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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BETRIXABANIN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

T. Hanuman[1*], T. Siva kumar[2], S. Sridhar[1]

[1]Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maissammaguda, Secunderabad – 500100
[2]Department of Pharmaceutical Chemistry, Annamalai University, Chidambaram, TN.

*Corresponding author E-mail: hanuman_elr2008@rediffmail.com

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation ofBetrixabanin pure and pharmaceutical dosage forms. A DenaliC_{18} column (150 x 4.6mm x 5µm)was used as a stationary phase with a mobile phase containing a mixture of buffer(accurately weighed and transferred 1.36gm of Potassium dihydrogenorthophosphate in a 1000mlof volumetric flask add about 900ml of milli-Q water and degass to sonicate and finally make up the volume with water, then pH adjusted to 3.0 with dil.Ortho phosphoric acid solution) and acetonitrile in the ratio of 60:40v/v. The flow rate was 1.0ml/min,the effluent was monitored at 292nm and eluted at 2.283min. Calibration curve was plotted with a range from 10-60µg/ml for Betrixaban and the correlation was found to be 0.9998.The accuracy range was found between 99.19% and 100.79%. The %RSD values for both intraday and interday precision were less than 2%.The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.08µg/ml and 0.24µg/ml respectively. The assay was validated for the parameters like specificity, system suitability, precision, accuracy, robustness and ruggedness parameters. The proposed method can be useful for the routine determination ofBetrixabanin pharmaceutical dosage form.

INTRODUCTION

Betrixaban, is chemically N- (5-chloropyridin-2-yl) -2- [4- (N, N-dimethyl carbamimidoyl) benzamido]-5-methoxybenzamide. The chemical formula is $C_{23}H_{22}ClN_5O_3$. Betrixaban is a cofactor-independent direct inhibitor of the Factor Xa and inhibits free and prothrombinase-bound Factor Xa. Betrixaban is an oral anticoagulant that excerts its action by preventing thrombin generation without having a direct effect on platelet aggregation [1]. Literature surveys reveal few methods for its determination [2]. The simple, accurate, precise and validated method for determination of BETRIXABAN was developed by RP-HPLC method.The present study was validated following the ICH guidelines [3].

MATERIALS AND METHODS

Materials: Betrixaban was purchased from Open Market.Acetonitrile, water(HPLC grade, Merck) andall the other reagents of AR grade were purchased from M R Enterprisers. A tablet (BEVYXXA) contains 40 mg of Betrixaban.

Methods

Instrumentation: The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals weremonitored and integrated using Empower 2 software.

Chromatographic conditions: The elution was isocratic and the mobile phaseconsisted of a mixture of buffer and acetonitrile (60:40 v/v).The mobile phasewas filtered through a 0.45-µm (HVLP, Germany)membrane filter prior to use. A Denali(150 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.0 ml/minand the column was operated at ambient temperature (~30^{o}C). The volume of sampleinjected was 10 µL.Prior to injection of the solutions, column was equilibrated for atleast 30min with mobile phaseflowing through the system. The UV detector was set at wavelength of 292nm. A typical chromatogram of Betrixabanis shown in (Figure:1).

Diluent: Acetonitrile and Water (50:50) v/v

Standard Preparation: Stock solution of Betrixaban was prepared by dissolving 100mg in 100 ml volumetric flask add few ml of diluent. Sonicate it for 30min and make up with diluent. Transfer 4ml from the above solution into 10ml volumetric flask to get concentration of 100 µg/ml.From the above solution, transfer 4ml from the above solution into 10ml volumetric flask to get concentration of 40 µg/ml.

Sample Preparation:

20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 100mgwas transferred to a 100 ml volumetric flask, dissolved in diluents. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 100 µg/ml.From the above solution, transfer 4ml from the above solution into 10ml volumetric flask to get concentration of 40µg/ml.

METHOD VALIDATION [4-7]

The developed method was validated as per ICH guidelines for its specificity, precision, linearity, accuracy, robustness, limit of detection and limit of quantification by using the following procedures.

System suitability:

System suitability and chromatographic parame ters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity: Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Betrixabanat different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug. The response was found to be linear in the range 10-60 µg/ml for Betrixaban.

