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A CRITICAL STUDY FOR PROCESS DEVELOPMENT AND QUALITY ASSURANCE OF TRANDOLAPRIL DOSAGE FORM

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ABSTRACT

Trandolapril is recognised by the British Pharmacopoeia, where its assay is calculated by non-aqueous titration, and by the US Pharmacopoeia, where comparable chemicals are evaluated by HPLC, which has a lengthy runtime and is incompatible with the MS detector. As it has been found that very little research has been done on the assay and organic impurities of trandolapril by UPLC-MS method, we devised a novel strategy for estimating trandolapril in the current study along with the construction of an appropriate solvent system. The stability analysis of trandolapril in its active pharmaceutical form (API) was carried out in accordance with ICH recommendations in order to assess the stability of the devised analytical method and identify factors impacting stability of the therapeutic product. The devised approach was found to be straightforward, accurate, stable, time-saving, and economical based on the experimental data collected.

KEY WORDS: *Process Development, Quality Assurance, Trandolapril, Dosage Form.*

INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitors include non-sulhydryl prodrugs such trandolapril, which is also known by the generic names (2S, 3aR, and 7aS). The compound 1-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] aminopropanoyl]Indole-2-carboxylic acid -ctahydro. The liver converts it to trandolapril, a physiologically active diacid form. Angiotensin II (ATII), which controls blood pressure and is a crucial part of the renin-angiotensin-aldosterone system (RAAS), is produced when angiotensin I (ATI) is converted to ATII by the enzyme ACE, which is inhibited by trandolapril. Trandolapril can be used to treat mild to moderate hypertension, to increase survival after myocardial infarction in clinically stable patients with left ventricular dysfunction, as a supplement to treatment for congestive heart failure, and to slow the progression of renal disease in hypertensive people with diabetes mellitus, micro albuminuria, or overt nephropathy.

PHARMACOLOGY

A prodrug called trandolapril is converted from trandolapril to trandolaprilat. The renin-angiotensin-aldosterone system is thought to be how it works to lower blood pressure. The half-life of trandolapril is approximately 6 hours, while that of trandolaprilat is around 10 hours. Trandolapril's activity is roughly eight times that of its parent medication. Trandolapril and its metabolites are eliminated in the faeces and the urine, respectively, in proportions of roughly one third and two thirds, respectively. About 80% of trandolapril's serum protein binding is reported.

A quick, easy, accurate, precise, economical, robust, and stability indicating reverse phase HPLC-PDA approach was reported for the detection of trandolapril. Transdolapril was separated isocratically on a Hypersil-Gold C18 column (250 mm 4.6 mm, 5 m) at a temperature of 25 2°C using a mobile phase that was split 50/50 acetonitrile and water with a triethylamine concentration of 0.025%. The medication had a retention time of 4.6 minutes. At 210 nm, the eluted chemicals were seen and identified. Over the concentration range of 1–24 g/mL, the method's linearity was excellent (r2 > 0.9999); the limits of detection (LOD) and quantitation (LOQ) were 0.0566 g/mL and 0.1715 g/mL, respectively. Less than 2% of the precision was overall. More than 99% of the drug was recovered on average; there was no interference from the preparation's ingredient. Studies on medication stability show that it was resistant to UV and daylight. When exposed to hydrogen peroxide, the medication produces 6 distinct oxidative products. Acidic conditions led to a slight deterioration being seen. In comparison to other situations, degradation was greater in the alkaline condition. A factorial design experiment was used to examine the method's robustness.

