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Development and validation of UV spectrophotometric method for the determination of Edoxaban Tosylate Monohydrate in pharmaceutical dosage form

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ABSTRACT

A precise, simple, cost effective, accurate Ultra violet spectrophotometric method has been developed for the determination of Edoxaban Tosylate Monohydrate (EDTM) in the Pharmaceutical dosage form. EDTM shows highest λmax at 291.2 nm. The EDTM follows linearity in the concentration range of 2-10 µg /mL with superior correlation coefficient value of 0.999. The precision of the method was studied as an intra-day and inter-day studies. The % RSD value is < 2 which indicates that the method is precise. The % recovery was found to be in the range lies between 99.75 - 99.85 %. Percentage assay of EDTM (Lxiana) obtained was 98.5 ± 1.85 %. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed UV method is accurate, precise and reproducible. Hence this rapid method can be viable for the quality control analysis of EDTM in pharmaceutical dosage form.

1. INTRODUCTION

The chemical name for Edoxaban Tosylate Monohydrate (EDTM) is 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;4-methyl benzene sulfonic acid. EDTM is generally used in the treatment of systemic embolism. Rogonic et al., [1] Edoxaban, an oral direct factor Xa inhibitor, has been found non-inferior to warfarin for prevent stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF), with a lesser rate of intracranial bleeding and stroke in adult patients with one or more risk factors like hypertension, congestive heart failure, non valvular arterial fibrillation (NVAF), diabetes mellitus, transient ischaemic attack.

Hitherto EDTM is not official in any pharmacopoeia. The literature is very poor as for as EDTM is concerned. Bathala MS et al.,[2] determined the pharmacokinetics, mass balance and biotransformation a selective, direct factor Xa inhibitor in humans by HPLC/tandem mass spectrometry method utilizing multiple reaction modes or LC radiometric method. This study was utilized to estimate the mass balance and pharmacokinetics of EDTM in humans. Gous etal.,[3] developed and validated a turbulent flow LC with high resolution MS for the assay of EDTM in human plasma. This method was used for the therapeutic drug monitoring of EDTM. Pasam Satyanarayana Reddy et al.,[4] developed and estimated a stability indicating isocratic HPLC method for the estimation of edoxaban in bulk and commercial tablet dosage form. In fact literature survey revealed that there is no UV spectrophotometric analysis for the determination of EDTM in pharmaceutical dosage form. So we felt it is necessary to develop a simple, precise and rapid UV method for quantitative determination of EDTM in tablet dosage form. The aim and objective of the present study was to develop and validate a precise, sensitive and simple cost effective UV spectrophotometric method for EDTM in its tablet dosage form. Chemical structure of EDTM is shown in fig1.

2. MATERIALS AND METHODS

Instrument: A double beam ELICO SL 210 UV spectrophotometer containing of two matched quartz cells with one cm light path was taken for measuring of absorbance of EDTM. Essaevibra AJ (0.1 mg sensitivity) balance was used for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this present study.

Chemicals and reagents: EDTM was procured from Hetero Drugs Ltd., Hyderabad, Telangana, India. The EDTM tablets containing 30 mg labelled claim of

EDTM (Lxiana) tablets were used for this study. CH_3CN and CH_3OH was procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of solvent: Plentiful trials were executed to find out the suitable solvent system for dissolving the EDTM. The solvents such as acetonitrile, DMSO, methanol and triple distilled water were tried based on the solubility of the drug. EDTM is soluble in solvents such as CH_3CN, methanol and DMSO thus methanol was selected right through the experiment.

Selection of detection wavelength: To determine the optimum λmax of EDTM, 10 µg /ml of the EDTM solution was prepared in CH_3OH and scanned in the Ultra Violet wavelength range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 291.2 nm, which was chosen as the detection wavelength for the estimation of EDTM.

