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International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Forced Degradation Studies on Trandolapril and Development of a Stability-indicating related Substances High Performance Liquid Chromatography Method in Presence of its Degradation Compounds

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ARTICLE INFO

Article history:
Received: 29 August, 2022
Revised: 05 December, 2022
Accepted: 13 December, 2022
Published: 30 January, 2023

Keywords:
High-performance liquid chromatography, Method development, Stability indicating, Trandolapril, Validation.

DOI:
10.25004/IJPSDR.2023.150105

ABSTRACT

In present studies high-performance liquid chromatography (HPLC) method for trandolapril is developed and subsequently validated. The method utilizes an Xterra MS C18 column at 35°C temperature, gradient elution with aqueous potassium dihydrogen phosphate having pH-3.0 by orthophosphoric acid and combination with acetonitrile as the mobile phase. We are reporting here simultaneously the separation of all related impurities and degradation products of trandolapril formed under different stress conditions as per ICH Q1A (R2) guideline. Trandolapril showed labile behavior in acidic, basic and oxidative stress conditions. The developed method was extensively validated and proved to be robust. The developed method has shown excellent linearity over the range from 0.10 to 3.0 µg/mL. The correlation coefficient(r) is 0.999. The % recovery value of trandolapril and their two impurities ranged from 97.98 to 100.55%, respectively.

Introduction

Stability indicating high-performance liquid chromatography (HPLC) method development consisting of forced degradation study is important for a drug. Forced degradation is the degradation of drug substance and drug product at conditions more severe than accelerated conditions. It demonstrates specificity of stability-indicating methods and provides insight into the drug's degradation pathway and degradation products. It also helps in the elucidation of the structure of the degradation products.

Impurity profile methods are standard tools in the pharmaceutical industry for characterizing active pharmaceutical ingredients and dosage form. The validation of these methods has been clearly expressed by United States Pharmacopeia[1] and international regulatory agencies.[2,3] The validation of an impurity profile method seeks to demonstrate that it is suitable for its intended use. The ability to consistently identify and quantify impurities is critical to establishing the properties of the drugs for safety and efficacy.

Trandolapril (Fig. 1) 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. Trandolapril is a long lasting angiotensin converting enzyme (ACE) inhibitor approved by U.S. Food and Drug

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

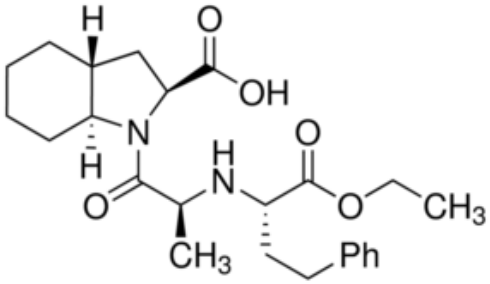

Fig. 1: Chemical Structure of Trandolapril

Administration (FDA) for lowering blood pressure in dosage up to 2 mg even after discontinuation of treatment.[4] Trandolapril is the rapidly absorbed and metabolized to its biologically active diacid form, trandolapril in liver which shows high lipophilicity compared to other ACE inhibitor. Literature survey reveals that few methods involving amperometric biosensors,[5] a potentiometric enantioselective membrane electrode,[6] liquid chromatography tandem mass spectrometric[7], HPLC,[8-12] however a very few stability indicating methods[13-15] have been reported, which provide varies level of degradation of trandolapril. Stability indicating method reported[13] by Manju *et al.*, does not produces any degraded product in different stressed conditions; although it is well documented that trandolapril is susceptible to hydrolysis. Sahu *et al.*,[15] have reported the validated stability indicating method which can separate the hydrolytic degraded product of trandolapril. Leena *et al.*,[16] have reported the validated stability indicating HPLC determination of trandolapril in bulk drug and pharmaceutical dosage forms. Laha *et al.*[17], reported RP-HPLC method for analysis of trandolapril in the pharmaceutical dosage form. Paraanath *et al.*[18], reported RP-HPLC method for analysis of trandolapril in tablet dosage form. Reddy CS *et al.*[19] reported stability-indicating related substances method of trandolapril by RP-HPLC and its degradation by using Agilent C18 column (L-150 × ID 4.6 mm, 3.5 micron particle size) with wavelength 213 nm, with very good run time. However, the literature survey reveals that it is impossible to elute the non-polar impurities such as impurity-D (Diketopiperazine) at early retention time about 4 minutes in reversed-phase HPLC. Also, the article does not confirm the separation of known impurities in the presence of forced degraded compounds of trandolapril.