The simultaneous measurement of trandolapril and verapamil in pharmaceutical dose form required the development of a straightforward, precise, and accurate reverse phase high performance liquid chromatographic approach. The column utilised was Hypersil BDS C18 (100mm x 4.6mm, 5), operating in isocratic mode, using phosphate buffer and acetonitrile (60:40 v/v) in the mobile phase. The buffer is made by placing 2.72 grammes of potassium dihydrogen ortho phosphate in a 1000 millilitre volumetric flask, adding 900 millilitres of millilitre water, degassing the mixture, sonicating it, and then adding 0.5 millilitres of triethylamine before adjusting the pH to 2.8 with dilute orthophosphoric acid solution. The effluents were seen at 240 nm and the flow rate was 0.8 ml/min. Trandolapril and verapamil were found to have retention times of 2.905 minutes and 3.481 minutes, respectively. Trandolapril and verapamil had linearities between 1-6 g/ml and 60-360 g/ml, respectively. Trandolapril and verapamil both had recoveries between 98.58% and 100.64% w/v and 98.28% and 100.82% w/v, respectively. The estimate of trandolapril and verapamil in mixed tablet dosage forms was successfully accomplished using the proposed method, which was validated.

The simultaneous measurement of verapamil hydrochloride and trandolapril in bulk and pharmaceutical dosage forms, a new reverse phase HPLC method was created. Using a symmetrical C18 column (4.6 x 150mm, 3.5m) at room temperature, the approach was created and tested. The UV detection wavelength was set at 230 nm, and the mobile phase was composed of potassium dihydrogen orthophosphate buffer (pH 2.2): acetonitrile [35:65 v/v] at a flow rate of 0.6 ml/min. Verapamil hydrochloride had a 2.5-minute retention time, while trandolapril had a 3.8-minute retention time. The linearity ranges for trandolapril and verapamil hydrochloride were 2 g/ml to 15 g/ml and 10 g/ml to 65 g/ml, respectively.

With the help of an ODS INERTSIL C18 column (250 x 4.6 mm, 5 m), developed a high-performance liquid chromatography method for quantifying trandolapril using UV detection. The mobile phase consisted of phosphate buffer and acetonitrile (1:1) and flowed at a rate of 1.0 ml/min. The approach was validated according to ICH requirements for several parameters, and it was discovered that they were all within acceptable limits. The monitoring wavelength utilised was 210 nm with UV detection.

RESEARCH METHODOLOGY

REAGENTS AND CHEMICALS

All of the compounds were HPLC grade. Trandolapril pure was received as a gift sample from Hyderabad, India's United States Pharmacopoeia (P) Ltd.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

On a Waters 2487UHPLC system equipped with Empower 3 software and an Acquity UPLC BEH C18 column of 100 mm x 2.1 mm x 1.7 m, ultra high-performance liquid chromatography (RP-UPLC) was carried out. The gradient method was used to carry out the elution. The gradient programme for the UHPLC was set to: Solution B time (min)/percentage values are 0/25, 2/25, 6/60, 8/60, 8.2/25, and 10/25.

INSTRUMENTATION

The system employed had a quaternary gradient pump with an inline degasser, an integral auto sampler, and Empower 3 software. It was a Waters H-Class UPLC system. Thermo Scientific's Ion trap LCMS System was employed for the LC-MS analysis in order to identify contaminants. During the trials, balances from Sartorius,

a photo stability chamber from SUNTEST XLS+, a hot air oven from Memmert, and an ESPEC humidity chamber were employed.

REAGENTS AND SAMPLES

The marketed formulation, related impurities, and pure trandalopril were all purchased from USP-India in Hyderabad, India. Acetonitrile and Trifluoroacetic Acid (TFA) of HPLC quality were purchased from Merck Chemicals in Mumbai, India. Purchased from Merck Chemicals (Mumbai, India) are additional analytical-grade chemicals such hydrochloric acid, sodium hydroxide, and hydrogen peroxide. A Milli-Q system was used to obtain HPLC-grade water.

PREPARATIONS FOR SAMPLES AND SOLUTIONS

Diluent: Throughout the experiment, the samples were dissolved in an 8:2 v/v solution of water and acetonitrile.

PREPARATION OF THE SAMPLE SOLUTION AND TRANDOLAPRIL ASSAY STANDARD

Approximately 10 mg of trandalopril were taken, dissolved, and diluted to volume (100 g mL) with diluent. Organic impurities (OI) standard preparation preparation 2.5 mg of Trandate, Trandate RC A, Trandate RC C, Trandate RC D, and Trandate RC E were taken, dissolved, and diluted to volume with diluent (250 g mL), then added to a 10 mL volumetric flask. The aforementioned stock solution in the diluent was used to prepare a working solution of 25 g mL-1 organic impurities.

