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Research Article

Analytical Method Development And Validation For The Terlipressin In Pharmaceutical Doasage Form By RP-HPLC

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ABSTRACT

Another methodology was set up for synchronous estimation of a Terlipressin by RP-HPLC system. The chromatographic conditions were viably created for the unit of Terlipressin by using Inertsil – ODS C18 (250 x 4.6 mm 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol:Acetonitrile (30:70), recognizable proof wave length was 225 nm.

INTRODUCTION

Terlipressin is a synthetic analogue of vasopressin, which is an endogenous neurohormone that accts as a vasoconstrictor. It is a prodrug of lypressin, or lysine vasopressin. Compared to endogenous vasopressin, Terlipressin has a longer half-life and increased selectivity for the V1 receptor. Molecular formula is C52H74N16O15S2.

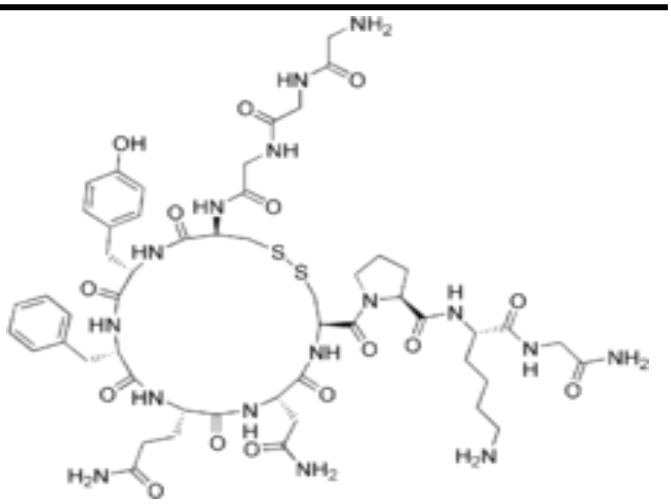

Figure no. - 1

The Literature survey indicates that there are no methods for the Estimation of Terlipressin.

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Therefore, an attempt was made to develop and validate a simple and economical RP-HPLC method as per ICH guidelines for the estimation of Terlipressin pharmaceutical dosage forms.

MATERIALS AND METHODS:

Instrument:

- HPLC waters model no. 2690/5 series compact system consisting of Inertsil-c18 ODS column
- Electronic balance
- Sonicator
- Chemical:
- Methanol HPLC grade
- Acetonitrile HPLC grade
- Buffer (KH2PO4) HPLC grade
- Water HPLC grade

Experimental conditions:

Quantitative HPLC was performed on isocratic HPLC of Waters model no. 2690/5 with software Empower-2 infinity isocratic LC manual injector with variable wavelength detector. For method development several trials were carried out. After many trials, the chromatographic conditions were decided. The separation was conducted by using column of Inertsil- ODS C18 (5µ, 4.6 mm×250) with mobile phase consisting of methanol and Acetonitrile in the ratio of (30:70). The mobile phase delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 225 nm and found a sharp and symmetrical peak with retention time of 3.68 min. The run time observed was 6 min.

Preparation of standard solution:

Take 100mg Terlipressin working standard in 100ml V.F add methanol sonicate it 30min, (That is 1000ppm solution).

Preparation of sample solution:

Take 10ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10min (That 100ppm solution)

Diluent:

The methanol was used as diluent.

Method validation:

Validation establish a documented evidence which provides which a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

1. System Suitability:

A Standard solution was prepared by using Terlipressin working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Terlipressin, retention times and peak areas.

2. Precision:

a. System precision: Standard solution prepared as per test method and injected five times.
b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

3.Accuracy:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Terlipressin into each volumetric flask for each spike level to get the concentration of Terlipressin equivalent to 50%, 100%, and 150% of the labelled amount as per the test method. The average % recovery of Terlipressin was calculated.

4.Linearity:

A Series of solutions are prepared using Terlipressin working standard at concentration levels from 20ppm to 70 ppm of target concentration.

