WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 1, 559-575. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT, VALIDATION AND STABILITY ESTIMATION OF TRANDOLAPRIL ASSAY AND ITS ORGANIC IMPURITIES BY RP-UPLC-MS

B. Anjaneyulu Reddy[1,2]*, P. Radhakrishnanand[2], Md. Irshad Alam, J. Satish and Arjun Siliveri

[1]Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.
[2]United States Pharmacopeia–India Private Limited, Research and Development Laboratory, ICICI Knowledge Park, Turkapally, Shameerpet, Hyderabad-500078, India.

Article Received on
06 November 2017,
Revised on 27 Nov. 2017,
Accepted on 18 Dec. 2017
DOI: 10.20959/wjpr20181-10291

***Corresponding Author**
B. Anjaneyulu Reddy
Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

ABSTRACT

A novel, rapid, specific and stable RP-UPLC-MS assay and its Organic Impurities method was developed and validated for the estimation of Trandolapril in Active pharmaceutical ingredient. The separation was carried out by using a mobile phase consisting of Solution A: 0.1% TFA in water, Solution B: 0.1% TFA in Acetonitrile.Solvent-Acetonitrile: water (2:8).The column used for separation wasColumn: Acquity UPLC BEH C18, 100 mm x 2.1 mm x1.7µm with flow rate of 0.4ml/min and wavelength at 210nm. The retention time for Trandolapril was 5.56. The stability of the developed method was estimated by stress testing of Trandolapril by exposing them to various forced degradation conditions. The method was also validated in terms of accuracy, precision, linearity, system suitability, robustness and ruggedness as per ICH guidelines.

KEYWORDS: Trandolapril, Assay, Organic Impurities, UPLC, LC-MS, ICH, Forced Degradation.

INTRODUCTION

Trandolaprilis a non-sulhydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of drugs, Trandolapril is the international nonproprietary name of (2S, 3aR,7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]–ctahydro-

1H–indole-2-carboxylic acid. It is metabolized to its biologically active diacid form, trandolapril, in liver. Trandolapril 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). 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 micro albuminuria or overt nephropathy. Trandolapril is official in British Pharmacopoeia and its assay estimated by non-aqueous titration and in United States pharmacopeiaassay and related substances determined by HPLC which are having long runtime and not compatible with the MS detector. As it has been investigated that very limited study was done on assay and organic impurities of trandolapril by UPLC-MS method, so in the present study we had developed a noveltechnique for estimation of trandolapril, with the development of a proper solvent system.As per ICH guidelines, the stability study of trandolapril in its active pharmaceutical form (API) was performed to validate the stability of developed analytical method and to identify factors influencing stability of the drug product. From the experimental data obtained it was observed that the developed method was simple, accurate, stable, time saving and economical.

MATERIALS AND METHODS

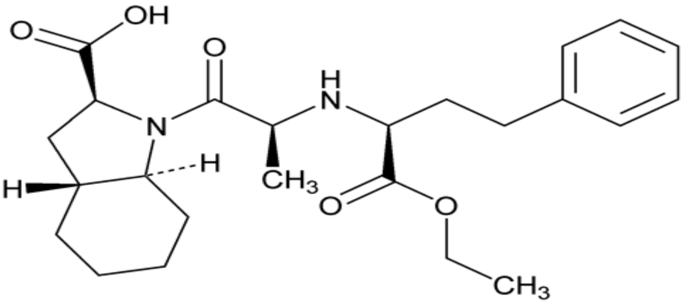
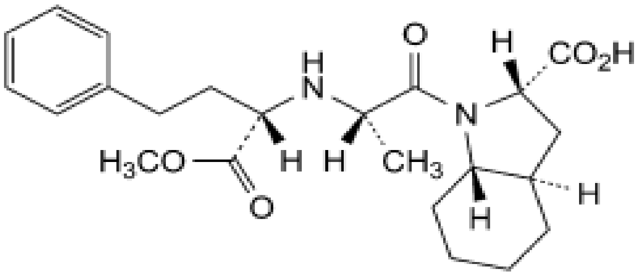
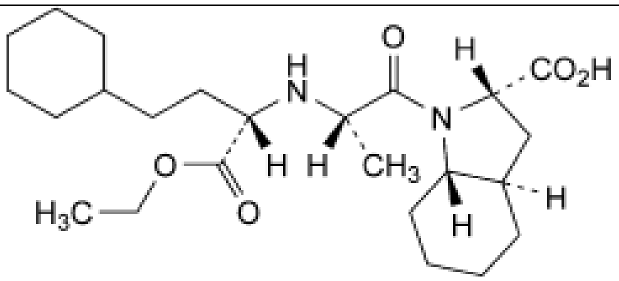
Reagents and Chemicals

