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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR EDOXABAN TOSYLATE MONOHYDRATE USING QBD APPROACH

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ABSTRACT

QbD(Quality by Design) has gained importance in the analytical method development. It involves the optimization of the critical parmaters and to evaluate their effect on the critical quality attributes. An attempt was to develop and validate a in bulk form. The Box-Behnken design was used for the Qbd approach in which the screening was done on the critical parameters i.e(Buffer pH, %ACN and flowrate) and their effects on the variable responses i.e(Retention time, NTP and Asymetry factor) was evaluated. The developed method was validated according to International Conference on Harmonization guideline with respect to accuracy, precision, specificity, linearity, solution stability and system suitability. For this, an isocratic condition of mobile phase comprising buffer of 0.01 M sodium acetate with pH 4.0 and acetonitrile in a ratio of 70:30, v/v at a flow rate of 1.0 mL/minute over Qualisil BDS C18, 250 mm×4.6 mm×5 µm column at 28 C temperature was maintained. The detection was done using a PDA detector at 290nm. The method showed excellent linear response with correlation coefficient (R2) values of 0.999 for Edoxaban tosylate monohydrate, which was within the limit of correlation coefficient (R2 ≥ 0.995). The percent recovery was found within the acceptance limit of 98.0% to 102.0%. The %RSD for precision studies was found to be less than 2%. The Limit of Detection(LOD) and Limit of Quantification(LOQ) was found be 0.2µg/ml and 0.5 µg/ml

respectively.

KEYWORDS: Edoxaban Tosylate Monohydrate, Stability indicating method, Validation, QbD.

INTRODUCTION

Quality by design (QbD) has become an important paradigm in the pharmaceutical industry since its introduction by the US Food and Drug Administration. The concept of Quality by design (QbD) has recently gained importance in the area of analytical method development by application of design of experiments approach.[1] QbD involves understanding of the critical factors and their interaction effects by a desired set of experiments.[1] The fundamental premise behind QbD is that quality is designed into the process at the onset to establish a thorough understanding of the response of the system quality to system parameters, leading ultimately to the establishment of the design space for the method.[2] QbD approach suggests looking into the quality of the analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into final results of analytical process.[2] Traditional chromatographic method development has always involved the time-consuming process of varying one system parameter at a time, examining its effect on the method and system operation.[2]

Edoxaban tosylate monohydrate is a member of the Novel Oral Anti-Coagulants (NOACs) class of drugs, and is a rapidly acting, oral, selective factor Xa inhibitor. By inhibiting factor Xa it prevents the binding of platelets to one another and hence is used in the treatment of stroke, pulmonary embolism and deep vein thrombosis.[3]

Chemically it is N-(5-Chloropyridin-2-yl)-N′-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl] oxamide mono (4-methylbenzenesulfonate) monohydrate (Fig no 1). The molecular formula is $C_{31}H_{40}ClN_7O_8S_2$. It has a molecular weight 738.272g/mol. It is a white to pale yellowish white non-hygroscopic powder. It is slightly soluble in water and Acetonitrile, Soluble in methanol and freely soluble in DMSO.[5]

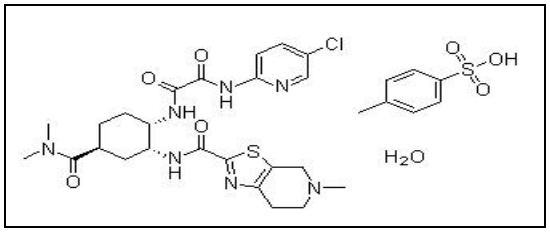

Fig No 1: Chemical Structure of the Drug.

Literature survey reveals that there are very few methods[6,7] available for the estimation of Edoxaban tosylate monohydrate. The available methods are on bioanalytical studies[6] and method available was assay method with no stability studies.[7]

Hence an attempt has been made to develop and validate chromatographic method for determination of Edoxaban tosylate monohydrate from bulk drug using High Performance Liquid Chromatography (HPLC). Attempt has also been made to indicate that method developed and validated is stability indicating method.

MATERIAL AND METHODS

Chemicals and Reagents: Edoxaban tosylate monohydrate was procured from an well established pharmaceutical company as a gift sample. HPLC grade water(SDFCL), Acetonitrile(Merck) and Methanol(Merck) were provided by Haffkine institute for training, research & testing.

Equipment: The estimation of Edoxaban tosylate monohydrate was carried using Thermoscientific Ulitmate 3000 HPLC system with Dionex PDA Detector using Chromeleon 6.80 software as an integrator, Analytical Balance(KERN ABT 320-4NM), pH meter (Eutech Instruments EC-PH 510/42S) and a sonicator (Grant XUBA3). The column used for separation of edoxaban tosylate monohydrate was QUALISIL BDS C18((250 mm×4.6 mm×5 um).

Solution Preparation: 100 mg of edoxaban tosylate monohydrate was accurately weighed and transferred to a 100 ml volumetric flask. The sample was dissolved in sufficient amount of methanol and volume was made up to the mark (1000µg/ml) (Solution A). 1 ml of this solution A was diluted to 10 ml with methanol (100µg/ml) (Solution B). Further, 1 ml of this solution B was diluted to 10 ml with diluent(mobile phase) (10µg/ml) (Solution C). The Solution C was used to carry out the validation parameters.

Preparation of mobile phase: 0.82gm of sodium acetate was weighed accurately and transferred in 1000ml beaker. 1000ml of water was added, sonicated and the pH was adjusted to 4.0 with glacial acetic acid. Mixed the buffer and acetonitrile in the ratio of 70:30(v/v) and sonicated for 15 minutes.

Initial Method Development

Choice of Wavelength: 10mg of Edoxaban tosylate monohydrate working standard was weighed accurately and transferred to 10ml volumetric flask and the volume was made up with methanol to give a concentration of 1000 µg/ml(Solution A). Solution A was further diluted with methanol to get the concentration of 10 µg/ml. Uv spectrum of a solution having a concentration of 10 µg/ml was recorded using methanol as blank. It showed absorbance at a wavelength of 290nm, so it was selected λmax for Edoxaban tosylate monohydrate.

Choice of Column: The selection of the column is given in Table No 1.

Table No 1: Experimental Trials For Choice of Columns.

Column	Observation	Inference
C_8	Poor retention of the analyte	Broad and poor peak shape
C_{18}	Improved retention of analyte	Improved peak shape

Choice of Mobile phase: The selection of mobile phase is given in Table No 2.

Table No 2: Experimental trials for selection of mobile phase.

