



RESEARCH ARTICLE

**Stability Indicating RP-HPLC Method Development and Validation of
Simultaneous Estimation of Trandolapril and Verapamil Hydrochloride with
Forced Degradation Studies in Bulk and Commercial Products**

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ABSTRACT

A simple reproducible and efficient isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of Trandolapril and Verapamil. All the drugs were separated on an Inertsil ODS – 3V 150 x 4.6mm, 5µm. The mobile phase, optimized through an experimental design, was a 40:60 (v/v) mixture of acetonitrile and triethylamine buffer (pH 3.0), pumped at a flow rate of 1.3 ml/min. UV detection was performed at 216 nm. The retention time of Verapamil Hydrochloride and Trandolapril was found to be 1.51 min and 3.43 min respectively. The method was validated in the sample concentration ranges of 1.6 - 25µg/ml for Trandolapril and 95-1530µg/ml for Verapamil Hydrochloride, The method demonstrated to be robust, resisting to small deliberate changes in pH and flow rate of the mobile phase. The LOD values were 0.26 µg/ml and 10.3 µg/ml, while the LOQ values were 0.87 µg/ml and 31.1 µg/ml for Trandolapril and Verapamil Hydrochloride respectively. The recoveries for all three levels were above 99%.

KEYWORDS

RP-HPLC, Tablet dosage form, Trandolapril and Verapamil

INTRODUCTION

Trandolapril is a non-sulphydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to its biologically active diacid form, Trandolapril at, in the liver. Trandolapril at inhibits 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) and chemically it is

(2S, 3aR, 7aS)-1-[(2S)-2-[[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl]-octahydro-1H-indole-2-carboxylic acid. Chemically Verapamil Hydrochloride is 2-(3, 4-dimethoxyphenyl)-5-[[2-(3, 4-dimethoxyphenyl) ethyl] (methyl) amino]-2-(propan-2-yl) pentane nitrile. Verapamil inhibits voltage-dependent calcium channels. Specifically, its effect on L-type calcium channels in the heart causes a reduction in ionotropy and chronotropy, thus reducing heart rate and blood pressure. Verapamil mechanism of effect in cluster headache is thought to be linked to its calcium-channel blocker effect. Literature survey reveals High Performance Liquid Chromatographic (HPLC)¹⁻⁶ for determination of Trandolapril and

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Verapamil Hydrochloride combination are not official in Pharmacopeias of USP and BP. And their determination is official as single compound in Pharmacopeias.

Various analytical methods have been reported for the assay of Trandolapril and Verapamil Hydrochloride alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV-VIS spectroscopy¹, high performance liquid chromatography²⁻⁶, high performance thin layer chromatography⁷⁻⁸ and LC - MS/ MS⁹.

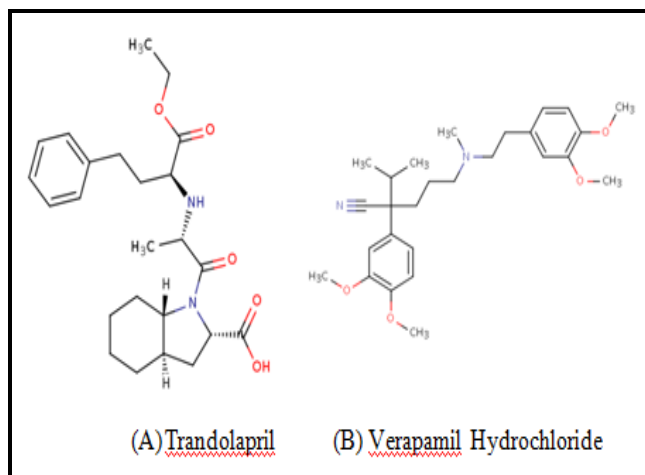


Figure 1: The Chemical Structures of Trandolapril potassium (A) and Hydrochlorothiazide (B)

As on only few methods is available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Trandolapril and Verapamil Hydrochloride in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, accurate method of Trandolapril and Verapamil Hydrochloride in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference on Harmonization (ICH).

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of Trandolapril and Verapamil Hydrochloride were obtained as a gift samples from Dr. Reddy's, Hyderabad used as such without further purification. A combination of Trandolapril and Verapamil Hydrochloride 4/240mg in tablet formulations (Tarka), HPLC grade methanol, Acetonitrile, water and triethylammonium phosphate buffer (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Waters 2695 separation module equipped with Empower-2 software and loop of injection capacity of 80 μ L, and waters-PDA detector set at 216 nm. Compounds were separated on an Inertsil ODS - 3V Column (150 \times 4.6 mm i.d., 5 μ m particle size) under reversed phase partition conditions. The mobile phase was an Acetonitrile and pH - 3.0 triethyl amine buffers (pH 3.0 \pm 0.05, adjusted with orthophosphoric acid).