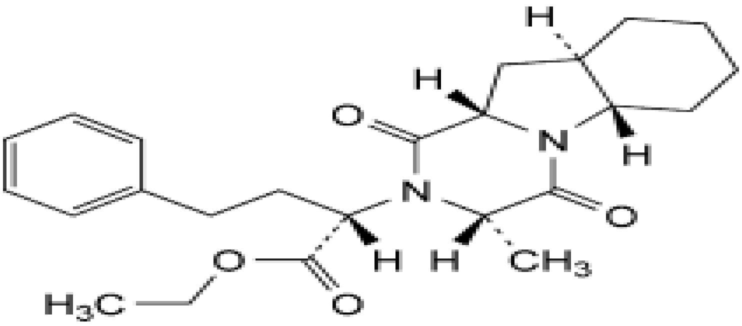
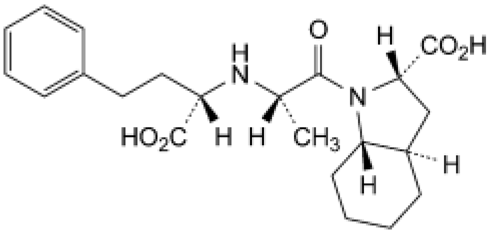
All chemicals used were of HPLC grade. Trandolapril pure was obtained as a gift sample from United states Pharmacopeia (P) Ltd, Hyderabad, India.

Instrumentation and Chromatographic Conditions

Ultra high performance liquid chromatography (RP-UPLC) was performed on Waters 2487UHPLC system that comprises with Empower 3 software, the column used for separation, Acquity UPLC BEH C18, 100 mm x 2.1 mm x1.7µm. The elution was carried out by gradient method. The UHPLC gradient program was set as: Time (min)/ % solution B: 0/25, 2/25, 6/60, 8/60, 8.2/25 and 10/25.

Fig 1: Chemical structure of Trandolapril and its impurities.

S.No	**Name**	**Structure**
1	**Trandolapril**	 1*H*-Indole-2-carboxylic acid, octahydro *N*-[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]-1-alanyl(2*S*,3a*R*,7a*S*); (2*S*,3a*R*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxy-3-phenylpropyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester Molecular Formula :$C_{24}H_{34}N_2O_5$ Molecular weight:430.54
2	**Impurity 1** **(Impuririy-A)**	 (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(methoxycarbonyl)-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (methyl ester derivative),
3	**Impurity 2** **(Impurity C)**	 (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (hexahydrotrandolapril),

4	**Impurity 3** **(Impurity D)**	 Ethyl(2*S*)-2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-ioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]-4-phenylbutanoate (trandolaprildiketopiperazine),
5	**Impurity 4** **(Impurity E)**	 (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (trandolaprilate),

EXPERIMENTAL

Instrumentation

Waters H-Class UPLC system with *Empower 3* software equipped with an integral auto sampler and quaternary gradient pump with an inline degasser was used. For LC-MS study, Thermo Scientific Ion trap LCMS System was used for identification of impurities. Balances from Sartorius, Photo stability chamber from SUNTEST XLS+, Hot Air Oven from Memmert and Humidity Chamber from ESPEC were used during experiments.

Samples and reagents

Pure trandalopril, its related impurities and marketed formulation (Tablets) were obtained from USP-India, Hyderabad, India. HPLC grade acetonitrile and Trifluoroacetic acid (TFA) was obtained from Merck chemicals (Mumbai, India). Other analytical grade chemicals like hydrochloric Acid, sodium hydroxide and hydrogen peroxide purchased from Merck chemicals (Mumbai, India). HPLC-grade water was obtained through a Milli-Q system (Sartorius, resistance 18.2 M.Ω-cm).

Sample and solution preparations

Diluent: A mixture of Water and Acetonitrile (8:2 v/v) was used as a diluent throughout the experiment for dissolving of the samples.

Preparation of trandolapril assay standard and sample solution

Taken about 10 mg trandalopril in to a 100 mL volumetric flask and dissolved and diluted to volume with diluent (100 µg mL^{-1}).

Preparation of organic impurities (OI) standard preparation

Taken about 2.5 mg trandalopril, trandaloprilRC A, trandalopril RC C, trandalopril RC D , trandalopril RC E in to a 10 mL volumetric flask and dissolved and diluted to volume with diluent (250 µg mL^{-1}). Working solution of organic impurities of 25 µg mL^{-1} was prepared from the above stock solution in the diluent.

Preparation of trandolapril organic impurities sample preparation

Taken trandolapril about 25mg to a 10 mL volumetric flask and dissolved and diluted to volume with diluent (2500 µg mL^{-1}).

Preparation of trandolapril and spiking with organic impurities preparation

Taken trandolapril about 12.5 mg to a 5 mL volumetric flask and added 0.5 mL of Organic impurities stock solution (25 µg mL^{-1}) dissolved and diluted to volume with diluent (API 2500 µg mL^{-1} and impurities with 2.5 µg mL^{-1}).