Mobile phase	Observation	Inference
Methanol:Water	High baseline noise	Interfernce at the Retention time of the analyte
ACN:Water	No precision in the Retention time of the analyte. Broad and poor peak shape	Use of buffer required

Method Optimization by QbD Approach

Software aided Method Development: A Qbd based RP-HPLC method was developed for the quantitation of Edoxaban tosylate monohydrate in bulk form. A Quality by deisgn with the Design experiments involves two phases which are as follows.

1. Screening phase.
2. Statistical Analysis and Final Optimization.

Screening Phase: The first phase of the method development involves the screening of the major effectors of selectivity and peak shape, primarily the buffer pH, flow rate and organic

modifier. This screening phase was carried out using Design Expert 11 software. In this software, Box-Behnken statistical screening design was chosen used to optimize the Critical Method Parameters wherein all the parameters were varied simultaneously unlike the conventional OFAT (one factor at a time) approach. The responses obtained after carrying out the 13 experimental trial runs under Box- Behnken design was fed back into DoE software.

Statistical Analysis and Final Optimization: Statistical Analysis was used to identify the significant influential chromatographic factors and their interaction impact on the three responses i.e. Retention time, Tailing factor, NTP of Edoxaban tosylate monohydrate. The analysis of 3D response surface plots and predicted vs actual plots were used to estimate as to which method parameter gave the most acceptable responses. Statistical analysis tool ANOVA was evaluated for each individual response to determine the most influential chromatographic parameter. Moreover, these statistical analysis tools were used to determine the significance of each method parameter selected for the study. The significance level for probability of a null hypothesis (H0) was defined at $p \geq 0.05$. Null hypothesis indicates variation in all factors which has no influence on the responses. The response variables i.e. Retention time, Asymetry factor and NTP were statistically evaluated and the results obtained were found within the acceptable criteria.

RESULTS AND DISCUSSION

Software Aided Method Optimization: The Box-Benhken design provided statistical analysis which was used to identify the chromatographic parameters and their interaction on the responses i.e Retention time, Asymetry factor and NTP for the analyte peak. The analysis of 3D response surface plots and predicted vs actual plots were used to evaluate as to which method parameter gave the most acceptable responses. The response variables, *i.e.* retention time, Asymetry factor and NTP was statistically evaluated.

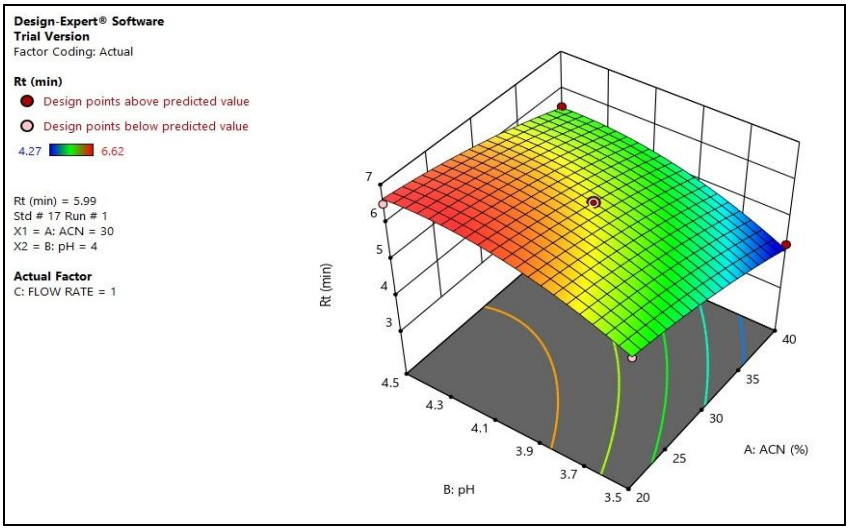

Fig. No. 2: Effect of %ACN and pH on Retention Time.

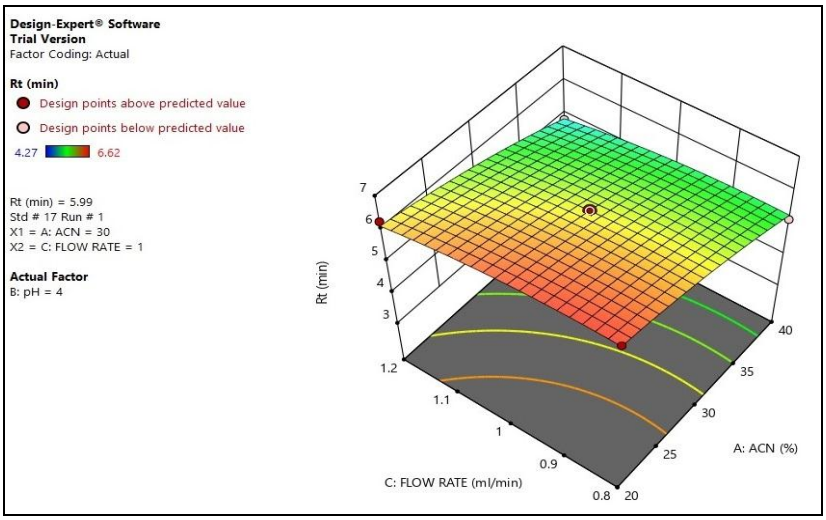

Fig. No. 3: Effect of %ACN and Flowrate on Retention Time.

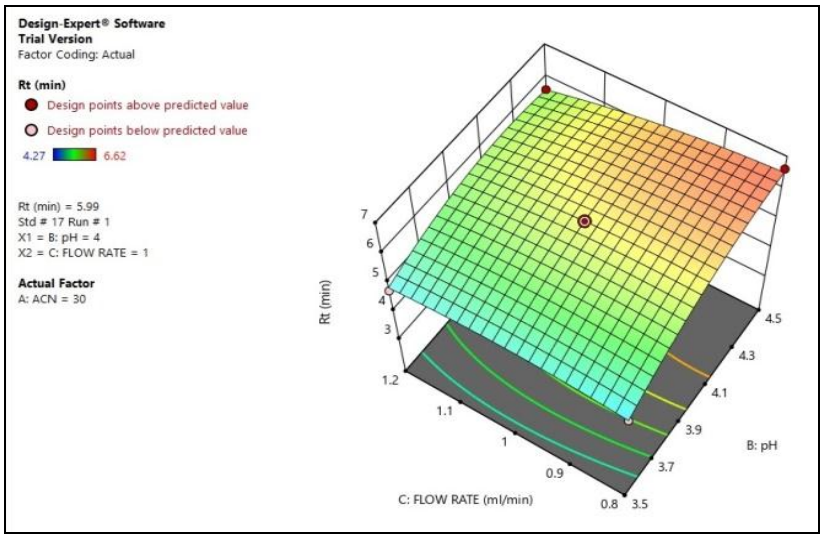

Fig. No. 4: Effect of pH and Flowrate on Retention Time.

