Stress degradation studies on trandolapril and development of a stability-indicating HPLC assay method for pharmaceutical dosage form

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ABSTRACT

A stability- indicating HPLC method was developed for the quantitative determination of trandolapril in pharmaceutical dosage forms in the presence of degradation products. It involved a X terra RP18 150 mm × 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^{-1} 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.
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INTRODUCTION

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[1] suggest that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aim of the present study was to establish inherent stability of trandolapril through stress studies under a variety of ICH recommended test conditions[1-3] and to develop a stability-indicating assay[4-6].

Trandolapril is in a class of drugs called angiotensin-converting-enzyme inhibitors (ACE inhibitors). It is chemically described as (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*) -1-(Ethoxycarbonyl)-3-phenylpropyl]amino] propanoyl] octahydro-1H-indole-2-carboxylic acid. It is used to lower high blood pressure. It is available in 1 mg, 2 mg and 4 mg tablets for oral administration (Figure 1).

In the literature there are limited reported methods referring to the determination of trandolapril in investi-
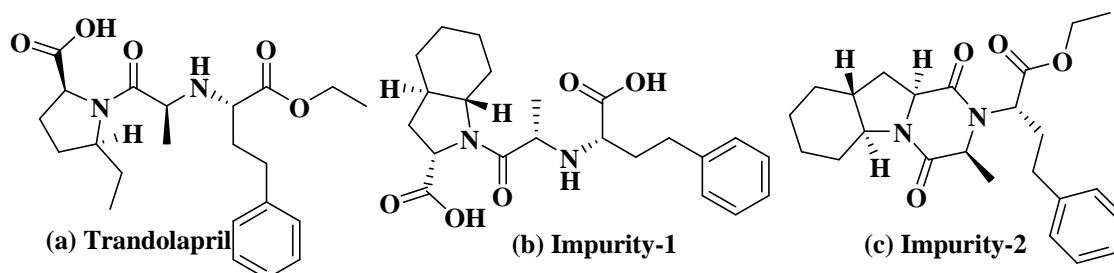
Note

Figure 1: Chemical structures of (a) Trandolapril (b) impurity-1 (c) Impurity-2

gation on the stereo chemical purity of trandolapril and octahydro-lH-indole-2-carboxylic acid by HPLC method[7]. A liquid chromatography and tandem mass spectrometry using solid-phase extraction has been reported for quantification of trandolapril and its metabolite trandolaprilat in human plasma[8]. A reversed-phase HPLC method with UV detection has been reported for the determination of trandolapril in capsules[9] and a reversed-phase HPLC method has been reported for the determination of trandolapril and verapamil in capsules[10]. A LC method for analysis of trandolapril has been reported for pharmaceutical dosage forms[11]. But in these procedures stability indicating nature of analytical method was not described.

So far, to our knowledge no stability-indicating LC assay method for trandolapril has been developed. Accordingly, the aim of the present study was to establish the stability of trandolapril through stress studies under a variety of ICH-recommended test conditions [1-3] and to develop a stability-indicating assay method[4-6].

Hence a rapid simple reproducible stability indicating RP liquid chromatography method was developed for the quantitative determination of trandolapril in pharmaceutical dosage forms in the presence of degradation products.

EXPERIMENTAL

Chemicals

Standards and tablets were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile and analytical grade triethyl amine and ortho phosphoric acid were purchased from Merck, Darmstadt, Germany. Water was prepared by using Millipore MilliQ Plus water purification system.

Equipment

The Waters HPLC system we used consists of a binary solvent manager, a sample manager and a UV detector. The out put signal was monitored and processed using empower software, water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber(Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic column used was a X terra RP18 150 mm4.6 mm i.d with 5μm particles. 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 of mobile phase was 1.2 mL min^{-1}. The HPLC gradient program was set as: time (min)/% solution B: 0/0, 8/0, 12/100, 16/0 and 20/0. The column temperature was maintained at 35^{0}C and the detection was monitored at a wavelength 210 nm. The injection volume was 10μL.

Preparation of stock solutions

A working solution of trandolapril standard and sample (0.08 mg mL^{-1}) was prepared by dissolving an appropriate amount in diluent (mixture of pH 3.0, 10mM Na_2HPO_4 buffer: acetonitrile 40:60, v/v).