5. Ruggedness:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analysed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variability

6.Robustness:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Terlipressin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

7.LODAnd LOQ (Limit Of Detection And Limit Of Quantitation):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\,\sigma}{S} = \frac{3.3\times 23.5654}{16054} = 0.0048$$

$$LOQ = \frac{10\,\sigma}{S} = \frac{10\times 23.5654}{16054} = 0.0146$$

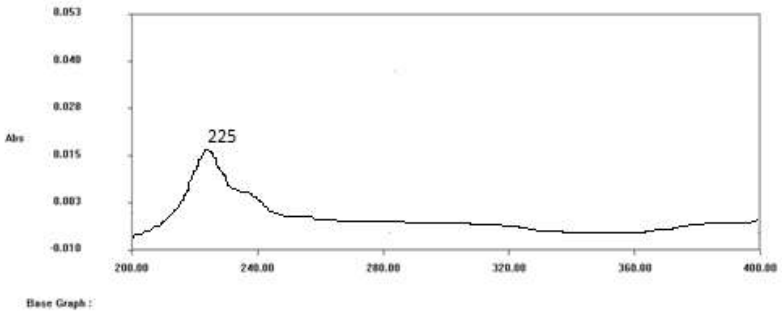

Figure no. 2 - chromatogram for diluent

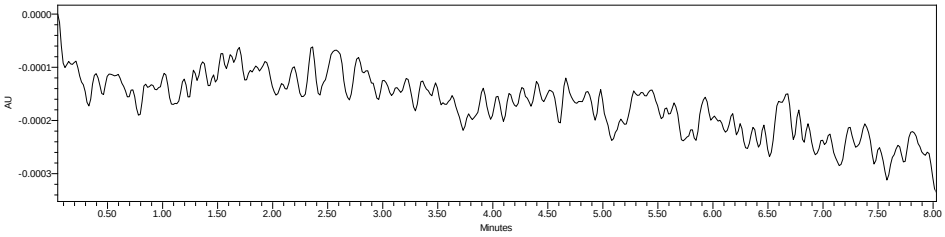

Figure no. 3 - Blank Chromatograph

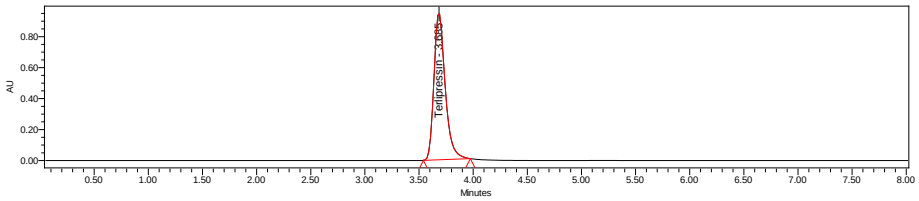

Figure no. 4 - Chromatogram Standard

RESULTS AND DISCUSSION:

Mobile Phase:

Methanol: Acetonitrile (30:70)V/V. Sonicate it 30min, Filter this mobile phase through 0.45micron filter paper.

Optimized Method Stock Solution Preparation : Take 100mg Terlipressin working standard in 100ml V.F add methanol sonicate it 30min, (That is 1000ppm solution).

Further Dilution (or) Optimized Method Solutions Preparation:

Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10min (That 40ppm solution).

Chromatographic conditions :

Table 1 : chromatographic condition

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (30:70)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	225nm

Table 2 : Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.683	645478.48	10621	1.101
2	3.684	645449.32	10630	1.103
3	3.684	645455.29	10632	1.101
4	3.683	645423.23	10645	1.103
5	3.685	645480.63	10650	1.102
Mean	3.6838	645457.39	10635	1.102
SD	0.000837	23.5654	-------	-------
% RSD	0.022712	0.00365	-------	-------

Table 3 : Data System precision

	Injection	Peak Areas of Terlipressin	%Assay
Concentration 40ppm	1	645440.56	100.22
	2	645480.24	100.22
	3	645471.28	100.22
	4	645423.23	100.21
	5	645462.64	100.22
Statistical Analysis	Mean	645455.59	100.22
	SD	23.3268	0.00363
	% RSD	0.00361	0.00362

Table 4 : Data Method precision

	Injection	Peak Areas of Terlipressin	%Assay
Concentration 40ppm	1	645522.29	100.23
	2	645499.64	100.23
	3	645531.85	100.23
	4	645481.56	100.22
	5	645539.93	100.23
	6	645510.45	100.23
	Mean	645514.28	100.23
	SD	21.5888	0.00336

