International Journal of Pharmaceutical Biological and Chemical Sciences (IJPBCS)
ISSN: 2278-5191 (Online)
IJPBCS | VOLUME 13| ISSUE 2 | APR-JUN 2024 | 30-38
Original Research Article – Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF TOLVAPTAN IN API FORM AND ITS MARKETED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Sumera Iram*, Shantanu Pal, Murtaja Ali, Md. Ali Zinnat, Marauf Ahamed, Samin Akther

Department of Pharmaceutics, Sree Dattha Institute of Pharmacy, Sagar Road, Sheriguda, Ibrahimpatnam, R.R.Dist.-501510

***Corresponding Author Email: sumeraeram9728@gmail.com**

ABSTRACT

A simple, rapid, specific, and accurate reverse phase high performance liquid chromatographic method has been developed for the validation of Tolvaptan in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm column with Acetonitrile, Methanol and 0.1% OPA in the ratio of 60:30:10 as mobile phase at a flow rate of 1.0 mL min^{-1} with UV detection at 235 nm; the constant column temperature was Ambient. The runtime under these chromatographic conditions was less than 6.0 min. The retention time of Tolvaptan was found to be 2.570min. The calibration plot was linear over the concentration range of 6–14 µg mL^{-1} with limits of detection and quantification values of 0.8 and 0.24ng mL^{-1} respectively. The mean % assay of marketed formulation was found to be 99.79%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for the estimation of Tolvaptan in bulk and marketed pharmaceutical dosage form dosage form.

Key words:
Tolvaptan, RP-HPLC, Validation, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION

Tolvaptan is a benzazepine derivative incorporating a benzene dicarboxamide function which is a selective vasopressin V2 receptor antagonist used to treat euvolemic and hypervolemic hyponatremia. It is also used in the treatment of rapidly progressing autosomal dominant polycystic kidney disease to slow the rate of cyst development and renal insufficiency. Tolvaptan[1] is a racemate consisting of a 1:1 mixture of 5R and 5S stereomers. It has a role as a vasopressin receptor antagonist and an aquaretic. It is benzazepine and a benzene di carboxamide. Tolvaptan is a Vasopressin V2 Receptor Antagonist. The mechanism of action of Tolvaptan is as a Vasopressin V2 Receptor Antagonist.Tolvaptan[2] is a vasopressin 2 receptor antagonist which is used for short term treatment of severe hyponatremia in patients with heart failure, cirrhosis or syndrome of inappropriate secretion of antidiuretic hormone (SIADH). It has been used experimentally to prevent progression of disease in autosomal dominant polycystic kidney disease (ADPKD). Tolvaptan recently has been implicated in causing serum aminotransferase elevations as well as clinically apparent acute liver injury during long-term use. Tolvaptan[3] 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). Also used to treat hypervolemic and euvolemic hyponatremia. Tolvaptan is used to treat low blood sodium levels (hyponatremia) associated with various conditions like congestive heart failure, cirrhosis, and the syndrome of inappropriate antidiuretic hormones (SIADH). FDA approved on May 19, 2009. The IUPAC Name of Tolvaptan is N-[4-[5R)-7-chloro-5-hydroxy-2, 3, 4, 5-tetra hydro-1-benzazepine-1-carbonyl]-3-methyl phenyl]-2-methyl benzamide.

The Chemical Structure of Tolvaptan is follows

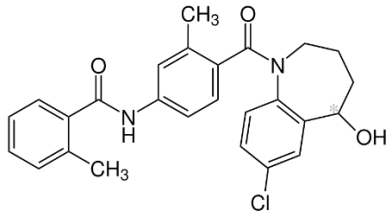

Fig-1: Chemical Structure of Tolvaptan

There are several HPLC methods [32-35]either in pharmaceutical products or biological samples reported in the literature for determination of Tolvaptan alone, with other agents. Some of these methods used isocratic elusion to separate the tested analytes.

The present study aims to develop and validate a simple, sensitive, rapid, and economic and isocratic HPLC method[4] for the determination of Tolvaptan on the same chromatographic run without the need for any costly treatment.

