WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 10, 1016-1025. Research Article ISSN 2277– 7105

QUANTITATIVE ESTIMATION OF EDOXABAN BY ZERO AND FIRST ORDER AREA UNDER CURVE SPECTROPHOTOMETRIC METHOD IN BULK AND *IN-HOUSE* TABLETS

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Article Received on
25 June 2019,

Revised on 15 July 2019,
Accepted on 04 August 2019,

DOI: 10.20959/wjpr201910-15643

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ABSTRACT

Aim: The aim of this work is to establish two simple, economical, and rapid spectrophotometric methods for the quantification of Edoxaban in bulk material and in tablets. Further, this study is designed to validate the developed methods as perICH guidelines. **Materials and Methods:** In Methods I and II, a stock standard solution was prepared by dissolving 10 mg of Edoxaban in 100 mL of 10% *v/v* Methanol to obtain a concentration of 100 µg/mL. After suitable dilution, 10 µg/mL of Edoxaban was prepared and scanned in the UV-visible range 400–200 nm; Edoxaban showed a maximum wavelength at 290 nm. In Method I, area under curve (AUC) of the zero-order spectrum was recorded between 283.00 and 300.00 nm. While, in Method II, zero-order spectra were derivatized into first-order, and the AUC was recorded between 299.00 and 314.00 nm. For a linearity study, series of dilutions were prepared from stock solutions. **Results:** In Method I, and II, Edoxaban followed linearity in the concentration range of 4-24 µg/mL with (r2>0.999). **Conclusion:** The developed methods are simple, precise, rugged, robust, and economical. Both these methods can be used for routine analysis of Edoxaban from its tablet formulation.

KEYWORDS: Edoxaban, UV Spectrophotometer, Derivative, AUC, Validation.

INTRODUCTION

Edoxaban (Molecular formula- $C_{24}H_{30}ClN_7O_4S$ M.W.- 548.06 g/mol.) Chemically isN'-(5-chloropyridin-2-yl)-N-[(1S,2R,4S)-4-(dimethylcarbamoyl)-2-{5-methyl-4H,5H,6H,7H[1,3] thiazolo[5,4-c]pyridine-2-amido}cyclohexyl]ethaniamide. Used as an anti-coagulants, anti-thrombin and factor Xa inhibitors.[3]

The literature review shows the various methods for the determination of Edoxaban by High Performance Liquid Chromatography(HPLC).[2] LC-MS method for estimation of Edoxaban in human plasma.[4]

The objective of this work is to establish zero and first derivative UV Spectroscopy and its AUC technique. The current works emphasize simple, precise, sensitive, and effective UV Spectroscopy method for estimation of Edoxban in bulk and *in-house* tablets. The method was validated as per ICH guidelines.

Figures

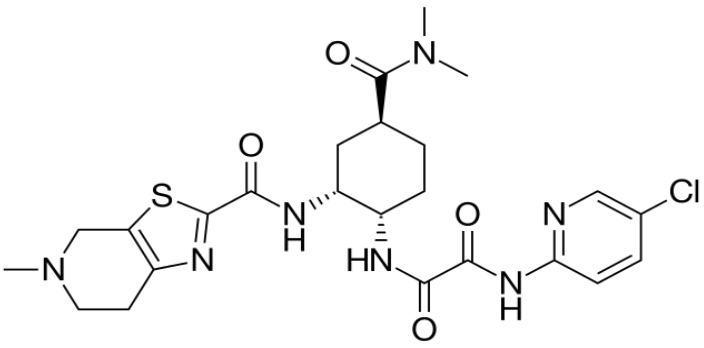

Figure 1: Chemical structure of Edoxaban.

MATERIALS AND METHODS

Materials

The drug was used without further purification. As the tablet formulation was not available in Indian market; tablet containing 15,30,60mg Edoxaban were prepared in-house using direct compression technique. Prepared tablets were used as pharmaceutical formulation for further analysis.

Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range:

200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: 1D (first order derivative, dA/dλ); band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of stock standard solution and selection of wavelengths

A stock standard solution was prepared by dissolving10 mg of Edoxaban in a 100 mL of 10% *v/v* water to obtain a concentration of 100 µg/mL. From it, anappropriate concentration of 10 µg/mL was preparedand scanned in the UV-visible range 400–200 nm; Edoxaban showed a maximum absorbance at290 nm. In Method I, area under curve (AUC) ofthe zero-order spectrum was recorded between the283.00 and 300.00 nm. While, in Method II, zero-orderspectra were derivatized into first-order and the AUC was recorded between 299.00 and 314.00 nm.

