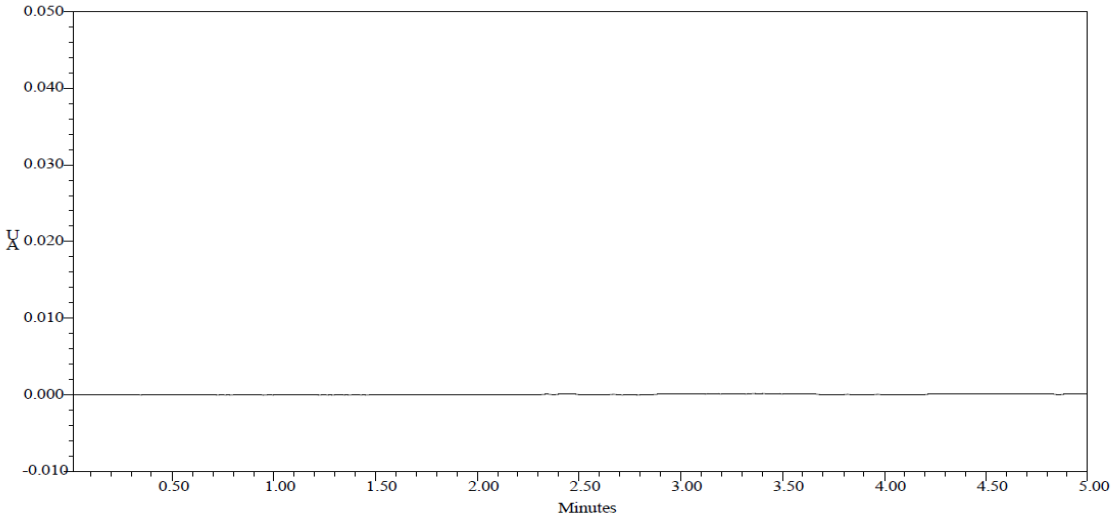

Figure 7.4: Chromatogram showing no interference of blank for Trandolapril

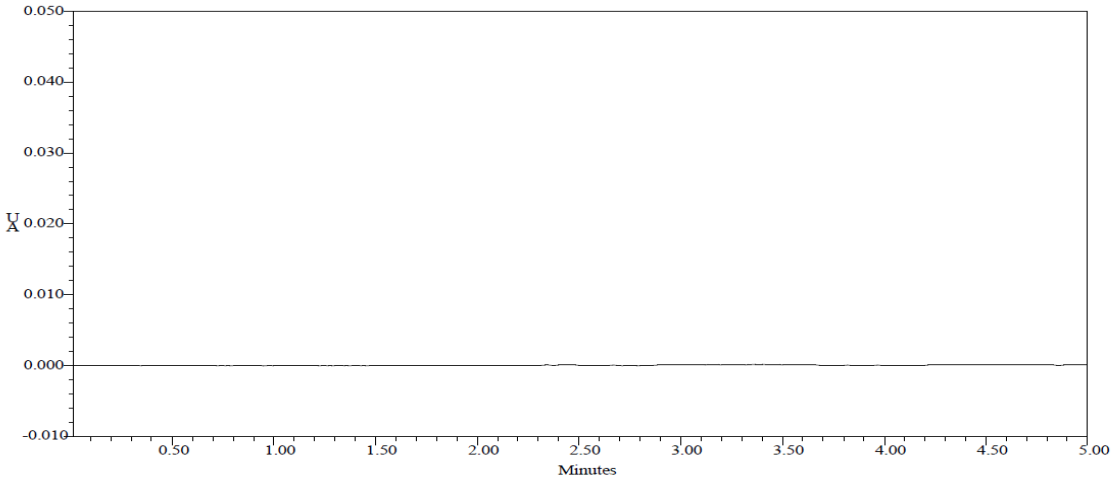

Figure 7.5: Chromatogram showing no interference of placebo for Trandolapril

5.2. Linearity

Linearity was performed by preparing standard solutions of Trandolapril at different concentration levels including working concentration mentioned in experimental condition from 20.0 to 60.0 µg/mL and twenty micro litres of each concentration was injected in duplicate into the HPLC system. The response was read at 210 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table 7.4 and linearity plots are shown in Figure 7.6.