MATERIALS AND METHODS

Instruments Used

Table-1: List of Instrument used

Instruments/Equipments/Apparatus
HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
T60-LAB INDIA UV – Vis spectrophotometer
Electronic Balance **(SHIMADZU ATY224)**
Ultra Sonicator **(Wensar wuc-2L)**
Thermal Oven
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
P^H Analyzer **(ELICO)**
Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table-2: List of Chemicals used

Name	Specifications		Manufacturer/Supplier
	Purity	Grade	
Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
Methanol	99.9%	HPLC	Loba Chem; Mumbai.
Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

METHOD DEVELOPMENT

Selection of Wavelength

The Standard & Sample Stock Solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis[5]. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Tolvaptan, so that the same wave number can be utilized in HPLC UV detector for estimating the Tolvaptan.

Sample and Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Tolvaptan standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution[6] was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions: The chromatographic conditions[7] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table-3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wavelength	Observation	Result
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile = 40:60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile = 55:45	1.0ml/min	235nm	Low response	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile: Water = 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN: Methanol: 0.1% OPA=70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN: Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Preparation of Mobile Phase:
600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration[8].

RESULTS AND DISCUSSION
Selection of Wavelength:

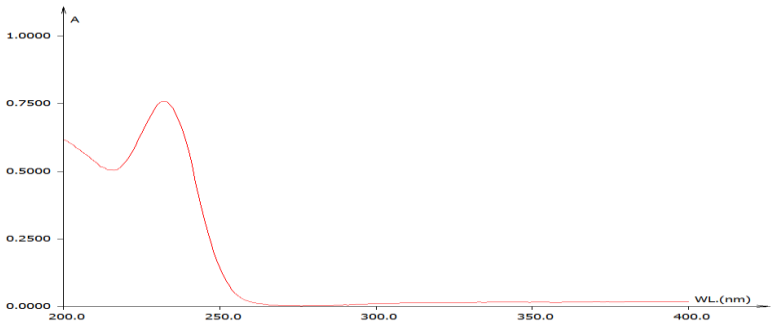

Fig-2: UV Spectrum for Tolvaptan

Observation: While scanning the Tolvaptan solution we observed the maximum at 235 nm. The UV spectrum has been recorded on T60-LAB INDIA make UV-Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions:
The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-4: Summary of Optimised Chromatographic Conditions

Mobile phase	ACN: Methanol: 0.1% OPA = 60:30:10
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto Sampler	Ambient

Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	2.570 minutes

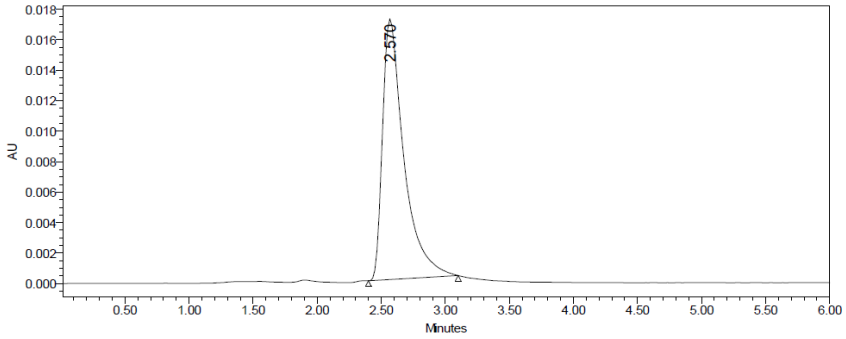

Fig-3: Chromatogram of Tolvaptan in Optimized Condition

Observation: The selected and optimized mobile phase[9] was ACN: Methanol: 0.1% OPA = 60:30:10 and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 06 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry[10]. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Method Validation

1. Accuracy:

Recovery Study:

To determine the accuracy[11] of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Tolvaptan were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation[12] y = 19423x + 5444.4. The results were shown in Table-5.

Table-5: Readings of Accuracy

Conc. In ppm	**Conc. Found**	**Peak Area**		**% Recovery**
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S. D	0.775
			%RSD	0.767046
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S. D	0.628835
			%RSD	0.628688
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S. D	0.987021
			%RSD	0.979894

2.Precision:

2.1. Repeatability

The precision[13] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Tolvaptan (API). The percent relative standard deviation[14] was calculated for Tolvaptan are presented in the Table-6.

Table-6: Readings of Repeatability

HPLC Injection Replicates of Tolvaptan	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

Observation: The repeatability study[15] which was conducted on the solution having the concentration of about 10µg/ml for Tolvaptan (n =6) showed a RSD of 0.768667% for Tolvaptan. It was concluded that the analytical technique showed good repeatability.