Validation of the method

Study of linearity curves

From the stock standard solution, an appropriateamount of aliquots portion in the range of 0.4–2.4 mLwere transferred into a series of 10 mL volumetric flasks and diluted up to mark using the same solvet to obtain a concentration in the range of 4-24 µg/mL. The solutions were scanned on a spectrophotometer inthe range of 400–200 nm. The calibration curves were plotted concentrations *versus* AUC between 283.00nm and 300.00 nm (Method I). While in Method II, anappropriate amount of aliquots portion in the range of 0.4-2.4 mL were transferred into a series of 10 mLvolumetric flasks and diluted up to the mark using the same solvent to obtain a concentration in the range of 4-24 µg/mL. The calibration curve was plotted as concentration *versus* AUC between 299-314nm (Method II).

Recovery studies

To the pre-analyzed sample solutions, a knownamount of stock standard solution was added at different levels, i.e. 80%, 100%, and 120%. Thesolutions were re-analyzed by the proposed methods.

Precision

The precision of the methods was studied as intra-dayand inter-day variations. In Method I, precision was determined by analyzing the 8, 16, and 24 µg/mL of Edoxaban solutions as intra-day and inter-day variations. In Method II, precision was determined by analyzing the 8,16 and 24 µg/mL of Edoxaban solutions as intra-day and inter-day variations.

Sensitivity

The sensitivity of measurements of Edoxaban by the use of the proposed methods was estimated in terms of the limit of quantification (LOQ) and the limit ofdetection (LOD). The LOQ and LOD were calculatedusing equation LOD=3.3 × *N*/*B* and LOQ=10 × *N*/*B*, where '*N*' is the standard deviation of the AUC of thedrugs (*n*=3), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 20µg/mL and 20 µg/mL concentration of Edoxaban solution for six times for Methods I and II, respectively.

Ruggedness

The ruggedness of the proposed methods was determined for 16µg/mL concentrations of Edoxaban by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions for Methods I and II, respectively.

Application of proposed method for pharmaceutical formulation

Twenty tablets were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder equivalent to one tablet was transferred into a 100 mL volumetric flask containing10 mL of Methanol, the volume was adjusted to the mark using the same solvent. An appropriate volume 1.2 mL was transferred into a 10 mL volumetric flask and the volume was adjusted to the mark to obtain the desired concentration of 12 µg/mL. The AUC was recorded at selected wavelengths for Method I. While in Method II, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges. The concentration of the drug was determined from the respective linear regression equations.

Determination of Edoxaban bulk

A quantity of powder equivalent to one tablet was transferred into a 100 mL volumetric flask containing10 mL of Methanol, the volume was adjusted to the mark using thesame solvent. An appropriate volume 1.2 mL was transferred into a 10 mL volumetric flask and the volume was adjusted to the mark to obtain the desired concentration of 12 µg/mL. The AUC was recorded at selected wavelengths for Method I. While in Method II, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges. The concentration of the drug was determined from the respective linear regression equations.

RESULTS AND DISCUSSION

Selection of wavelengths

Figures 2 and 3 show the selection of wavelengthsin Methods I and II, respectively. The selection of wavelengths in both the methods is based on the reproducibility of the results.

Linearity studies

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 4-24 µg/mL for Method I and 4-24 µg/mL for Method II (Figure 4 and 5). The results are expressed in Table 1.

Accuracy

The pre-analyzed sample used in Methods I and II. In Method I, themean % recovery was found to be in zero order 99.43%. While in Method II, it was found to be in first order 99.07%. The results are expressed in Table 2.

Precision

The precision of the developed method was expressedin terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 indicate that the methods were precise for the determination of drugs in formulation. The results are expressed in Table 3.

Sensitivity

The LOD and LOQ for Edoxaban were found to be 0.1330 and 0.4030 µg, respectively, for Method I. For Method II, they were found to be 0.2586 and 0.7835 µg, respectively. The results are expressed in Table 1.

Repeatability

Repeatability was determined by analyzing 20 µg/mL(Method I) and (Method II) concentrations of Edoxaban solution for six times and the % amount determined with % RSD<2 for both the methods. The results are expressed in Table 5.

Ruggedness

The peak area was measured for the same concentration solutions, six times for both methods. The results were in the acceptable range for both the drugs. The results showed that the % RSD was less than 2% [Table 4].

Determination of Edoxaban in bulk

The concentrations of the drug were calculated from linear regression equations. The % RSD was founds to be in method I-0.4023 and in method II-0.1713 (Table 6).

Application of proposed method for pharmaceutical formulation

The spectrum was recorded at 290 nm. The concentrations of the drug were calculated from linear regression equation. The % RSD was found to be in Method I- 0.9011% and Method II- 0.9300% (Table 7).

