Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TOLVAPTAN AND ITS RELATED SUBSTANCES IN DRUG PRODUCT BY RP – HPLC METHOD

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ABSTRACT

The developed method was a simple, efficient, economical method for the Validation of Tolvaptan and its related substances in Drug product by RP- HPLC. In this method Inertsil ODS-3V column (250×4.6mm, 5µm) as column. All the parameters used in this method were validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool. Parameters are like Linearity, Specificity, Accuracy, System suitability, Robustness, Ruggedness and Method precision. The results obtained were well within the acceptance criteria.

KEYWORDS

Tolvaptan, Validation, RP-HPLC and Inertsil ODS-3V column.

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INTRODUCTION[1,2,3]

Tolvaptan is used to treat congestive heart failure (CHF), cirrhosis, and syndrome of inappropriate antidiuretic hormones (SIADH). The drug is also used to maintain the blood sodium levels.

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. High performance liquid Chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase passed through the column at rapid speed as a result, the analysis time is reduced by 1-2

orders of the magnitude relative to classical column chromatography and fine particles of adsorbent or support used which makes the column efficient. The importance of chromatography is increasing rapidly in pharmaceutical analysis is to separate closely relate compound and to identify them specifically with quantitative estimation. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. The reasons for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry.

In the present work, attempts were made to develop analytical method and validation of Tolvaptan and its related substances in drug product by RP - HPLC method.

MATERIAL AND METHODS[4,5]

List of instruments used in the method development and validation are placed in the Table No.1 column details are placed in Table No.2 and list of chemicals used in this work were placed in the Table No.3.

Preparation of solutions for estimation of tolvaptan[6,7,8]

Mobile Phase a Preparation

Mixed 1.0 ml of orthophosphoric acid into a 1000 ml water. Filtered through 0.45 µ membrane filter paper and degas.

Mobile Phase A Preparation

Mixture of Acetonitrile and methanol in the ratio of 900:100 v/v respectively. Filtered through 0.45 µ membrane filter paper and degas.

Stock solution preparation

Weighed accurately about each 10 mg of TVP VIII and Tolvaptan standards into a100 ml volumetric flask, dissolved and make upto volume with diluent.

Reference solution preparation

Transferred 0.5 ml of above stock solution into a 50 ml volumetric flask, and diluted to volume with diluent.

Test solution

Weighed accurately about 25mg of test sample into a 25 ml volumetric flask, dissolved and make upto volume with diluent.

METHOD DEVELOPMENT

Trail I

Chromatographic Conditions

Column: Inertsil-ODS 3V C-18 5µ (250 x 4.6mm)
Flow Rate : 1 ml/Min
Column Oven Temperature : 35°C
Wave Length : 254 nm
Injection Volume : 10µl
Run Time : 45 Minutes
Buffer: 0.1% H_3PO_4 Solution in 1000 ml H_20
Mobile Phase -A : Buffer
Mobile Phase-B : Methanol
Diluent: 1:1 (ACN: H_2O)

Trail II

Chromatographic Conditions

Column: Inertsil-ODS 3V, 5µ (250 x 4.6mm)
Flow Rate : 1ml/Min
Column Oven Temperature : 35°C
Wave Length : 254nm
Injection Volume : 10µl
Run Time : 45Minutes
Buffer: 0.1 %H_3PO_4 Solution In 1000 ml H_20
Mobile Phase-A : Buffer
Mobile Phase-B : Methanol: ACN (50:50)
Diluent: 1:1 (ACN: H_2O)

Trail III

Chromatographic Conditions

Column: Symmetry C-18 5 µ (250 x 4.6mm)
Flow Rate : 1ml/Min
Column Oven Temperature : 35°C
Wave Length : 254 nm
Injection Volume : 10µl
Run Time : 45Minutes
Buffer: 0.1 %H_3PO_4 Solution In 1000 ml H_20
Mobile Phase -A : Buffer
Mobile Phase-B : Methanol
Diluent : 1:1 (H_2O: ACN)

Optimized method

Chromatographic conditions

Column: Inertsil-ODS 3V, 5µ (250 x 4.6mm)
Flow Rate : 1 ml/Min
Column Oven Temperature : 35°C
Wave Length : 254 nm
Injection Volume : 10µl
Run Time : 45 Minutes
Buffer: 1 ml H_3PO_4 Solution In 1000 ml H_20
Mobile Phase-A : Buffer
Mobile Phase-B: ACN: Methanol (90:10)
Diluent : 1:1 (ACN: H_2O)

METHOD VALIDATION[9,10,11]

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the Procedure meet the requirements for the intended analytical applications.