The flow rate was 1.3 ml/min and the run time was 7 minutes. Samples were injected using Rheodyne injector with 10 μ L loop and detection was carried out at 216 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45 μ nylon filter. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in column temperature maintained at 30 \pm 5 $^{\circ}$ C.

The UV spectrum of Trandolapril and Verapamil Hydrochloride selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solutions

Preparation of Standard Stock Solution – I

Weigh and transfer about 8 mg of Trandolapril working standard or reference standard into a 50 ml volumetric flask, add about 20 ml of diluent and sonicate for 3 min to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard Stock Solution – II

Weigh and transfer about 50 mg of Verapamil HCl working standard or reference standard into a 25 ml volumetric flask, add about 10 ml of Diluent and sonicate for 3 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard Stock Solution – III

Pipette out 20 ml of above stock solution -II in to 25 mL volumetric flask and diluted up to the volume with diluent.

Preparation of Standard Solution

Pipette out 24 ml of standard stock solution - I & 4 ml of diluted standard stock solution -III into 50 mL volumetric flask and diluted up to the volume with diluent.

Procedure for Analysis of Tablet Formulation

Accurately transfer ten (for 4/240 mg) intact tablets in to a 250 ml volumetric flask. Add 70 ml of diluent and sonicate to disperse the tablets completely. Add about 100 ml of diluent and sonicate for 30 min with intermittent vigorous shaking and stir with the aid of magnetic stirrer for 30 min and dilute to volume with diluent and mix and allow the sample solution to settle down. Dilute 4 ml of supernatant solution to 50 ml with diluent and mix. Filter the solution through the 0.45N nylon filter.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Degradation Study

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain Trandolapril (12.5 ppm) and Verapamil Hydrochloride (765ppm) concentration 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample. Specific degradation conditions were described as follows.

Acidic Degradation Condition

To 4 ml of stock solution 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C.

Alkali Degradation Condition

To 4 ml of stock solution 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C.

Oxidative Degradation Condition

To 4 ml of stock solution of 1 ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60°C.

Thermal Degradation Condition

The standard drug solution was placed in oven at 105 °C for 6hr to study dry heat degradation.

Photolytic Degradation Condition

The photochemical stability of the drug was also studied by exposing the sample solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m² in photo stability chamber.

RESULTS AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation-resolution Trandolapril and Verapamil Hydrochloride in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be an Acetonitrile and

triethyl amine buffer (pH 3.0 ±0.05, adjusted with orthophosphoric acid). This mobile phase used gave a very satisfactory and good resolution of Trandolapril and Verapamil Hydrochloride. Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of Trandolapril and Verapamil Hydrochloride on the analytical column was evaluated at a flow rate of 1.3 ml/min. The injection volume was 10 µL. The retention time of standard and sample for Trandolapril and Verapamil Hydrochloride were satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions were mentioned on below Table-1.

Table 1: Finalized chromatographic conditions

Flow rate: 1.3 ml/min	Wave length: 216 nm	Injection Volume: 10µL
Column temperature: 30±5°C	Sample temperature: Ambient	Run time: 7 minutes
Column : Inertsil ODS -3V Column (150 × 4.6 mm i.d., 5µm particle size)		
Mobile phase ratio	Mobile phase-A (%v/v) (pH 3.0 buffer)	Mobile phase-B (%v/v) (Acetonitrile)
	60	40

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table-2.

Table2: Results from system suitability study of Trandolapril and Verapamil Hydrochloride

System Suitability Parameters	Results		Acceptance Criteria
	Trando-lapril	Verapamil HCl	
Retention time	3.43	1.51	
% RSD for area of Trandolapril and Verapamil Hydrochloride for five replicate injections of standard solution	0.03	0.12	NMT 2.0
Tailing factor for Trandolapril and Verapamil Hydrochloride peak	1.0	1.1	NMT 2.0
Theoretical plates for Trandolapril and Verapamil Hydrochloride	5838	5265	NLT 2000