Preparation of sample solution

20 tablets, containing 1 mg of trandolapril were weighed and transferred to a 250 mL standard volumetric flask. The drug was finally dissolved in 250 mL
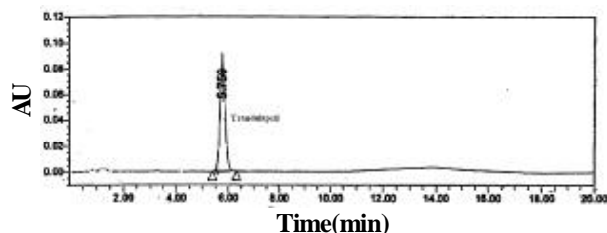
Figure 2: Chromatogram of trandolapril from tablets

of diluent. The solution was filtered through 0.45 μm Millipore PVDF filter. Then 10 μL of these solutions were injected in the column and chromatogram was recorded and shown in figure 2. The retention time of trandolapril was found to be 5.7 min.

Stress studies

Specificity is the ability of the method to measure the analyte response in the presense of its potential impurities[4]. Stress testing of a drug substance can help to identify the likely degradation products, which can in tern help establish the degradation pathways and the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 0.08 mg mL^{-1}. Acid hydrolysis was performed in 0.01N HCl at 50^{0}C for 15 min. The study in basic solution was carried out in 0.01N NaOH at 50^{0}C for 30 min. For study in neutral solution, drugs dissolved in water was heated at 50^{0}C for 90 min. Oxidation studies were carried out at 40^{0}C in 1% hydrogen peroxide for 2 h. Photo degradation studies were carried out at according to option 2 of Q1B in ICH guidelines[3]. Samples were exposed to light for an overall illumination of 1.2 million lux h and an integrated near ultraviolet energy of 200 watt hm^2. The drug product was exposed to dry heat at 105^{0}C for 12 h. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution (0.08 mg mL^{-1}).

Method validation

Precision

The precision of the assay method was evaluated by carrying out six independent assays of trandolapril (0.08 mg mL^{-1}) test samples against qualified reference standard. Different analyst from the same laboratory evaluated the intermediate precision of the method. The percentage of RSD of six assay values was calculated.

Linearity

Linearity solutions were prepared from stock solution at five concentration levels from 12.5% to 150% of analyte concentrations (from 10 to 120 μg mL^{-1}). The slope, Y-intercept and correlation coefficient were calculated.

Accuracy

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration in tablets. The percentages of recoveries were calculated.

Solution stability and mobile phase stability

The solution stability of trandolapril was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 48 h. The same sample solution was assayed for a 24 h interval up to the study period against freshly prepared standard solution of trandolapril. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 24 h interval up to 48 h. The mobile phase preparation was kept constant during the study period. The percentage of RSD of assay of trandolapril was calculated for the study period during mobile phase and solution stability experiments.
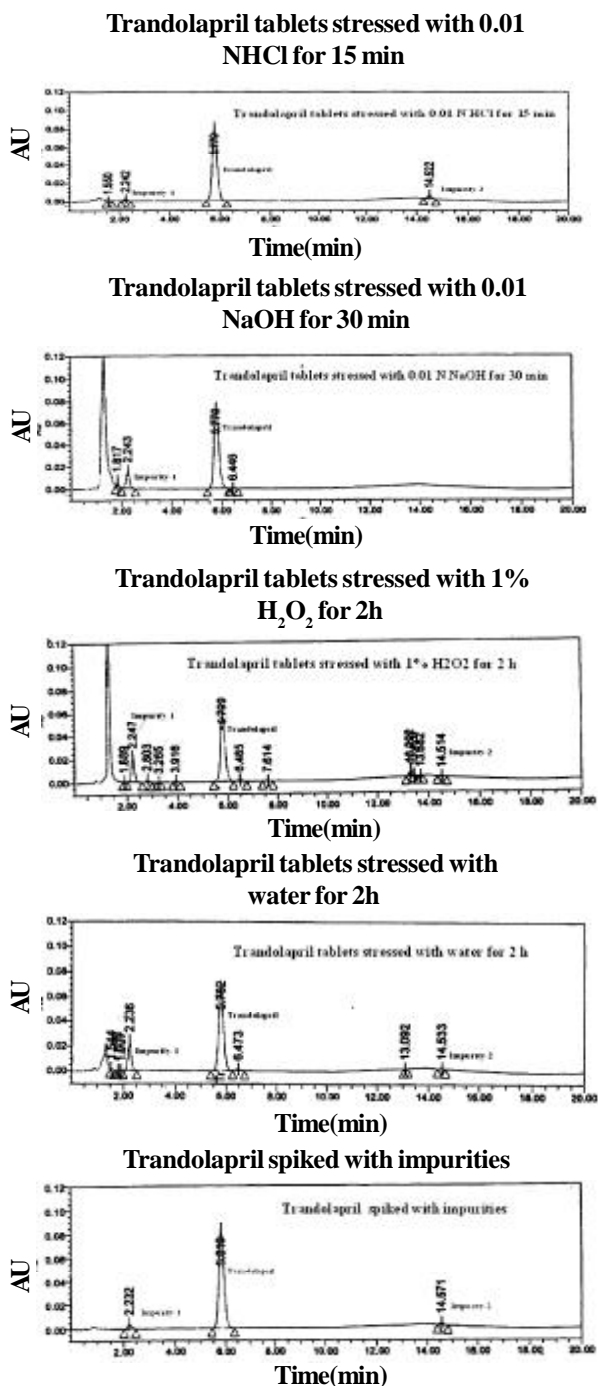
Robustness