Standard preparation solution: A stock solution of EDTM at 1,000 µg/mL is prepared in CH_3OH by sonication. Dilution in CH_3OH are made up at 2 µg/ ml-10 µg/ ml concentrations.

Preparation of Calibration curve: From the above prepared EDTM stock solution, appropriate dilutions were prepared to get the ultimate concentration of 2, 4, 6, 8, and 10 µg/ ml and absorbance was taken at λ_{max} 291.2 nm. Average of such five sets of values was taken for standard calibration plot, and the calibration curve was plotted. Calibration curve was done by plotting EDTM concentration on X-axis and their respective absorbance's on Y-axis.

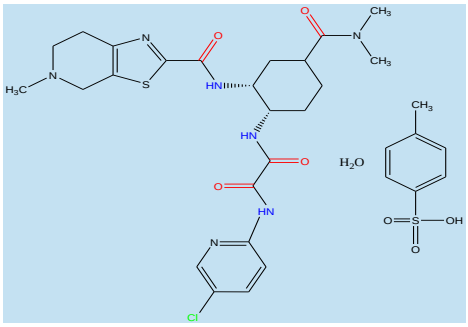

Figure.1.Chemical structure of EDTM

Table.1. Calibration data of EDTM

Concentration (µg/ml)	Absorbance
2	0.0727
4	0.1412
6	0.1974
8	0.2724
10	0.3399

Table.2. Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	291.2 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	24,2889.083
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.0303951
Regression equation (Y = mx+ c)	0.0336x + 0.0024
Slope (m)	0.0336
Intercept (c)	0.0024
Standard error of slope (S_m)	0.0005399
Standard error of intercept (S_c)	0.0032694
Standard error of estimate(S_e)	0.0045174
Correlation coefficient (r^2)	0.999

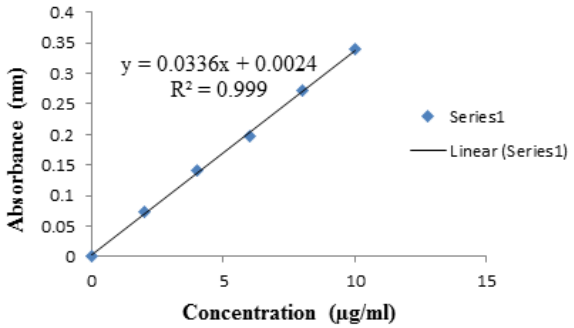

Figure.2. Calibration curve of EDTM

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.99948517							
5	R Square	0.998970605							
6	Adjusted R Squa	0.998713257							
7	Standard Error	0.004517411							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	0.079215472	0.079215472	3881.779389	3.97506E-07			
13	Residual	4	8.1628E-05	0.000020407					
14	Total	5	0.0792971						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	0.0024	0.003269462	0.734065755	0.50361643	-0.006677481	0.011477481	-0.00667748	0.011477481
18	X Variable 1	0.03364	0.000539934	62.30392756	3.97506E-07	0.032140903	0.035139097	0.0321409	0.035139097
19									

Table.3.Summary output of EDTM (ANOVA)