Validation parameters:

- System Suitability
- Specificity/selectivity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantification Stability
- Robustness
- Ruggedness

All the parameters are done and the results are placed in the Table No.7.

Table No.1: Instruments used

S.No	Name of the instrument	I. D. Number	Make	Model
1	HPLC with PDA	NRC\QC\I\074	Waters	2998 PDA, 2695 pump
2	HPLC	NRC\QC\I\050	Waters	2489 UV, 2695 pump
3	Electronic Balance	NRC\QC\I\061	Mettler-Toledo	XS-205 dual range

Table No.2: Columns used

S.No	Column details: Column	I. D. Number	S.No	Make	Dimensions
1	Inertsil ODS-3V	306	1C184036	GL Sciences	250×4.6mm, 5µm
2	Inertsil ODS-3V	323	7CS70017	GL Sciences	250×4.6mm, 5µm

Table No.3: Materials used

S.No	Name	Grade	Supplier
1	Ortho phosphoric acid	HPLC	Merck
2	Acetonitrile	Gradient	JT Baker
3	Methanol	Gradient	JT Baker
4	Water	Milli-Q	…..

Table No.4: Working Standards/Impurity Standards used

S.No	Name	Grade	Lot No/B. No
1	Tolvaptan	1H*	TVP/RS/001/12
2	TVP-VIII	1H*	TVP-VIII/RS/001/11

Table No.5: Gradient table

S.No	Time (Min)	Mobile Phase-A(%V/V)	Mobile Phase-B(%V/V)
1	0	40	60
2	5	40	60
3	20	25	75
4	30	15	15
5	32	40	60
6	40	40	60

Table No.6: Gradient table for Optimized

S.No	Time (Min)	Mobile Phase-A(%V/V)	Mobile Phase-B(%V/V)
1	0	50	50
2	5	50	50
3	10	40	60
4	20	40	60
5	25	20	80
6	35	20	80
7	40	50	50
8	45	50	50

Table No.7: Results of validation parameters

S.No	Validation Parameter	Acceptance Criteria	Results	
			Tolvaptan	TVP -VIII
1	System Suitability	RSD% Standard solution should be not more than 10%.	0.9	0.009
		Theoretical plate count should not be less than 3000.	23239	8743
		The tailing factor [Asymmetry] should be NMT 2.	0.9	0.55
		Resolution should be NLT 2.	3.54	2.15
2	Specificity	The peaks of diluents, placebo and known impurities should not interfere with the main peaks	The peaks of diluents and placebo did not interfering with the peaks of Tolvaptan and TVP -VIII	
3	Precision			
4	Method Precision	The% RSD calculated on 6 determinations should be less than 2%	Tolvaptan	TVP -VIII
			1.0	0.4
	System Precision	The% RSD calculated on 6 determinations should be less than 2 %	Tolvaptan	
			0.9	
5	Linearity	The correlation coefficient should be ≥0.99	Tolvaptan	TVP-VIII
			0.999	0.999
6	Accuracy	Mean %recovery at each level should be between 90%-110%	Tolvaptan	TVP -VIII
			98.73-100.30	97.35-99.47
7	Robustness	The system suitability parameters should pass for all conditions	The system suitability parameters passed for all the conditions	
8	LOD	S/N ratio should be about3:1.	Tolvaptan	TVP-VIII
			3.8:1	3.2:1
9	LOQ	S/N ratio should be about10:1.	Tolvaptan	TVP-VIII
			10.6:1	10.1:1