PREPARATION OF ORGANIC CONTAMINANTS IN TRANDOLAPRIL SAMPLE PREPARATION

Trandolapril was added to a 10 mL volumetric flask, dissolved, and then diluted (2500 g mL) to capacity.

THE PROCESS OF MAKING TRANDOLAPRIL AND ADDING ORGANIC CONTAMINANTS

A 5 mL volumetric flask containing 12.5 mg of trandolapril was then filled with 0.5 mL of an organic impurities stock solution (25 g/mL), which had been dissolved and diluted to volume with diluent (2.5 g/mL for the impurities and 2500 g/mL for the API).

MAKING TRANDOLAPRIL TABLETS FOR ORAL INFUSION SAMPLES

Twenty tablets were weighed, and the average tablet weight was determined. 560 mg of tablet powder was put into a 5 mL volumetric flask. 2 mL of the diluent was added to this, and it was then sonicated for 30 minutes with periodic shaking. After being diluted to 5 mL with diluent, the solution was centrifuged at 3000 rpm for 10 minutes. In order to calculate OI, the supernatant (2500 g mL-1) was collected, filtered through a 0.22- filter, and utilised as the sample solution.

MAKING A PILL OF TRANDOLAPRIL PREPARATION OF THE TEST SAMPLE

Twenty tablets were weighed, and the average tablet weight was determined. 22 mg of tablet powder were placed into a 5 mL volumetric flask. 2 mL of the diluent was added to this, and it was then sonicated for 30 minutes with periodic shaking. After being diluted to 5 mL with diluent, the solution was centrifuged at 3000 rpm for 10 minutes. In order to determine the assay, the supernatant (1000 g mL1) was collected, filtered through a 0.22 filter, and utilised as the sample solution.

ROBUSTNESS SOLUTION CREATION

A 10 mL volumetric flask was filled with 2.5 mg of trandolapril, trandolapril RC A, trandolapril RC C, trandolapril RC D, and trandolapril RC E, which was then dissolved and diluted to volume with diluent (250 g mL1). The aforementioned stock solution in the diluent was used to prepare a working solution of 25 g mL-1 organic impurities. (This solution contains trandalopril, trandalopril RC A, trandalopril RC C, trandalopril RC D, and trandalopril RC E in diluent at a concentration of each 25 g mL1). On the Thermo Scientific Iontrap LCMS System, compounds were detected. The following operating source conditions were optimised for the usual MS scan of trandolapril and its contaminants in positive HESI mode: 250 C for the capillary, 300 C for the source heater Sheath Gas flow: Aux, 45 20 gas flow The source voltage is 3.00 kV.

MODEL VALIDATION

Regarding system applicability, specificity, precision, sensitivity, linearity, accuracy, and robustness, the approach was validated.

SYSTEM APPROPRIATENESS

System performance was checked using system suitability parameters. Six replicate injections of the Organic Impurity (OI) standard preparation and the assay standard preparation were used to determine the system

precision. The resolution, theoretical plate number, peak tailing, relative standard deviation, and other crucial chromatographic parameters were all measured. The system, technique, and column performance were all covered by these system suitability characteristics.

SPECIFICITY

By contrasting the chromatograms acquired from drug standards, the specificity of the approach was evaluated. There was no evidence of any coeluting peaks from the diluents, and the retention periods of the drug from standard solutions and capsule content were identical, confirming a particular approach for quantitative determination of the medication for commercial use.

PRECISION

By injecting six separate formulations of Trandalopril and Trandalopril Tablets (2500 g mL1) spiked with each 0.1% of impurity-1, impurity-2, impurity-3, and impurity-4, the accuracy of the assay and organic impurities method was examined. Another analyst in the same lab rated the intermediate precision (ruggedness). Six independent assays of a sample solution of trandalopril were run against a certified standard to evaluate the accuracy of the assay method, and the RSD (%) was calculated. In the same facility, a different analyst assessed the assay method's intermediate precision.