Literature survey reveals that, no reference has been found for the simultaneous determination of trandolapril and its related impurities in the presence of forced degradation compounds of trandolapril. So it is felt to develop and validate stability indicating HPLC method for estimation of trandolapril and its related compounds in bulk drug in presence of degradation products as per International Council for Harmonisation (ICH) guidelines.

Material And Methods

Chemical and Reagents

Trandolapril standard drug substance was received as gratis samples from Lupin pharmaceutical Ltd (Tarapur, India). Analytical reagent (AR) grade hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), potassium phosphate, phosphoric acid, acetonitrile (ACN), and methanol (MeOH) were purchased from Rankem fine chemicals Ltd. (Mumbai, India). Ultra-pure HPLC grade water was obtained from a millipore water purification system (Merck, Germany)

Equipments

A (HPLC) system from water corporation (USA) was used for the present studies which consisted of an on-line degasser, autosampler injector, an ultraviolet UV-visible detector, a quaternary pump (four port), spectra recorded on a computer system loaded with Empower software. Degradation experiments were performed using water bath on Labline Sun Scientific Pvt. Ltd. Mumbai, India). All the separation was achieved on Xterra RP-18 column (Water Inc. USA). Photolytic degradation was carried out in photo stability chamber from Thermolab, 95 Th-400G, Mumbai, India.

Chromatographic Conditions

Chromatographic separation was achieved on Xterra MS C18, (100 x 4.6 mm i.d. with 3.5 micron particle size) (Water technology USA) column under gradient mode elution for HPLC as T (min), %B(V/V); 0/01, 22/01, 45/90, 50/90, 60/01 and 70/01. The mobile phase A consist of buffer and acetonitrile 80:20,v/v. the mobile phase B consist of buffer and acetonitrile 45:55,v/v. Separation was performed at 35°C using 1-mL/min flow rate with a run time of 70 minutes the injection volume was 20 µL and the detection was performed using a photodiode array detector.

Stress Studies

As per the ICH guidelines, the stress degradation studies were carried out under hydrolytic, photolytic, oxidative, and thermal conditions. The stock solution of the drugs was prepared in acetonitrile at the concentration of 1000 µg/mL. HCl, NaOH, H_2O_2 the stressor and the drug solution were used under all the stress conditions. Degradation studies of trandolapril were carried out from lower concentrations of degradative solvents or acids at minimum temperature to achieve about 5–15% degradation. In the initial alkali stress experiment, the trandolapril showed higher degradation (above 40%) at 5 mL, 1 N NaOH at 60°C, hence the concentration of alkali was gradually decreased to get optimum degradation, while in acid stress experiments, the trandolapril showed different stability behavior. The acidic stressor (HCl) was increased from lower concentration to higher concentration to

achieve optimum degradation. The oxidative stress condition was optimized from lower hydrogen peroxide (30%) concentration (1 mL) to high concentration (30%, 3 mL at 50°C). For base and acid hydrolysis were finally carried out with 1N NaOH and 1N HCl, at room temperature and 70°C for 30 and 45 minutes, respectively. The potential for oxidative decomposition was studied in 3 mL, 30% H_2O_2 at 50°C for 25 minutes.

Sample Preparation

The degraded stressed samples of trandolapril were used for preparation of sample solutions. Sample solutions of each individual degraded sample were prepared with a concentration 2500 µg/mL by using water: acetonitrile (50:50, v/v). Adjusted the pH of final solution with equal volume of acid and base. All impurities spiked stock solution of 1.0 mg/mL was prepared by dissolving an appropriate amount in diluents. Further weighed accurately about 125 mg of trandolapril in to 50 mL volumetric flask, mixed, dissolved and made up to the mark after adding 1.25 mL of impurity stock solution.

HPLC Method Development

The HPLC method development of related impurities focused on separating related impurities and degradation products in the drug substance. The trandolapril drug substance solution was scanned from 200 to 400 nm at 100 µg/mL concentration. The obtained UV spectra observed that absorbance at 210 nm wavelength was maximum. Hence 210 nm wavelength was selected for further analysis. To achieve separation between trandolapril related impurities (Trandolapril related compound-E and trandolapril related compound-D) and degradation products, various ratio of phosphate buffer with acetonitrile were tried. pH of phosphate buffer solution was also varied. Initially, separation between trandolapril and it's related impurities was achieved to check the retention time by using binary HPLC system. Then all degraded solutions were injected individually into HPLC binary system. The test procedure parameters were optimized to achieve the base to base separation for all the degradation products of trandolapril and its related impurities.

