Analytical Method Development and Validation of Assay of Anticoagulant drug Edoxaban by RP-HPLC method

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Abstract:

The aim of this study is to develop a sensitive, specific, rapid, and precise reverse phase high performance liquid chromatography (RP-HPLC) method and validate it for Edoxaban an anticoagulant drug. The chromatographic separation was achieved on the analysis of the drug was carried out on Agilent 1100 Series HPLC Isocratic System with Auto injector, UV (PAD) detector, C18 column (4.6mm x 250 mm; 5 µm), a 20µl injection loop and running Chemstation software. Different solvents of varying polarity in different proportions were tried as trial and error as Mobile phase for development of the chromatogram. The selection of the wavelength was based on λ_{max} obtained scanning of Sample solution in MeOH. The system gave the good optimized result, resolution & optimum retention time with an appropriate purity, tailing factor, Theoretical plates and symmetry. Therefore optimized method with HPLC column C8 along with runtime of 7 min, Mobile phase Methanol, flow rate 1.5ml/min, The column oven temperature maintain at 35 °C, the PAD detector maintain at 289 nm with good optimized result, effectively, successfully, efficiently, resolution and Retention time 2.058, Theoretical Plates 5685, Symmetry Factor 0.81 and purity 996.41. The developed method was validated as per ICH guidelines and found to be specific, precise, sensitive, and robust.

Keywords: RP-HPLC, Methanol, C18 column, Edoxaban, Validation, Optimization.

Introduction:

Edoxaban (EXN), chemically known as N'-(5- chloropyridin-2-yl)-N-[(1S,2R,4S)-4- (dimethylcarbamoyl)- 2- [(5-methyl-6,7-dihydro-4H- [1,3] thiazolo [5,4-c] pyridine-2-carbonyl)amino] cyclohexyl] oxamide, is an oral anticoagulant that acts as highly specific direct factor Xa inhibitor, with Molar mass 548.0 g mol-[1]. In 2011, Japan approved EXN for prevention of venous thromboembolism after total hip arthroplasty with no increased risk of bleeding. Food and Drug Administration approved EXN for the prevention of stroke and non central nervous system systemic embolism in patients having nonvalvular atrial fibrillation in 2015. So far EXN is not official in any pharmacopeias. The literature is poor regarding the reports on the assay of EXN.

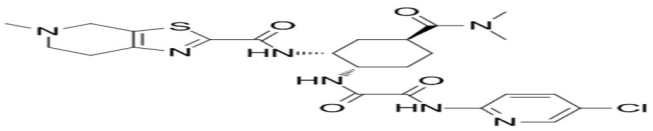

Fig no 1: Structure of Edoxaban

The HPLC has capability to separate, identify, and quantify the mixtures that are present in any of the sample that dissolved in a liquid. High performance liquid chromatography is the perfect analytical methods broadly used for the quantitative and the qualitative analysis of drug product. The principle of HPLC separation is the affinity between polar mobile phase and non-polar stationary phase. When mixture of compound is introduced into the HPLC column, they pass according through to their relative affinities towards the stationary phase.

The developed method was validated in agreement with International Conference on Harmonization guidelines.

MATERIAL AND METHOD

Chemical and Reagent: A pharmaceutical grade sample of Edoxaban (assigned purity 99.98%) was obtained as gift from Chemstation Nashik. Acetonitrile HPLC grade, Water HPLC grade and Methanol HPLC grade were purchased from Finar ltd. Ahmedabad.

Instruments: The analysis of the drug was carried out on Agilent 1100 Series HPLC Isocratic System with Auto injector, UV (DAD) & Isocratic Detector. Equipped with Reverse Phase C_8 column (4.6mm x 250mm;5µm), Temperature was set at 30°C, Initial run time 10 min, flow rate 1ml/min 20µl injection loop and DAD detector and running Chemstation software.

Experiment Work:

Selection of stationary phase**:** The column used in this method C8. The configuration of the column is 4.6 x 250 mm, particle size 5 µm. C8 column gives high non polar retentively, symmetric peak shape, highly reproducible and stable ideal for HPLC method.

Selection of mobile Phase: The selection of Mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey and finally mobile phase was selected.

Preparation Sample Solution Stock: (Edoxaban)

Stock Solution: Precisely weight and transfer 50 mg drug working standard into 100 ml volumetric flask as about dilute completely and make volume up with the same solvent to get 500 µg/ml standard &15 min Sonicate to dissolve it.

