Development and Validation of Spectrophotometric Method for the Estimation of Edoxaban Tosylate Monohydrate in its Synthetic Mixture

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ABSTRACT

Edoxaban Tosylate Monohydrate (EXN) is an oral anticoagulant drug indicated to reduce the risk of stroke and systemic embolism (SE) in patients with nonvalvular atrial fibrillation (NVAF) and for the treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE). Sensitive and reproducible UV-Visible spectrophotometric method has been developed and validated for the estimation of Edoxaban Tosylate Monohydrate in its synthetic mixture. Methanol was used as a solvent. Developed method has been validated for linearity range, precision, accuracy, limit of detection, and limit of quantification as per ICH Q2(R1) guidelines. The method was found to be linear in the range of 5-25 µg/mL at λmax 289 nm and the regression coefficient value was found to be 0.9999. For Edoxaban LOD and LOQ values were found to be 0.654 µg/mL and 1.982 µg/mL. The method was successfully applied for estimation of Edoxaban Tosylate Monohydrate in its synthetic mixture and results were found to be in good agreement with the amount of Edoxaban Tosylate Monohydrate present in synthetic mixture.

Keywords: Edoxaban Tosylate Monohydrate, UV-visible Spectrophotometric method, Validation

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INTRODUCTION

Edoxaban Tosylate Monohydrate chemically \( N'-(5\text{-chloropyridin-2-yl})-N-(1S,2R,4S)-4\text{-}(\text{dimethylcarbamoyl})-2\text{-}[(5\text{-methyl-6,7\text{-dihydro-}4\text{-H}[1,3]thiazolo[5,4-c]pyridine-2}\text{carbonyl})amino]cyclohexyl\text{]oxamide;4\text{-methylbenzenesulfonic acid; monohydrate. \) Edoxaban is a member of the novel oral anti-coagulants (NOACs) class of drugs, and is a rapidly acting, oral, selective factor Xa inhibitor. It does not require antithrombin III for antithrombotic activity. Edoxaban inhibits free FXa, and prothrombinase activity and inhibits thrombin-induced platelet aggregation. Inhibition of FXa in the coagulation cascade reduces thrombin generation and reduces thrombus formation. It is soluble in methanol; slightly soluble in water, ethanol and acetonitrile and molecular structure of Edoxaban Tosylate Monohydrate has been presented in Figure 1.

![Molecular structure of Edoxaban Tosylate Monohydrate](image)

Edoxaban is not official in any pharmacopeias. Literature survey revealed that various liquid chromatographic methods have been reported for the estimation of Edoxaban. Bathala et al., analyzed Edoxaban and its four metabolites in human plasma, urine and fecal samples, after oral administration of \([^{14}\text{C}]\) EXN to 6 healthy male subjects, by either high-performance liquid chromatography/tandem mass spectrometry or a liquid chromatography radiometric method. This study was used to determine the mass balance and pharmacokinetics of EXN in humans. Gous et al., developed and validated a turbulent flow liquid chromatography with high-resolution mass spectrometry for assay of EXN in human plasma. This method was applied for the therapeutic drug monitoring of EXN. Zhang et al., developed and validated UPLC/MS-MS method for determination of EXN in rat plasma. This method was successfully applied to a pharmacokinetic study of EXN in rat plasma. Reddy et al., developed and validated stability indicating high performance liquid chromatography for determination of EXN in its tablet dosage form. Le-Roy et al., developed and validated a common dosage method in plasma to four direct anticoagulant using UHPLC-DAD. In this a single assay method for four molecules (apixaban, rivaroxaban, edoxaban and dabigatran) has been developed for their anticoagulant
activity. Wiesen et al., evaluated two independent extraction methods and developed a multi-analyte ultrahigh performance liquid chromatography tandem mass (UHPLC-MS/MS) method for the quantification of apixaban, dabigatran, edoxaban and rivaroxaban in human plasma.