2.2. Intermediate Precision/Ruggedness:

2.2.1. Intra-Day & Inter-Day:

The intra & inter day variation[16] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Tolvaptan revealed that the proposed method is precise.

Intra Day/Day-1/Analyst-1:

Table-7: Results of Intermediate Precision Analyst 1 for Tolvaptan

S.No.	PeakName	RT	Area (µV*sec)	USP Plate count	USP Tailing
1	Tolvaptan	2.580	206587	3102	1.16
2	Tolvaptan	2.597	206859	2986	1.18
3	Tolvaptan	2.581	207854	3054	1.13
4	Tolvaptan	2.573	208965	3154	1.14
5	Tolvaptan	2.590	206547	3157	1.12
6	Tolvaptan	2.572	209865	3268	1.18
Mean			**207779.5**		
Std. Dev.			**1381.9336**		
%RSD			**0.665**		

Inter Day/Day-2/Analyst-2:

Table-8: Results of Intermediate Precision Analyst 2 for Tolvaptan

S. No.	Peak Name	RT	Area (µV*sec)	USP Plate count	USP Tailing
1	Tolvaptan	2.580	215263	3215	1.17
2	Tolvaptan	2.597	214235	3652	1.19
3	Tolvaptan	2.581	213254	3496	1.15
4	Tolvaptan	2.573	212367	3258	1.16
5	Tolvaptan	2.590	213698	3365	1.17
6	Tolvaptan	2.572	217456	3524	1.14
Mean			**214378.8**		
Std. Dev.			**1791.516**		
%RSD			**0.835678**		

Observation: Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, the method at selected wavelength[17] was found to be precise.

3. Linearity & Range:

The calibration curve[18] showed good linearity in the range of 6 – 14 µg/ml, for Tolvaptan (API) with correlation coefficient (r^2) of 0.999 (Fig-4). A typical calibration curve has the regression equation of y = 19423x + 5444.4 for Tolvaptan.

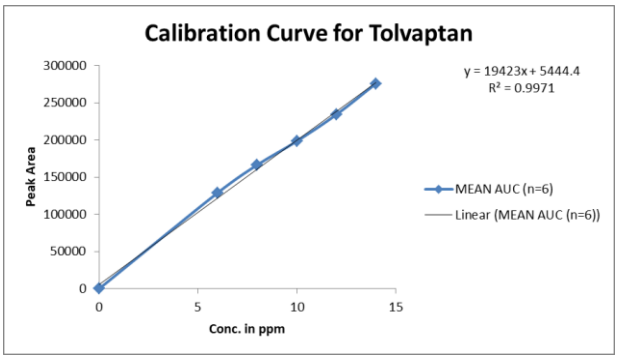

Fig-4: Calibration Curve of Tolvaptan (API)

Table-9: Results of Linearity

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Tolvaptan is a straight line.

Y = mx + c

Slope (m) = 19423

Intercept (c) = 5444.4

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if Correlation Coefficient[19] is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 5444.4. These values meet the validation criteria.

4. Specificity:

The system suitability for specificity[20] was carried out to determine whether there was any interference of any impurities in the retention time of the analytical peak.