LIMITS OF QUANTIFICATION (LOQ) AND DETECTION (LOD)

The peak heights of the known amounts of each organic contaminant were compared to the baseline noise obtained from the blank samples to determine the signal-to-noise ratio. In general, a signal to noise ratio of 3:1 is seen to be suitable for determining the detection limit. For estimating the quantification limit, a signal to noise ratio of 10:1 is typically regarded acceptable.

PRECISION AND SCOPE

From stock solutions, impurity linearity solutions were created at five concentration levels, ranging from 0.05 to 1.0% of analyte concentration. The least-squares linear regression analysis was performed on the peak area versus concentration data. Plotting impurity regions versus concentration expressed in g mL1 resulted in the calibration curve. Five concentration levels of assay linearity solutions, ranging from 50% to 150% of analyte concentration, were made from stock solution. The least-squares linear regression analysis was performed on the

peak area versus concentration data. Trandolapril areas were compared to concentrations expressed in g mL1 to create the calibration curve.

ACCURACY

The degree of agreement between the true value and the observed value is expressed by the analytical procedure's accuracy. The method's impurity accuracy was shown at four different concentration levels. All of the contaminants on the API samples were spiked during the examination at 0.05, 0.10, 0.2, and 1.0% of the trandolapril concentration (2500 g mL1). Calculations were made to determine the percentage mean recoveries for all contaminants at each level. The method's assay accuracy was proven at three distinct concentration levels.

RESULTS AND DISCUSSION

DEVELOPMENT AND IMPROVEMENT OF PROCEDURES

Polar and medium-polar molecules make up trandolapril as well as its impurities. Reverse phase conditions are more ideal for the development of analytical methods. To separate Trandalopril and its impurities, we first attempted a variety of reverse phase columns, including C8, C18, cyano, and phenyl. But owing of the significant silonol interactions, the phenyl, cyano column displayed poor separation and a wider peak shape. The spacing in C8 columns is decent, but the peak shape is wider.

We therefore limited our optimisation investigation to C18 columns. The overlay UV spectra of the drug Trandolapril and its impurities, demonstrating that 210 nm is the best wavelength for detecting the drug and its impurities with a good response and the least amount of baseline noise. Due to variations in molecule pKa, the mobile phase pH is a significant component that affects the method's selectivity. Based on the literature study, the initial technique development was tested on three distinct pH levels: 2.5, 4.5, and 6.8. High tailing (>2) and an improper peak shape were seen between 4.5 and 6.8, though. We noted the peak form and strong resolution at pH 2.5. Base line drift, however, was inappropriate. Then, we experimented with Trifluoroacetic Acid (TFA) of 0.05 to 0.1% strength, and found that the baseline concentration of 0.1% TFA is adequate. Acetonitrile, methanol, and occasionally n-butanol and tetrahydrofuran are the main organic modifiers for reversed-phase. Tetrahydrofuran and n-butanol were avoided when choosing an organic modifier due to their high UV cutoff and the presence of peroxide contaminants that impair the stability of analytes. Acetonitrile is what we utilised because it is inexpensive, easily soluble in all buffers, has a greater dipole moment, and is primarily acidic (a

hydrogen bond donor). Finally, within 10 minutes, we noticed a strong baseline, better peak forms, and better peak separation. The mobile phase-A contains 0.1% trifluoroacetic acid (TFA) and the mobile phase-B contains 0.1% trifluoroacetic acid (TFA) in acetonitrile, which produced the best results. The column oven temperature was set at 50°C, the optimal flow rate was 0.4 mL min-1, and the elution took place for up to 10 minutes. The time (min)/% solution B gradient programme was set to 0/25, 2/25, 6/60, 8/60, 8.2/25, and 10/25. a typical chromatogram where the separation was accomplished. Acuity Reversed-phase packing, UPLC BEH C18, 100 mm x 2.1 mm x 1.7 m column, can be utilised for basic, neutral, or acidic samples. The most common mobile phases can be accommodated by these columns, which can be employed for a variety of applications throughout a pH range of 1 to 12. In the current investigation, the four process impurities were specified at 0.10% with respect to 2.5 mg/mL. Thermo ion trap LCMS instrument was utilised in conjunction with the same mobile phase to confirm the masses of known process contaminants and trandolapril. No tablet formulation excipient interference was noticed.

