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Bioanalytical method development and Validation of tolvaptan

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Abstract

This study aims to develop and validate RP-HPLC bioanalytical method for Tolvaptan in bulk and Pharmaceutical formulation in human plasma. Chromatographic separation was achieved on (250 x 4.6 mm) column. The mobile phase was developed for assessment of drug in human plasma consists of methanol and 0.1% acetic acid (75:25) at a flow rate of 0.7 mL min^{-1}. Detection was done at 254 nm. Calibration curve for Tolvaptan was linear in the range of 2 to 10 µg mL^{-1} with correlation coefficient (r^2) of 0.999. Accuracy for Tolvaptan studied in the range of 80 – 120% QC standard levels. A validated bioanalytical method was found to be accurate, reproducible, linear, precise & robust.

Key words : Bioanalytical method**,** Reverse phase, Tolvaptan, Method optimization.

Objectives

Analytical method development and validation has crucial place in the origination and production of drug products. Method validation ensures that test is reasonable for its expected use. Results from method validation can be utilized to acquire the quality, reliability and consistency of analytical results.

Analytical methods working for quantitative determination of drugs and their metabolites in biological fluids are the key determinants in generating reproducible and dependable data that in turn are used in the evaluation and elucidation of bioavailability, bioequivalence and pharmacokinetics.

Acetic acid offers several advantages over other solvents. For the preparation of the manuscript relevant literature[1-12] has been consulted.

P^H *adjustment:* Enhance LC separations

Resolution Enhancement: increase the resolution of certain compounds

Compatibility: Glacial acetic acid is often used with acetonitrile and methanol,

Cost effective: relatively inexpensive

Objectives : To develop cost effective, simple, robust and reliable bioanalytical method suitable for almost all labs in India.

All chemicals were purchased from Jinendra Scientific, Jalgaon, M.S.

Preparation of Standard Solution : 10 mg Amount of standard + 2 ml of human plasma (untreated) was mixed upto100 ml methanol, then vertically shaked for 30 min then centrifuged at 5000 rpm for 1 hr .Then it was filtrated using membrane filters to get clear organic solution .Then it can be filled in to the sample vials of HPLC and loaded on to HPLC for Run. From the above solution 0.2, 0.4, 0.6, 0.8 and 1.0 mL were taken in different 10 mL volumetric flasks and volume was made to make 2, 4, 6, 8 and 10 µg mL^{-1} Tolvaptan solutions.

Twenty µg mL^{-1} Tolvaptan solution was scanned in UV region 200- 400 nm.

Different mobile phase compositions were studied for better elution of Tolvaptan.

Assay for marketed Formulation : (NATRISE 15mg SUN PHARMA LTD.)

Tablets (20) were crushed & weighed equivalent to about 10mg of Tolvaptan + 2ml of human plasma (untreated) was taken up to 100ml methanol. Then vertically shaked for 30 min then centrifuged at 5000 rpm for 1 hr .Then it was filtrated using membrane filters to get clear organic solution. From this stock solution 0.2 ml was taken and volume was made with mobile phase (0.1% Acetic Acid: Methanol (25:75 V/V) in 10 ml volumetric flask to get the final concentration of 20 µg mL^{-1} Tolvaptan.

Analytical Method Validation :

The method developed was validated as per ICH guidelines Validation of Analytical Procedures: Text and Methodology Q2 (R1)[2-4].

Specificity : It was measured by injecting drug solution (10µg mL^{-1}) and comparing the chromatograph with that obtained from blank run.

Linearity : Method's linearity was determined by spiking known concentrations (2, 4, 6, 8 and 10 µg mL^{-1}), peak areas were recorded. Calibration curve was constructed with drug response on Y-axis and concentration on X-axis. The correlation was evaluated through a regression analysis. The correlation coefficient (R^2) was calculated.