To determine the robustness of the method the experimental conditions were deliberately changed and the % RSD for five injectins area of standard was evaluated. The mobile phase flow rate was 1.2 mL min^{-1}; to study the effect of flow rate on resolution it was changed to 1.0 and 1.4 mL min^{-1}. The effect of pH was studied at pH 2.8 and 3.2 (instead of pH 3.0). The effect of column temperature was studied at 30 and 40^{0}C (instead of 35^{0}C). In all these experiments the mobile phase components were not changed.

RESULTS AND DISCUSSION

Degradation in acidic solution

In 0.01 N HCl at 50^{0}C for 15 min, drug underwent degradation, forming degradation products at RRTs of 0.27, 0.39 and 2.51. The total degradation was about 6.1% (Figure 3).

Note

Trandolapril tablets stressed with 0.01 NHCl for 15 min

Trandolapril tablets stressed with 0.01 NaOH for 30 min

Trandolapril tablets stressed with 1% H₂O₂ for 2h

Trandolapril tablets stressed with water for 2h

Trandolapril spiked with impurities

Figure 3: Chromatograms indicating the degradation of Trandolapril in acid, base, peroxide, water and spiked with related impurities

Degradation in basic solution

In 0.01 N NaOH at 50^0C for 30 min, drug underwent degradation, forming degradation products at RRTs of 0.31, 0.39 and 1.11. The total degradation was about 13.2% (Figure 3).

Oxidative conditions

The drug was exposed to 1% hydrogen peroxide at 50^0C for 2 h. Major degradation was observed at RRTs of 0.39, 1.10 and 2.51. The total degradation was about 27% (Figure 3).

Degradation in neutral aqueous solution

The drug was exposed to water at 50^0C for 90 min. Major degradation was observed at RRTs of 0.39 and 1.10. The total degradation was about 21% (Figure 3).

Photolytic conditions

The drug was stable against the effect of photolysis. When the powdered drug was exposed to light for an overall illumination of 1.2 million lux h and an integrated near ultraviolet energy of 200 watt h m^2 in a photostability chamber, no degradation was observed.

Thermal degradation

When the drug powder was exposed to dry heat at 105°C for 24 h, no decomposition was observed.

The impurity which was formed at RRT 0.39 in acid, base, peroxide and water degradation was '(2S,3aR, 7aS)-1-[(2S)-2-[[(1S)-1-carboxy-3-phenylpropyl] amino] propanoyl]octahydro-1H-indole-2-carboxylic acid' (Impurity-1). This was confirmed with spiking analysis with qualified reference standard of Impurity-1 and also with spectral matching and with mass spectroscopy. The mass number of peak at 0.39 RRT observed is 403.The impurity which was formed at RRT 2.51 in acid and peroxide degradation was the 'ethyl (2S)-2-[(3S,5aS,9aR,10aS)-3-methyl-1,4-dioxo decahydropyrazino[1,2-a]indol-2(1H)-yl]-4-phenyl butanoate' (Impurity-2). This was confirmed with spiking analysis with qualified reference standard of Impurity-2 with spectral matching and with mass spectroscopy. The mass number of peak at 0.39 RRT observed is 413.