Preparation of trandolapril tablet OI sample preparation

Twenty tablets were weighed and average weight of tablet was calculated. Tablet powder equivalent to 560 mg of was transferred in to a 5 mL volumetric flask. To this added 2 mL of the diluent and sonicated for 30 minutes with intermediate shaking. The solution was then diluted to 5 mL with diluent and centrifuged at 3000 rpm for 10 min. The supernatant (2500 µg mL^{-1}) was collected, filtered through 0.22 µ filter and used as the sample solution to determine OI.

Preparation of trandolapril tablet Assay sample preparation

Twenty tablets were weighed and average weight of tablet was calculated. Tablet powder equivalent to 22 mg of was transferred in to a 5 mL volumetric flask. To this added 2 mL of the diluent and sonicated for 30 minutes with intermediate shaking. The solution was then diluted to 5 mL with diluent and centrifuged at 3000 rpm for 10 min. The supernatant (1000 μg mL^{-1}) was collected, filtered through 0.22 μ filter and used as the sample solution to determine assay.

Preparation of Robustness solution

Taken about 2.5 mg trandolapril, trandolapril RC A, trandolaprilRC C, trandolaprilRC D, trandolapril RC E in to a 10 mL volumetric flask and dissolved and diluted to volume with diluent (250 μg mL^{-1}). Working solution of organic impurities of 25 μg mL^{-1} was prepared from the above stock solution in the diluent. (This solution contains each 25 μg mL^{-1} of trandalopril, trandalopril RC A, trandalopril RC C, trandalopril RC D, trandalopril RC E in diluent). Compounds were identified on Thermo Scientific Iontrap LCMS System. The typical operating source conditions for MS scan of trandolapril and its impurities in positive HESI mode were optimized as follows : Capillary temp(C) 250, Source heater temp(C) 300, Sheath Gas flow:45, Aux Gas flow:20,Source voltage(kV):3.00.

Method Validation

The method was validated with respect to system suitability, specificity, precision, sensitivity, linearity, accuracy and robustness[10to1]

System suitability

System suitability parameters were performed to verify the system performance. System precision was determined on six replicate injections of Organic Impurity (OI) standard preparation and assay standard preparation. All the important chromatographic characteristics, including the relative standard deviation, peak tailing, theoretical plate number and resolution were measured. These all system suitability parameters covered the system, method and column performance.

Specificity

Specificity of the method was assessed by comparing the chromatograms obtained from drug standards. The retention times of drug from standard solutions and from capsule content were identical and no coeluting peaks from the diluents were observed, indicating specific method for quantitative estimation of drug in the commercial purpose.

Stress study

Stress studies were performed at an initial concentration of 100 μg mL^{-1} of trandolapril in active pharmaceutical ingredients (API) to provide the stability-indicating property and specificity of the method. Initial degradation was attempted by the stress conditions of exposed to acid (0.1N HCl for 1 hour reflux at 80°C), base (0.1 N NaOH for 10 minutes at room temperature), oxidation (6% peroxide for 24 hours at room temperature), Thermal (Exposed at 105°C for 48 h) and photolytic stress (1.2 million lux hours followed by 200watt-hours per square meter).

Oxidative Stress

Trandolapril (5 mg) was weighed accurately and transferred to 50 mL volumetric flask. 5 mL of 6% hydrogen peroxide was added to it. It was stirred for one hour, and then the contents were diluted to 50 mL with diluent. Replicate solutions of concentration 100 µg/mL were prepared and chromatograms of these solutions were recorded and compared with the chromatograms obtained from the fresh solution of trandolaprilhaving the same concentration and the chromatogram of the blank (solution containing only hydrogen peroxide).

Effect of Acid Media

Trandolapril (5 mg) was weighed accurately and transferred to 50 mL volumetric flask. It was shaken for one hour with 5 mL of either 0.1 M hydrochloric acid (HCl). After one hour the content was diluted to 50 mL with diluent. Replicate solutions of concentration 100 µg/mL were prepared and chromatograms of these solutions were recorded and compared with the chromatograms obtained from the fresh solution of trandolapril having the same concentration and the chromatogram of the blank.

Effect of Alkaline Media

Trandolapril (5 mg) was weighed accurately and transferred to 50 mL volumetric flask. It was shaken for 10minutes with 5 mL of 0.1 M sodium hydroxide (NaOH). After one hour the content was diluted to 50 mL with diluent. Replicate solutions of concentration 100 µg/mL were prepared and chromatograms of these solutions were recorded and compared with the chromatograms obtained from the fresh solution of trandolapril having the same concentration and the chromatogram of the blank.

Effect of UV Light or Sunlight

Trandolapril API (50 mg) was placed in photo stability chamber for (1.2 million lux hours followed by 200watt-hours per square meter) After that 5 mg of trandolapril was weighed and transferred to 50 mL volumetric flask dissolved and diluted to the mark with diluent. Chromatograms were recorded and compared with the chromatogram of unexposed API.

