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METHOD DEVELOPMENT AND VALIDATION OF TOLVAPTAN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A new, simple, specific, accurate, precise, and rapid reverse phase high performance liquid chromatographic method was developed and validated for the determination of Tolvaptan in pure and tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on Symmetry C18 (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. The Tolvaptan was quantified at 254nm. The retention time of Tolvaptan was 7.419 min. 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. Accuracy (recoveries: 98.48 - 101.60 %.) and reproducibility were found to satisfactory. The developed method was found to be cost effective and was successfully employed for the determination of Tolvaptan in various pharmaceutical preparations.

KEYWORDS

Method development, Tolvaptan, RP-HPLC and Validation.

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INTRODUCTION

Tolvaptan is an orally administered non-peptide vasopressin (VP) V2 receptor antagonist that inhibits water re-absorption in the kidney by competitively blocking VP binding, resulting in water diuresis without significantly changing total electrolyte excretion[1-2]. Chemically (±)-4'- [(7-chloro-2, 3, 4, 5-tetrahydro -5 – hydroxyl - 1H - 1- benzazepin-1-yl) carbon yl] -*o* tolu *m*toluidide[3]. Chemical structure of

Tolvaptan is shown in Figure No.1. It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Tolvaptan[4-5].

MATERIALS AND METHODS

Chromatographic conditions

A prominence isocratic HPLC system (Waters high-performance liquid chromatography with Auto Sampler and UV detector) column Symmetry C18 (150 x 4.6mm; 5µm). A 20µL Rheodyne injection syringe was used for sample injection. HPLC grade, Acetonitrile: Methonol 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 254nm.

Preparation of Phosphate buffer

Weigh 680 mg of Potassium dihydrogen phosphate into a 500ml beaker, dissolve and diluted to 500ml with HPLC water. Adjusted the pH to 3 with Orthophosphoric 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 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Mobile phase as diluents.

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.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

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 C18 column with UV detector at 254 nm. The typical chromatogram of Tolvaptan was shown in Figure No.3. The optimal retention time found to be 7.419 minute.

METHOD VALIDATION

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 8.25µg - 57.75µg were prepared. The chromatograms were recorded and the peak areas were given in Table No.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 No.2.

Precision Method

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 was made and the response factor of drug peak and % RSD were calculated and present in Table No.2. The chromatogram was shown in Figure No.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 No.2. From the data obtained, the developed method was found to be precise.

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 No.3. The mean recoveries of Tolvaptan from spiked were found to be in the range of 98.48-101.60 %.

Table No.1: Linearity Range of Tolvaptan

S.No	% Level	Concentration (µg/mL)	Peak area (mv)
1	25	8.25	457726
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	3180191

Table No.2: Precision results for Tolvaptan

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
Std.Dev		0.1844	0.1222
% RSD		1.0000	0.9999

Table No.3: Accuracy results for Tolvaptan

S.No	Spike level	µg/mL Added	µg/mL found (Recovered)	% of Recovery	Mean % recovery
1	50%	16.5	16.32	98.90	98.48
	50%	16.5	16.26	98.54	
	50%	16.5	16.17	98.00	
2	100%	33	33.64	101.93	101.60
	100%	33	33.42	101.27	
	100%	33	33.53	101.60	
3	150%	49.5	49.21	99.41	99.30
	150%	49.5	49.17	99.33	
	150%	49.5	49.08	99.15	