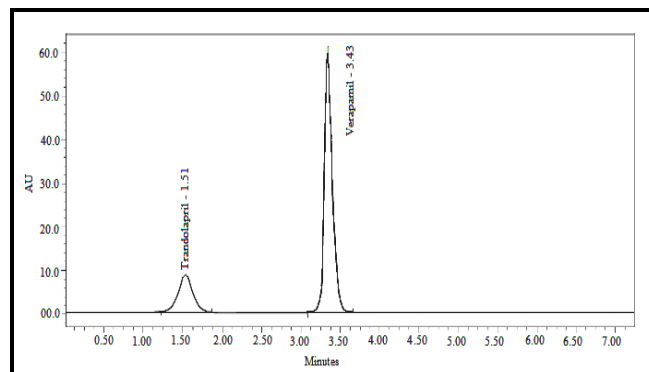


Figure 2: Optimized chromatograms for Trandolapril (12.5 ppm) and Verapamil Hydrochloride (765ppm)

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

Aliquots 2.5, 2.5, 5.0 ml of Working standard solution Trandolapril and Verapamil Hydrochloride were transferred in a series of 20 mL, 10 mL, 10 mL volumetric flasks respectively for 12.5, 25, 50% levels and Aliquots 1.6 ml, 2.4 ml, 3.2 ml of standard stock solution - I & 9.6 ml of standard stock solution – II, 11.5 ml, 15.4 ml of standard stock solution – III were transferred in three different 20 ml volumetric flasks for 100, 150 and 200% levels. Finally the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of Trandolapril and Verapamil Hydrochloride were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for Trandolapril and Verapamil Hydrochloride were plotted separately of response against respective concentration of Trandolapril and Verapamil Hydrochloride. The slope and intercept value for calibration curve were $y = 180784x - 7907.1$ ($R^2 = 0.9998$) for Trandolapril and $y = 25971x - 15546$ ($R^2 = 0.9999$) for Verapamil Hydrochloride where Y represents the peak area of analyte and X represents analyte concentration.

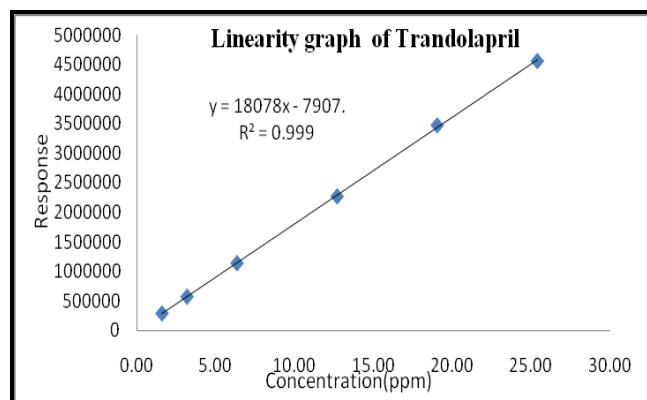


Figure 3: Linearity curve for Trandolapril

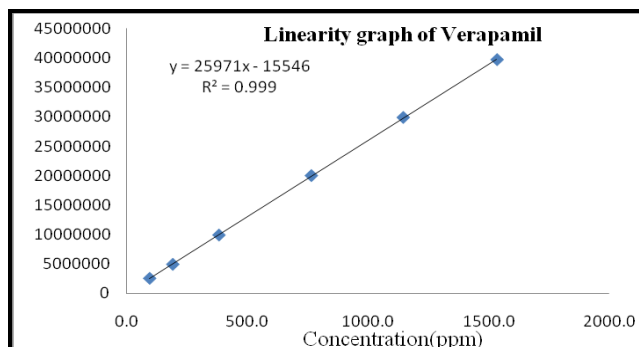


Figure 4: Linearity curve for Verapamil Hydrochloride

The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of Trandolapril and Verapamil Hydrochloride are given in Figures 3 and 4 respectively.

Precision

Precision of the method was confirmed by the repeated analysis of formulation for six times. The %RSD values were found to be satisfactory. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method. Intraday and Interday precision was determined by preparing six ($n=6$) replicate samples and analyzed on same day for intraday and on different days for interday precision. (Table3). The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. For strength 4/240 mg, the %RSD of intraday precision of Trandolapril and Verapamil Hydrochloride are 1.7, 1.2 respectively. The %RSD of interday precision of Trandolapril and Verapamil Hydrochloride are 0.7, 0.2 respectively and overall %RSD for Trandolapril and Verapamil Hydrochloride are 1.3, 0.8 (Table3)

Accuracy

To check the accuracy of the method, recovery studies were carried out by the addition of standard drug solution to pre-analyzed sample solution at three different levels 25%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