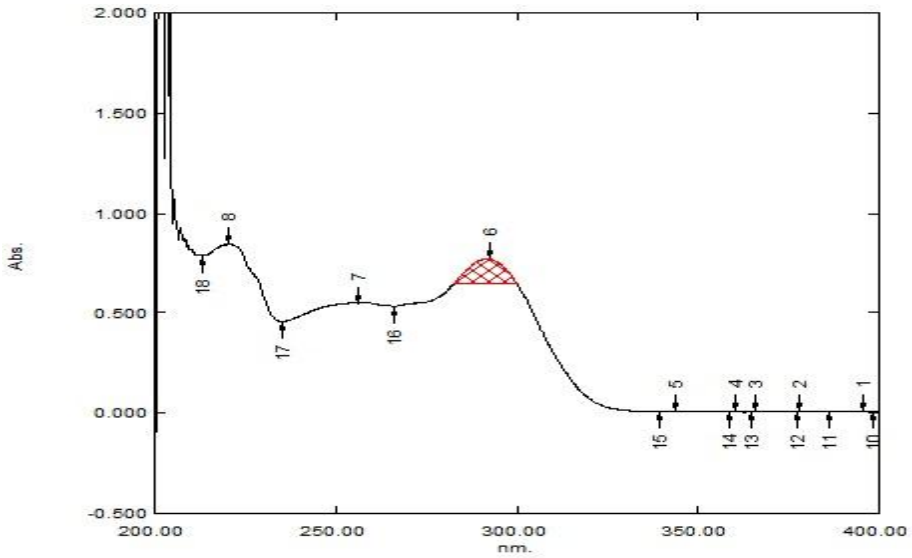

Figure 2: UV-spectrum Edoxaban in methanol.

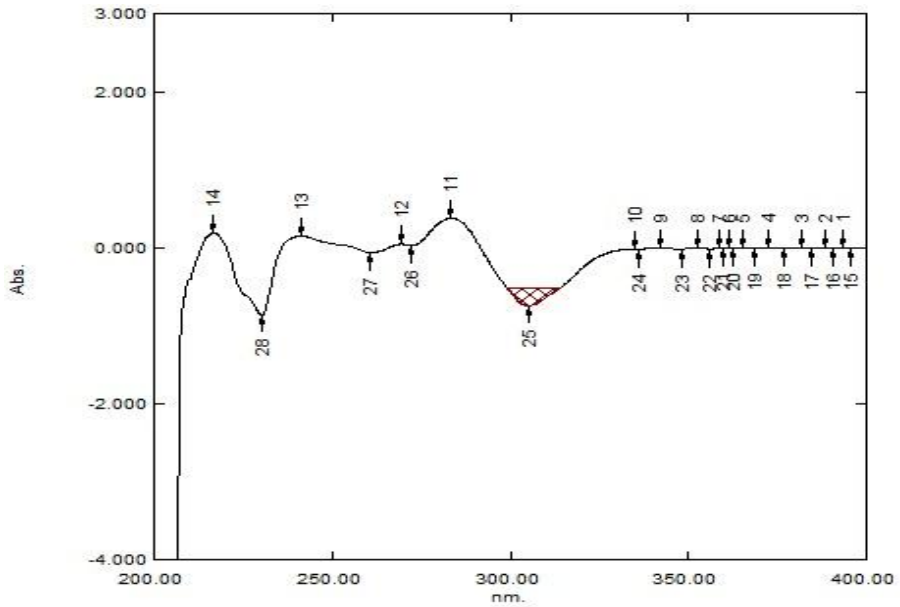

Figure 3: First-order derivative spectrum of Edoxaban in Methanol.

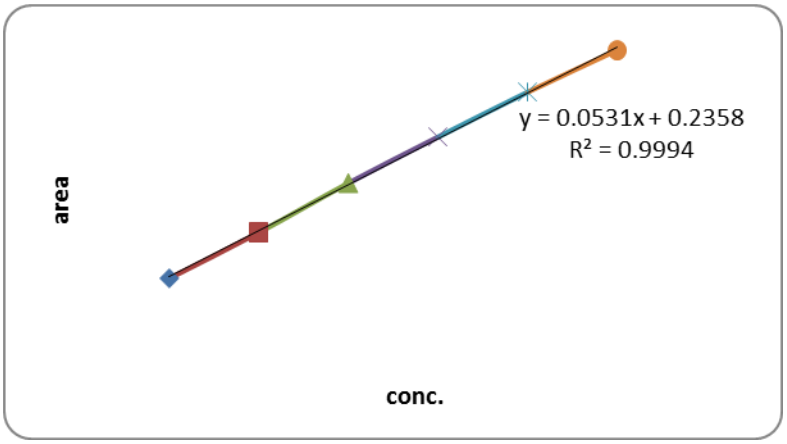

Figure 4: Linearity curve of Edoxaban by AUC.