Mass balance (% assay + % degradents + % impurities) is calculated for stress sample. The mass balance of stressed sample was > 99.0% for all stress conditions (TABLE 3).

Method development and optimization of stability indicating assay method

The method was optimized to separate major degradation products formed under varies stress condi-

tions. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly the degradation products at 1.10 and 2.51 RRT. The degradation samples were run using different stationary phases like C18, C8, Cyano and Mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-8) and using organic modifiers like acetonitrile, tertahydrofuran and methanol in the mobile phase and different ratio's of solution B. But the separation was satisfactory in the adopted chromatographic conditions only.

Interference of excipients was also checked by injecting sample solutions of these excipients. There was no interference of excipients with degradation peaks or with the trandolapril peak.

Validation of developed stability-indicating method

Precision

The percentage RSD value for the precision study was 0.4% (inter-day precision) and 0.2% (intra-day precision). This is confirming good precision of the method (TABLE 1).

Linearity

Linearity calibration plots for this method was obtained over the calibration ranges tested; i.e. 10 to 120µg mL^{-1} trandolapril and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte. The slope and Y-intercept of the calibration curve were calculated. The mean regressions equation was found as A =16777 C - 4445 (r^2 = 0.9999, n = 6). A = aC + b, where A is the peak area ratio of the drugs, a is the slope, b is the intercept and C is concentration of the measured solution in µg mL^{-1}. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Accuracy

The percentage recovery of trandolapril in pharmaceutical dosage forms ranged from 99.8 to 101.6. Excellent recoveries were made at each added concentration (TABLE 2).

Solution stability and mobile phase stability

The solution stability and mobile phase stability ex-

TABLE 1: Precision results of trandolapril from tablets

Precision	% Assay						% RSD	% Mean
	1	2	3	4	5	6		
Intra-day	101.7	102.7	102.2	102.5	103.0	102.7	0.4	102.5
Inter-day	102.8	102.4	102.3	102.7	102.3	102.5	0.2	102.5

TABLE 2: Percentage recovery of trandolapril from tablets

Added (µg)	Recovered (µg)	Recovery (%)	RSD (%)
41.0	41.0	100.0	0.21
82.0	81.8	99.8	0.18
123.0	125.0	101.6	0.05

TABLE 3: Summary of results from forced degradation experiments

Stress condition	Time	Assay of active substance (%)	Mass balance (% assay + % impurities + % degradation products)	Remarks (Major degradation products)
Acidic hydrolysis (0.01 N HCl)	15 min	93.0	99.1	Degraded to Impurity-1 and Impurity-2
Basic hydrolysis (0.01 N NaOH)	30 min	86.4	99.6	Degraded to Impurity-1
Oxidation (1% H$_2$O$_2$)	2 h	72.4	99.8	Degraded to Impurity-1
Aqueous hydrolysis	2 h	78.5	99.3	Degraded to Impurity-1
Thermal treatment (60^{0}C)	10 days	98.5	99.6	No degradation products formed
Light (photolytic degradation)	10 days	98.9	100.1	No degradation products formed

TABLE 4: Results from study of robustness

Condition	Variation	%RSD for five injections area for standard
Temperature (± 5ºC of optimum temp.)	30ºC	0.1
	40ºC	0.2
Flow rate (± 0.2ml/min of optimum flow rate)	1.0 mL min^{-1}	0.1
	1.4 mL min^{-1}	0.3
pH (± 0.2 unit of set pH)	2.8	0.2
	3.2	0.0

periment data confirms that sample solution and mobile phase used during the assay were stable up to 48 h.

Note

Robustness

When mobile phase flow rate, pH and column temperature were deliberately varied % RSD for area of five replicate injections of standard was less than 1.0, illustrating the robustness of the method (TABLE 4).

Tablet application

Analysis was performed for commercially available trandolapril 4 tablets. The Mean assay (n = 6) for trandolapril was 99.8%. The percentage RSD value for the six assay values was 0.5%.

CONCLUSION

A rapid gradient HPLC method prove to be simple, linear, precise, accurate and specific. The total runtime was 20 min within which active drug and their degradation products were separated. The method was completely validated showing satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for the quantitative determination of the trandolapril in presence of degradation products in stability by the industry.

ACKNOWLEDGMENTS

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