Section A-Research paper

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TOLVAPTAN IN BULK AND ITS TABLET DOSAGE FORM

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ABSTRACT

Back ground: The discovery and development of novel medications requires a methodical strategy based on analysis. CGMP and FDA rules require the adoption of more sensitive and reproducible techniques of analysis. Analytical techniques are developed using the same or equivalent equipment. There are very few new HPLC methods that don't resemble or compared to present, literature based HPLC methods.

Aim: To develop and validate a new, simple, rapid, precise, and accurate RP-HPLC method development and validation for an estimation of tolvaptan in bulk and tablet dosage form.

Method: The HPLC separation was carried out by reverse phase chromatography on Symmetry C_{18} (150 x 4.6mm; 5µm) with a mobile phase consist of acetonitrile:methanol:buffer (680 mg potassium dihydrogen phosphate in 500 ml water, pH-3

adjusted with ortho-phosphoric acid) in the ratio of 40:10:50 v/v delivered in isocratic mode at a flow rate of 1.5 ml/min.

Results: The tolvaptan was quantified at 260 nm. The retention time of tolvaptan was 7.419 min. The coefficient of correlation (r^2) was 0.9998. The developed method was validated according to ICH guidelines. The interday and intraday precision was found to be within limits. The developed method has adequate sensitivity and specificity for the determination of tolvaptan in bulk and its tablet dosage forms.

Conclusion: The present proposed RP- HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of tolvaptan in bulk and pharmaceutical dosage form. The method is useful in the quality control of bulk and pharmaceutical formulations.

Key words: Reversed-Phase High-Performance Liquid Chromatography, tolvaptan, method validation, International Conference on Harmonization (ICH), hyponatremia, vasopressin receptor antagonist.

INTRODUCTION

The discovery and development of novel medications requires a methodical strategy based on analysis. CGMP and FDA rules require the adoption of more sensitive and reproducible techniques of analysis. Analytical techniques are developed using the same or equivalent equipment. There are very few new HPLC methods that don't resemble or compared to present, literature based HPLC methods currently HPLC may be used to study both degradation products and intact medicines (High performance liquid chromatography). If you use the right selection and chromatographic conditions, HPLC can be used to its utmost potential. For drug testing, UV spectroscopy and other standard procedures are equally simple. Consequently, HPLC and UV spectroscopy will be utilised in the provided procedures to analyse the selected medications.

Pharmaceutics analysis is increasingly an essential part of many academic disciplines. A wide range of scientific disciplines have contributed to the development of pharmaceutical analysis. In addition, there are pharmacology and microbiology. A wide range of scenarios can benefit from analytics, including quantitative and qualitative data.

Tolvaptan, a selective vasopressin receptor antagonist, is a Class IV agent of Biopharmaceutical Classification System (BCS). Tolvaptan is classified as a BCS Class IV drug with low solubility (50 ng/mL, 25 ^{0}C, pH 2-12) in aqueous solution and low

permeability. The bioavailability was extremely low, 0.63% and 2% in rats and dogs, respectively, after oral dosing of power prepared using a jet-mill. Tolvaptan is indicated for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (serum sodium < 125 mEq/L or less marked hyponatremia that is symptomatic and has resisted correction with fluid restriction), including patients with heart failure, cirrhosis, and syndrome of Inappropriate anti-diuretic hormone.

Chemically tolvaptan is (±)-4'-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-o-tolu-m-toluidide. Tolvaptan is a white to off white crystalline powder with a molecular weight 448.94 g/mol. Tolvaptan is soluble in benzyl alcohol and methanol, practically insoluble in water and hexane. Tolvaptan melting point was approximately 224 ^{0}C. Its empirical formula is $C_{26}H_{25}ClN_2O_3$.

Tolvaptan is a selective arginine vasopressin (AVP) V2 receptor blocker used to induce free water diuresis in the treatment of euvolemic or hypervolemic hyponatremia. Currently, the orally active medication is in the final stages before approval by the FDA for outpatient therapy. It appears to be safe and effective at promoting aquaresis and raising serum sodium levels in both short- and long-term studies. Tolvaptan is also effective for the treatment of congestive heart failure (CHF) exacerbation, but whether there are long-standing beneficial effects on CHF is still controversial. Prolonged use of tolvaptan leads to increased endogenous levels of AVP and perhaps over-stimulation of V1A receptors. Theoretically, this activation could lead to increased afterload and cardiac myocyte fibrosis, causing the progression of CHF. However, after 52 weeks of tolvaptan therapy, there was no worsening of left ventricular dilatation. In addition, tolvaptan is metabolized by the CYP3A4 (Human Cytochrome P450 3A4) system; thus, physicians should be aware of the potential for increased interactions with other medications. Tolvaptan is a breakthrough in the therapy of hyponatremia as it directly combats elevated AVP levels associated with the syndrome of inappropriate secretion of antidiuretic hormone, congestive heart failure, and cirrhosis of the liver. The empirical structure of tolvaptan is shown in Figure 1.