Precision

The precision of the assay and organic impurities method was checked by injecting six individual preparations of Trandalopril and Trandalopril Tablets (2500 µg mL^{-1}) spiked with each 0.1% of impurity-1, impurity-2, impurity-3 and impurity-4. Intermediate precision (ruggedness) was evaluated by a different analyst in the same laboratory. Assay method precision was assessed by performing six independent assays of a sample solution of Trandalopril and against a qualified standard and calculation of the RSD (%). The intermediate precision of the assay method was evaluated by a different analyst in the same laboratory.

Limit of detection (LOD) and limit of quantification (LOQ)

The signal-to-noise ratio was determined by comparing the peak heights of the known concentrations of each organic impurity with that of the baseline noise obtained from the blank samples. A signal to noise ratio 3:1 is generally considered acceptable for estimating the detection limit. A typical signal to noise ratio is 10:1 is generally considered acceptable for estimating the quantification limit.

Linearity and Range

Impurity linearity solutions were prepared from stock solutions at five concentration levels from 0.05 to 1.0 % of analyte concentration. The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting impurity areas against the concentration expressed in µg mL^{-1}. Assay linearity solutions were prepared from stock solution at five concentration levels from 50% to 150 % of analyte concentration. The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting trandolapril areas against the concentration expressed in µg mL^{-1}

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the observed value. Impurity accuracy of the method was demonstrated at four different concentration levels. The analysis was carried out by spiking all the impurities on the API samples at 0.05, 0.10, 0.2 and 1.0 % of the trandolapril concentration (2500 µg mL^{-1}). The percentage mean recoveries at each level for all the impurities were calculated. Assay accuracy of the method was demonstrated at three different concentration levels.

RESULTS AND DISCUSSION

Method Development and Optimization

Trandolapril and its impurities are polar and medium-polar compounds. Analytical method development is more preferable reverse phase conditions. Initially, we tried different reverse phase columns like C8, C18, cyano, and Phenyl for separation of Trandalopril and its impurities. But phenyl, cyano column showed poor separation and broader peak shape because of high silonol interactions. In C8 columnseparation is good but broader peak shape.

Therefore, we considered only C18 column for optimization study. Fig.2 shows the overlay UV spectra of Trandolapril and its impurities, which indicates that 210 nm is the optimum wavelength to detect Trandolapriland its impurities with good response as well as minimum base line noise. The mobile phase pH is an important factor that drives the selectivity of the method due to differences in the pKa of molecules. Initial method development was tried on three different pH 2.5, 4.5 and 6.8 based on the literature report. However, high tailing (>2), no proper peak shape was observed at 4.5 and 6.8. We observed at pH 2.5 peak shape and resolution is good. But Base line drift was not appropriate. Then we have tried with 0.05 to 0.1 % strength of Trifluoroacetic acid (TFA), and it was observed at 0.1 % Trifluoroacetic acid(TFA) baseline is appropriate. Mainly organic modifiers forReversed-phase include acetonitrile, methanol, and in some cases, n-butanol and tetrahydrofuran. Due to the high UV cut off as well as presence of peroxide impurities in tetrahydrofuran that affect the stability of analytes, hence n-butanol and tetrahydrofuran was avoided for selection of organic modifier. We have used Acetonitrile because of its cost, good solubility in all buffers and it has a higher dipole moment and is more predominantly acidic (hydrogen bond donor). Finally we have observed good baseline, better peak shapes and separation within 10 minutes. Best results were obtained with the mobile phase-A consists of 0.1 % Trifluoroacetic acid(TFA) and mobile phase-B consists of 0.1% Trifluoroacetic acid(TFA) in Acetonitrile. The optimized flow rate was 0.4 mL. min-1, column oven temperature was set at 50°C and the elution was carried out up to 10 min. The UHPLC gradient program was set as: time (min)/% solution B: 0/25, 2/25, 6/60,8/60, 8.2/25 and 10/25 . The typical chromatogram where the separation was achieved is shown in *Fig. 2*.Acuity UPLC BEH C18, 100 mm x 2.1 mm x1.7μm column is a reversed-phase packing that can be used for basic, neutral or acidic samples. These columns can be used for a wide range of applications over a pH range of 1–12, accommodating most popular mobile phases. In the present study 0.10% specification was kept for the four process impurities with respect to 2.5 mg/mL. To confirm the masses of known process impurities and trandolapril Thermo ion trap LCMS instrument was used with same mobile phase.Fig.3 shows work flow of systematic development approach for LC method development. No formulation excipient interference observed from Tablets Fig.4.