Accuracy: Accuracy was performed in triplicatefor various concentrations of Betrixaban equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated.

Precision

A) System Precision: Six standard solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

B) Method Precision: Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

C) Intermediate Precision (Day to Day variability)

Intraday: Two sample solutions have analysed in the same day as per test method conducted the study. For '0' hourand '24' hour, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

Inter day: Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD and LOQ were found to be 0.08 µg/ml and 0.24 µg/ml respectively.

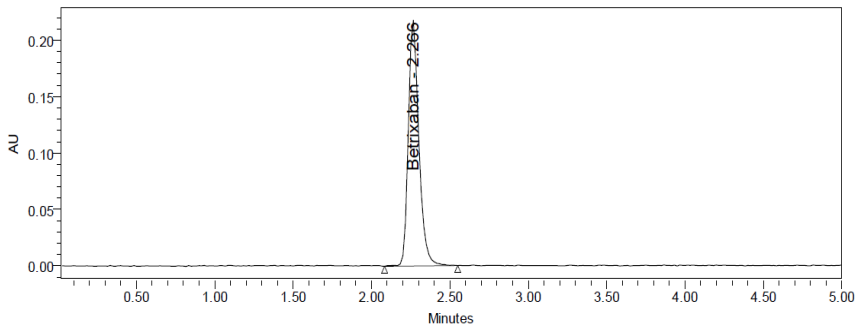

Fig.1: HPLC Chromatogram of Betrixaban

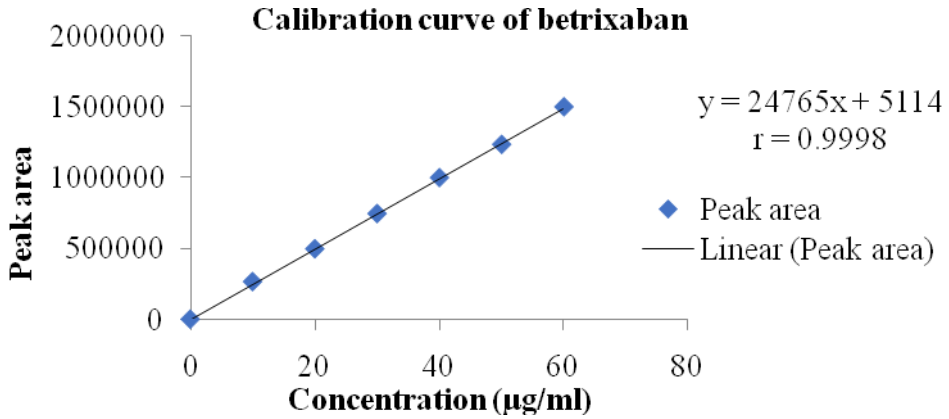

Fig. 2: Calibration curve of Betrixaban

Table 1: Observed data for calibration curve of Betrixaban

S.No	Concentration (µg/ml)	Injection	Retention time (mins)	Area
1	10	1	2.256	264946
2	20	1	2.258	496271
3	30	1	2.274	744848
4	40	1	2.266	998798
5	50	1	2.264	1233238
6	60	1	2.267	1498374

Table 2: Accuracy data

S.No.	Spiked level	Amount Added(µg/ml)	Amount Found(µg/ml)	Average %Recovery*	Std.Dev	%RSD
1(n=3)	50%	10	10.91	99.62	0.05	0.05
2(n=3)	100%	20	20.56	101.12	0.38	0.38
3(n=3)	150%	30	29.89	99.86	0.33	0.33

*n=3 (Average of 3 determinations)

Table 3: System Precision data of 40 µg/ml

S.No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	40	1	2.634	999246
2	40	1	2.656	999876
3	40	1	2.672	998379
4	40	1	2.644	999622
5	40	1	2.698	998852
6	40	1	2.623	990934
Mean				997818
Std.Dev				3415
%RSD				0.34

Table 4: Method Precision data of 40µg/ml

S.No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	40	1	2.628	999457
2	40	1	2.641	999699
3	40	1	2.642	998727
4	40	1	2.647	999143
5	40	1	2.595	998017
6	40	1	2.651	990187
Mean				997538
Std.Dev				3650
%RSD				0.37