Table 7.4: Linearity study of Trandolapril

Level	**Concentration of Trandolapril (µg/mL)**	**Mean peak area**
Level-1	20	1029710
Level-2	30	1502392
Level-3	40	1935168
Level-4	50	2474039
Level-5	60	2936930
Slope		**47861**
Intercept		**61213**
Correlation Coefficient		**0.9995**

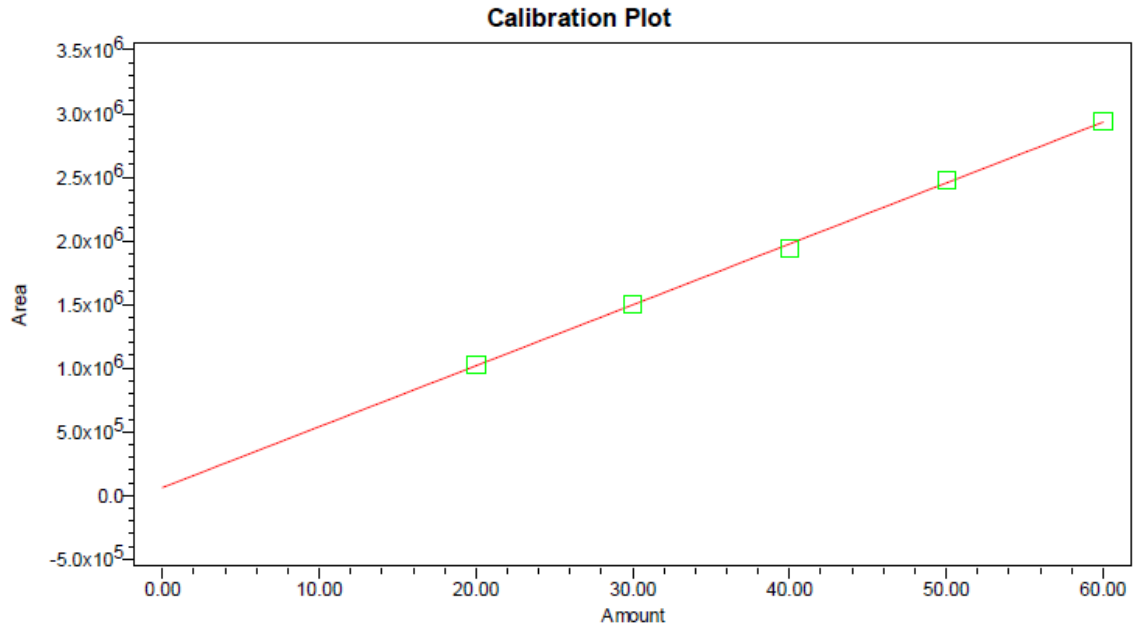

Figure 7.6: Linearity plot of Trandolapril

5.3. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as system precision, method precision and intermediate precision.

5.3.1. System precision

To study the system precision, five replicate standard solutions of Trandolapril was injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.47 for Trandolapril, which is well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 7.5.

Table 7.5: System precision

Injection number	Area of Trandolapril	Acceptance criteria
1	2021079	The %RSD of peak area of Trandolapril should not be more than 2.0
2	2019713	
3	2020269	
4	2031519	
5	2041268	
Mean	**2026770**	
SD	**9450**	
%RSD	**0.47**	

5.3.2. Method precision

The method precision study was carried out on five preparations from the same tablet samples of Trandolapril and percent amount of both were calculated. The %RSD of the assay result of five preparations for Trandolapril in method precision study was found to be 0.95 which is well within the acceptance criteria of not more than 2.0. The results obtained for assay of Trandolapril is presented in Table 7.6.