Table 3: Precision studies

No. of Tablets	% Assay			
	Trandolapril		Verapamil Hydrochloride	
	Intraday precision	Interday precision	Intraday precision	Interday precision
1	99.5	99.3	99.8	99.9
2	97.5	100.8	99.1	99.8
3	98.8	100.0	101.3	99.8
4	101.8	101.3	99.4	100.1
5	101.3	100.8	101.6	100.1
6	101.5	101.0	101.6	100.1
Mean	100.1	100.5	100.5	100.0
%RSD	1.7	0.7	1.2	0.2
Over all % RSD (n=12)	1.3		0.8	

Table 4: Recovery studies of Trandolapril and Verapamil Hydrochloride

Level of % Recovery	% Mean Recovery*		% R.S.D.*	
	Trandolapril	Verapamil HCl	Trandolapril	Verapamil HCl
25	100.4	99.6	0.40	0.12
100	100.0	99.8	0.41	0.13
150	100.7	99.8	0.26	0.04

*Avg. of six determinations for 25 & 150, three determinations for 100%, R.S.D. is relative standard deviation

Table 5: Forced Degradation Studies

Drug substance	Sample treatment	% assay	% degradation	Purity Angle	Purity Threshold
Trandolapril	Acid	97.65	2.35	0.185	0.311
	Base	97.95	2.05	0.289	0.342
	Peroxide	95.00	5.00	0.181	0.311
	Thermal	96.65	3.35	0.175	0.30
	UV	98.44	1.56	0.200	0.314
Verapamil Hydrochloride	Acid	97.79	2.21	0.106.	0.275
	Base	97.77	2.23	0.102	0.270
	Peroxide	94.53	5.47	0.168	0.274
	Thermal	95.62	4.38	0.093	0.261
	UV	98.34	1.66	0.102	0.271

LOD and LOQ

LOD and LOQ were calculated as $3.3 \sigma / S$ and $10 \sigma / S$ respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected standard and sample solutions by small variation in the chromatographic conditions and found to be unaffected by small variations like $\pm 2\%$ variation in volume of mobile phase composition with respect to acetonitrile, ± 0.2 mL/min in flow rate of mobile phase, ± 0.5 variation in pH, different type of filters and ± 5 column temperature variation. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both Trandolapril and Verapamil Hydrochloride in sample solution.

The degradation study indicated that Trandolapril and Verapamil Hydrochloride were susceptible to acid, base, oxidation, photo and thermal degradation. Typical chromatograms of stressed samples are shown in figs. 6-11. In all degradations the drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non-degraded drug, without giving any additional degradation peaks. Both the drugs showed no degradation at 0 h, in all the degradation conditions. In that percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under non-degradation

condition. It also showed retention time of degraded products which were observed in different degradation conditions for both drugs.

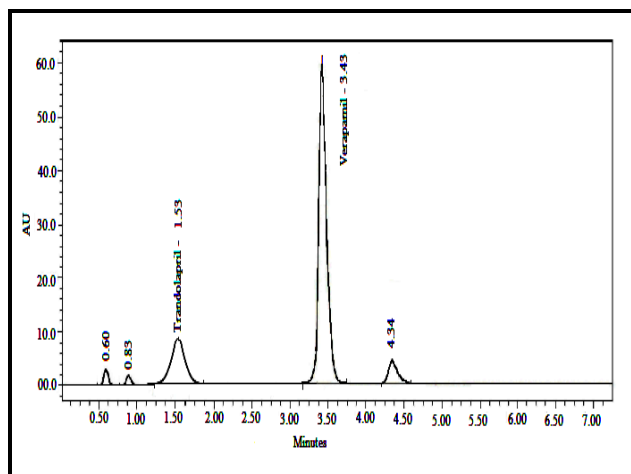


Figure 3: Acid degradation chromatograms for Trandolapril and Verapamil Hydrochloride

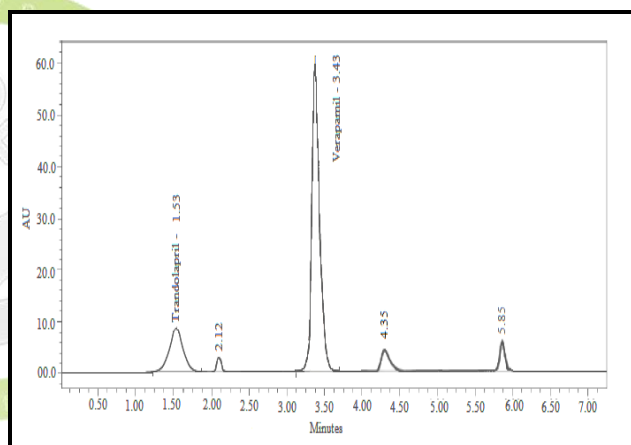


Figure 4: Base degradation chromatograms for Trandolapril and Verapamil Hydrochloride