Table 5: Intra-day data relating to change in the day

S.No	Intra-day Precision		
	Peak Area		
	Concentration (µg/ml)	'0'hour	'24' hour
1	40	994147	996847
2	40	998474	999283
3	40	989283	998391
4	40	998511	999037
5	40	985833	993821
6	40	991837	995382
Mean		993014	997127
SD		5063	2188
%RSD		0.51	0.22

Table 6: Inter-day data relating to change of day

S.No	Inter-day Precision		
	Peak Area		
	Concentration (µg/ml)	Day – 1	Day – 2
1	40	994657	993584
2	40	992358	998543
3	40	999125	996837
4	40	993574	992394
5	40	996542	991652
6	40	997521	997568
Mean		995630	995096
SD		2550	2915
%RSD		0.26	0.29

Table 7: Robustness data relating to change in flow rate (1.0ml/min)

S.No	Flow rate (ml/min)	Average Peak Area*	SD	%RSD
1	0.9ml/min	992466	5832	0.39
2	1.0ml/min	992411	4861	0.32
3	1.1ml/min	991465	3825	0.25

*n=3 (Average of 3 determinations)

Table 8: Robustness data relating to change in mobile phase composition

S.No	Mobile Phase Variation (%)	Average Peak Area*	SD	%RSD
1	M.P-1-(Buffer:ACN::61:39)	991452	4217	0.28
2	M.P-2-(Buffer:ACN::60:40)	992919	3307	0.22
3	M.P-3-(Buffer:ACN::59:41)	990950	3800	0.25

*n=3 (Average of 3 determinations)

Table 9: Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Purity ± RSD*
Brand-1 (BEVYXXA)	40mg	40.08mg	100.03± 0.12

*n=3 (Average of 3 determinations)

Table 10: Results of acid degradation studies of Betrixaban

S.No	Betrixaban				
	Concentration (µg/ml)	Time (h)	Area	%Assay	%Degradation
1	40	0	1497636	100	
2	40	24	1390048	92.77	-8

Table 11: Results of base degradation studies of Betrixaban

S.No	Betrixaban				
	Concentration (µg/ml)	Time (h)	Area	%Assay	%Degradation
1	40	0	1497636	100	
2	40	24	1406536	93.87	-8

Table 12: Results of peroxide degradation studies of Betrixaban

S.No	Betrixaban				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	40	0	1497636	100	
2	40	24	1421094	94.84	-6

Table 13: Results of thermal degradation studies of Betrixaban

S.No	Betrixaban				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	40	0	1497636	100	
2	40	24	1434604	95.74	-5

Table 14: Results of UV degradation studies of Betrixaban

S.No	Betrixaban				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	40	0	1497636	100	
2	40	24	1476310	98.53	-2

Table 15: System suitability parameters

Validation parameters	Results
Linearity range (µg/ml)	10 – 60
Regression equation	Y = 24765x +5114
Correlation Coefficient(r^2)	0.9998
Accuracy	99.48% to 101.54%
Precision (%RSD)	0.37
Flow rate (0.9ml/min & 1.1ml/min)	NMT 0.39
Mobile phase – ACN : H_2O(61:39 & 59:41)	NMT 0.28
Intraday – (‘0’hour&‘24’ hour)	NMT 0.51
Interday – (Day 1 & Day 2)	NMT 0.29

Robustness: Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Betrixaban was noted. The factors selected were flow rate and variation in the mobile phase composition.

Assay: The assay & % purity was performed by taking brand BEVYXXA with label claim 40mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form.

Degradation studies: The standard solution injected in the chromatographic system and the standard chromatogram was compared with different stress degradation conditions chromatogram.

RESULTS AND DISCUSSION

A reverse-phase column procedure was proposed as a suitable method for the determination of Betrixaban dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 60:40v/v was used as mobile phase, which showed good resolution of Betrixaban peak. The wavelength of detection selected was 292nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Betrixaban was about 2.283minute and none of the impurities were interfering in its assay. The chromatogram of the drug is shown in Fig. 1 and calibration curve is shown in Fig. 2 respectively. The observed peak area values for respective concentrations are shown in Table 1. The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Betrixabanin marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Betrixabanin bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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