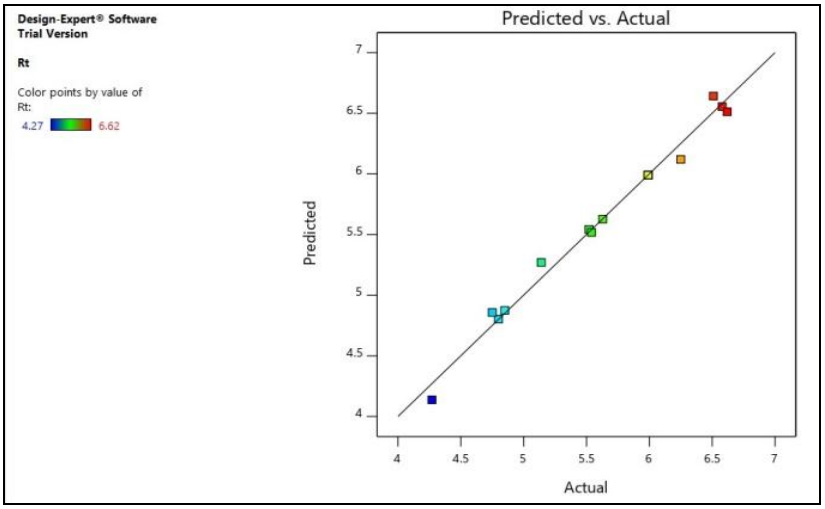

Fig. No. 5: Predicted vs Actual Plot for Retention Time.

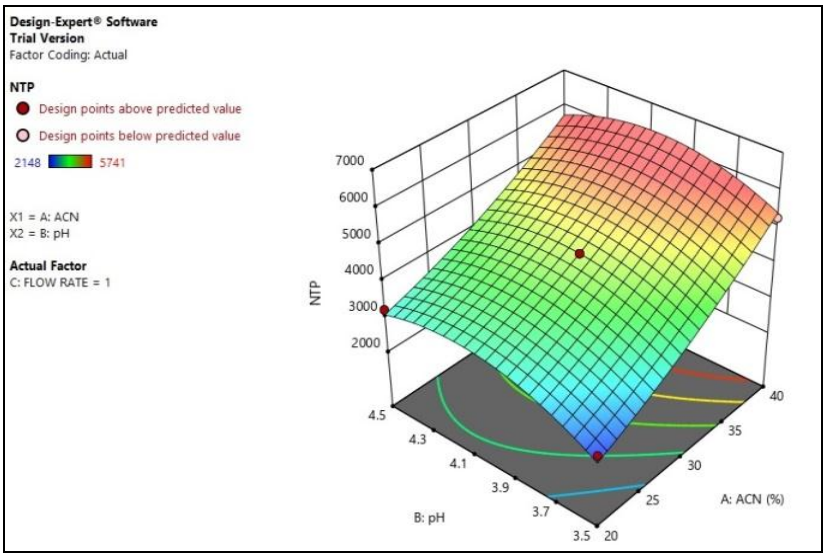

Fig. No 6: Effect of %ACN and pH on NTP.

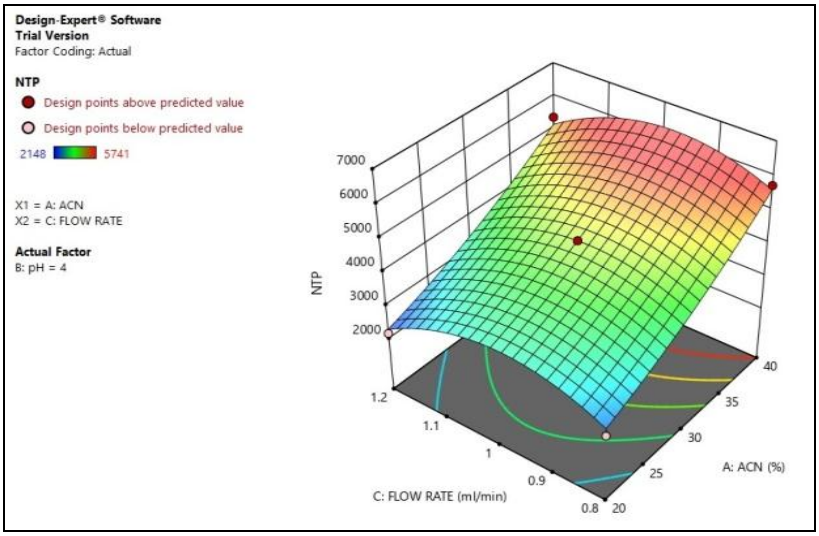

Fig. No 7: Effect of %ACN and Flowrate on NTP.

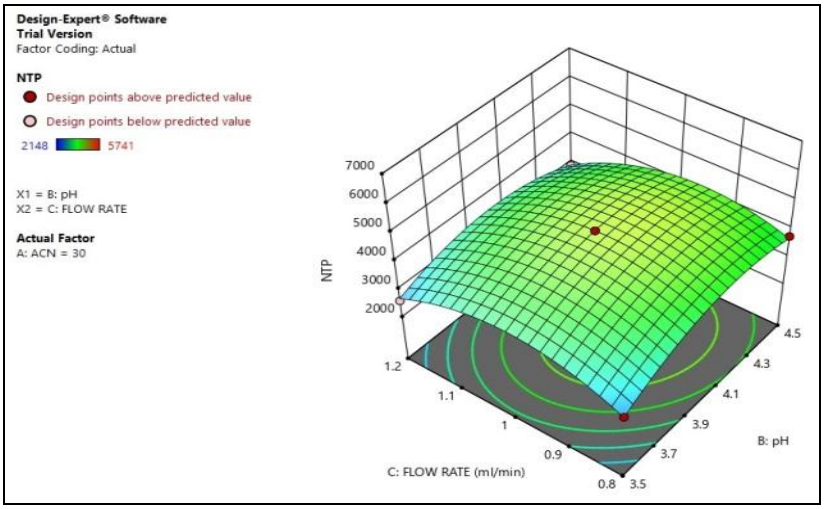

Fig. No 8: Effect of pH and Flowrate on NTP.