Table 7.6: Method precision

Sample number	%assay
	Trandolapril
1	100.7
2	100.8
3	99.1
4	101.5
5	99.7
Mean	**100.3**
SD	**0.9528**
%RSD	**0.95**

5.3.3. Intermediate precision

The intermediate precision study was carried out by different analysts, different columns, different reagents using different HPLC systems from the same tablet of Trandolapril and the peak area of Trandolapril was calculated. The %RSD of the peak areas of five preparations in intermediate precision study of Trandolapril was 0.12 which is well within the acceptance criteria of not more than 2.0. The results of intermediate precision study are reported in Table 7.7.

Table 7.7: Intermediate precision study of Trandolapril

Injection number	Area of Trandolapril	Acceptance criteria
1	2069536	The %RSD of peak area of Trandolapril should not be more than 2.0
2	2063371	
3	2065251	
4	2063285	
5	2065260	
Mean	**2065421**	
SD	**2463**	
%RSD	**0.12**	

5.4. Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels of 50%, 100% and 150%. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD at each level was calculated and results are presented in Table 7.8. The percent recovery of Trandolapril was 100.5% to 101.5% and the mean recovery was found to be 100.6% by the proposed method. This indicates that the proposed method was accurate.

Table 7.8: Recovery study for Trandolapril

%Concentration (at specification Level)	Mean peak area	Amount of Trandolapril spiked (mg)	Amount of Trandolapril recovered(mg)	%Recovery	Mean Recovery
50%	1035894	20.13	20.23	100.5%	100.6%
100%	1965887	40.01	39.91	99.7%	
150%	2945821	60.05	60.92	101.5%	

5.5. Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase and composition of the mobile phase. Sample of Trandolapril at 40 µg/mL concentration were analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 7.9.

Table 7.9: Robustness study for Trandolapril

Condition	Mean Peak area	%assay	%difference
Unaltered	1965381	99.9	-
Flow rate at 0.6 mL/min	1995622	101.5	1.6
Flow rate at 1.0 mL/min	1985547	101.0	1.1
Mobile phase:			
• Buffer(55):Acetonitrile(45)	1926865	98.0	1.9
• Buffer(45):Acetonitrile(55)	1948823	99.1	0.8

5.6. System suitability

System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table 7.10.

Table 7.10: System suitability for Trandolapril

Parameter	Tailing factor	Theoretical plates
Specificity study	1.50	2990
Linearity study	1.12	2987
Precision study	1.12	2857
Robustness study		
Flow rate at 0.6 mL/min	1.32	2856
Flow rate at 1.0 mL/min	1.22	2584
Mobile phase:		
• Buffer(55):Acetonitrile(45)	1.05	2637
• Buffer(45):Acetonitrile(55)	1.09	2549

5.7. Limit of detection and Limit of quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Trandolapril are given in Table 7.11.

Table 7.11: LOD and LOQ of Trandolapril

Parameter	Measured value (µg/mL)
Limit of detection	0.02
Limit of quantification	0.08

5.8. Solution stability

To determine the stability of Trandolapril in solution, the standard and sample solution were observed under room temperature. Any change in the retention time, peak shape and variation in response was compared to the pattern of chromatogram of freshly prepared solution. The solution stability results are shown in the Table 7.12.

Table 7.12: Solution stability of Trandolapril

Standard solution			Sample solution		
Time (hours)	**Response**	**%variation**	**Time (hours)**	**Response**	**%variation**
Initial	1965381	-	Initial	1960986	-
12	1945871	1.0	12	1948624	0.6
24	1927854	1.9	24	1928697	1.6

5.9. Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hours at room temperature. The results show that for both solutions, the retention time and peak area of Trandolapril (%RSD less than 2.0) has no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hours, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of proposed method. The results of the degradation studies are shown in the Table 7.13.