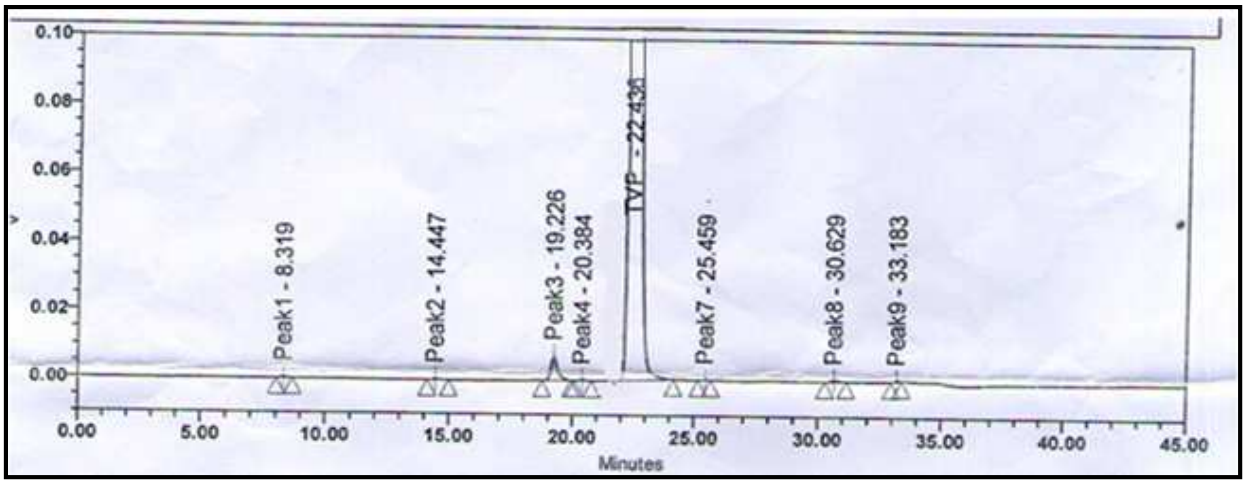

Figure No.1: Method Development Chromatogram_1
Remarks: Retention time is more and impurity peaks are not eluted

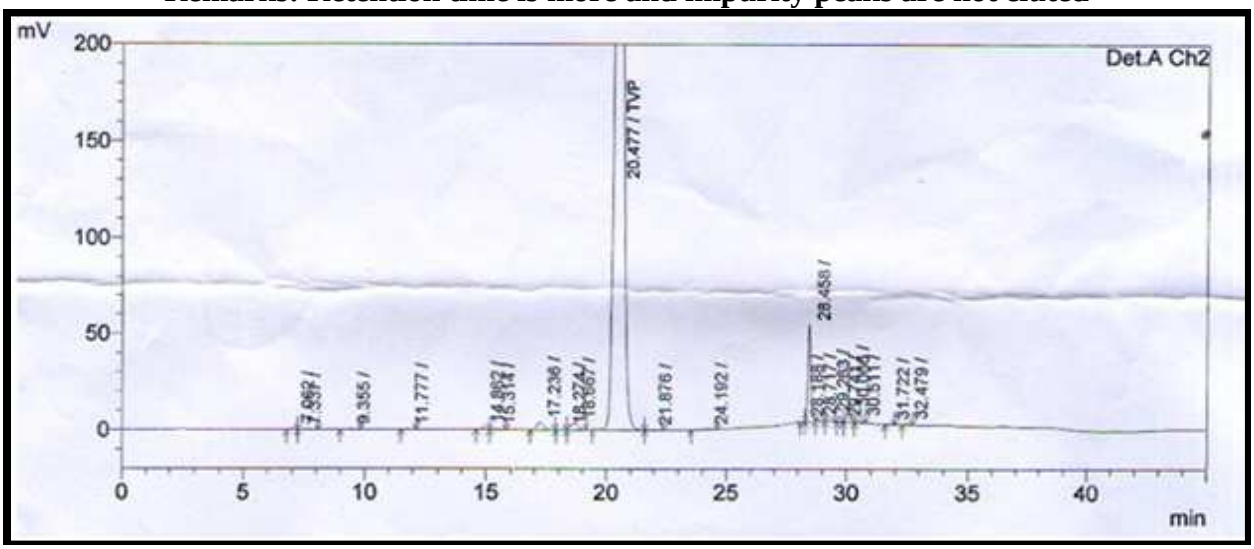

Figure No.2: Method Development Chromatogram_2
Remarks: Retention time is more and All Peaks are merged