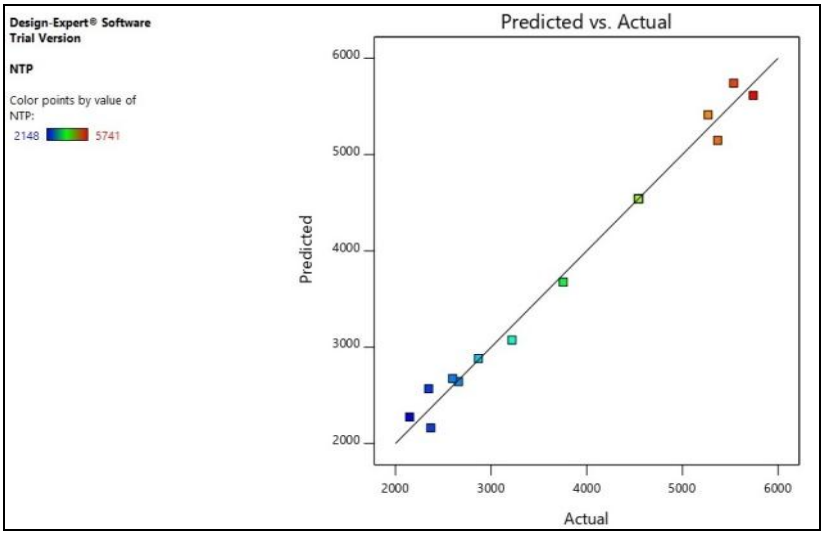

Fig. No 9: Predicted vs Actual plot for NTP.

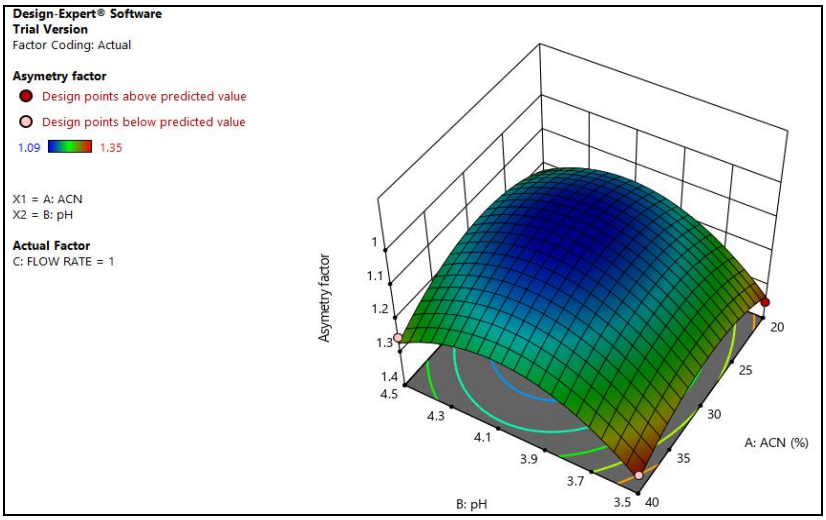

Fig. No 10: Effect of %ACN and pH on Asymetry Factor.

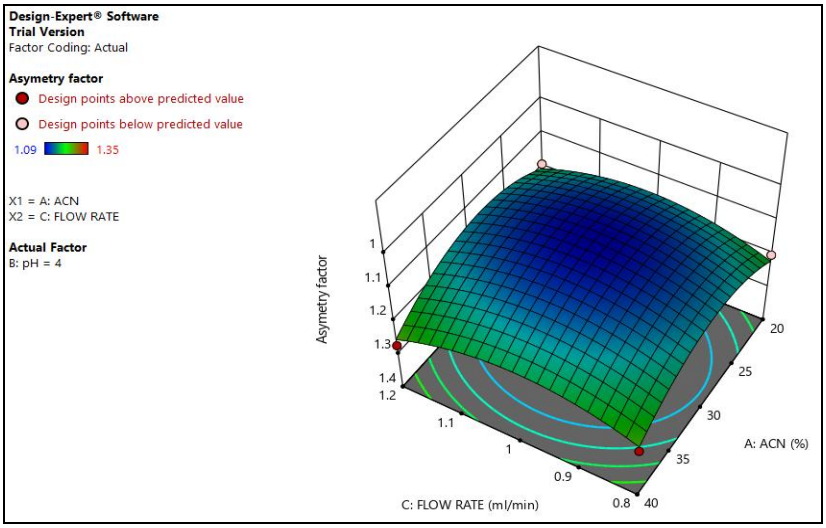

Fig. No 11: Effect of %ACN and Flowrate on Asymetry Factor.

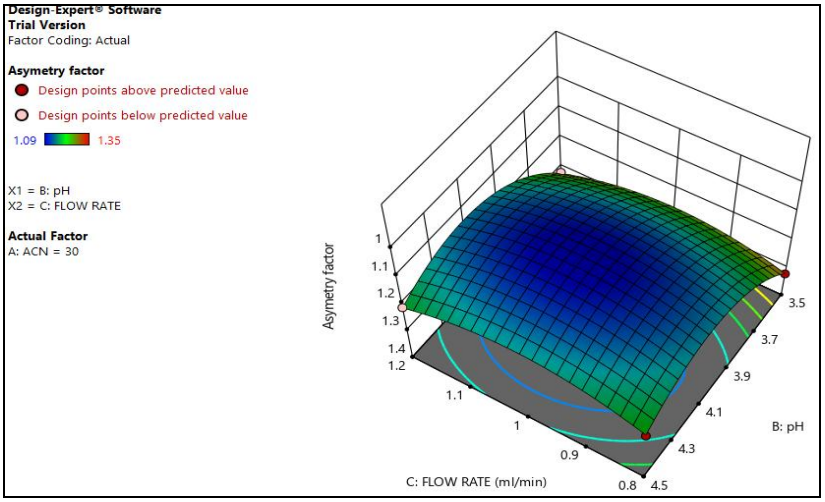

Fig. No 12: Effect of pH and Flowrate on Asymetry Factor.

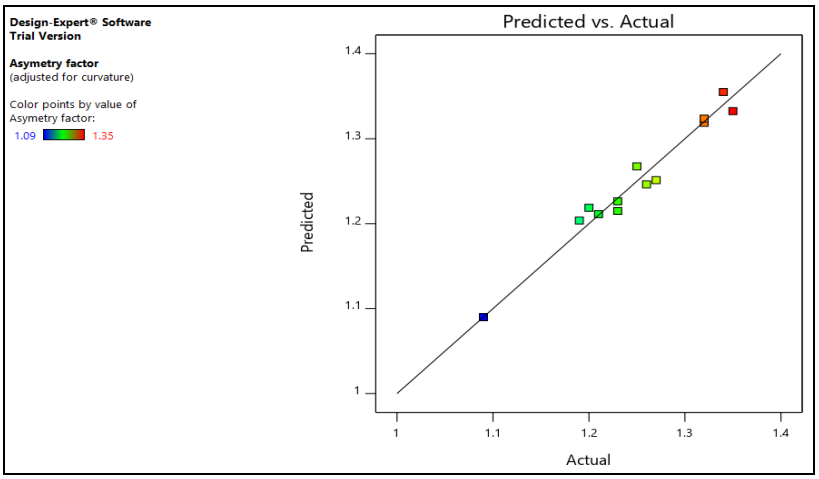

Fig No 13:- Predicted vs Actual plot for Asymetry Factor.