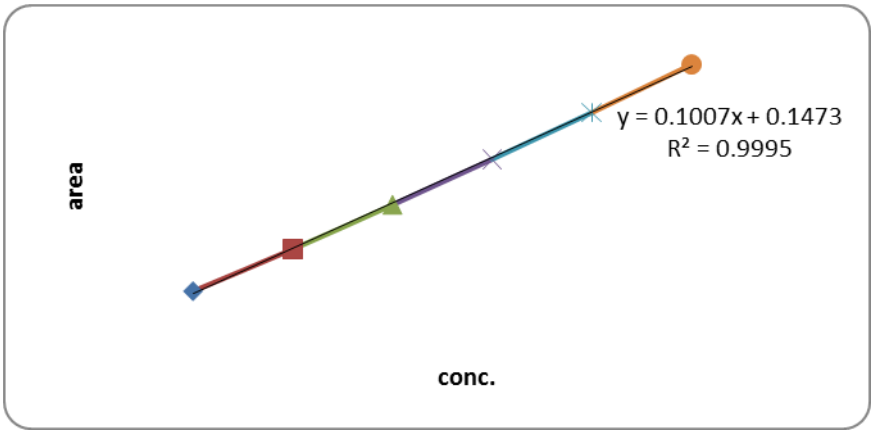

Figure 5: Linearity curve of Edoxaban by First order Derivatives.

Table 1: Optical characteristics and linearity data of Edoxaban.

Parameters	Method I	Method II
Linearity range (µg/mL)	4-24	4-24
Selected range (nm) for AUC	283-300	299-314
Slope	0.053	0.100
Intercept	0.235	0.147
Correlation coefficient	0.999	0.999
Limit of detection (µg)	0.1330	0.2586
Limit of quantitation (µg)	0.4030	0.7835

Table 2: Accuracy.

S	Initial amount (µg/mL)	Amount added (µg/mL)	Method I (n=3)		Method II (n=3)	
			% Recovery[a]	% RSD	% Recovery[a]	% RSD
80	20	17	97.2561	1.6400	98.1483	0.1238
100	20	20	100.1406	1.4355	99.2031	0.1361
120	20	23	100.8963	0.2443	99.8637	0.0762

Table 3: Precision.

Conc. (µg/mL)		Intra-day[a]		Inter-day[a]	
Method I	Method II	% RSD		% RSD	
		Method I	Method II	Method I	Method II
8	8	0.5956	0.1635	1.5083	0.2076
16	16	0.3031	0.2546	0.6145	0.2465
24	24	1.3916	0.1453	0.9342	0.1798

[a]Average of three estimates

Table 4: Ruggedness.

Method	Amount taken (µg/mL) (n=3)	Amount found (%)[a]	
		Analyst I	Analyst II
Method 1	16	99.0902	99.2331
Method 2	16	99.5540	99.5934

[a]Average of Six estimations

Table 5: Repeatability.

Method	Amount taken (µg/mL) (n=6)	Amount found[a] (%)	% RSD
Method 1	20	99.1471	0.5938
Method 2	20	100.1953	0.9783

[a]Average of six estimations.

Table 6: Analysis of Bulk.

Method	Concentration (µg/mL) (n=6)	Amount found (µg/mL)(n=6)	Amount found[a] (%)	% RSD
Method 1	12	49.5590	99.8130	0.4023
Method 2	12	49.4787	99.5955	0.1713

[a]Average of six estimations.

Table 7: Analysis of Formulation Brand name- Lixiana 30mg.

Method	Concentration (µg/mL) (n=6)	Amount found (µg/mL)(n=6)	Amount found[a] (%)	% RSD
Method 1	12	49.8780	99.8342	0.9011
Method 2	12	49.6706	98.5672	0.930

[a]Average of six estimations

Table 8: Summery of validation parameter.

Parameters	Method 1	Method 2
Linearity Range (µg/mL)	4-24	4-24
Correlation coefficient	0.999	0.999
Limit of Detection LOD (µg/mL)	0.1330	0.2586
Limit of Quantification LOQ (µg/mL)	0.4030	0.7835
% Recovery	99.43	99.07
Precision (%RSD) Intra-day Inter-day	 0.7634 1.0190	 0.1878 0.2113
Ruggedness (% Amnt Found) Analyst- I Analyst- II	 99.0902 99.2331	 99.5540 99.5934
Repeatability (%RSD)	0.5938	0.9783
Analysis of Bulk (%RSD)	0.4023	0.1713
Analysis of Formulation (%RSD)	0.9011	0.9300

CONCLUSION

Both the developed methods are economical, simple, accurate, precise and rugged, and can be used for the usual study of Edoxaban from its pharmaceutical formulations. The methods are developed for quantification of Edoxaban tablets. It is also used in routine quality control of the formulations containing Edoxaban.

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