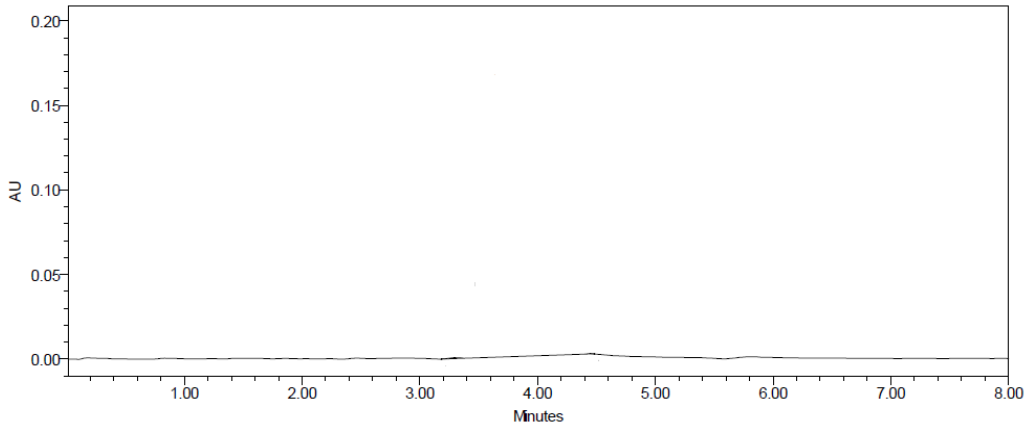

Fig-5: Chromatogram for Blank Solution

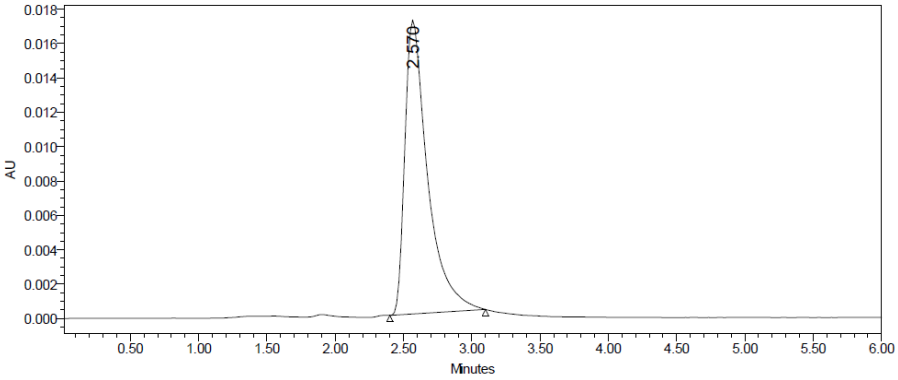

Fig-6: Chromatogram of Tolvaptan Standard Solution

Observation: The study was performed by injecting blank and standard into the system. There was no interference of any peak in the blank with the retention time of the analytical peaks.

5. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection[21] (±2nm) & Organic phase in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-10, % RSD < 2%) the developed RP-HPLC method[22] for the analysis of Tolvaptan (API).

Table 10: Results for Robustness for Tolvaptan

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	203654	2.570	2915	1.16
Less Flow rate of 0.9 mL/min	265876	2.573	3652	1.19
More Flow rate of 1.1 mL/min	298653	2.631	3854	1.20
Less Organic Phase	315874	2.590	3945	1.17
More Organic Phase	326985	2.602	3487	1.19

6. LOD & LOQ:

LOD: The detection limit[23] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10×σ/S

Where,

σ = Standard deviation[24]of the response

S = Slope of the calibration curve

Observation: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified[25] (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

7. System Suitability Parameter: System suitability[26-28] was carried out with six injections of solution of 100% concentration having 10µg/ml of Avapritinib into the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in Table-11.

Table-11: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Tolvaptan=0.23
2	Theoretical plate	N > 2000	Tolvaptan=2987
3	Tailing Factor	T<2	Tolvaptan=1.17

8. Estimation of Tolvaptan in Pharmaceutical Dosage Form

Label claim: 15mg

Each tablet contains: 15mg

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after deciding the normal weight.

The above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused into five reproductions into the HPLC framework[29] and the perceptions were recorded.

A copy infusion of the standard arrangement was additionally infused into the HPLC framework, and the peak regions were recorded. The information[30] appeared in Table-12.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt.} = \text{mg/tab}$$

Where:

AT = Peak Area of medication acquired with test arrangement

AS = Peak Area of medication acquired with standard arrangement

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table-12: Recovery Data for estimation of Tolvaptan in Resodim-15 Tablets

Brand Name of Tolvaptan	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Resodim-15 Tablets (Lupin Limited)	15mg	14.569 (± 0.526)	99.735 (±0.347)

RESULT AND DISCUSSION:

The amount of drug in Resodim-15 Tablets was found to be 14.569 (± 0.526) mg/tab for Tolvaptan & % assay[31] was 99.735%.

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Tolvaptan, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Tolvaptan it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1.0 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Tolvaptan in various details.