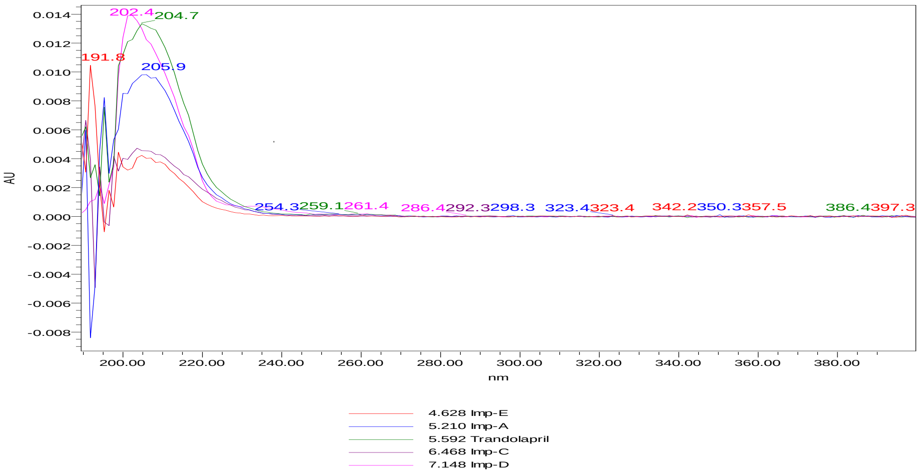


Fig 2: UV Spectra for organic impurities standard solution at 210 nm.

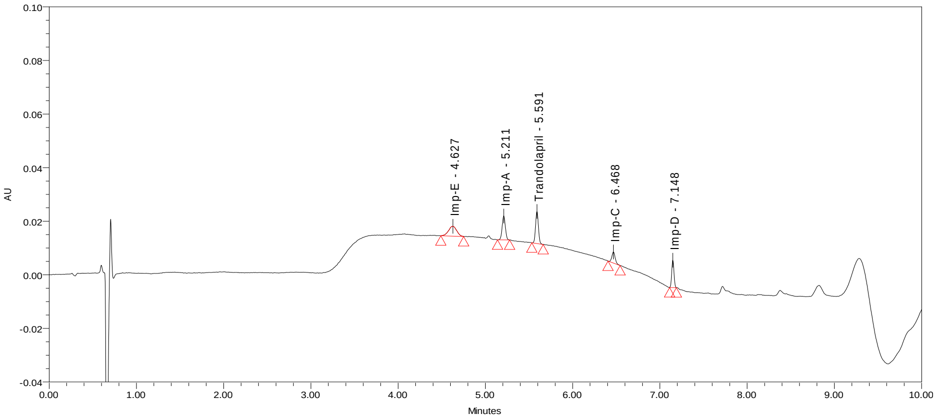


Fig3: Chromatogram for organic impurities and standard preparation.

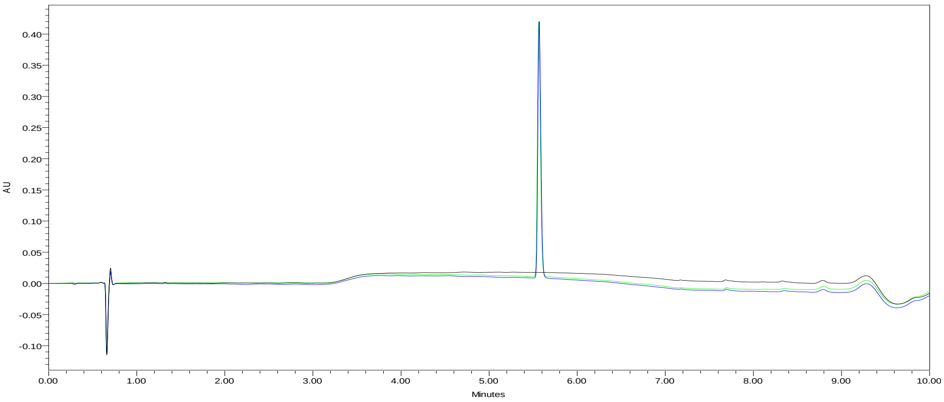


Fig. 4: Overplayed Chromatogram for Blank, assay sample and Stadard (Tablet) preparation.

System suitability

Results from system suitability study were given in Table.1.

The typical chromatogram of blank, assay standard, assay sample, tablet assay is shown in Fig. 4 and the typical chromatogram of organic impurity standard, spiked sample and LOQ is shown in Fig. 5.

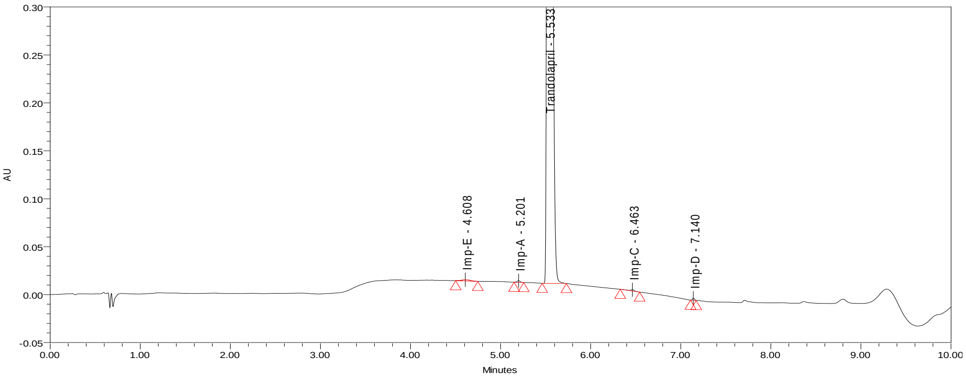


Fig. 5: Chromatogram for spiked sample standard preparation.