PRECISION AND INTERMEDIATE PRECISION

During the assay precision investigation, the peak RSD (%) for trandolapril was less than 0.5%. In the examination of organic impurity method precision, the RSD (%) of peak area for impurity-1, impurity-2, impurity-3, and impurity-4 was 5.0% or less. These findings attest to the analytical method's high degree of precision. The RSD (%) of the assay results obtained in the research of intermediate precision was 0.5% or below, and the RSD (%) for the impurities 1, 2, 3, and 4 were all well within 5.0%, demonstrating the robustness of the approach.

TABLE -1: PRECISION DATA FOR TRANDOLAPRI ASSAY AND ORGANIC IMPURITIES

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

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

For a 2 L injection, the limits of quantification for impurity-1, impurity-2, impurity-3, and impurity-4, respectively, were 0.02, 0.007, 0.02, and 0.006% of the analyte concentration (i.e., 2500 g mL1). For a 2 L injection, the limits of detection for impurity-1, impurity-2, impurity-3, and impurity-4 were, respectively, 0.0055, 0.0023, 0.0055, and 0.0020% of the analyte concentration (i.e., 2500 g mL1).

LINEARITY

For the assay procedure over the calibration range tested, 0.05 -25g mL-1, a linear calibration plot was produced. The Y-intercept was 245360.x, the slope was 1218.2, and the regression coefficient (R2) was 0.99998. For impurity-1, impurity-2, impurity-3, and impurity-4, a linear calibration plot was obtained for the OI method for the calibration range examined, i.e. from the 0.05 to 1.0%. For all impurities, the regression coefficient was 0.999. This result shows a strong association between the peak area and the impurity concentrations in Table for impurity-1, impurity-2, impurity-3, and impurity-4.

TABLE 2.: LINEARITY DATA FOR TRANDOLAPRIL ASSAY AND ORGANIC IMPURITIES.

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

SOLUTION STABILITY

When solution stability and mobile phase stability experiments were conducted, no appreciable changes in the trandalopril method's composition were found. The results of the experiments on the stability of the sample solution and mobile phases show that they were stable for at least 48 hours at room temperature during the assay and impurity detection.

ACCURACY

Trandalopril recovery (%) for samples ranged from 100 to 101.0%. The percentage of impurities 1, 2, 3, and 4 that were recovered from API samples ranged from 99.5 to 101.0% Sample.

TABLE -3. 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 -4: ACCURACY DATA FOR TRANDOLAPRIL ORGANIC 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			

CONCLUSION

For trandolapril and its contaminants, a unique, straightforward, and sensitive reverse phase stability indicating UHPLC-MS method has been created. The new method is able to quantify trandolapril and all probable impuritiesin a faster (10 min) run time than previously described methods, which exclusively addressed the quantification of trandolapril alone with longer gradients utilising conventional columns (250 and 150 mm, 5 m). planning experiments, A key strategy for maximising selectivity-controlling parameters for the assay and identifying organic contaminants in the pharmaceutical formulation of trandalopril API was statistically based experimental designs. By applying central composite design and response surface methods, the significant factors were optimised. For the quantitative determination of all its organic impurities and degradants of trandolapril, the DOE predicted and confirmed the retention time, retention time ratio, resolution, USP tailing, and%RSD. In contrast to previously described procedures, this method uses orthogonal detection techniques to separate and quantify BH organic contaminants. The current study will help trandolapril suppliers and producers quantify and categorise purity based on degradation data. According to ICH recommendations, the UHPLC-MS method is validated and determined to be more specific, exact, linear, accurate, tough, and most robust. Therefore, this approach would be appropriate for process development and quality assurance of trandolapril in both formulation and bulk medication.

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