Accuracy : The accuracy was measured by using three QC samples of different concentrations of Tolvaptan 80% (8 µg mL^{-1}), 100% (10 µg mL^{-1}) and 120% (12 µg mL^{-1}). Three injections of each concentration of Tolvaptan were chromatographed. The percent accuracy was calculated using formula.

Precision : The intraday precision was determined by analyzing control standards of Tolvaptan (4, 6, 8 µg mL^{-1}) three times over one day in no fixed order. Inter-day precision was determined from the analysis of each control standards of Tolvaptan (4, 6, 8 µg mL^{-1}) one on each of three different days. The % RSD was calculated for day-to-day and within-day variations and reported as a measure of reproducibility.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The Limit of Detection and Limit of Quantitation studies of Tolvaptan were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of diluted solutions with known concentrations. LOD and LOQ were calculated using the formulae.

Robustness : To determine the Robustness, in this study, composition of mobile phase, flow rate, wavelength parameters were chosen.

Method development : The UV scan (Shimadzu UV 1700 Pharmaspec) of pure Tolvaptan in mobile phase showed a λ_{max} of 254 nm. Hence UV detector was set at this wavelength for the entire analysis. The mobile phase optimized after several experimental evaluations was Methanol: 0.1 % acetic acid in the ratio of 75:25 and 0.7 ml/min flow rate was found to be the most suitable for best elution of Tolvaptan. (Fig. 1).

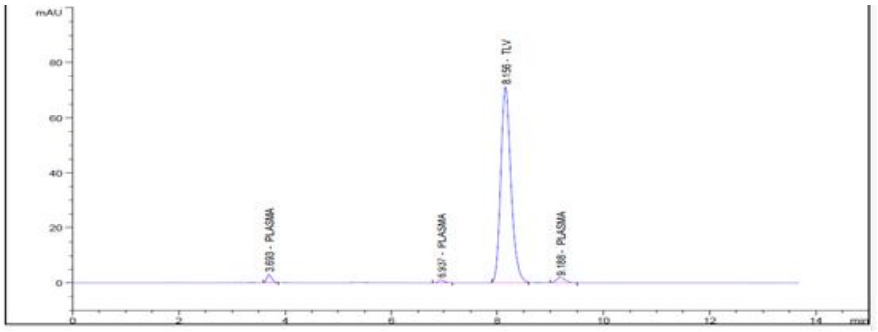

Figure 1: Optimized HPLC Graph

Method Validation :

Specificity: Under the developed condition Tolvaptan had a retention time of 8.1 min. The results showed that the developed HPLC method was selective for Tolvaptan.

Linearity : The peak area response was linear over the concentration range studied. Linearity equation & correlation coefficient 'r^2'obtained were found to be y = 39.9x + 197 and r^2=0.999 for Tolvaptan. (Fig. 2).

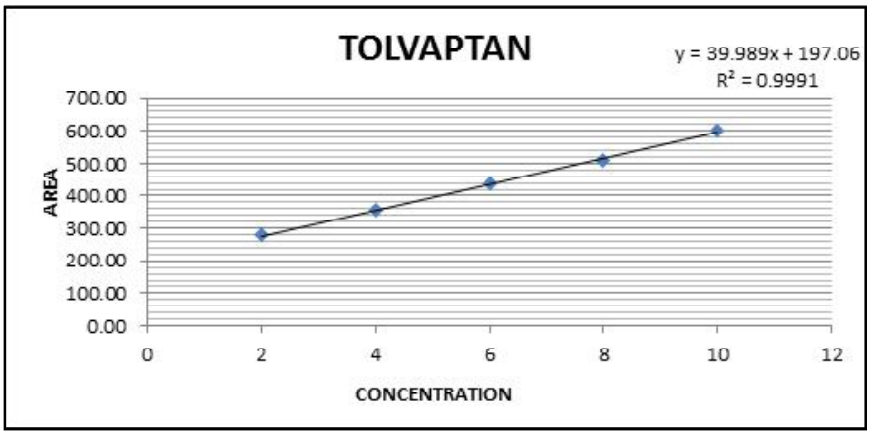

Figure 2: Calibration Curve

Accuracy: For 80%, 100% & 120% recoveries for Tolvaptan were 98.68 %, 98.67 % & 99.88 % respectively. (Fig. 3-5)