Table.1. System suitability results.

System suitability from Organic impurities solution					
Compound name	**RT***	**RRT#**	**USP Resolution**	**% RSD**	**m/z value**
Impurity-E	4.63	0.83	-	3.87	
Impurity-A	5.21	0.93	4.58	2.45	
Trandalopril	5.59	1.00	NA	1.72	431.26
Impurity-C	6.47	1.16	13.08	4.27	
Impurity-D	7.15	1.28	11.09	4.58	
System suitability from assay standard					
Compound name	**RT***	**USP Tailing**	**USP Plate count**	**% RSD**	**m/z value**
Trandalopril	*5.57*	*1.23*	*122119*	*0.22*	*431.26*

***RT: Retention time.**

Precision and intermediate precision

The RSD (%) of Trandolapril peak during the study of assay precision was below 0.5%. The RSD (%) of peak area for impurity-1, impurity-2, impurity-3 and impurity-4 in the study of organic impurity method precision was within 5.0%. These results confirm the precision of the analytical method is good. The RSD (%) of the assay results obtained in the study of intermediate precision was within 0.5% and RSD (%) for impurity-1, impurity-2, impurity-3 and impurity-4 were well within 5.0%, confirming the ruggedness of the method. Table. 2

Table 2. Precision data for Trandolapri assay and organic impurities.

Trandolapril API			
S.No	**Parameter**	**% RSD of The assay**	**% RSD of The Organic impurity**
1	Precision Day-1	0.5	<5.0
2	Intermediate Precision different analyst and different day	0.4	<5.0

Limit of detection and limit of quantification

The limits of quantification for impurity-1, impurity-2, impurity-3 and impurity-4 were 0.02, 0.007, 0.02, 0.006 % of the analyte concentration (i.e. 2500µg mL^{-1}), respectively, for a 2 µL injection. The limits of detection for impurity-1, impurity-2, impurity-3 and impurity-4 were 0.0055, 0.0023, 0.0055 and 0.0020% of the analyte concentration (i.e. 2500 µg mL^{-1}), respectively, for a 2 µL injection.

Table 3. Linearity data for Trandolapril assay and organic impurities.

S.No	**Name**	**Concentration range (µg mL-1)**	**Equation of regression linearity**	**R^2 value**
1	Trandolapril assay	50-150	y = 1E+07X-7945.8	0.9997
2	Impurity-E	0.05-1.0	y = 245306 x-1218.2	0.9994
3	Impurity-A	0.05-1.0	y = 227121x-29.481	0.9998
4	Impurity-C	0.05-1.0	y =100812x+34.48	1.0000
5	Impurity-D	0.05-1.0	y = 6806.6x-337.11	0.9996

Linearity

A linear calibration plot was obtained for the assay method over the calibration range tested, i.e. 0.05 -25µg mL^{-1}. The regression coefficient (R^2) was 0.99998, the slope 1218.2, and the *Y*-intercept 245360.x. A linear calibration plot was obtained for OI method over the calibration range tested, i.e. from the 0.05 to 1.0 % for impurity-1, impurity-2, impurity-3 and impurity-4. The regression coefficient was 0.999 for all impurities. This result is indicative of excellent correlation between the peak area and concentrations of impurity-1, impurity-2, impurity-3 and impurity-4 in Table 3.

Solution stability

No significant changes were observed in the content of trandalopril method during solution stability and mobile phase stability experiments when performed. The solution stability and mobile phase stability experiment data confirms that the sample solution and mobile phases used during the assay and impurity determination were stable for at least 48 h at room temperature.

Accuracy

Recovery (%) of Trandalopril ranged from 100 to 101.0% for samples. Recovery of Impurity-1, impurity-2, impurity-3 and impurity-4 from API samples ranged from 99.5 to 101.0% Sample. Table. 5.

Table 4. Accuracy data for Trandolapril assay.

Results of Trandolapril assay Accuracy Study			
S.No	Level (%) (n=3)	% Recovery	% RSD
1	50	100.2	0.5
2	100	100.5	0.3
3	150	100.7	0.2
n= Number of determinations			

Table 5. Accuracy data for Trandolapriorganic impurities.

