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INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 01, January 2025, pp : 1124-1129

e-ISSN : 2583-1062
Impact Factor : 7.001

DEVELOPMENT AND VALIDATION OF NOVEL DRUG RP-HPLC METHOD FOR QUANTIFICATION OF TOLVAPTAN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A method was set up for synchronous estimation of a Tolvaptan by RP-HPLC. This was simple, rapid, effective and efficient technique for the validation of Tolvaptan in bulk and Pharmaceutical dosage form by RP-HPLC. A Thermosil C18 Column (100mm x 4.6mm) 5µm in isocratic mode, with mobile phase was Phosphate buffer: Methanol pH 2.5 (35:65 v/v) were used. The flow rate was 1ml/min. UV recognition at 254 nm. The correlation coefficient is 0. 999.The method was validated for system suitability, Linearity, precision, accuracy, ruggedness, robustness, LOD & LOQ. The recovery studies were found to be in the range 99.1-100.11% and showing linearity in the range of 20-60µg/ml. Proposed method can be successfully applied for the quantitative determination for Tolvaptan in Bulk and Pharmaceutical dosage form.

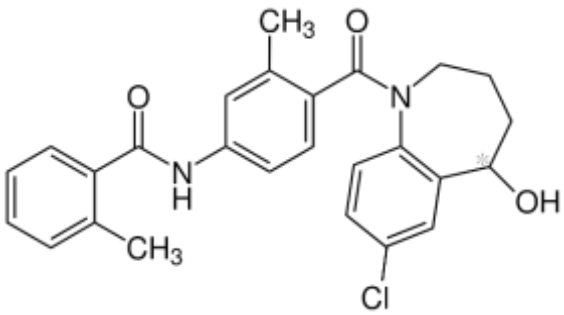
Keywords: Tolvaptan, RP-HPLC, Method validation

1. INTRODUCTION

Tolvaptan is a selective vasopressin V2-receptor antagonist to slow kidney function decline in patients at risk for rapidly progressing autosomal dominant polycystic kidney disease (ADPKD). Tolvaptan is used to treat low blood sodium levels (hyponatremia) associated with various conditions like congestive heart failure, cirrhosis, and syndrome of inappropriate antidiuretic hormones (SIADH). Tolvaptan is a selective and competitive arginine vasopressin receptor 2 antagonist. Vasopressin acts on the V2 receptors found in the walls of the vasculature and luminal membranes of renal collecting ducts. By blocking V2 receptors in the renal collecting ducts, aquaporins do not insert themselves into the walls thus preventing water absorption. This action ultimately results in an increase in urine volume, decrease urine osmolality, and increase electrolyte-free water clearance to reduce intravascular volume and an increase serum sodium levels. Chemically (±)-4'-[(7-chloro-2, 3, 4, 5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-o tolu-mtoluidide

2. DRUG PROFILE

TOLVAPTAN



IUPAC Name: N-{4-[(5R)-7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1H-1-benzazepine-1-carbonyl]-3-methylphenyl}-2-methylbenzamide

Chemical formula: $C_{26}H_{25}ClN_2O_3$

Molecular weight: 448.941

Solubility: Soluble in methanol and Chloroform

3. MATERIALS & METHOD

The strategy improvement and approval of Tolvaptan require a more prominent goal. Consequently, extraordinary dissolvable frameworks were attempted.

The path is utilizing UV 3000+ hardware with a PDA locator and isocratic siphon. The framework is constrained by LC arrangement programming. The portable stage comprises of water: methanol HPLC grade were used.

4. METHOD DEVELOPMENT

Choice of stream rate:

The streaming pace of Tolvaptan was attempted from 0.8 ml to 1.5ml.

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Preliminary 1

Cushion readiness:

About 7.0g of potassium dihydrogen orthophosphate was broken up in 1000ml of HPLC grade water and PH 2.5 was changed with ortho-phosphoric corrosive. It was sifted through a 0.45µm nylon film channel and degassed with a sonicator. It was utilized like a diluent for the arrangement of test and standard arrangement.