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation

HPLC, Model No	Agilent Tech. Gradient System with Auto injector, 1100
Detector	UV (DAD) G13148 S.NO. DE71365875
Pump	Quaternary Gradient (G130A) S.NO.DE9180834)
Software	CHEMSTATION 10.1
Column & Particle size	4.6 x 250 mm, 5 µm
Stationary Phase	C18 (AGILENT) P.No.f18-050502, SN-BO5151408-1
Mobile Phase, Flow rate	Methanol: 0.1 % acetic acid (75:25 V/V), 0.7 ml/min
Detection Wavelength	254nm
Temperature	25^{0}C
Sample size	20 µl

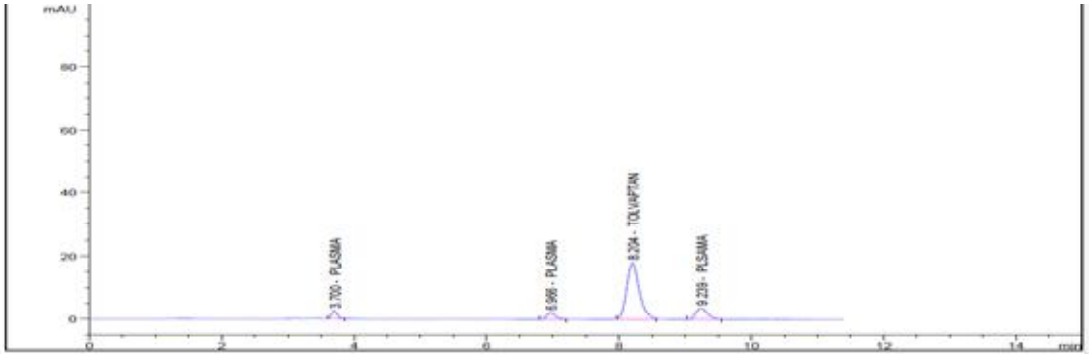

Figure 3. Accuracy (80%)

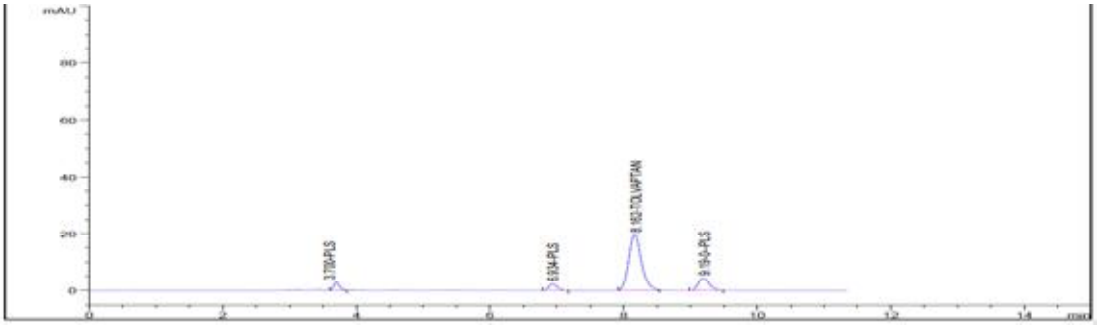

Figure 4. Accuracy (100%)