Trandolapril			
Results of Impurity-E Accuracy Study			
S.No	Level (%) (n=3)	% Recovery	% RSD
1	0.05	99.5	1.88
2	0.10	99.8	1.5
3	0.2	100.0	1.0
4	1.0	99.8	0.7
n= Number of determination's			
Results of Impurity-A Accuracy Study			
S.No	Level (%) (n=3)	% Recovery	% RSD
1	0.05	99.1	1.7
2	0.10	99.4	1.6
3	0.2	98.9	1.2
4	1.0	99.3	0.9
n= Number of determinations			
Results of Impurity-C Accuracy Study			
S.No	Level (%) (n=3)	% Recovery	% RSD
1	0.05	100.2	1.8
2	0.10	100.4	1.3
3	0.2	99.6	1.1
4	1.0	101.5	0.8
n= Number of determinations			
Results of Impurity- D Accuracy Study			
S.No	Level (%) (n=3)	% Recovery	% RSD
1	0.05	100.7	1.6
2	0.10	99.1	1.4
3	0.2	100.2	0.8
4	1.0	100.1	0.5
n= Number of determinations			

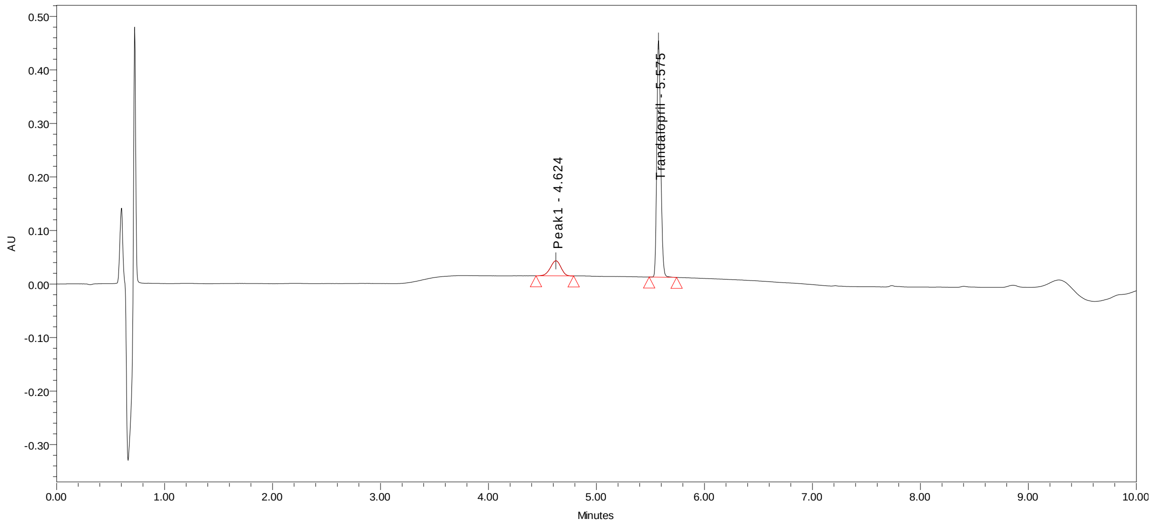


Fig 5: Chromatogram for base degradation sample.