Table No 3: Factor screening by Box Behken Design.

Factor screening by Box Behnken Design						
Run	**Factor %ACN**	**Factor pH**	**Factor Flow rate**	**Response Rt**	**Response NTP**	**Response Asymetry factor**
1	40	4	1.2	4.85	5369	1.27
2	20	3.5	1	5.52	2368	1.35
3	**30**	**4**	**1**	**5.99**	**4540**	**1.09**
4	30	4.5	1.2	5.63	2867	1.21
5	40	4	0.8	5.14	5741	1.26
6	30	3.5	0.8	4.8	2659	1.32
7	20	4.5	1	6.51	3218	1.23
8	20	4	1.2	6.25	2148	1.19
9	30	3.5	1.2	4.75	2597	1.32
10	40	3.5	1	4.27	5269	1.34
11	20	4	0.8	6.58	2347	1.20
12	30	4.5	0.8	6.62	3754	1.23
13	40	4.5	1	5.54	5536	1.25

Validation of the Optimized Method

Specificity: The specificity was carried out by injection a blank solution(diluent) and no interefering peak at the Retention time of the analyte was seen.

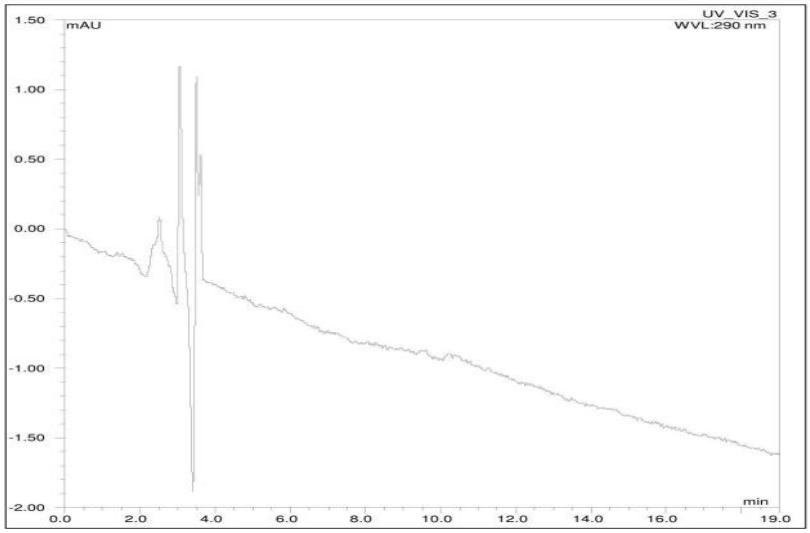

Fig. No. 14: Chromatogram of Blank.

System suitability: System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (2.0 µg/ml of Edoxaban tosylate monohydrate) was injected six times, and the chromatograms were recorded. The % RSD for area response obtained from six replicate injections of Standard solution should be NMT 2.0%, Asymetry factor should be between 0.8 to 2.0. Theoretical plates should be > 2000.

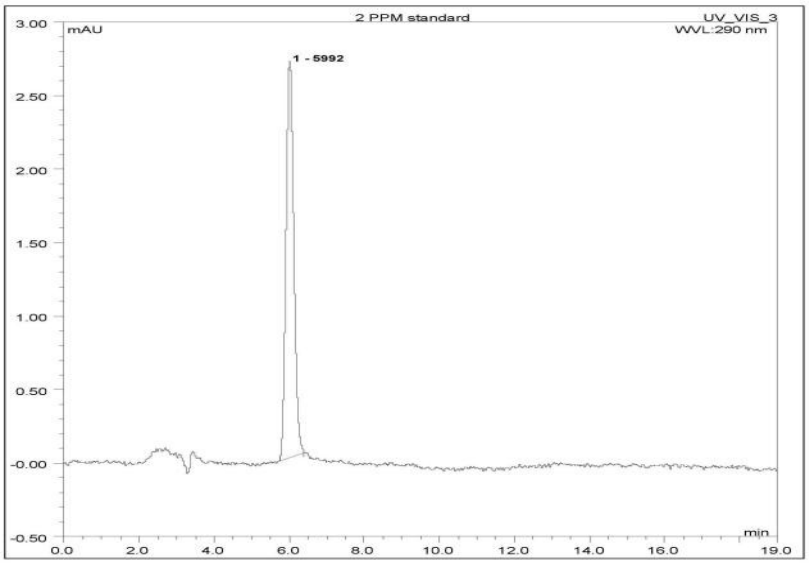

Fig No 15: Chromatogram of sytem suitability.

Table No. 4: System Suitability Data for Edoxaban Tosylate Monohydrate.

Sr.No	System suitability parameters	Observations	Acceptance criteria
1	Edoxaban tosylate monohydrate standard solution	2.0µg/ml	----
2	Area %RSD	0.75	NMT 2.0%
3	Retention time	5.98	----
4	NTP	4734	>2000
5	Asymetry factor	1.20	0.8-2.0

Linearity: The linearity was studied over the concentration range of 1.0 µg/ml to 3.0 µg/ml by injecting the standard solutions of the drug and injecting six times into the HPLC system by keeping the injection volume constant. The peaks were plotted against the concentrations to obtain the linearity graphs and the correlation coefficient was determined.

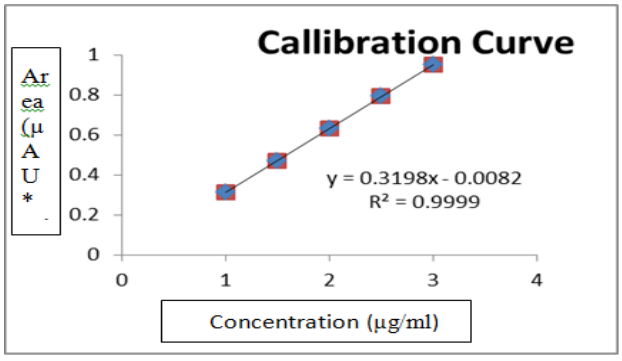

Fig. No. 16: Callibration Curve for Edoxaban Tosylate Monohydrate.

Precision: The precision studies were carried out for the developed analytical method by injecting three replicate injections of the concentration 1.0µg/ml, 2.0µg/ml, 3.0µg/ml(50%,

100%, 150% of the working level). Intra and Interday precision studies were carried out by estimating the corresponding responses for the solutions of above three concentrations levels on the same day and on a different day respectively.

Accuracy: % Recovery study was performed using a minimum of 3 concentration levels, each in triplicate determinations. It was carried out by spiking blank concentrations of 50%, 100% and 150% of working level(1.0 µg/ml, 2.0 µg/ml, 3.0 µg/ml) and obtaining the percent recovery by putting the values of the areas of the peak obtained in the calibration curve to obtain the values of the concentration injected.

