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Stability indicating RP-HPLC method for simultaneous determination of Terlipressin in pure and pharmaceutical formulation

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ABSTRACT

A simple, selective, precise and stability indicating High Performance Liquid Chromatographic (HPLC) method of analysis of Terlipressin in pure and pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed-phase C_{18} column (250 x 4.6 mm), 5 µ with a mobile phase consisting of a mixture of Acetonitrile-monobasic potassium phosphate solution (35:65v/v) and pH adjusted to 3.5. Flow rate was 1.5 mL / min. Detection was carried out at 280 nm. The retention time of Terlipressin was 10.05 min. Terlipressin was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 2-12µg/ml. The value of correlation coefficient, slope and intercept were, 0.9995, 1848.2and 62.786, respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Key words: Terlipressin, RP-HPLC, degradation studies.

INTRODUCTION

Terlipressin is described chemically as 1-{[(4R, 7S, 10S, 13S, 16S, 19R)-19 {[({[(amino acetyl)amino]acetyl}amino) acetyl] amino}-7-(2-amino-2-oxo ethyl)-10-(3-amino-3-oxo propyl)-13-benzyl-16-(4-hydroxy benzyl)-6, 9, 12, 15, 18-penta oxo-1,2-dithia-5, 8, 11, 14, 17-penta azacycloicosan-4-yl]carbonyl}-L-prolyl-N-(2-amino-2-oxoethyl)-L-lysinamide (Figure: 1) is an analogue of vasopressin used as a vasoactive drug in the management of hypotension [1, 2].

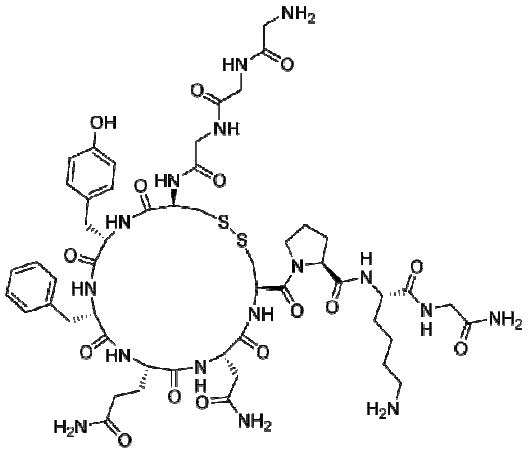

Figure: 1 Chemical Structure of Terlipressin

The objective of this work was to develop an analytical HPLC procedure, which would serve as stability indicating assay method for Terlipressin. A thorough literature search revealed none of the reported analytical procedures describe a stability indicating method for simultaneous determination of stability indicating HPLC method for Terlipressin [3, 4, 5]. To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, photolytic, acid and basic hydrolytic and oxidative), and stressed samples were analysed by the proposed method [6].

In this paper, we describe a reversed-phase HPLC method for the assay of Terlipressin and estimation of its related compounds. This method has been demonstrated to be sensitive, accurate, linear, precise, reproducible, repeatable, specific, and robust, and therefore suitable for routine analysis of Terlipressin in quality control laboratories. This method is also demonstrated to be stability indicating because it can separate the degradation peaks from the Terlipressin peaks that are present in typical stability samples of Terlipressin. From the best of our knowledge via literature search, this is the first known RP-HPLC method that can separate all the related compounds of Terlipressin from each other and from Terlipressin and is therefore suitable to conduct stability studies of Terlipressin.

EXPERIMENTAL SECTION

Materials

Terlipressin was supplied by Sun Pharma and Ampoule, Product Name: Terlyz (100 mcg x 1 mL x 10ml) and Manufacturer: Sun Pharma was procured from the market. Acetonitrile, Monobasic potassium phosphate AR Grade, Methanol, were purchased from RFCL Ltd., New Delhi, India. High purity water was prepared by using Millipore Milli-Q plus water purification system.