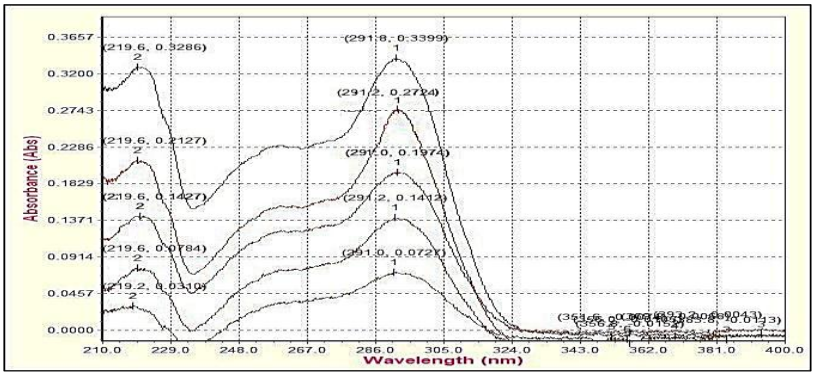

Figure.3.Ultra violet Overlain Spectra of EDTM

Method development and validation[5-7]: A lot of solvents were tested for solubility for EDTM solvents such as ethanol, DMSO, acetonitrile and distilled water at 10 µg/ml concentrations. Nevertheless EDTM was soluble and stable in methanol for minimum 24 hours at room temperature. Therefore methanol solvent was used for the detection of wavelength and preparation of standard and working concentration. In order to ensure the planned method to the pharmaceutical formulation, an assay of EDTM 30 mg tablets was utilized at working concentration. Assay for working concentration of the sample at 291.2 nm was analyzed. A UV spectrophotometric method is validated according to ICH Q2 (R1) guidelines for validation of analytical procedures. The method was validated for parameters such as precision, accuracy, specificity, linearity, ruggedness, robustness, LOD and LOQ.

Precision:

System precision: In system precision 10 µg/ml concentrations of six reproduce recordings of absorbance at 291.2 nm were experiential on the same day and corresponding responding responses were studied. The mean, SD and % RSD were calculated.

Method precision: Method precision was estimated by conducting the assay of sample under the test of repeatability (intraday precision) and intermediate precision performed during three successive days for three times. Eventually the mean, SD and % Relative standard deviation were determined.

Table.4. Results of system precision

S. No	Absorbance
1	0.197
2	0.196
3	0.195
4	0.197
5	0.196
Mean	0.1962
Standard deviation	0.000837
% Relative Standard deviation	0.426432

Table.5.Results of method precision (Intraday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD
4	0.141 0.140 0.142	0.141 ± 0.001	0.70922
6	0.197 0.196 0.195	0.196 ± 0.001	0.510204
8	0.274 0.273 0.272	0.273 ± 0.001	0.3663

Table.6.Results of method precision (Interday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD (n=3)
4	0.142 0.141 0.144	0.142 ± 0.001	0.704
6	0.197 0.195 0.198	0.1966 ± 0.001	0.776
8	0.275 0.274 0.276	0.275 ± 0.001	0.363

Accuracy (recovery studies): Recovery studies of EDTM were carried out by utilizing standard addition method in which estimation of % mean recovery of sample by % method at 3 different levels (80 %, 100 % and 120 % i.e., 4.8 µg/ml, 6 µg/ml, 7.2 µg/ml). These 80 to 120 levels of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (6 µg/ml). At each level, 3 analyses were performed. % mean recovery was calculated as shown in table 6. The accepted limits of recovery are 98 % - 102 %. Infact, from the amount of EDTM was found and % recovery was estimated.

Table.7. Accuracy of results

Level (%)	Absorbance	% Recovery	Mean % Recovery	% RSD
80	0.139	98.81	99.82	0.192
80	0.141	100.82		
80	0.146	100.34		
100	0.192	98.92	99.92	0.195
100	0.197	99.34		
100	0.199	101.39		
120	0.269	99.075	99.99	0.198
120	0.272	100.18		
120	0.276	101.65		

Ruggedness: Ruggedness is done by performing proposed method on different instruments. In addition to that this method is carried out by two different analysts and performing the method on different days to check the reproducibility.

Table.8. Results of ruggedness

Parameter	Absorbance for 10 µg/mL			
	Analyst-1	Analyst-2	Instrument-1	Instrument-2
SD	0.003	0.004	0.003	0.004
Mean	0.2055	0.206	0.2055	0.206
% RSD	0.198	0.082	0.198	0.082

Analysis of marketed formulation: The validated method was applied to the estimation of EDTM (Lxiana) tablets. 20 tablets were assayed and the results are represented in table 9 which indicates that the amount of drug in the tablet sample was in good agreement with label claim of the formulation as indicated by percentage recovery 99.83 %.