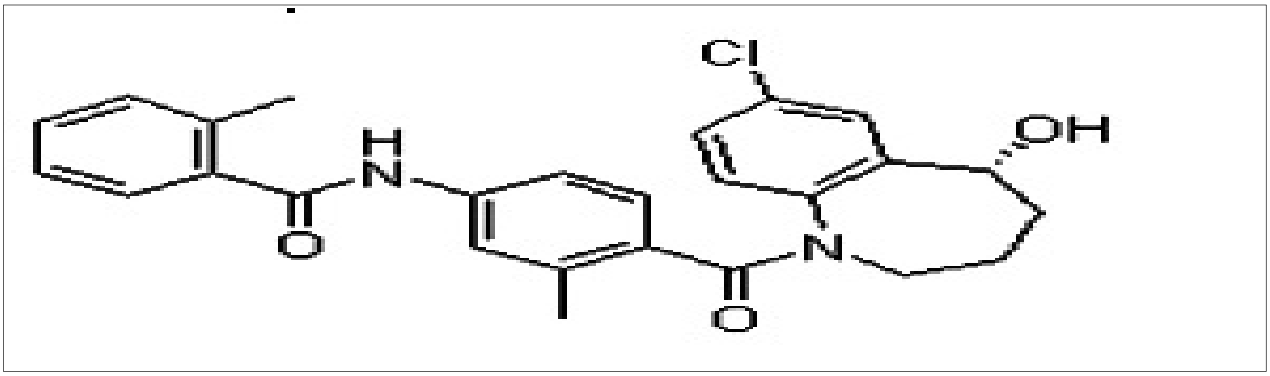

Figure No.1: Chemical structure of Tolvaptan

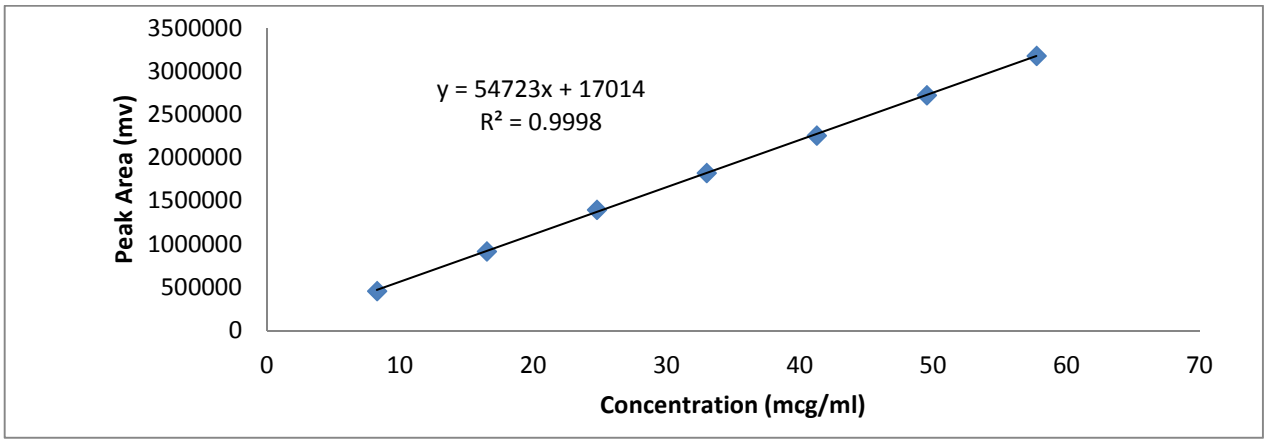

Figure No.2: Linearity curve of Tolvaptan

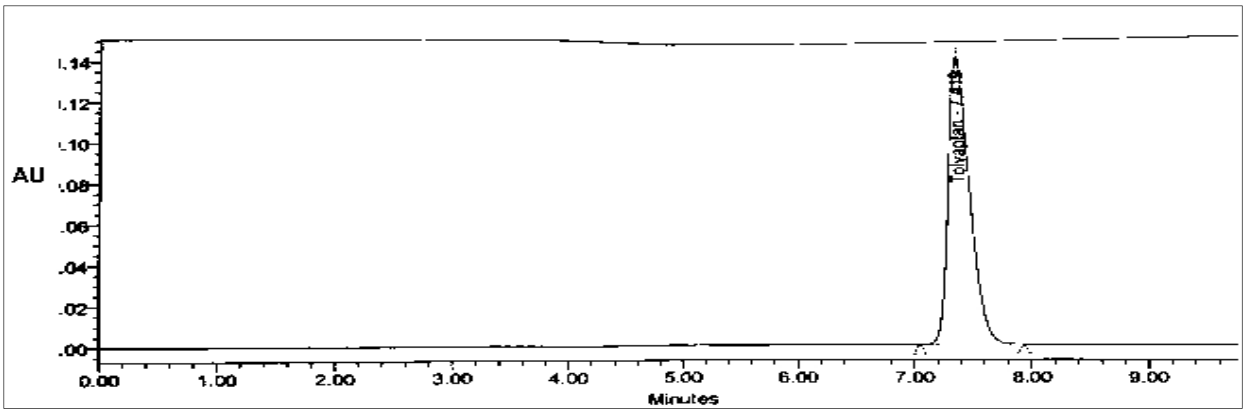

Figure No.3: Chromatogram for Tolvaptan

CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Tolvaptan in bulk and tablet dosage form. Hence, this method can easily and conveniently adopt for routine analysis of Tolvaptan in pure and its tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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