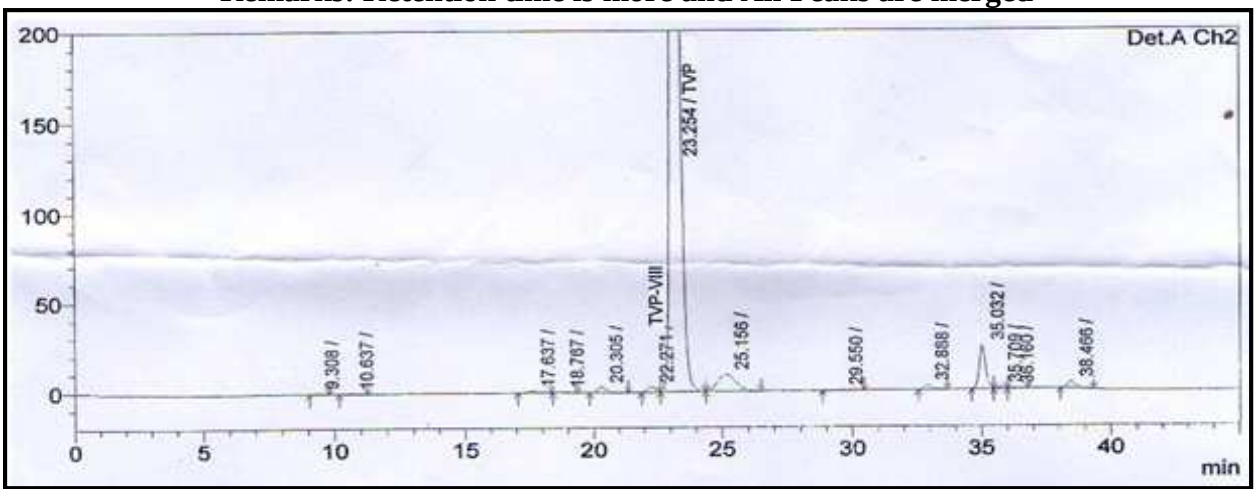

Figure No.3: Method Development Chromatogram_3

Remarks: Resolution was not good.

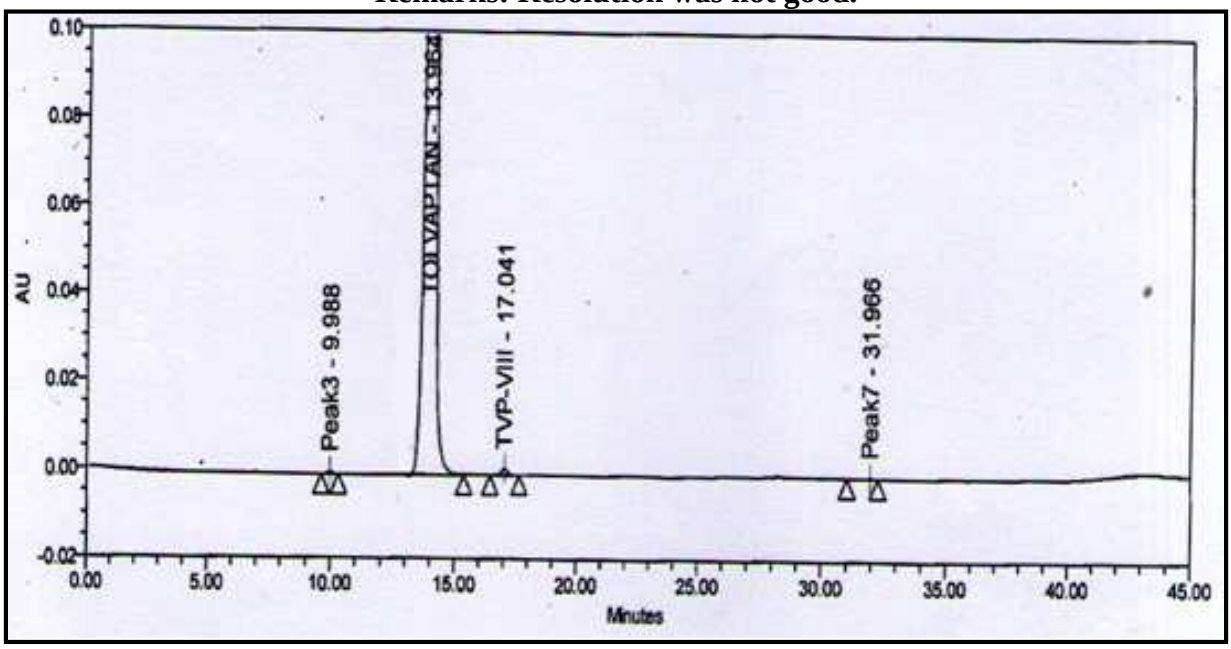

Figure No.4: Optimized method Chromatogram
Status: ok. Peak shape is good and all peaks are resolved

CONCLUSION

The Developed and validated method for estimation of Tolvaptan and it's Related Substances was found to be simple, precise, accurate and rapid. The mobile phase is simple to prepare and economical. The proposed method was simple and did not involve laborious time-consuming sample preparation. Short run time and the possibility of analysis of a large number of samples. Hence, the methods were easily and conveniently adopted for routine analysis of Tolvaptan and it's Related Substances.

ACKNOWLEDGEMENT

The author thankful to the Principal, Guide, HOD and Management of QIS College of Pharmacy for provide all the facilities and supports for accomplishment and completion of this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Sk. Chandmalin and CH. 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.