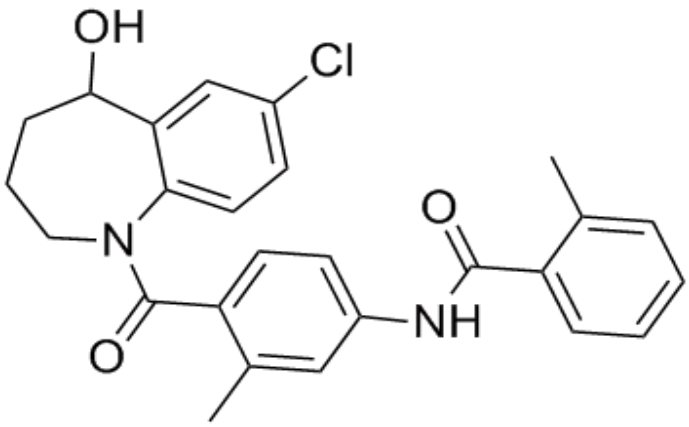

Figure 1: Chemical structure of tolvaptan

High Performance Liquid chromatography is one of the effective separation analytical tools to determine and quantitate the impurities. By using HPLC, we can separate a mixture of compounds to identify and quantify into individual components. Literature survey reveals few UV spectrophotometers, HPLC, UPLC, LC/MS-MS methods for compound estimation in bulk and pharmaceutical dosage form. Based on the literature review, stability-indicating RP-HPLC method for the estimation of tolvaptan was not found. Hence, it was felt that there is a need for a new analytical method. The main objective of the present research work is the development and validation of a method for the estimation of tolvaptan in bulk and tablet dosage form by RP-HPLC.

MATERIALS AND METHODS

Chemicals and reagents

Tolvaptan standard drug (API) was procured from Hetero Drugs Pvt. Ltd., Hyderabad, Telangana, India as gift sample. Tolvaptan tablets was purchased from local pharmacy near Uppal, Hyderabad, Telangana. And, throughout the study HPLC grade solvents were used. Sodium dihydrogen phosphate and ortho-phosphoric acid bought from Merck, Mumbai, Maharashtra, India.

Instrumentation

The method development and validation were done by using Shimadzu HPLC system (LC-20AD multi-solvent delivery system, SPD-20A, UV-Visible Detector, LC solution software), UV-Visible Spectrophotometer (Shimadzu- 1800 double beam, with UV Probe 2.33). Ultra sonicator was used for sonication of the sample solution. Thermo scientific pH meter was

used to measure pH. A vacuum pump filter was used for the filtration of mobile phase solvents. Used electronic digital balance to weigh the samples throughout the experiment.

Chromatographic conditions

A prominence isocratic HPLC system (Waters high performance liquid chromatography with Auto Sampler and UV detector) column Symmetry C_{18} (150 x 4.6mm; 5µm). A 20µL Rheodyne injection syringe was used for sample injection. HPLC grade, acetonitrile, methanol and phosphate buffer were used for the preparing the mobile phase. A freshly prepared, acetonitrile:methanol:potassium dihydrogen phosphate buffer (pH -3) (40:10:50 v/v) was used as the mobile phase. The solvent was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.5 mL/min, column temperature was maintained at room temperature and the detection of the drug was carried out at 260 nm.

Preparation of phosphate buffer

Weigh 680 mg of potassium dihydrogen phosphate into a 500ml beaker, dissolve and diluted to 500ml with HPLC grade water. Adjusted the pH to 3 with ortho-phosphoric acid.

Preparation of mobile phase

Mix a mixture of above buffer 500mL (50 %), acetonitrile 400ml (40 %) and 100ml of methanol HPLC (10 %) and degas in ultrasonic water bath for 5 min. Filter through 0.45 µ filter under vacuum filtration.

Diluent preparation

Mobile phase as diluent.

Standard solution preparation

Accurately weigh and transfer 33mg of tolvaptan working standard into a 50mL volumetric flask add about 35 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 5 ml of the above stock solution into a 100mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Sample solution preparation

Weigh 5 tolvaptan tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 33 mg of Tolvaptan into a 50mL volumetric flask. Add about 35mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 15 ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Method validation

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyte in samples within a given range.

Precision

The degree of closeness of the agreement among individual test results when the method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.