Sensitivity: The Limit of Detection(LOD) and Limit of Quantification(LOQ) for the developed method were determined by progressively injecting low concentrations of the Standard solution of Edoxaban tosylate monohydrate using the developed HPLC method. This was carried out until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ respectively.

Solution stability

The solution stability studies was evaluated for three different concentrations (low, medium and high) i.e. 1.00 µg/ml, 2.00 µg/ml and 3.00 µg/ml and six replicate each which were stored for 3 days at refrigerated temperature of 10ºC-15 ºC. The sample analysis was performed at initial time zero and after those 6, 9, 24, 48 and 72 hours.

Table No. 5: Statistical data of Validation.

Sr.No	Validation Parameters	Edoxaban tosylate monohydrate
1	Sytem suitability	NTP-4734 %RSD-0.75% Asymetry factor-1.20
2	Specificity	No interference was found at the Rt of the analyte
3	Linearity	R^2=0.9999 (Linear) y = 0.3198x - 0.0082
4	Accuracy	99.91%
5	Interday precision(%RSD)	1.0%
6	Intraday precision(%RSD)	1.05%
7	LOD	0.2µg/ml
7	LOQ	0.5µg/ml
8	Solution Stability(%RSD)	0.47%

Force Degradation: To prove the stability indicating nature of the method, forced degradation studies were carried out by exposing the stock solution of the drug to the following conditions:

1. Acid hydrolysis.
2. Base hydrolysis.
3. Oxidation degradation.
4. Thermal degradation.
5. Photolytic degradation.

Acid Hydrolysis: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with three different concentrations of HCL i.e 0.1N,0.5N and 1.0N separately and then neutralized with the same Molar concentrations of Sodium Hydroxide solution i.e 0.1N,0.5N and 1.0N separately and the volumes were made up with the diluent(mobile phase) to give a solution of 200 µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

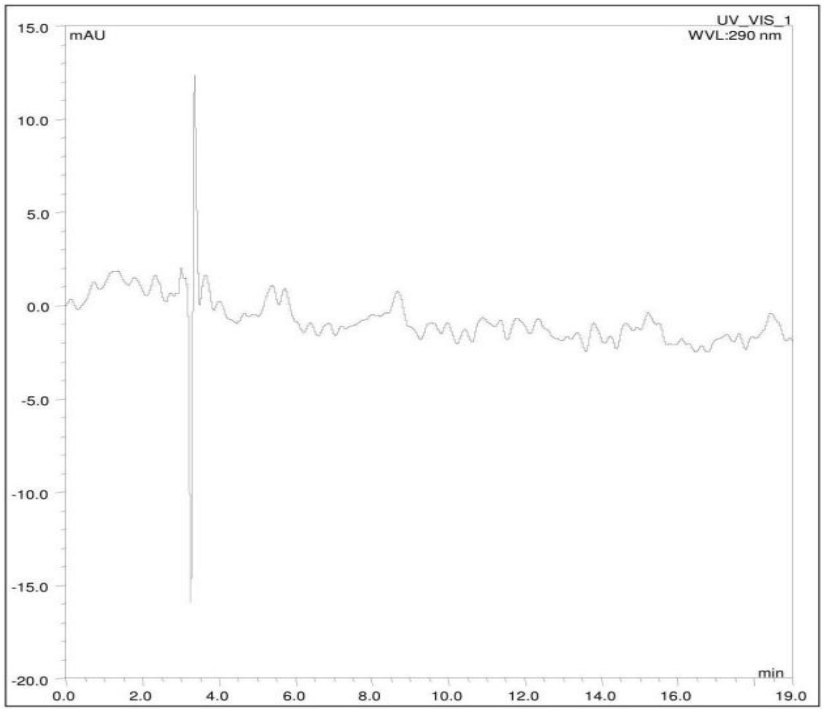

Fig. No 17: Chromatogram of Blank for Acid hydrolysis.

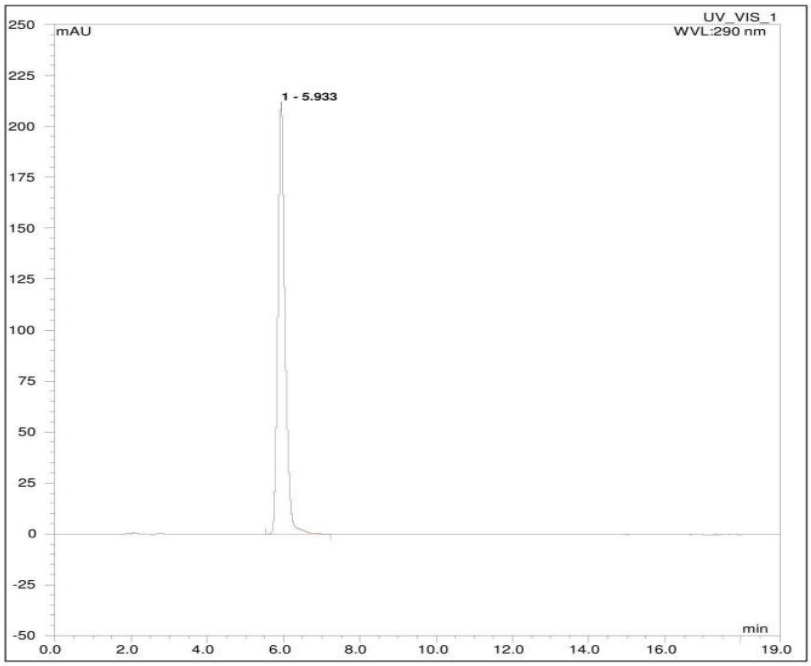

Fig No 18:- Chromatogram of Acid hydrolysis.