Table 7.13: Forced degradation study results for Trandolapril

Stress Conditions	Degradation Time (Hrs)	Trandolapril	
		%Assay	%Degradation
Control	--	99.9	--
Acid	1	92.0	-7.9
Base	1	90.9	-9.0
Peroxide	1	87.0	-12.9
Thermal	48	87.9	-12.0

Control sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with

further quantity of diluent. Then this mixture was filtered through 0.45 µ membrane filter. 20.0 mL of this filtrate was further diluted to 50 mL with mobile phase.

Acid degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 10 mL of 5N acid (Hydrochloric acid) was added, refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralized with 5N base (Sodium hydroxide) and diluted to volume with diluent. About 25 mL of the above sample solution was filtered through 0.45 µ membrane filter. Pipetted 20 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of acid degradation for Trandolapril is shown in Fig. 7.7.

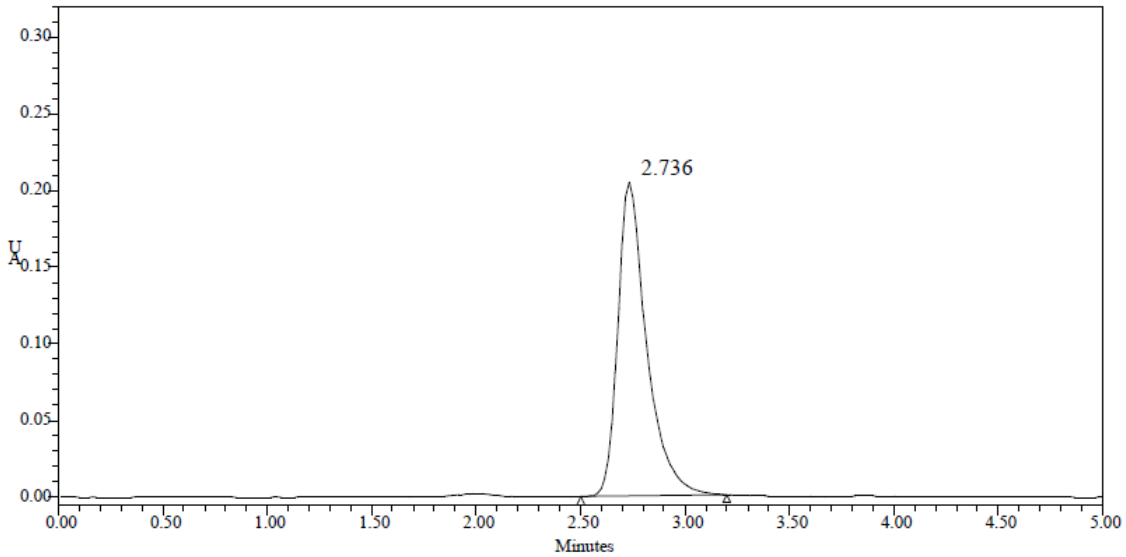

Figure 7.7: Chromatogram of acid degradation showing Trandolapril

Base degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 10 mL of 5N base (Sodium hydroxide) was added, refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralized with 5N acid (Hydrochloric acid) and diluted to volume with diluent. About 25 mL of the above sample solution was filtered through 0.45 µ membrane filter. Pipetted 20 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of base degradation for Trandolapril is shown in Fig. 7.8.