Instrument used

The HPLC used was a shimazdu HPLC LC-20AT series with SPD-20A UV photodiode array detector and LCsolution software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were performed in a dry air oven (Thermo labs, India). Micrositer syringer-50 µL (Hamilton Company. USA).

Methodology

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column. The mobile phase consisted of Acetonitrile-monobasic potassium phosphate solution (35:65v/v) at a flow rate of 1.5 ml/min. Monobasic potassium phosphate solution was prepared by dissolving 1400 mg KH_2PO_4 in 100ml double distilled water. Final pH of the mobile phase was adjusted to 3.5 by diluted orthophosphoric acid. The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1.5 mL / min was maintained. Detection was carried out at 280 nm. The injection volume was 20 µL for assay and degradation level.

Standard preparation

10 mg of Terlipressin working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase. A series of standard solutions in the concentration range of 2, 4, 6, 8, 10 and 12µg/ml were prepared followed by a suitable dilution of stock solution with the mobile phase.

Sample preparation

Solution equivalent to 50 mcg of Terlipressin was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 RPM for 10 min.

Method Validation

Linearity

The linearity of response for Terlipressin assay method was determined by preparing and injecting solutions with concentrations of about 2, 4, 6, 8, 10 and 12µg/ml of Terlipressin.

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (6 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (6 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Terlipressin.

Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Terlipressin working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\% \text{ Recovery} = \frac{\%Amount\ \text{Recovered}}{\%SumLevel} \times 100$$

Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

Analysis of marketed formulation

Solution was equivalent to 50 mcg of Terlipressin to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

Forced degradation studies

Preparation of acid and based- Induced degradation product

Solution equivalent to 50 mcg of Terlipressin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Preparation of hydrogen peroxide - induced degradation product

Solution equivalent to 50 mcg of Terlipressin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Photodegradation product

Solution equivalent to 50 mcg of Terlipressin (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Thermal degradation product

Solution equivalent to 50 mcg of Terlipressin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min

and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Terlipressin and its degradants in pharmaceutical dosage form is given in Table: 5 & 6. The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples (Figures: 2 to 5) is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

Detection of the related impurities

Solution equivalent to 50 mcg of Terlipressin is transferred to a 50 mL volumetric flask. Add about 30 mL of mobile phase and sonicate for 20 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 rpm for 10 min. inject the clear supernatant solution into the HPLC system.

RESULTS AND DISCUSSION

Method of development

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). XTerra® RP18 column had given a good peak shape with response at affordable retention time than Luna C8. The chromatographic conditions finally comprised of Acetonitrile-monobasic potassium phosphate solution (35:65v/v) at a flow rate of 1.5 ml/min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 280 nm.

Validation of the method

Linearity

These results indicate that the response is linear over the range of 2, 4, 6, 8, 10 and 12µg/ml of Terlipressin with coefficient of regression, R^2, value as 0.9995. The value of correlation coefficient, slope and intercept were, 0.9995, 1848.2 and 62.786, respectively the results were shown in **Table: 1.**

Table: 1 Regression characteristics of the proposed RP-HPLC method

S. No	Drug	Terlipressin
1.	Range (µg/ml)	2-12µg/ml
2.	Detection wave length (λ max)	280 nm
3.	Mean **'R^2'** value	0.9995
4.	Slope (m)	1848.2
5.	Intercept (c)	62.786
6.	Run time	20min
7.	Retention Time (min)	10.05
8.	Theoretical Plates (N)	10326
9.	Tailing Factor	0.94

Precision

The %RSD for repeatability of sample preparation is 0.89%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. **Table: 2** represent the precision of method.

Table: 2 Method precision of Terlipressin

Sample Preparation	% Assay Terlipressin	% Deviation From Mean Assay value Terlipressin
1	99.59	0.34
2	98.65	-0.6
3	100.05	0.8
4	97.57	-1.68
5	99.78	0.53
6	99.87	0.62
Mean	99.25	
±SD	0.88	
%RSD	0.89	

Ruggedness and Robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst The deliberate aforementioned changes in parameters alters the result of Terlipressin 0.01% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. **Table: 3** represent the ruggedness and robustness of the method.