Statistical Analysis	% RSD	0.00334	0.00335

Table 5 : Data of Accuracy

Concentration % of spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	322742.02	20	19.98	99.94	MEAN	100.32
50% Sample 2	322769.61	20	20.10	100.52	%RSD	0.336
50% Sample 3	322728.59	20	20.10	100.51		
100 % Sample 1	645512.85	40	40.09	100.23	MEAN	100.42
100 % Sample 2	645489.56	40	40.20	100.51		
100% Sample 3	645530.51	40	40.20	100.52	%RSD	0.1651
150% Sample 1	968148.53	60	60.19	100.31	MEAN	100.44
150% Sample 2	968125.94	60	60.30	100.50	%RSD	0.1104
150%Sample 3	968165.54	60	60.30	100.51		

Table 6 : Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	16054
20	322716.85	y-Intercept	1854
30	484074.36	Correlation Coefficient	0.999
40	645432.45		
50	806790.56		
60	968148.84		
70	1120456.85		

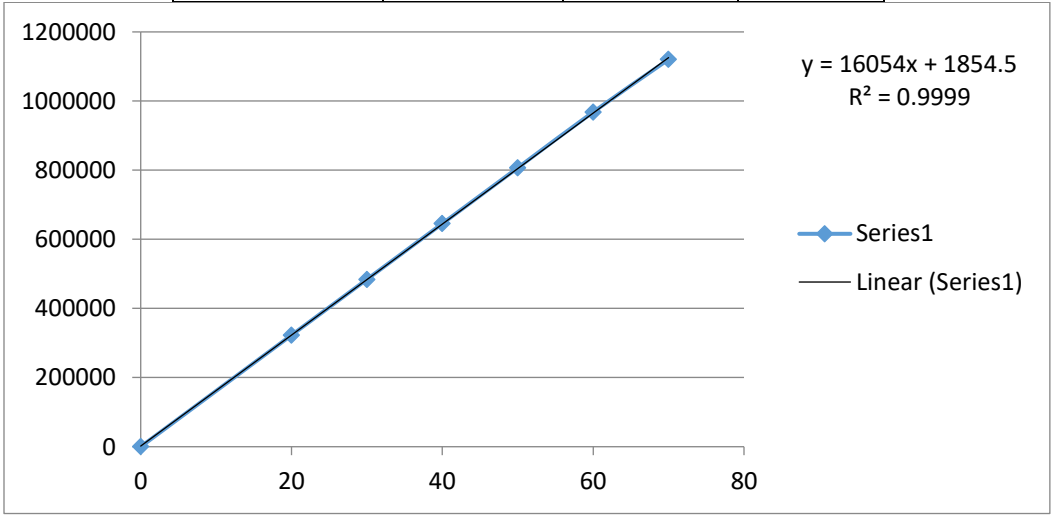

Fig no. 5 - Linearity Plot (Concentration Vs Response)

Table 7 : Data on System Variability System(Ruggedness)

Sr. No:	Peak area	Assay % of Terlipressin
1	645488.73	100.22
2	645410.82	100.21
3	645466.89	100.22
4	645419.83	100.21
5	645452.88	100.22

6	645492.21	100.23
Mean	645455.22	100.22
%RSD	0.005302	0.00531

Table 8 : Data for Effect of variation in flow rate(Robustness)

Flow 0.8 ml	**Std Area**	**Tailing factor**	**Flow 1.0 ml**	**Std Area**	**Tailing factor**	**Flow 1.2 ml**	**Std Area**	**Tailing factor**
	640125.54	1.119		645444.52	1.123		650184.85	1.136
	640181.45	1.123		645489.23	1.125		650136.85	1.138
	640144.44	1.125		645512.12	1.123		650148.36	1.137
	640138.84	1.129		645463.52	1.124		650163.36	1.137
	640152.15	1.131		645475.57	1.125		650196.65	1.136
Avg	640148.48	1.125	Avg	645476.99	1.124	Avg	650166.01	1.137
SD	20.8325	0.0047	SD	25.6012	0.001	SD	24.8123	0.0008
%RSD	0.003254	0.4242	%RSD	0.00396	0.0889	%RSD	0.00381	0.0735

Table 9 : Assay of formulation

Injection	**Peak Areas of Terlipressin**	**%Assay**
1	645415.33	100.22
2	645231.08	100.19
3	646087.47	100.32
4	649175.34	100.8
5	645671.08	100.26
Mean	646316.06	100.358
SD	1630.3551	0.251833
% RSD	0.25225	0.250935

CONCLUSION :

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of Terlipressin was discovered to be 225nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Acetonitrile (30:70) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase. The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For both medicines, the analytical approach was shown to be linear throughout a range of 20-70ppm of the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

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