Arrangement of portable stage:

The portable stage comprises of water: methanol HPLC of PH 2.5 (30:70) was taken sonicated and degassed for 10 min and sifted through 0.45µm nylon layer channel.

Standard Preparation:

Weigh precisely 10mg Tolvaptan working Reference Standard and 15mg of Tolvaptan working reference standard is taken into 100ml volumetric flask and afterwards, it was disintegrated and weakened to volume with portable stage sufficient. After that 50ml of the above arrangement was taken into a 100ml standard carafe and made up with a versatile stage. (Stock arrangement)

Further pipette 0.5ml of the above stock arrangement into a 10ml volumetric jar and weaken sufficiently with diluent.

Chromatographic conditions:

Column : Thermosil C18 Column (100mm x 4.6mm) 5µg.
Mobile phase : Phosphate buffer: Methanol PH 2.5 (35:65 v/v)
Flow rate : 1ml/ min
Detector wavelength : 254 nm
Injection mode : Auto injector (vial)
Injection volume : 20µl

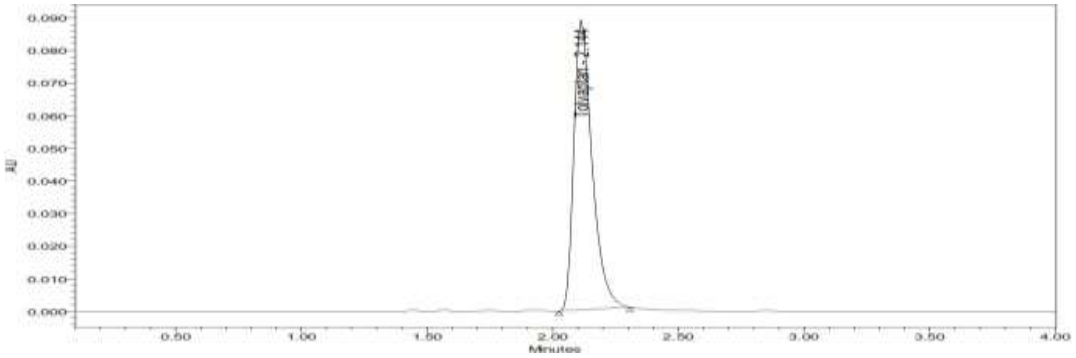

Figure:01 Optimised Chromatogram

S.No	Peak Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Tolvaptan	2.605	2233704	365596	4456	1.4

5. METHOD VALIDATION

Specificity: A measure of 352.6 mg of the container powder was taken into a 100ml standard jar. A volume of 70ml of the portable stage was added and sonicated for 30min. Then the arrangement was cooled and weakened to volume with versatile stage and sifted through 0.45µm layer channel. Further pipette out 0.25ml of Tolvaptan of the above stock solution into a 10ml Volumetric flask.

Standard Arrangement

Weigh precisely 10mg Tolvaptan Working Reference Standard is taken into 100ml volumetric cup and afterwards, it was broken down and weakened to volume with versatile stage sufficient. After that 50ml of the above arrangement was taken into a 100ml standard jar and made up with a versatile stage. Further pipette out 0.5ml of Tolvaptan of the above stock solution into a 10ml Volumetric flask.

Test Arrangement

A measure of 352.6 mg of the tablet powder was taken into a 100ml standard jar. A volume of 70ml of the versatile stage was added and sonicated for 30min. Then the arrangement was cooled and weakened to volume with a portable stage and sifted through 0.45µm film channel. Further pipette out 0.25ml of Tolvaptan of the above stock solution into a 10ml Volumetric flask.

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Linearity and Range

Arrangement of stock arrangement

Weigh precisely 10mg Tolvaptan Working Reference Standard is taken into 100ml volumetric flagon and afterwards it was disintegrated and weakened to volume with portable stage sufficient. After that 50ml of the above arrangement was taken into a 100ml standard jar and made up with a versatile stage.