Base Hydrolysis: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with 2ml of 0.1N Sodium hydroxide for 10minutes. This solution was then neutralized with 2ml of 0.1N Hydrochloric acid and the volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

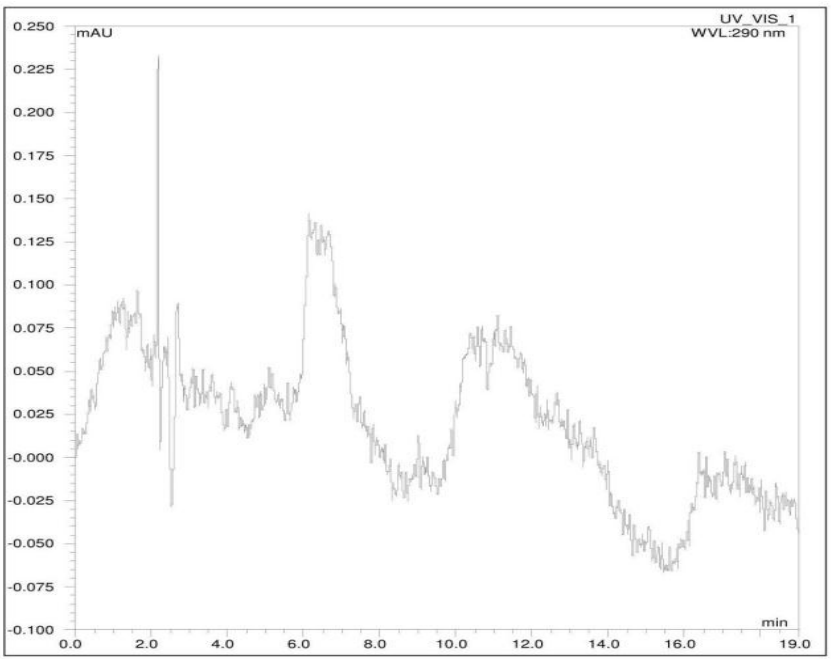

Fig. No 19: Chromatogram of Blank for Base hydrolysis.

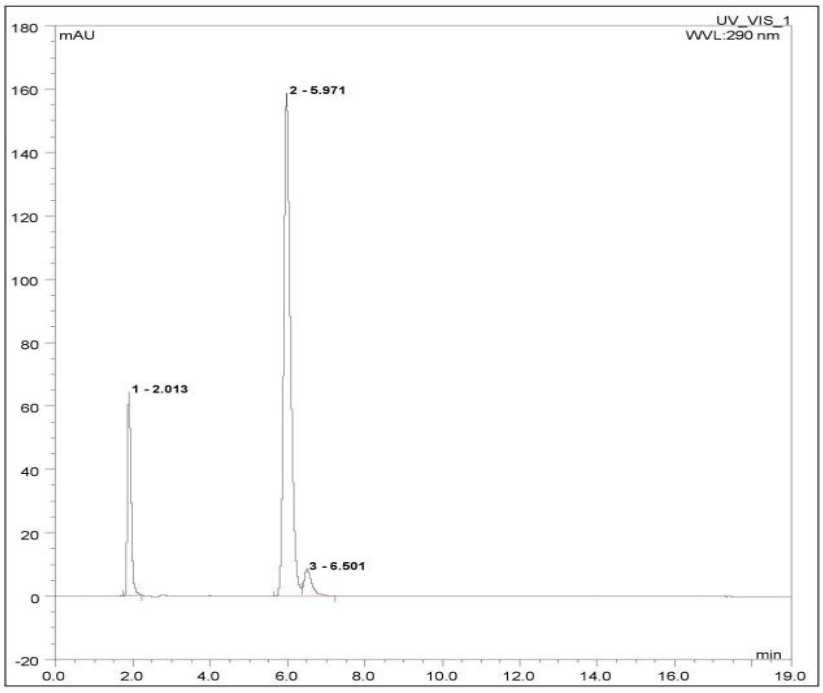

Fig No 20:- Chromatogram of Base Hydrolysis.

Oxidative degradation: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with 1ml of 3% H_2O_2 solution kept as such at room temperature for 2 hours. The volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

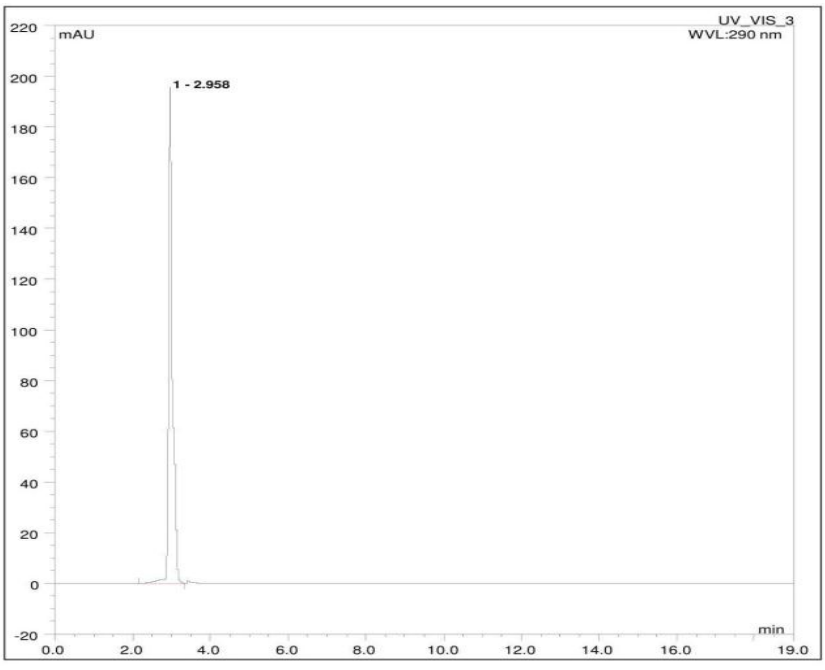

Fig No 21: Chromatogram of Blank for Oxidative degradation.

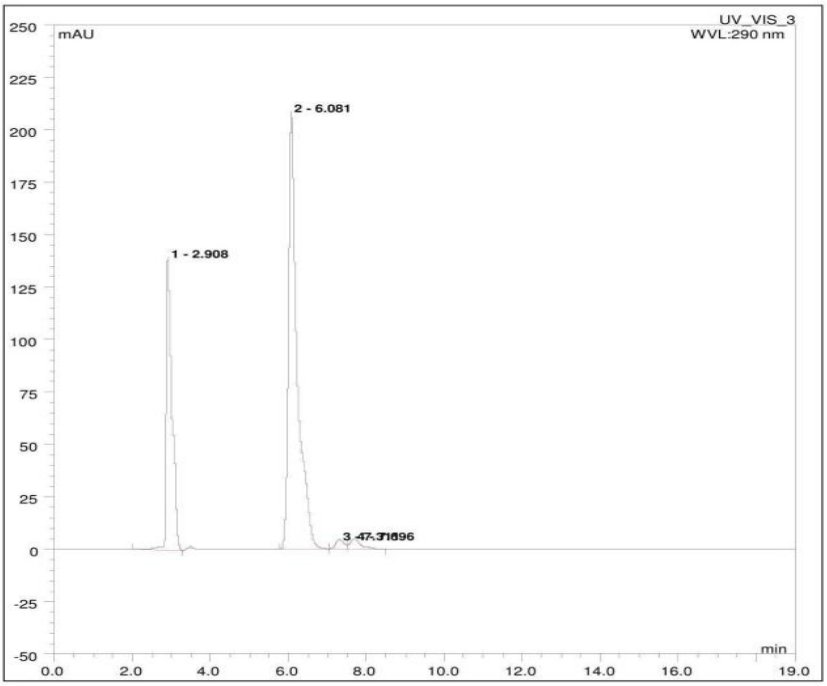

Fig No 22: Chromatogram of Oxidative Degradation.