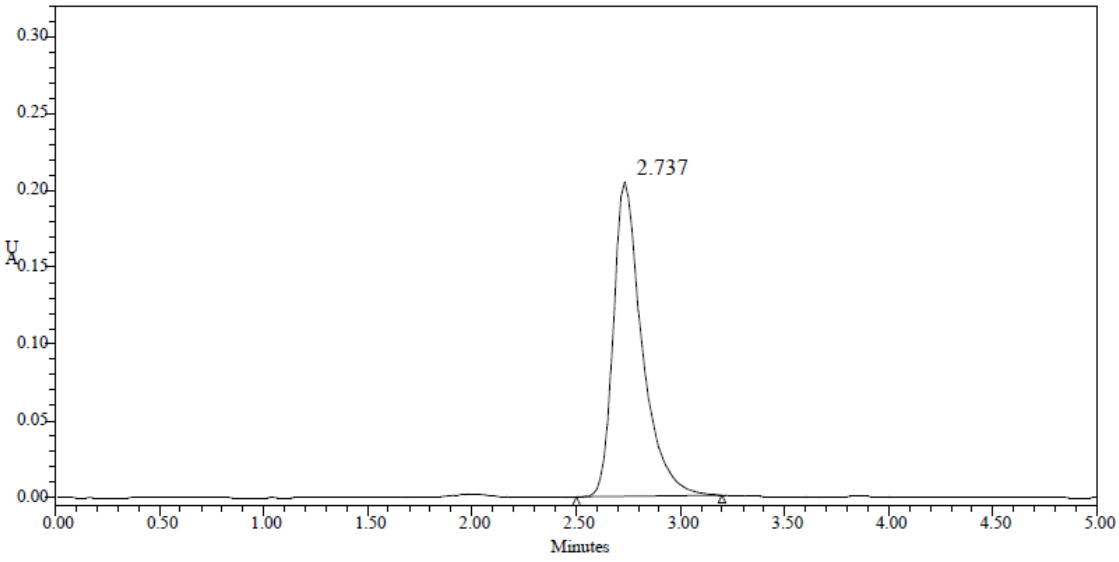

Figure 7.8: Chromatogram of base degradation showing Trandolapril

Peroxide degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 50 mL

volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 4 mL of 30 % hydrogen peroxide was added, refluxed for 60 minutes at 60°C, then cooled to room temperature and diluted to volume with diluent. About 25 mL of the above sample solution was filtered through 0.45 µ membrane filter. Pipetted 20 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of peroxide degradation for Trandolapril is shown in Fig. 7.9.

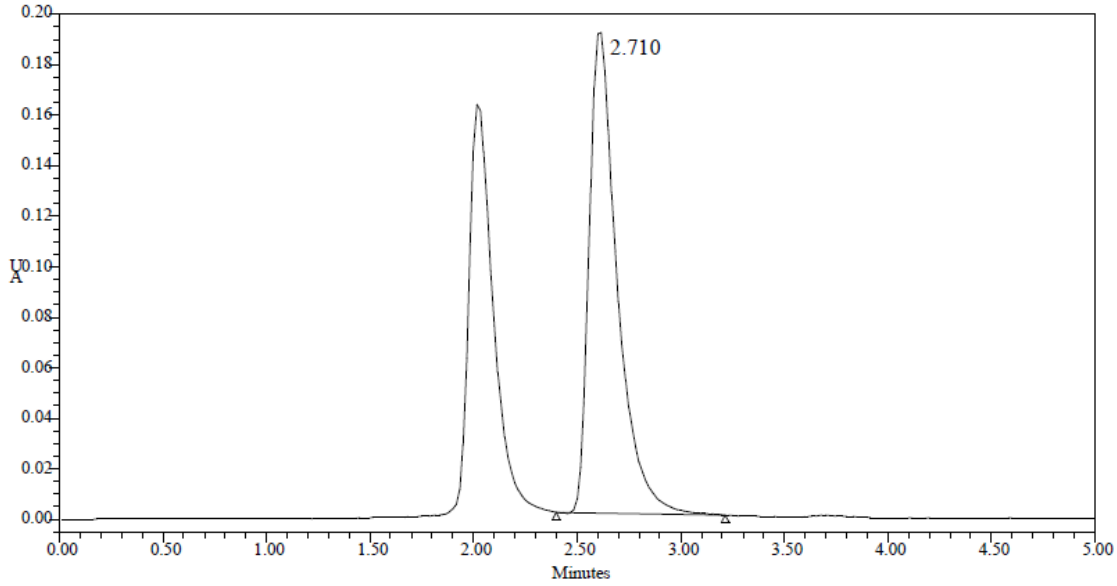

Figure 7.9: Chromatogram of oxidative degradation showing Trandolapril

Thermal degradation sample

Twenty tablets were weighed and finely powdered. The powder is exposed to heat at 105°C for about 2 days. An accurately weighed portion of powder sample equivalent to 10 mg of Trandolapril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. About 25 mL of the above sample solution was filtered through

0.45 µ membrane filter. Pipetted 5 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of thermal degradation for Trandolapril is shown in Fig. 7.10.