Test Solution: The resulting solution 5 ml was subjected to 50 ml volumetric flask and the volume was made up to the mark with Solvent. The resulting 50 µg/ml solution was added to analyses using mobile phases of different strengths with chromatographic conditions mentioned below in trial and error.

Result and Discussion :

Optimized Chromatographic Condition

Reverse Phase C8 column (4.6mm x 250mm;5µm),a 20µl injection loop and DAD detector and running Chemstation software. HPLC column with a runtime of 7 min, Mobile phase Methanol HPLC grade, Flow rate 1.5 ml/min, respectively The column oven temperature maintain at 30 °C, and Absorbance at 289 nm.

Table no 1: System Suitability of Edoxaban

Sr.no	Components	RT	Theoretical Plate	Symmetry Factor	Purity factor	Purity Threshold
1	EDOXABAN	2.059	5685	0.81	992.96	996.41

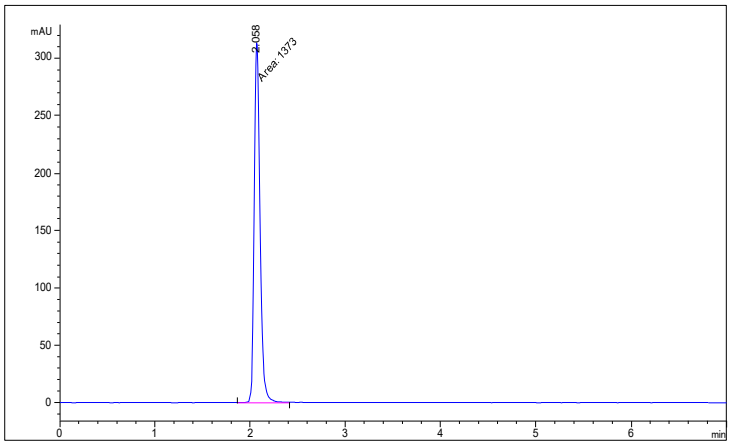

Fig no 2: Chromatogram of Edoxaban

Peak was eluted at Retention time 2.08 purity and Symmetry was within the range.

Accuracy

Several methods are available for determination of accuracy: Application of an analytical procedure to an analyte of known purity. Comparison of the results of the proposed analytical procedure with those of a second well characterized procedure. Accuracy may be inferred once precision, linearity and specificity have been established.

Specificity:

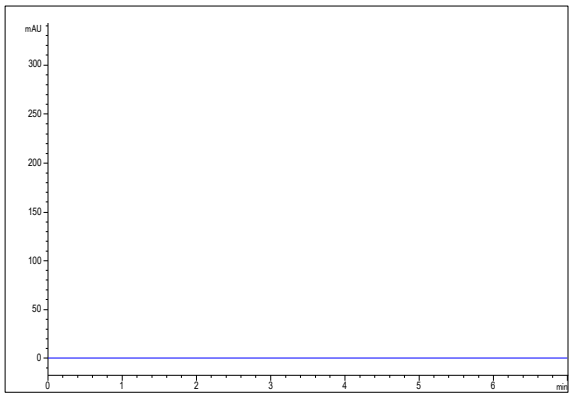

Fig no 3: Chromatogram of Blank

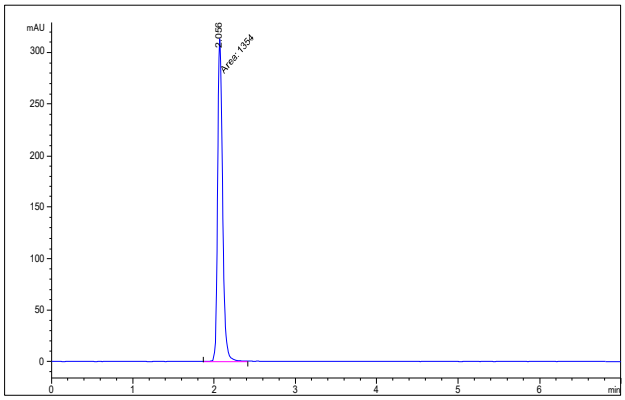

Fig no 4: Chromatogram of Standard

Discussion:

The data demonstrate that there is no interference of blank with Edoxaban at 289 nm