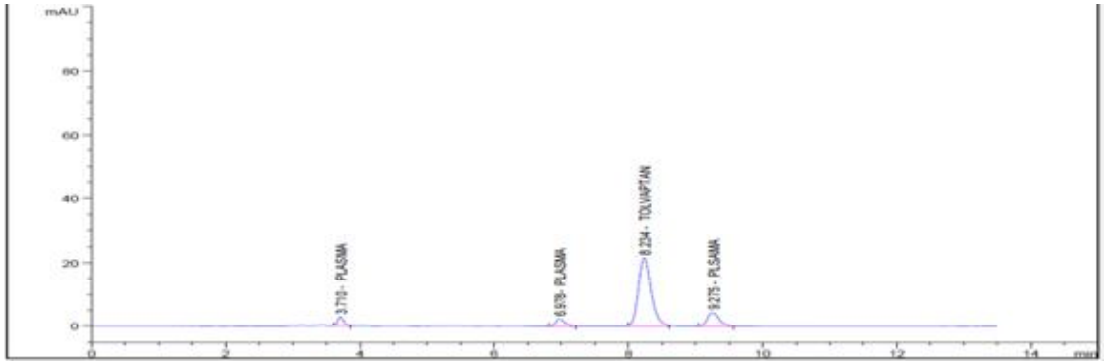

Figure 5. Accuracy (120%)

Precision: Intra-day precision % RSD for Tolvaptan was 0.31, 0.05 and 0.18 for the spiked concentration at 4, 6 & 8 µg mL^{-1} respectively. Inter-day precision % RSD for Tolvaptan was 0.65, 0.23 and 0.11 for the spiked concentration at 4, 6 & 8 µg mL^{-1} respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ for Tolvaptan were determine for concentration range 4, 6 & 8 µg mL^{-1} solutions respectively at 0.7 ml/min flow rate as determined in linearity study and LOD and LOQ value determined as per their equation. The LOD and LOQ were found to be 0.066 µg mL^{-1} and 0.20 µg mL^{-1} respectively for Tolvaptan. Robustness: It was measured by changing flow rate *i.e.* 0.6 and 0.8 ml for Tolvaptan and by changing mobile phase ratio for Tolvaptan *i.e.* 1% Acetic Acid: Methanol (74:26 V/V) and 1% Acetic Acid: Methanol (76:24 V/V) and by changing detection wavelength for Tolvaptan *i.e.* 253nm and 255 nm. % RSD was found to be in between 0.04 to 0.66 which is less than 2%. (Fig. 6-10).

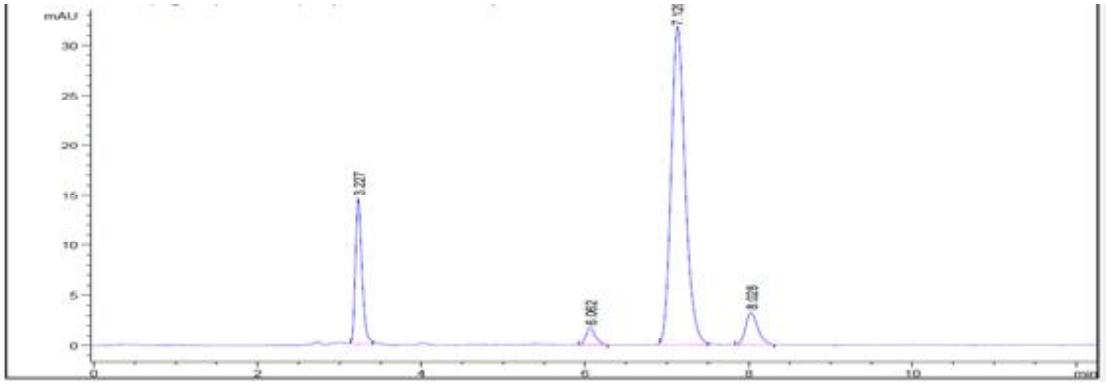

Figure 6: Robustness (0.8 ml)