Table: 3 Ruggedness and robustness of Terlipressin

Parameter	Normal (Original)	Changed conditions
Column make	X Terra® RP18 column; 250 x 4.6 mm; 5 µ	Luna C_8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1.5 mL/min	1.2 mL/min
Mobile phase Composition	Acetonitrile: Buffer (mono basic KPO4) (35:65)	Acetonitrile: Buffer (mono basic KPO_4) (45:55)
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
Analyst	Kalyan.B	Narasimha.V
% assay of Terlipressin	99.25%	99.22%
% deviation from mean assay value obtained in method precision studies for Eprosartan : 0.01%		

Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Terlipressin ranges from 100.6% to 101.8% with mean recovery of 101.1% and % relative standard deviation of 0.37%. The recovery of Terlipressin by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 99.0 - 102.0%. **Table: 4** represent the accuracy of method.

Analysis of the marketed formulation

The drug content was found to be 99.22% with a % RSD of 0.87%. It was noted that no degradation of Terlipressin had occurred in the marketed formulation that were analysed by this method. The low RSD value indicated the suitability of this method for routine analysis of Terlipressin in pharmaceutical dosage form.

Table: 4 Recovery of Terlipressin

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery
Preanalysed sample				99.25
1	80	80.23	81.17	101.2
2	80	79.86	81.32	101.8
1	100	100.32	101.24	100.9
2	100	100.02	101.19	101.2
1	120	120.45	121.12	100.6
2	120	120.12	121.23	100.9
Mean				101.1
± standard deviation				0.374
% relative standard deviation				0.369

Stability- indicating property

The chromatogram of no stress treatment sample (as control) showed no additional peak (**Figure: 2& 3**). The retention time (RT) of standard and sample were 10.5 min and 10.1 min respectively.

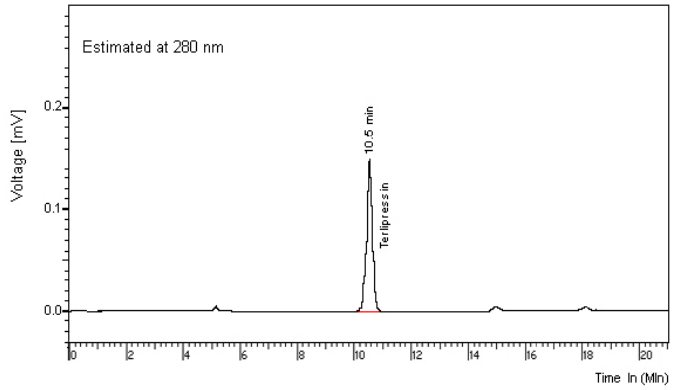

Figure: 2 The simple chromatogram of standard Terlipressin

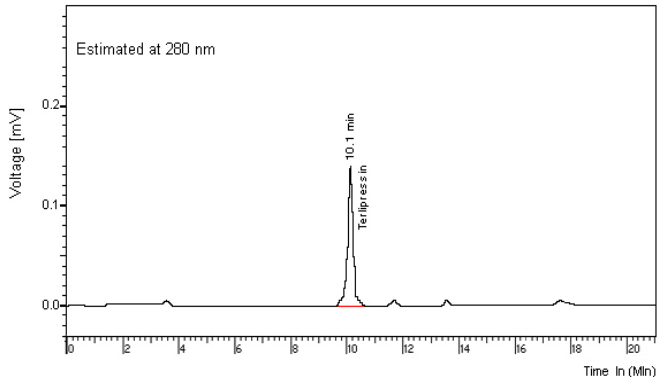

Figure: 3 The simple chromatogram of test Terlipressin

The chromatogram of acid degraded sample showed no additional peaks. The chromatogram of alkali degraded sample showed no additional. The chromategram of hydrogen peroxide degraded sample showed additional peaks at RT of 7.12 and 8.98 min respectively (**Figure: 4**).