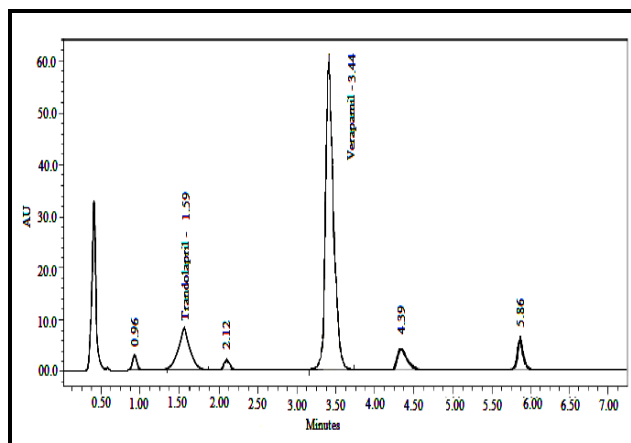


Figure 5: Oxidative degradation chromatograms for Trandolapril and Verapamil Hydrochloride

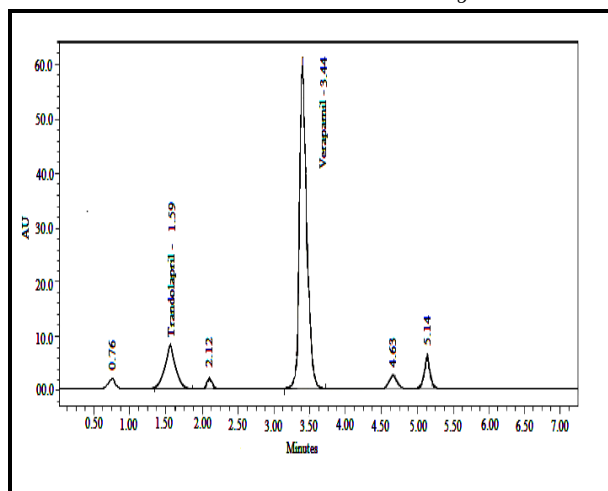


Figure 6: Thermal degradation chromatograms for Trandolapril and Verapamil Hydrochloride

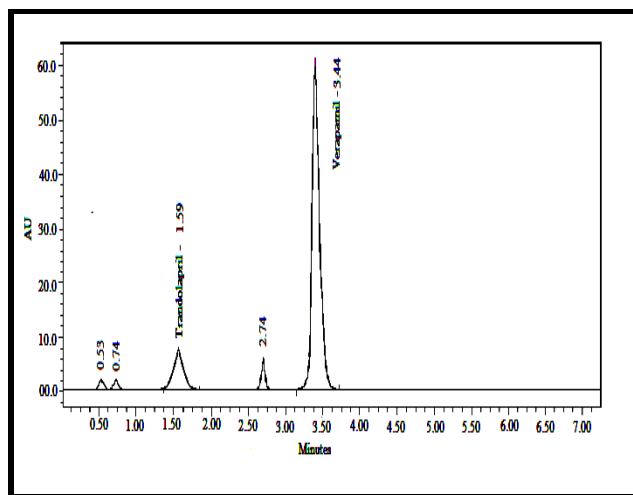


Figure 7: Photo degradation chromatograms for Trandolapril and Verapamil Hydrochloride

Table 6: Summary of validation parameters of proposed RP-HPLC method

Parameters	Trandolapril	Verapamil Hydrochloride
Linearity range ($\mu\text{g/mL}$)	1.6 – 25.4	95.7-1534.6
Correlation co-efficient	0.9998	0.9999
LOD ^a ($\mu\text{g/mL}$)	0.26	10.3
LOQ ^b	0.87	31.1

($\mu\text{g/mL}$)		
Accuracy (% Recovery)	100.0 - 100.7	99.6 – 99.8
Precision (% RSD)		
Intraday ($n^d = 6$)	1.7	1.2
Interday ($n^d = 6$)	0.7	0.2

^a LOD = Limit of detection.

^b LOQ = Limit of quantitation.

^c RSD = Relative standard deviation.

^d n = Number of determination

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Trandolapril and Verapamil Hydrochloride in combined tablet dosage form.

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