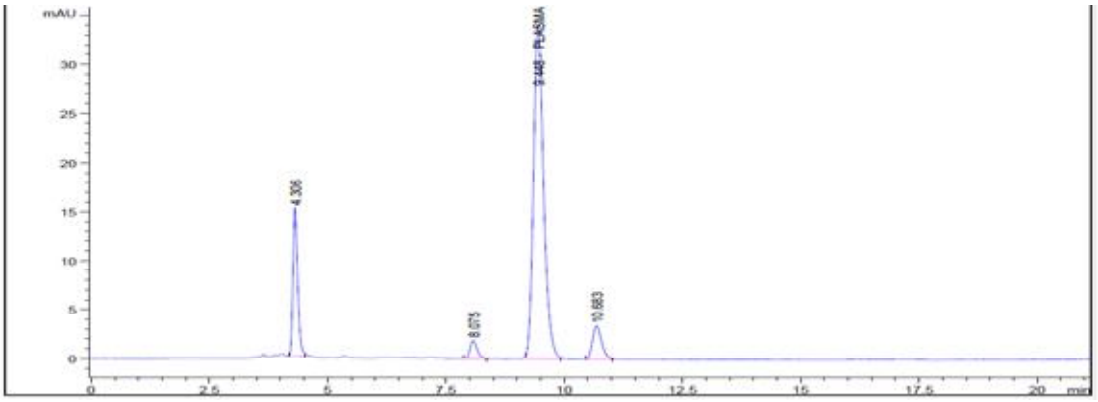

Figure 7. Robustness (0.6 ml)

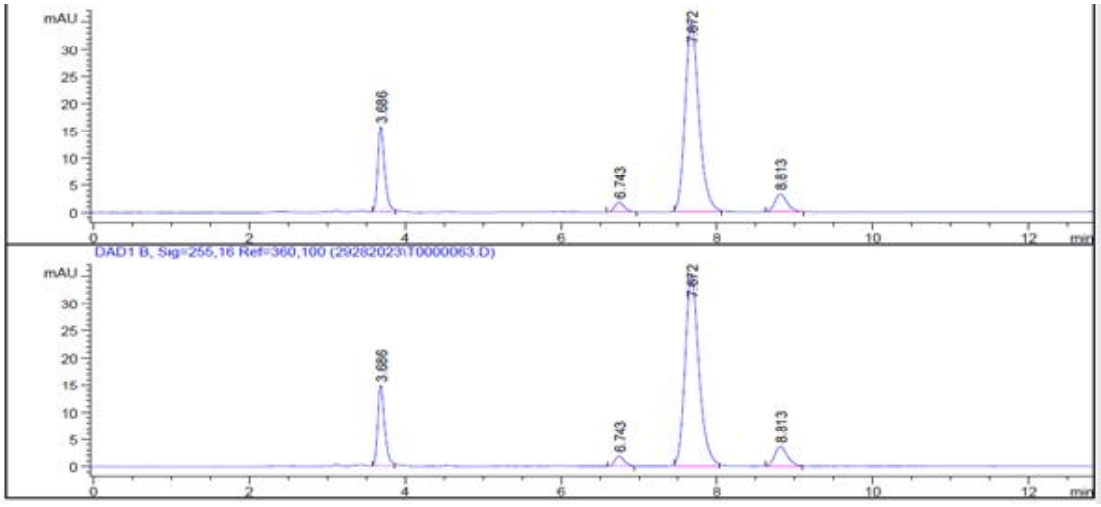

Figure 8. Robustness (253 nm & 255 nm)

ASSAY: Assay was performed for Tolvaptan and amount was found to be 98.56%, SD was 0.307 and % RSD was 0.311 for Tolvaptan.

RP-HPLC bioanalytical method has been developed and validated for the estimation of Tolvaptan in bulk and tablet dosage form. Calibration curves at 5 levels were Linear in the range of 2 to 10 µg mL^{-1}. Accuracy for Tolvaptan was studied in the range of 80 – 120% quality control standard levels and % recoveries were 98.68 %, 98.67 % & 98.88 % respectively. The validated RP-HPLC bioanalytical method for the determination of Tolvaptan in bulk and tablet dosage form was found to be sensitive, accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase preparation, rapid analysis,

easy sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of Tolvaptan in formulation.

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