BIBLIOGRAPHY

1. https://go.drugbank.com/drugs/DB06212
2. https://pubchem.ncbi.nlm.nih.gov/compound/RTolvaptan
3. https://en.wikipedia.org/wiki/Tolvaptan
4. *Morgan, David J.*, "Fraction collector (post on Flickr)". *Flickr*. Retrieved, 28 October 2015.
5. Karger, Barry L. "HPLC: Early and Recent Perspectives". Journal of Chemical Education. 74: 45. Bibcode: 1997JChEd.74...45K, 1997.
6. Henry, Richard A., "The Early Days of HPLC at Dupont". Chromatography Online. Avanstar Communications Inc, 1 February 2009.
7. Iler, R.K., the Chemistry of Silica. John Wiley & Sons. New York, 1979.
8. Karger, B. L.; Berry, L. V. "Rapid liquid-chromatographic separation of steroids on columns heavily loaded with stationary phase". Clin. Chem. 17 (8): 757–64, 1971. S
9. Giddings, J. Calvin, Dynamics of Chromatography, Part I. Principles and Theory. Marcel Dekker, Inc., New York. p. 281, 1965.

10. *Ettre, C.*, "Milestones in Chromatography: The Birth of Partition Chromatography" (PDF). LCGC, Volume: 19 (5), Pg no: 506–512, *2016*, and 2001.
11. *Martin, A J P; Synge, R L M,* "Separation of the higher monoamino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool". *Biochemical Journal. Volume: 35 (1–2), Pg no: 91–121, 1941.*
12. *Lindsay, S.; Kealey, D.,* High performance liquid chromatography. Wiley. *from review* Hung, L. B.; Parcher, J. F.; Shores, J. C.; Ward, E. H. (1988). "Theoretical and experimental foundation for surface-coverage programming in gas–solid chromatography with an adsorbable carrier gas". J. Am. Chem. Soc. Volume: 110(11), Pg no: 1090–1096, 1987.
13. Journal of Pharmaceutical and Biomedical Analysis Volume 21, Issue 2, Pages 371–382, 1 November 1999.
14. Tropical Journal of Pharmaceutical Research, © Pharmacotherapy Group, Volume:8(5), Pg no: 449-454, October 2009.
15. Rabi Sankar, Instrumental Method of Analysis, P-18-6, P-18-3.
16. Lloyd R. Snyder, Practical HPLC Method Development, 2nd edition, P-503.
17. Guidance for industry, Analytical Procedure and Method Validation, U.S. Department of Health and Human Services FDA, August 2000.
18. Validation of analytical procedures, methodology, ICH harmonized tripartite guideline, 108, 1996.
19. B.K. Sharma, Instrumental Methods of Chemical Analysis, pp.75-78, 113-115.
20. Galen W. Ewing, Instrumental Methods of Chemical Analysis, Vth Ed., 1.
21. Takeru Higuchi, EinarBrochmann, HanffenHanssen, Pharmaceutical Analysis, 1st edition, 1-10.
22. A.H. Beckett, J.B. Stenlake, Practical Pharmaceutical Chemistry, IV edition, Volume II, Pg no: 275-298.
23. Quality Assurance, worth the effort, Inforum, volume 7; number.4, October 2003.
24. P.D. Sethi, Quantitative Analysis of drugs in pharmaceutical formulation, III Ed., pp.1-21, 51-56.
25. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
26. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
27. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
28. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
29. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
30. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
31. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
32. 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 Chemistry, Vol.4, No.1 (2011), 165-171.
33. K Bhavyasri, B Aishwarya, Anushree Hari and M Sumakanth, A New Approach for Analytical Method Development and Validation for Quantification of Tolvaptan Using RP-HPLC in Bulk and in its Tablet, Journal of Pharmaceutical Research & Reports, Volume 4(2): 1-5, 2023, SRC/JPRSR-147.

DOI: doi.org/10.47363/JPRSR/2023 (4)134.

34. B. Mohan Gandhi, A. Lakshmana Rao, and J. Venkateswara Rao, A New Stability-Indicating and Validated RP-HPLC Method for the Estimation of Tolvaptan in Bulk and Pharmaceutical Dosage forms, Asian J. Research Chem. 7(7): July 2014; Page 628-633.
35. S. Murugan, V. Rajasekhar reddy, P. Sirisha, N. Pravallika, K. Chandrakala, Method Development and Validation of Tolvaptan in Bulk and Tablet Dosage Form by RP-HPLC Method, International Journal of Research in Pharmaceutical and Nano Sciences, 2(1), 2013, 135- 139.