However, to the best of our knowledge, there are no reports published on the quantitative analysis of Edoxaban by UV-Visible spectrophotometric method. The developed method has been validated in agreement with International Council for Harmonization guidelines by assessing its linearity, precision, robustness, limit of detection, limit of quantitation, and accuracy.

MATERIALS AND METHOD

Instrumentation

A double beam UV-Visible spectrophotometer (Lab India, UV 3092) was used for all absorbance measurements with matched quartz cells. Electronic balance (AUX-220, Shimadzu), Ultrasonicator (Janki Impex) and Centrifuge (REMI, R-8C Centrifuge).

Materials

All chemicals and reagents were of analytical or HPLC grade. Analytical grade methanol was purchased from Loba chemie. Edoxaban in the form of Edoxaban tosylate monohydrate was provided as gift sample by Cadila Pharmaceuticals Ltd., Ahmedabad.

Preparation of working standard solutions

Accurately weighed 10 mg of standard EXN was transferred to 10 mL volumetric flask, dissolved in 5 mL methanol and diluted up to the mark with same to get stock solution having strength of 1000 μg/mL. Aliquot of 1 mL from the standard stock solution, transferred into 10 mL volumetric flask and the volume was made up to the mark with methanol to get a strength of 100 μg/mL. Aliquot of 1 mL of resulting solution, further diluted to 10 mL with methanol to get a solution having strength of 10 μg/mL.

Preparation of Synthetic mixture

Synthetic mixture for EXN was prepared by physically mixing commonly used excipients for tablet formulation with drug. All ingredients were accurately weighed and physically mixed in poly bag for 10 min to make synthetic mixture and placebo. List of ingredients for preparation of synthetic mixture and placebo has been shown in table 1.

Table 1: List of ingredients for preparation of synthetic mixture and placebo

| Ingredient                  | Quantity to be taken (mg) |
|-----------------------------|---------------------------|
| Edoxaban Tosylate Monohydrate | Synthetic Mixture | Placebo |
| Starch                      | 20.0 | 00.0 |
| Sodium starch glycolate     | 10.0 | 10.0 |
| Hydroxypropyl cellulose     | 10.0 | 10.0 |
| Titanium dioxide            | 2.0  | 2.0  |
Selection of wavelength of detection
The working standard solution of EXN was scanned in the range of 200-400 nm keeping methanol as blank and wavelength of maximum absorption was determined from the spectrum.

Preparation of Calibration Curve
From the working standard solution (10μg/mL) 0.5mL, 1.0mL, 1.5mL, 2.0mL and 2.5 mL were pipetted into 10 mL volumetric flasks and volume was made up to the mark with methanol to produce the concentrations ranging from 5-25 μg/mL respectively. The absorbances were measured at 289 nm and calibration curve was plotted of absorbance against concentrations.

Solution Stability Study
The freshly prepared working standard solution of EXN (10ug/mL) was stored at room temperature for 24 hour. The solution was analyzed immediately after preparation (0 hr) and after (24 hr) using optimized chromatographic conditions. Absorbance of EXN obtained at 0 hour and 24 hour were compared to check the stability of solution.

Validation of Developed Method
The developed method was validated as per the International Council for Harmonization,Q2R1 guidelines guidelines with respect to linearity, precision, robustness, specificity, limit of detection, limit of quantification, and recovery.

Linearity and Range
From the working standard solution containing 10μg/mL of EXN, dilutions were made to prepare range of standard solutions having different concentrations of EXN (5, 10, 15, 20 and 25 μg/mL). The absorbances were measured at 289 nm. The five representative calibration curves for EXN in range of 5-25 μg/mL were plotted and from average data of five calibration curves correlation coefficient of regression and regression line equations were computed.

Precision
Repeatability of method was checked by measuring absorbance of working standard solution in seven replicates. Precision was carried out by performing interday variation and intraday variation. In interday variation the sample was checked by repeating procedure of calibration curve on five consecutive days. In intraday variation the absorbances were measured by repeating procedure of calibration curve five times in a day. The % RSD of the results was used to evaluate the method precision.
Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was carried out by performing wavelength variation of ±2 nm.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The solutions of placebo and synthetic mixture (15μg/mL) were scanned in the range 200-400 nm and compared to assess the interference between placebo and synthetic mixture.