Table.9. Result of assay of pharmaceutical formulation (EDTM)

Sample no. (n=6)	Label claim EDTM (mg/tab)	Amount found EDTM (mg/tab)	Percent of label claim EDTM %
1	30	29.92	99.7
2	30	29.95	99.8
3	30	29.97	99.9
4	30	28.85	96.16
5	30	28.87	96.23
6	30	29.93	99.7
Mean ± SD		29.58 ± 0.55	98.5 ± 1.85

*mean of 6 determinations

3. RESULTS AND DISCUSSION

The ultraviolet spectra of EDTM were scanned in the region between 200-400 nm. The overlay spectra of EDTM at different concentrations were absorbed maximum at 291.2 nm, which was selected as the detection wavelength. The response of the EDTM was found to be linear in the concentration range of 2-10 µg/ml with a good correlation coefficient of $r^2 = 0.999$ and the fig 2 shows the EDTM linearity calibration curve and the table 1 shows the calibration data of EDTM. Table 2 lists the linear regression data of the proposed UV method. Fig. 3 displays the overlay spectra of EDTM. The summary output (ANOVA) results of EDTM are summarized in table 3. The system precision, intermediate precision results, i.e., inter-day and intra-day precision of EDTM are tabulated in tables 4 - 6 respectively. The % RSD less than 2 in all precision results cases which indicates that the method was precise. In this recovery study accuracy was carried out by using a standard addition method at three different concentration levels (80 %, 100 %, and 120 %). The mean percentage recovery at each level should be 99.82-99.99 %. All the results are well within the acceptance criteria and results indicate that the method is accurate. Results are excellent and displayed in table 7. Ruggedness was performed to check the reproducibility which showed the % RSD less than 2 which indicates that the method was rugged (table 8). The developed method was eventually applied for quantification of EDTM in tablets. The mean % assay values were found to be 98.5 ± 1.85. The amount of the drug in the tablet sample was in good agreement with label claim of the formulation. The assay results are shown in table 9.

4. CONCLUSION

The UV method was developed for the determination of EDTM. In this study, the precision and accuracy was < 2 % RSD. This method provides reproducible results with high precision, accuracy and was capable of analyzing EDTM in low concentrations. However, this UV method is simple, quick, sensitive. The results proved that this method is successfully ideal for routine quality control testing of EDTM samples.

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REFERENCES

1. Rognoni C, Marchetti M, Quaglini S, Liberato NL, Edoxaban versus warfarin for stroke prevention in non-valvular atrial fibrillation: a cost- effectiveness analysis, J Thromb Thrombolysis, 39, 2015, 149-154.

2. Bathala MS, Masumoto H, Oguma T, He L, Lowrie C, Mendell J, Pharmacokinetics, biotransformation, and mass balane of edoxaban, a selective, direct factor Xa inhibitor, in humans, Drug Metab Dispos, 40, 2012, 2250-2255.

3. Gous T, Couchman L, Patel JP, Paradzai C, Arya R, Flanagan RJ, Measurement of the direct oral anticoagulants apixaban, dabigatran, edoxaban, and rivaroxaban in human plasma using turbulent flow

liquid chromatography with high- resolution mass spectrometry, Ther Drug Monit, 36, 2014, 597-605.

4. Satyanarayana Reddy P, Shanmukha Kumar Jagarlapudi, Chandra Bala Sekharan, Determination of edoxaban in bulk and in tablet dosage form by stability indicating high performance liquid chromatography, Pharmaceutical sciences, 22,2016,35-41.

5. Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation. IOSR Journal of Pharmacy, 5,2015,7-19.

6. Ravisankar P, Gowthami S, Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and Biotechnology, 2, 2014, 1183-1195.

7. ICH Q2 (R1), Validation of analytical procedures, Text and methodology. International Conference on Harmonization, Geneva, 2005, 1-17.