Method Validation

The developed test procedure was validated with respective system precision, specificity, method precision, linearity, Limit of Detection (LoD), Limit of Quantitation (LoQ) and accuracy.

Specificity

Specificity of the method was confirmed by injecting diluent as a blank solution and individual trandolapril 2500 µg/mL solution. The spiked solution of trandolapril with two impurities at 1% of specification level were prepared and injected.

Precision

The method precision experiment was performed by injecting six individual preparation of trandolapril test sample solution. The % RSD (relative standard deviation) of six results were calculated.

Method Precision (Intermediate Precision)

The intermediate precision experiment was performed by injecting six individual preparation of trandolapril test sample with different system and on a different day. The overall % RSD (relative standard deviation) of six results was calculated.

Linearity

Linearity of the developed test procedure was performed from LoQ to 120% of the specification levels of all two impurities with trandolapril (with respective 2.5 mg/mL of trandolapril test solution) LoQ to 120% of 2500 µg/mL of the analyte. The standard solution 0.10, 0.15, 0.20, 0.25, 0.50, 1.0, 1.5, 2.5, and 3.0 µg/mL were injected. The calibration curve was obtained by plotting peak area response verses concentration of impurities and trandolapril drug substance. Slope, y-Intercept, residual sum of square and correlation coefficient were calculated.

LoD and LoQ

LoQ and LoD for trandolapril and its related compounds (impurities) were predicted by linearity curve by using residual standard deviation and slope. The precision of predicted LoQ was established by performing six replicate analysis of standard solution at LoQ level. %RSD (relative standard deviation) was calculated.

Accuracy/Recovery

The accuracy of test procedure was performed by spiking known amounts of impurities in a pre-analysed trandolapril drug substance. Actual % amount found was calculated. The recovery was tested at three levels 50, 100, and 150% of the specification limit.

Robustness

The robustness of the test procedure was performed by making the deliberate variations in the chromatographic conditions. The mobile phase flow rate was 1.0 mL/min; to study the effect of flow rate on resolution between trandolapril and its impurity. Related compound-E, it was changed to ± 0.1 units from 1.0. The robustness of HPLC column temperature on the resolution was also studied at 33 and 37°C.

Solution Stability

The analytical solution stability was performed at room temperature. A spiked sample solution with all known impurities is analyzed initially and periodically up to 24 hours. The cumulative % RSD (relative standard deviation) of peak area response were calculated.

Table 1: Stress conditions and % degradation of trandolapril

Stress conditions	*Exposure condition*	*Duration*	*% degradation*	*Peak purity*
Acid	2 mL, 1.0 N HCl	70°C, 45 minutes	10.5	997.215
Base	1 mL, 1.0N NaOH	at room temperature, 30 minutes	18.2	998.551
Oxidative	3 mL, 30% H_2O_2	25 minutes	6.65	997.233
Thermal	80°C	24 hours	3.5	998.156
UV-short	254 nm	24 hours	-	999.216
UV-long	366 nm	24 hours	-	999.771
Photo	1.2 lux hours	24 hours	-	998.857

Table 2: System suitability, precision and recovery parameters of trandolapril and related substances

Parameters	*Trandolapril*	*Di-acid*	*Di-ketopiprazine*
Retention time	14.87	3.81	40.78
Tailing factors	0.97	1.01	1.03
Resolution	-	4.8	12.5
Method precision (%RSD)	0.58	0.72	0.83
Intermediate precision(overall %RSD)	0.67	0.82	0.99
Recovery (%)	99.99	97.82	100.15

Table 3: Linearity, LoQ and LoD data

Analyte name	*LoQ(ppm)*	*LoD(ppm)*	*Correlation coefficient*
Trandolapril	0.24	0.07	0.9998
Trandolapril related compound E	0.01	0.003	0.9997
Trandolapril related compound D	0.26	0.08	0.9999

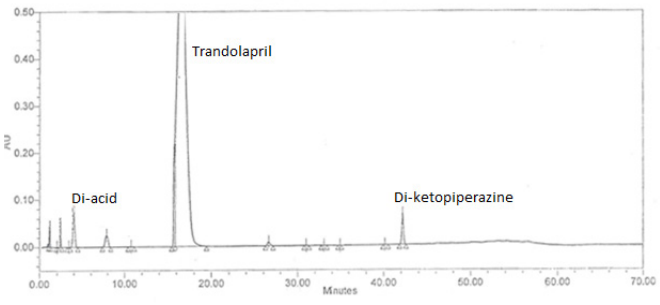

Fig. 2: Trandolapril acid degradation product

Results And Discussion

The trandolapril drug substance was found stable under all other stress conditions, including photo and thermal stress conditions. The degradation impurities were formed in acid, base and oxidative stress conditions. The chromatograms observed the degradation behavior of trandolapril shown in Figs 2 and 3. Total of 14 degradation products were generated from the drug. The major degradation products were generated from acid and alkali stress conditions. Four minor degradation products