Accuracy
The accuracy of the developed method was determined by recovery on standard addition method. Recovery study was carried out by spiking three different known amounts (80%, 100% and 120%) of the standard drug to the placebo mixture. Solutions were prepared in methanol. Absorbance of each solution was measured. Percent recovery was determined for 3 times and % mean recovery was calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)
LOD and LOQ were calculated from the data obtained from the linearity studies. The slope and intercept of the linearity plot were determined. LOD and LOQ were calculated using the following formula:

\[
\text{LOD} = 3.3 \times \frac{\text{Std. deviation of y intercept}}{\text{Mean of slope}}
\]

\[
\text{LOQ} = 10 \times \frac{\text{Std. deviation of y intercept}}{\text{Mean of slope}}
\]

Analysis in Synthetic mixture
From the Synthetic mixture equivalent to 10 mg of EXN was accurately weighed and transferred into a 10 mL volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredient was extracted into methanol by vortex mixing followed by ultrasonication. The above solution centrifuged for 30 minutes at 2000 rpm. Aliquot of 1 mL from the supernatant layer was transferred into 10 mL volumetric flask and diluted up to mark. From above solution, aliquot of 1.5 mL was transferred into 10 mL volumetric flask and diluted up to the mark. Absorbance of the solution was measured at 289 nm. Concentration of EXN was determined from calibration curve equation and % labelled claim of EXN was computed.

RESULTS AND DISCUSSION
Selection of wavelength
The working standard solution of EXN was scanned in the range of 200-400 nm keeping methanol as blank and wavelength of maximum absorption was determined from the spectrum
and the $\lambda_{\text{max}}$ was found to be 289 nm. Spectrum of selection of wavelength is depicted in Figure 2.

![Figure 2 UV Spectrum of EXN of 10 μg/mL ($\lambda_{\text{max}}$=289)](image)

**Preparation of calibration curve**

Calibration curve was prepared in range of 5-25 μg/mL for EXN. Absorbance of EXN increases linearly with concentration. Calibration curve of EXN is shown in figure 3. Data of calibration curve is shown in Table 2.

**Table 2: Data for calibration curve**

| Concentration (5-25 μg/mL) | Absorbance |
|----------------------------|------------|
| 5                          | 0.162      |
| 10                         | 0.323      |
| 15                         | 0.487      |
| 20                         | 0.642      |
| 25                         | 0.795      |

![Figure 3: Calibration curve of EXN (5-25 μg/mL) in methanol](image)

$$y = 0.0317x + 0.0063$$

$R^2 = 0.9998$
Figure 4: Linearity Curve for EXN (5-25µg/mL) in methanol

Table 3 Data for Linearity curve

| Sr.no | Concentration (µg/mL) | Absorbance (mean ± SD) (n=5) | % RSD |
|-------|-----------------------|-------------------------------|-------|
| 1     | 5                     | 0.162 ± 0.0023                | 1.42  |
| 2     | 10                    | 0.321 ± 0.0023                | 0.73  |
| 3     | 15                    | 0.478 ± 0.0063                | 1.32  |
| 4     | 20                    | 0.631 ± 0.0087                | 1.38  |
| 5     | 25                    | 0.787 ± 0.0112                | 1.43  |

Method validation

Linearity: Representative calibration curve of EXN was obtained by plotting the mean absorbance of EXN against concentration over the range 5-25 µg/mL (n=5) Figure 3. It was found to be linear in the above mentioned range with regression coefficient of 0.9999. The % RSD for each level of EXN was found to be in range of 0.73-1.43%. The average linear regressed equation for calibration curve was y =0.0312x+0.0075. Linearity data is depicted in Table 3 and summary data depicted in Table 4. Calibration curve of EXN is shown in Figure 4.