Table:02 Data for Linearity Results

Tolvaptan	
Concentration (µg/ml)	**Area**
20	1224140
30	1595681
40	1992966
50	2356546
60	2797214

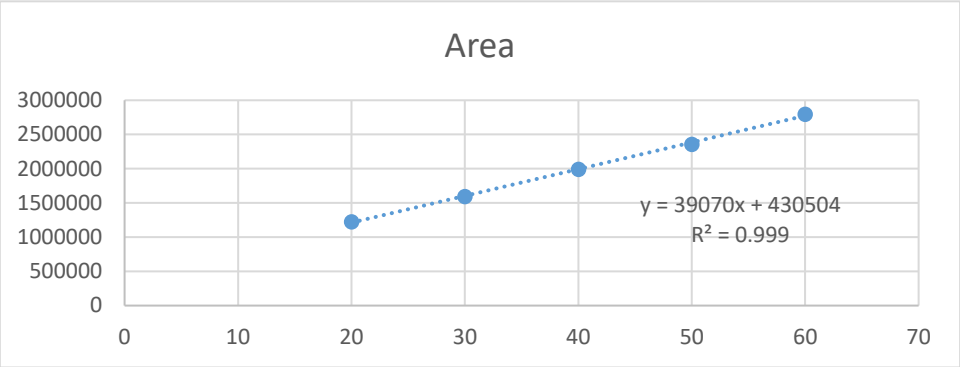

Figure:02 Linearity curve for Tolvaptan

Accuracy Studies

Table-3: Accuracy for Tolvaptan

Recovery level	**Accuracy of Tolvaptan**					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
150%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

Precision:

S.No	Injection	Peak Name	Rt	Area	Height
1	Injection-1	Tolvaptan	2.112	2010800	92856
2	Injection-2	Tolvaptan	2.122	2002956	95705
3	Injection-3	Tolvaptan	2.113	2012800	90602
4	Injection-4	Tolvaptan	2.115	2005243	91610

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5	Injection-5	Tolvaptan	2.136	2011092	89754
6.	Injection-6	Tolvaptan	2.124	2011054	94584
Average				2008991	
Standard Deviation				3922.241	
%RSD				0.195234	

Ruggedness

Injection	Area
Injection-1	2005053
Injection-2	2007362
Injection-3	2007473
Injection-4	2009153
Injection-5	2012800
Average	2008368.1
Standard Deviation	2874.8
%RSD	0.10

Robustness: more flow rate 1.2 ml/min

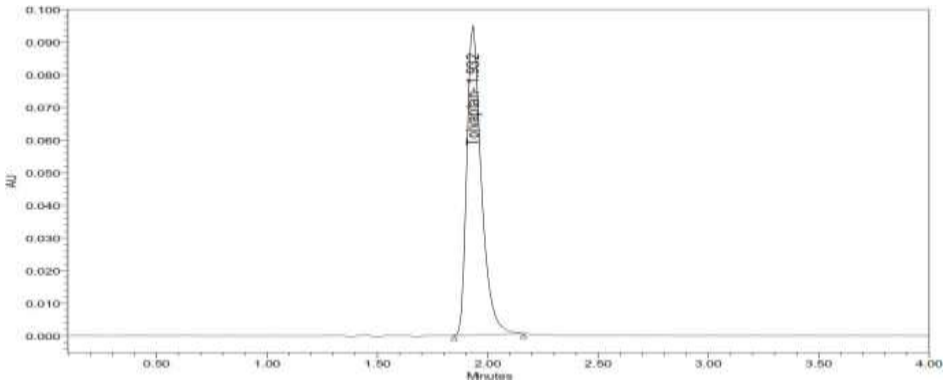

Figure. 3

S.No	Peak Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Tolvaptan	2.168	1676589	321224	4207	1.3