Accuracy

The closeness of results was obtained by a method to the true value. It is a measure of the exactness of the method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection of limit and quantification limit for each analyte were determined based on a signal-to-noise concept, as the lowest concentration. The ICH indicates that LOD (which they call DL, the detection limit) can be calculated as LOD = 3.3Xσ/S, and the limit of quantification (which they call QL, the quantitation limit) LOQ = 10Xσ/S. Here σ is the standard deviation of the response and S is the slope of the calibration curve.

The developed and optimized method has been validated according to the guidelines of the ICH (International Conference on Harmonization) concerning precision, linearity, accuracy, optimized chromatographic conditions are developed for tolvaptan.

RESULTS AND DISCUSSION

The aim of this study was to develop a simple, accurate and precise HPLC method for the analysis of tolvaptan in bulk and tablet dosage forms using mobile phase and commonly employed Symmetry C_{18} column with UV detector at 260 nm. The typical chromatogram of tolvaptan was shown in Figure 2. The optimal retention time found to be 7.419 min.

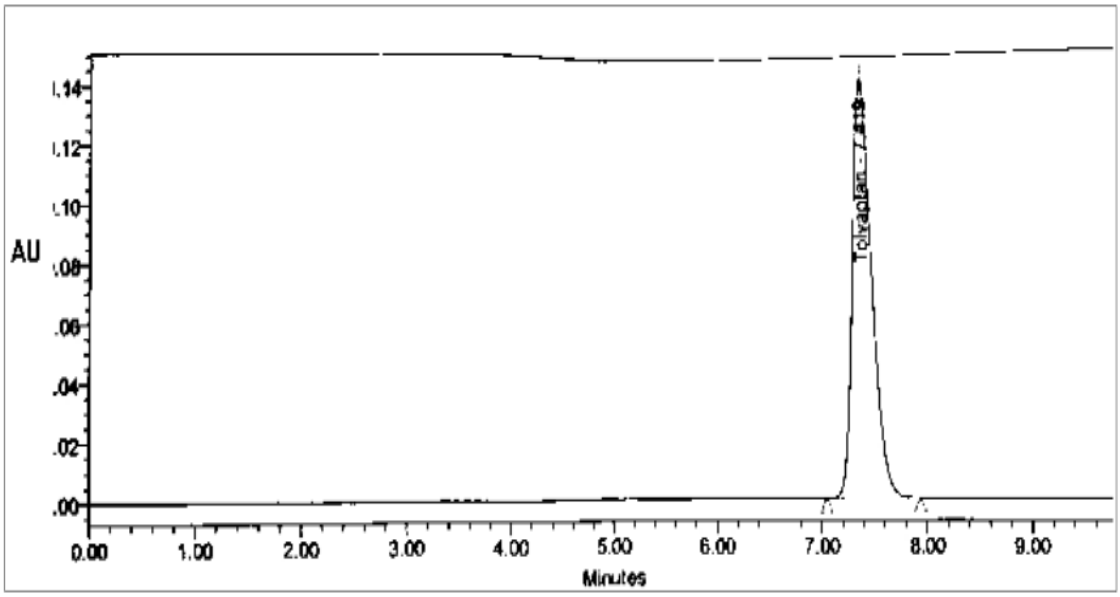

Figure 2: Chromatogram of tolvaptan

Method validation

In this method, linearity, precision, accuracy, robustness, LOD and LOQ were validated for the selected tolvaptan drug by RP-HPLC.

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 8.25µg/mL - 57.75µg/mL were prepared. The chromatograms were recorded, and the peak areas were given in Table 1. A linear relationship between areas versus concentrations was observed in about linearity range. This range was selected as linear range for analytical method development of tolvaptan. Linearity graph was shown in Figure 3.

Table 1: Linearity of tolvaptan

S. No.	% Level	Concentration (µg/mL)	Peak area (mv)

1	25	8.25	457725
2	50	16.5	916517
3	75	24.75	1400270
4	100	33	1823026
5	125	41.25	2257751
6	150	49.5	2724541
7	175	57.75	3180192