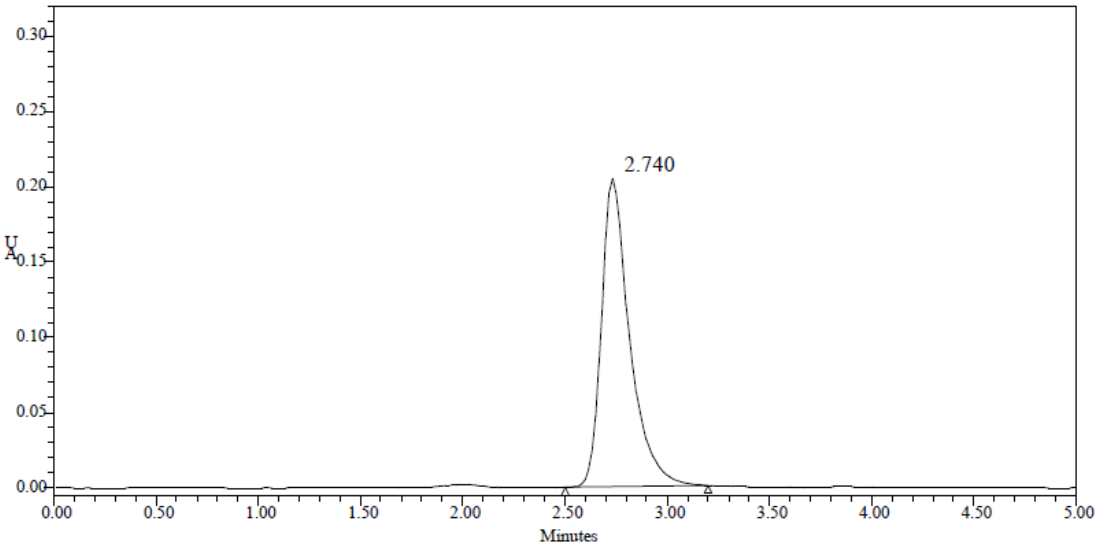

Figure 7.10: Chromatogram of thermal degradation showing Trandolapril

6. RESULTS AND DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the estimation of Trandolapril from bulk samples and tablet dosage forms. A non-polar C18 analytical chromatographic column was chosen as the stationary phase for the separation and determination of Trandolapril. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and acetonitrile in the ratio of 35:65 v/v was

proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention time of Trandolapril was found to be 2.73 min.

The linearity was found satisfactory for the drug in the range 20.0-60.0 µg/mL (Table 7.4). The regression equation of the linearity curve of Trandolapril between concentrations over its peak areas was found to be Y=47860X+61215 (where Y is the peak area and X is the concentration of Trandolapril in µg/mL). Precision of the method was studied by repeated injection of tablet solution and results showed lower %RSD values (Table 7.5-7.7). This reveals that the method was quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the % RSD at each level were within the acceptable limits (Table 7.8). This indicates that the method was accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method was specific.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method was robust (Table 7.9). The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor (Table 7.10). The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method was sensitive (Table 7.11). The solution stability studies indicate that both the drugs were stable up to 24 hours (Table 7.12). The forced degradation studies indicate that both the drugs were stable in stability studies (Table 7.13).

7. CONCLUSION

The proposed stability-indicating RP-HPLC method was simple, specific, sensitive, accurate and precise and can be used for analysis of Trandolapril in bulk samples and its tablet dosage forms.

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