less flow rate 0.8ml/min

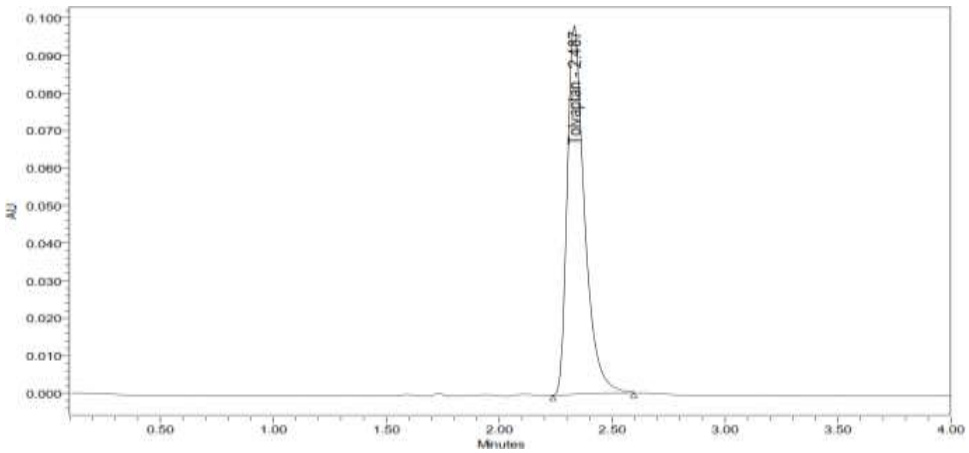

Figure:04 Chromatogram of Tolvaptan Flowrate

S.No	Peak Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Tolvaptan	3.215	2492492	372153	5752	1.4

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Flow rate results for Tolvaptan:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5752	1.4
2	1.0	5026.5	1.3
3	1.2	4476	

Organic phase results for Tolvaptan

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6498	1.2
2	*Actual	5026.5	1.3
3	5 % more	6471	1.2

LIMIT OF DETECTION:

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Tolvaptan	618048	39092	0.001

The LOD was performed for Tolvaptan was found to be 0.001.

QUANTITATION LIMIT

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Tolvaptan	618048	39092	0.004

The LOQ was performed for Tolvaptan was found to be 0.004

Assay

The results show that the %purity was found to be 99.7% which indicates that the value was within the specified range and hence meets the necessary criteria.

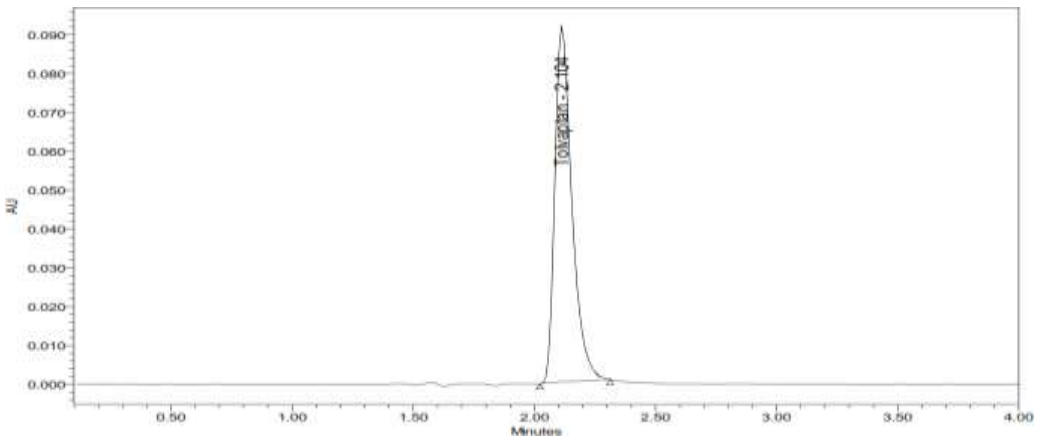

Figure:05 Assay Chromatogram for Tolvaptan

Calculation: (For Tolvaptan)

2005829 15 0.5 100 10 99.89 694.2

---------- x ----------x ---------- x---------- x---------- x---------- x ---------- x 100