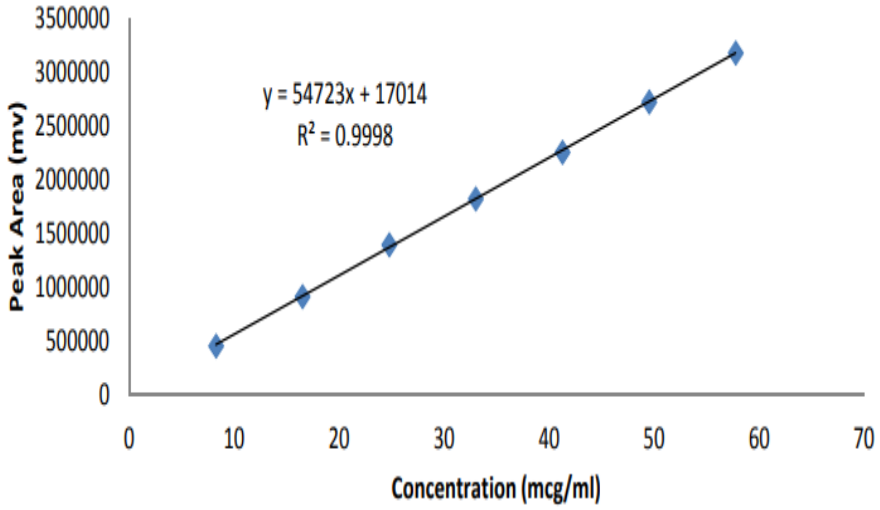

Figure 3: Linearity

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated and present in Table 2. The chromatogram was shown in Figure 3. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drugs peak and % RSD were calculated shown in Table 2. From the data obtained, the developed method was found to be precise.

Table 2: Precision results

S. No.	Concentration (µg/mL)	Intraday precision (Area)	Interday precision (Area)
1	33	1814150	1830132

2	33	1835138	1817119
3	33	1823121	1828120
4	33	1813726	1829171
5	33	1831030	1830018
6	33	1828125	1819071
Mean		**1824215**	**1825605**
SD		**0.1844**	**0.1222**
% RSD		**1.0000**	**0.9999**

Accuracy

A study of recovery of tolvaptan from spiked placebo was conducted at three different spike levels i.e., 50, 100 and 150%. Samples were prepared by mixing placebo with tolvaptan raw material equivalent to about the target initial concentration of tolvaptan. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table 3. The mean recoveries of tolvaptan from spiked were found to be in the range of 98.48 - 101.60 %.

Table 3: Accuracy results

S. No.	Spike level	µg/mL Added	µg/mL Recovered	% Recovery	Mean % recovery
1	50%	16.5	16.32	98.90	**98.48**
	50%	16.5	16.27	98.54	
	50%	16.5	16.17	98.0	
2	100%	33	33.64	101.93	**101.60**
	100%	33	33.42	101.54	
	100%	33	33.53	101.6	
3	150%	49.5	49.21	99.41	**99.30**
	150%	49.5	49.17	99.33	
	150%	49.5	49.09	99.15	

Robustness

Robustness is carried out by changing the parameters from the optimized chromatographic conditions such as changes in flow rate and wave length. Such small changes in the optimized method shows very little change in the results. The degree of reproducibility of the results proven that the method is robust. The results are explained in Table 4.

Table 4: Robustness (n=2) results

Parameter	Level	Peak area	% RSD
Flow rate (ml/min)	1.3	916439	1.005
	1.5	916517	1.000
	1.7	916587	1.112
Wavelength (nm)	255	916489	0.989
	260	916517	1.000
	265	916535	1.118

Limit of Detection and Limit of Quantification

The quantitation limit is considered as the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under the stated operational conditions of the method. The LOD and LOQ values obtained for tolvaptan are shown in Table 5. The LOD and LOQ values were recorded from signal to noise ratio method.

Table 5: LOD and LOQ

Drug	LOD	LOQ
Tolvaptan	4.679 µg/mL	9.237 µg/mL

CONCLUSION

The proposed RP-HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines and was found to be applicable for routine quantitative analysis of tolvaptan by HPLC in pharmaceutical dosage form. The results of linearity, precision, and accuracy was proved to be within the limits. The method provides selective quantification of tolvaptan with no interference from other formulation excipients. The

proposed method was highly reproducible, reliable, rapid, robust, and specific. The developed chromatographic method was simple and reliable for quantification of tolvaptan from bulk and pharmaceutical dosage form which requires less time and less mobile phase consumption. % RSD values for accuracy and precision studies obtained were not more than 2.0% which revealed that developed method was accurate and precise. The validated HPLC method was found to be robust and can be successfully applied to estimate tolvaptan in bulk and pharmaceutical dosage form in routine analysis. The present proposed methodology makes is cost effective which can be implemented for routine analyses in pharmaceutical industry.

The method does not involve any tedious procedural steps; do not require any extra reagents or longer analysis time and a very simple instrument are required. The method can be used to determine the purity of the drug available from various sources. Because of cost-effective and minimal maintenance, the present HPLC method can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of tolvaptan in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

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Declarations

Authors contribution

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

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Ethical approval

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Consent

It is not applicable.

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