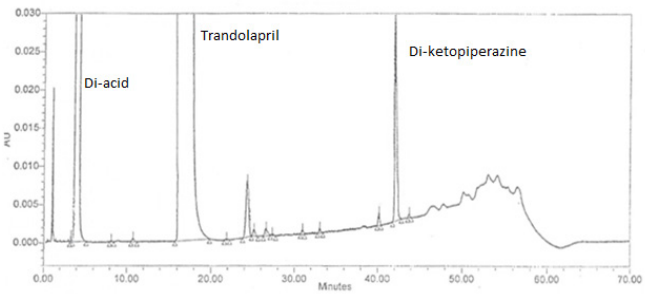

Fig. 3: Trandolapril alkali degradation product

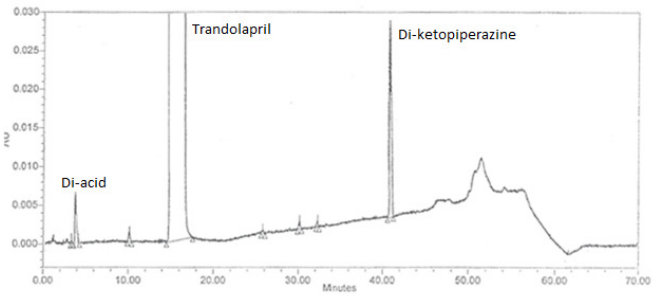

Fig. 4: Trandolapril spike solution

generated from per-oxide stress conditions. This shows that the drug substance trandolapril is acid, base and oxidative labile to certain extent and an indication to the API and drug product manufacturers to optimize the critical quality attributes (CQA) to avoid the hydrolysis and oxidative exposer during manufacturing process.

The degradation conditions, percentage, and peak purity results of trandolapril were determined. These are shown in Table 1.

In specificity study, all degradation products of analyte and all known related compounds were separated and no interference of degradation products were observed. The retention time of trandolapril was found to be 15.58 minutes, while the tailing factor was recorded as 0.97 to 1.03. the other parameters such as resolution between trandolapril and all degradation products found more than 1.6 respectively. Trandolapril peak, while theoretical plates was found to be 9822, respectively (Fig. 4). The peak purity of trandolapril stressed samples checked using photodiode detector. The system suitability parameters and recovery values are shown in Table 2

The resolution between trandolapril and related compound-E was not less than 3.0 in the robustness study.

The equation of the calibration curve for trandolapril Y=7955.5x+4458.4, trandolapril related compound-E, Y=2588.2x+1782.3, and trandolapril related compound-D Y=6844.1x+3244.5 the correlation coefficient are 0.9998, 0.9997 and 0.9999 respectively.

The LoD for trandolapril and Related compounds (impurities) are 0.07, 0.003 and 0.08 µg/mL, respectively and the LoQ is 0.24, 0.01 and 0.26 µg/mL, respectively. The parameters are shown in Table 3.

The experimental data and results yielded new and useful information yet not reported in the literature on the simultaneous separation of trandolapril and their related substance as well as their degradation products by reverse phase HPLC (RP-HPLC) method. The developed HPLC test procedure was observed to be suitable for the separation and base to base resolution of all impurities and degradation products formed under specified stress conditions. In line with the reported method, for first time we have developed a simultaneous separation and validation of related substances and degradation products test procedure of trandolapril active pharmaceutical ingredients is different from the reported methods. The advantages of this method over existing reported methods are that it can be used in the quality control department and stability study of drug substances and pharmaceutical drug products.

Acknowledgement

Authors are thankful to Lupin pharmaceutical Ltd (Tarapur, India) for providing gratis samples for the present studies.

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HOW TO CITE THIS ARTICLE: Chavan RS, Thakur A, Ullah Q, Yameen S, Arif PM. Forced Degradation Studies on Trandolapril and Development of a Stability-indicating related Substances High Performance Liquid Chromatography Method in Presence of its Degradation Compounds. Int. J. Pharm. Sci. Drug Res. 2023;15(1):34-38. **DOI:** 10.25004/IJPSDR.2023.150105