2008408 10 10 694.2 0.25 100 200

= 99.77%

6. CONCLUSION

In this research, we have effectively established and thoroughly validated a high-performance liquid chromatography with ultraviolet detection (RP-HPLC) UV- technique for the accurate quantification of Tolvaptan in biological samples. The method exhibited outstanding performance across multiple validation criteria, such as linearity, precision, accuracy, recovery, stability, specificity, and robustness. The established method demonstrated a broad linear range from 20 to 60

µg/ml, rendering it appropriate for the quantification of Tolvaptan in pharmaceutical formulations. The method's precision, assessed through both intraday and interday evaluations, fell within acceptable parameters, thereby ensuring dependable and reproducible outcomes Recovery studies revealed consistent and reproducible recovery rates, further affirming the method's reliability in drug quantification.

7. REFERENCES

[1] Skoog, West, Holler. "*Fundamental of Analytical chemistry"*. 7th eds. USA: Saunders college publishing, 1992; 3-4.

[2] Furniss BS, Nash RA. "*Vogel's Textbook of practical organic chemistry"*. 5th eds.London: Longman group LTD, 1989; 165-169.

[3] Kealey D, Haines PJ. Instant Notes "*Analytical chemistry",* UK: BIOS Scientific Publishers LTD, 2002; 1-2.

[4] Kealey D, Haines PJ. Instant Notes Analytical Chemistry, UK: BIOS Scientific Publishers LTD, 2002: pp. 3-4.

[5] Remington. The Science & Practice of a pharmacy Vol. I, 20TH Edn.B.I.publication PVT. Ltd. pp. 587-613.

[6] Synder L.R., Kirkland, J.L.Glajch, "*practical HPLC Method Development"*. John Wiley and Sons, INC; 2ND Edn. 1997: 98-102.

[7] Ahuja S, Dong MW, editors. "*Handbook of Pharmaceutical Analysis by HPLC".*

[8] Dong MW. "*Modern HPLC for practising Scientists"*. John Wiley& Sons, New Jersey; 2006: 17-27.

[9] Marvin C.McMaster. "*HPLC-A Practical User's Guide*". 2nd eds. New Jersey: John Wiley & sons. Inc. Hoboken-2007.

[10] V. Kalyana Chakravarthy* and D.Gowri Shankar, Development And Validation Of Rp-Hplc Method For Estimation Of Tolvaptan In Bulk And Its Pharmaceutical Formulation, *Rasayan Journal of Chem* -Vol.4, No.1 (2011), 165-171.

[11] K. Vijaya Sri, S. Sruthi, M.A. Madhuri. Rapid RP-HPLC Method Development and Validation of Tolvaptan in Bulk and Pharmaceutical Dosage Form for an Internal Standard. Asian J. Pharm. Ana. 2017; 7(1): 36-40. DOI: 10.5958/2231- 5675.2017.00007.2.

[12] Sk. Chandmalin and CH. M. M. Prasada Rao. Analytical method development and validation of Tolvaptan and its related substances in drug product by RP- HPLC method, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, 4(4), 2016, 194-200

[13] Ranga BG, Sankepally R, Sollu S, Pragada VR, Haque MA, Boggul N, et. al. Analytical method development and validation of Tolvaptan in bulk and its tablet dosage form by UV- Spectrophotometry. IAJPS. 2022; 09 (02):186-193.

[14] Ahmed A, Khan GJ. An eco-friendly RP-HPLC and UV-method development and validation for an estimation of Tolvaptan in bulk and tablet dosage form followed by forced degradation studies. JPRI. 2021; 33(42B):271-86.

[15] Chakravarthy VK, Shankar DG. Development and validation of RP-HPLC method for estimation of Tolvaptan in bulk and its pharmaceutical formulation. Rasayan J. Chem. 2011; 4(1):1165-71.

[16] Gandhi BM, Rao AL, Rao JV. A New Stability Indicating and Validated RP-HPLC Method for the Estimation of Tolvaptan in Pharmaceutical Dosage Forms. Asian J. Research Chem. 2014; 7(7): 628-633.