Table no 2: Specificity of Standard and Edoxaban

Sr.no	Components	RT	Theoretical Plate	Symmetry Factor	Purity factor	Purity Threshold
1	STANDARD	2.054	5872	0.81	413.07	998.54
2	EDOXABAN	2.059	5685	0.81	992.96	996.41

Linearity:

Table no 3: Linearity of Edoxaban

Level (In %)	Concentration (ppm)	Response		
		1	2	Mean
60%	30 ppm	816	801	**808**
80%	40 ppm	1087	1077	**1082**
100%	50 ppm	1355	1341	**1348**
120%	60 ppm	1610	1630	**1620**
140%	70 ppm	1887	1877	**1882**
Correlation coefficient (R)				**0.999**
(r^2)				**1**
SLOPE				**26.8523**
Y- INTERCEPT				**5.223**

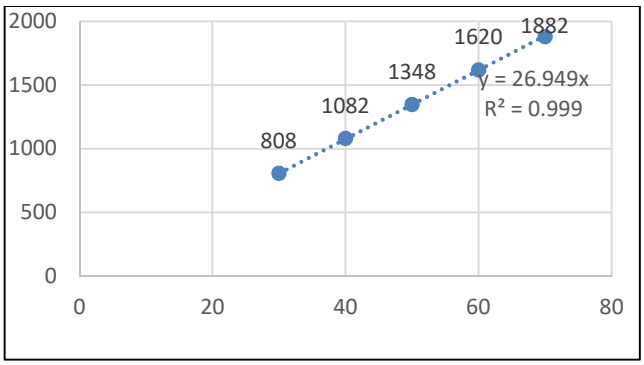

Fig no 5: Linearity Graph of Edoxaban

Discussion: The data was obtained specified range for intended application 60% to 140%of working level concentration.The data was obtained that response found is Linear ; correalation coeficient (R) not less than 0.999, r^2 is more than 0.999.

Precision :

Table no 9: Method Precision of Edoxaban

Sample No.	Area Mean	% Assay
1	**1330**	**98.98**
2	**1317**	**98.05**
3	**1330**	**99.01**
4	**1335**	**99.35**
5	**1332**	**99.15**
6	**1324**	**98.55**
Average	**98.85**	
Std. Relative deviation	**0.47**	
%RSD	**0.48**	

Table no 10: Intermediate Precision of Edoxaban

Sample No.	Test	RESPONSE	
		Area mean	%Assay
1	Precision (Analyst 1)	**1330**	98.98
2		**1317**	98.05
3		**1330**	99.01
4		**1335**	99.35
5		**1332**	99.15
6		**1324**	98.55
7	Precision (Analyst 2)	**1332**	99.12
8		**1343**	99.93
9		**1342**	99.89
10		**1341**	99.84
11		**1351**	100.55
12		**1354**	100.80
	Average		**99.44**
	Std.Relative deviation		**0.80**
	%RSD		**0.80**

Discussion: The data was obtained that system suitability is fulfilled. The data was obtained that %RSD for % assay is within acceptance criteria and hence method is precise.

Robustness

Table no 11: Robustness of Edoxaban

Changes in Parameters	Values	Retention Time	Theoretical Plates	Symmetry factor	% RSD of Standard Area	% Assay
***Control**	As per method	2.06	6545	0.82	0.56	99.95
Flow Rate (1.5ml/min)	1.3ml	2.267	6566	0.82	0.47	100.14
	1.7ml	1.902	5715	0.82	0.32	100.22
Temperature (30°C)	28°C	2.061	5719	0.82	0.38	99.34
	32°C	2.061	5716	0.82	0.23	99.86

Discussion: The overall percentage relative standard deviation of method precision and robustness results should not be more than 1.0%,. The overall percentage relative standard deviation of method precision and robustness results should not be more than 1.0%

CONCLUSION:

This technique was employed in the present investigation for assay of Edoxaban. The analysis of the drug was carried at Agilent 1100 Series HPLC Isocratic System with Auto injector & UV (DAD). Equipped with Reverse Phase C8 column (4.6 mm x 250 mm; 5 µm), a 20µl injection loop and running Chemstation software. HPLC column with a runtime of 7 min, Mobile phase Methanol, The column oven temperature maintain at 30 °C, Absorbance at 289 nm. The system gave the good optimized result, resolution & optimum retention time 2.058 with an appropriate purity factor 992.96, Theoretical plates 5685 and symmetry factor 0.81. The developed method was validated as per ICH guidelines & establishes to be specific, precise, sensitive, and robust.

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