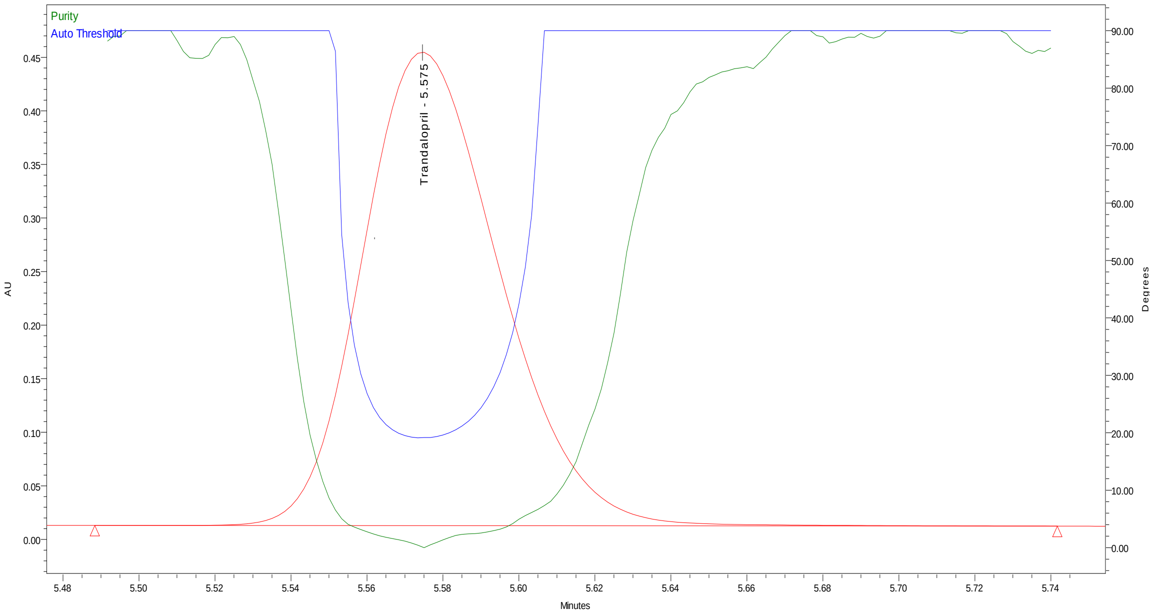
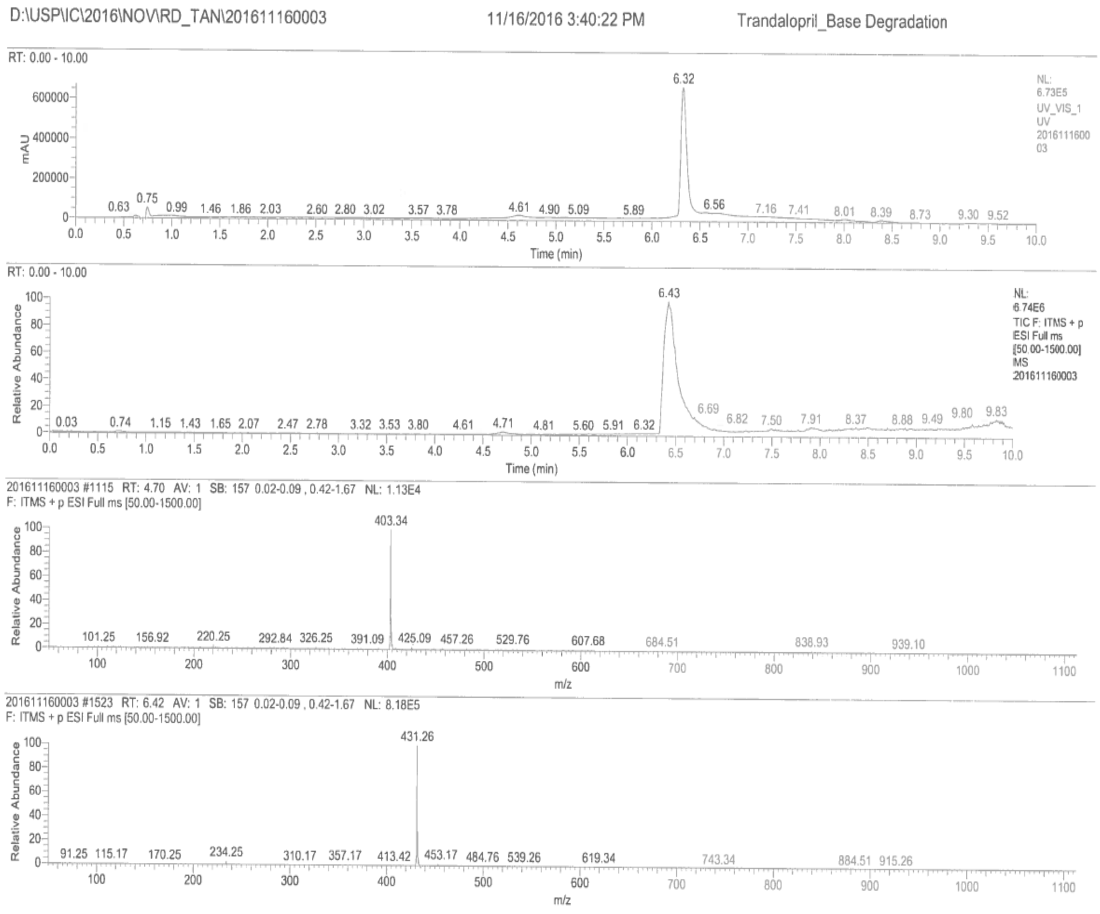


Fig 5: Purity Flag and MS Spectra for Base degraded sample (Trandalopril).



CONCLUSION

A novel simple and sensitive reverse phase stability indicating UHPLC-MS method has been developed for trandolapril and its impurities using systematic approach, Previously reported methods addressed only quantification of Trandolapril alone with longer gradients by using regular columns (250 and 150 mm, 5µm), while the present method is able to quantify trandolapril and all potential impuritiesin a shorter (10 min) run time. Design of Experiments, Statistically based experimental designs proved to be an important approach in optimizing selectivity-controlling parameters for the assay and Organic impurities determination in trandalopril API and Pharmaceutical formulation. The significant factors were optimized by applying central composite design and response surface methodology. The retention time and Retention time ratio, Resolution, and USP tailing and %RSD were predicted by DOE and validated for the quantitative determination of all its organic impurities and degradants of trandolapril. Compared with the previously reported methodologies, this method provides for separation and quantification of BH organic impurities with orthogonal detectors techniques. The present study will aid the manufacturers and suppliers of trandolapril to quantify and qualify the purity based on degradation data. The UHPLC-MS method is validated as per ICH guidelines and found to be more specific, precise, linear, accurate, rugged, and most robust. Hence, this method would be suitable for process development and quality assurance of Trandolapril in the bulk drug as well as formulation.

ACKNOWLEDGEMENTS

The authors wish to thank the management of United States pharmacopeia-India (P) Ltd for facilities and cooperation. The authors have declared no conflict of interest.

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