Thermal Degradation: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was kept in water bath at 80 degree celicius for 2 hours. The volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

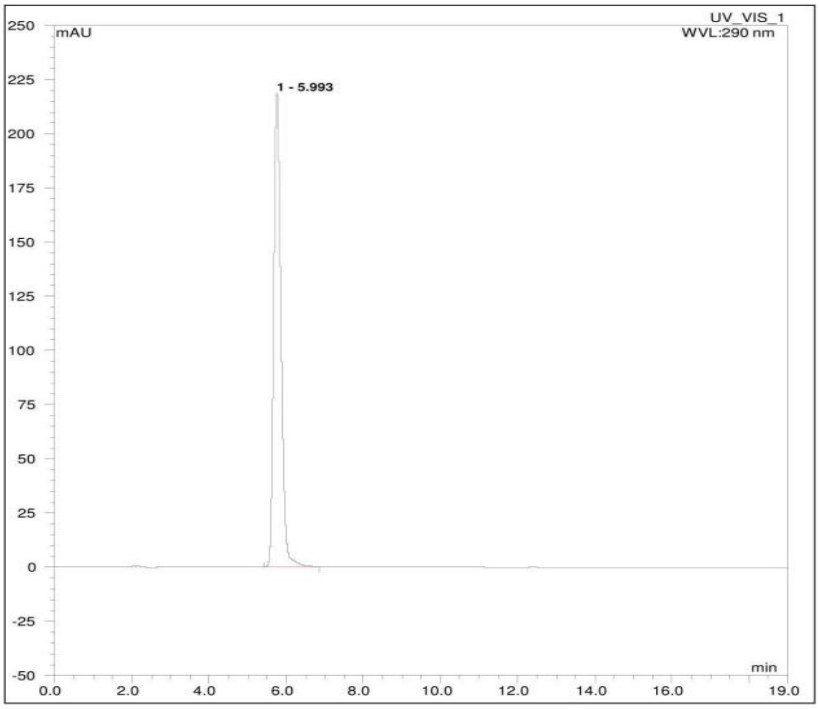

Fig No 23: Chromatogram of Thermal Hydrolysis.

Photolytic Degradation: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask The solution was kept in sunlight for 2 hours. The volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

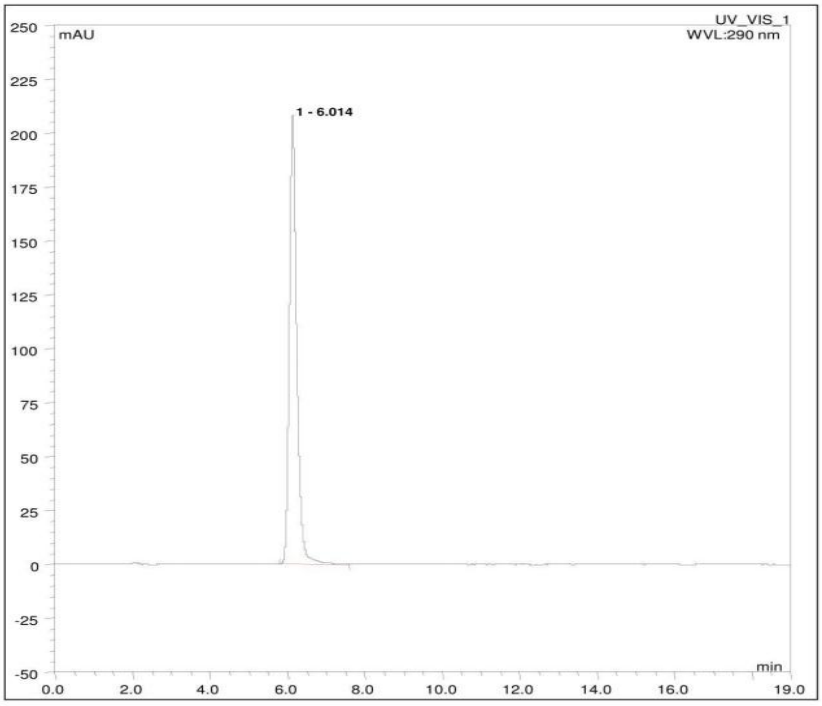

Fig No 24: Chromatogram of Photolytic Degradation.

Table No 6: Results for Forced Degradation Studies.

Sr.No	Degradation Condition	Retention time for degradation products(min)	Edoxaban tosylate monohydrate Percent degradation
1	Acid Hydrolysis	--	No degradation
2	Base Hydrolysis	2.01,6.50	7.58%+1.81%=9.39%
3	Oxidative Degradation	7.31,7.70	3.83%+3.87%=7.7%
4	Photolytic Degradation	--	No Degradation
5	Thermal Degradation	--	No Degradation
6	Total Degradation		17.09%

RESULT AND DISCUSSION

Selection of the column and mobile phase

The drug is basic in nature. It contains cyclohexane ring and heterocyclic rings containing nitrogen. So reverse phase chromatography was found to be best choice for its analysis. The effectiveness of C_8 and C_{18} Columns were evaluated and it was found that C_{18} Column showed better retention of the drug. The method development was initiated using methanol as

organic modifier but was found to produce baseline noise leading to interference of the analyte causing broad and peak asymetry. Hence the organic modifier was replaced with ACN which showed no baseline noise which intereferd with analyte peak. Ammonium acetate, formic acid were added to adjust the mobile phase pH to have a stable retention time but these buffers were not able to provide a stable retention time for the analyte peak hence sodium acetate was used which provided stable retention time and a good peak shape with minimum asymetry factor. As the drug is a base a basic pH woud led to its more retention on the column leading to a longer run time, hence the pH was adjusted to a acidic pH two units below the pka of the drug such that it would get eluted at a suitable retention time which provides a suitable run time for routine analysis of the drug.

Forced Degradation studies

The forced degradation studies indicate that the drug is susceptible to base hydrolysis and oxidative degradation. The degradation observed for base hydrolysis was found to be 9.39% and for oxidative degradation was found to be 7.7%. The representative chromatograms for forced degradation studies reveal that the method is a stability indicating method and can be used for the routine analysis of drug in bulk form.

CONCLUSION

The developed and validated RP-HPLC method was found to simple, precise, accurate and was found to meet the validation parameters as per current guidelines. The developed method using Qbd approach was found to be more reproducible, decreases the number of trials as well as failure. The developed method can be successfully applied for the routine analysis of drug in bulk form.

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