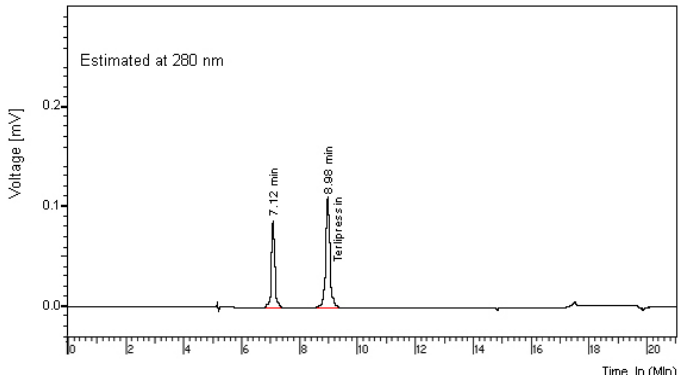

Figure: 4 The simple chromatogram of Hydrogen Peroxide degraded sample.

The chromatogram of UV degraded sample showed no additional peaks. The chromatogram of thermal degraded sample showed additional peak at RT of 7.99 min (**Figure: 5**) and the values were shown in **Table: 5& 6**.

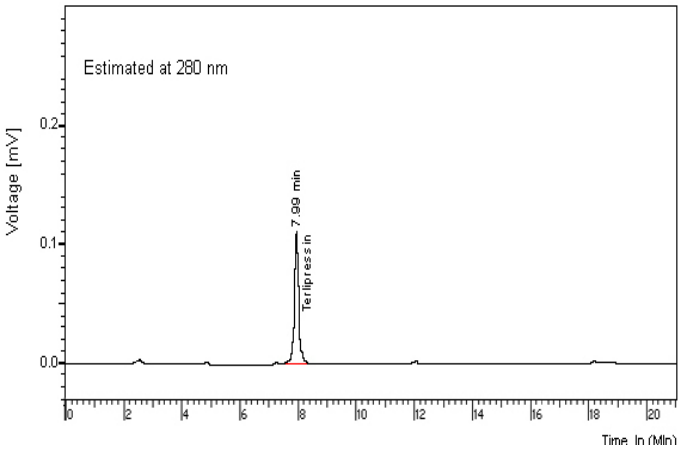

Figure: 5 The simple chromatogram of Thermal degraded sample

Table: 5 Stressed study data of Terlipressin

S. No	Condition	% assay Terlipressin	Retention time of drug	% Degradation
1	No stress treatment	99.25	10.5	Nil
2	Acid	-	-	Nil
3	Alkali	-	-	Nil
4	H_20_2	98.57	7.12, 8.98	0.08
5	UV	-	-	Nil
6	Thermal	99.64	7.99	0.06

Table: 6 Summary of forced degradation results

S. No	Stress condition	Time	%Assay of active substance	Mass balance (%assay + %degradation products)	Remarks
1	Acid degradation (1 N HCl)	1/2 hr	-	-	-
2	Alkali degradation (1 N NaOH)	1/2 hr	-	-	-
3	H_2O_2 degradation (3%)	1/2 hr	98.57	98.7	Mild degradation formed
4	UV degradation	24 hr	-	-	-
5	Thermal degradation (60 ^{0}C)	1/2 hr	99.64	99.7	No degradation products formed

Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation sample were additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to hydrogen peroxide degradation and thermal degradation but not susceptible to acid-base hydrolysis degradation and UV degradation. The lower RT of the degraded component indicated that they were more polar than the analyte itself.

Detection of the related impurities

The sample solution showed no additional peak other than principal peak. Hence, related impurities are not present in the market sample.

CONCLUSION

Introducing HPLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis of Terlipressin as bulk drug and in pharmaceutical formulation without any interference from the excipients. This study is a typical example of a stability-indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. It is proposed for the analysis of drug and degradation products in stability samples obtained during industrial production.

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