Table 4: Summary of linearity data for estimation of EXN

| Sr. No. | Parameter                     | Result                      |
|---------|-------------------------------|-----------------------------|
| 1       | Linearity(µg/mL)              | 5-25 ug/mL                  |
| 2       | Regression line equation      | Y=0.0312x+0.0075            |
| 3       | Slope                         | 0.0312 ±0.0014              |
| 4       | Intercept                     | 0.0075±0.0061               |
| 5       | Correlation Coefficient       | 0.9999                      |

Precision:

Repeatability Repeatability of the instrument was checked by measurement of absorbance (working standard solution) seven times. The % RSD for absorbance was found to be 0.100.% RSD is less than 2% hence method is repeatable. The data for repeatability of measurement is depicted in Table 5.
Table 5: Repeatability for EXN

| Drug                                      | Concentration (µg/mL) | Absorbance (Mean ± SD; n=7) | % RSD     |
|------------------------------------------|-----------------------|-----------------------------|-----------|
| Edoxaban Tosylate Monohydrate            | 15                    | 0.4872±0.0004               | 0.100136  |

Table 6: Data for Intermediate precision for estimation of EXN

| Concentration (µg/mL) | Intra-day precision Absorbance Mean ± SD (n=5) | % RSD | Inter-day precision Absorbance Mean ± SD (n=5) | % RSD |
|-----------------------|-----------------------------------------------|-------|-----------------------------------------------|-------|
| 5                     | 0.158±0.0028                                   | 1.818 | 0.158±0.00230                                  | 1.453 |
| 10                    | 0.320±0.0031                                   | 0.970 | 0.325±0.00516                                  | 1.588 |
| 15                    | 0.476±0.0073                                   | 1.553 | 0.487±0.00659                                  | 1.354 |
| 20                    | 0.627±0.0104                                   | 1.658 | 0.639±0.0101                                  | 1.592 |
| 25                    | 0.782±0.0098                                   | 1.283 | 0.783±0.0112                                   | 1.430 |

Intermediate precision

The % RSD for intra-day and inter-day precision of EXN was found to be in range of 0.970-1.818% and 1.354-1.592%, respectively. The data for intra-day and inter-day precision for EXN is depicted in Table 6. % RSD is less than 2% hence method is precise for measurement of EXN.

Robustness:

The % RSD for robustness of EXN was found to be in range of 0.671-1.498% which is less than 2% hence method is robust for measurement of EXN. The data for robustness for EXN is depicted in Table 7.

Table 7: Robustness for EXN

| Concentration (µg/mL) | Absorbance(Mean ± SD) | % RSD |
|-----------------------|------------------------|-------|
| 5                     | 0.149±0.0010           | 0.671 |
| 10                    | 0.315±0.0047           | 1.498 |
| 15                    | 0.464±0.0045           | 0.970 |
| 20                    | 0.612±0.0060           | 0.993 |
| 25                    | 0.778±0.0055           | 0.707 |

Specificity

An overlain spectrum of placebo and synthetic mixture of EXN is depicted in Figure 4. Placebo showed no absorbance in range of 200-400nm, this indicates excipient did not interfere for estimation of EXN. Overlain spectra of synthetic mixture and placebo mixture (15 µg/mL) is shown in Figure:5.
Limit of Detection:
The limit of detection was found to be 0.654 μg/mL. Limit of quantification was found to be 1.982 μg/mL.

Accuracy:
The proposed method was applied for estimation of EXN in its placebo mixture after spiking with known quantity of standard drug. The percentage recovery was found to be 98.85-102.87. All results are in the range of 98 -102 % hence method is accurate in measurement of EXN. Data of recovery studies is depicted in table 8.

| Concentration taken (μg/mL) | Spiked level (%) | Standard added (mg) | Amount found (mg) | % Recovery |
|----------------------------|------------------|---------------------|-------------------|------------|
| 15                         | 80               | 8                   | 8.2               | 102.87     |
| 15                         | 100              | 10                  | 10.1              | 101.10     |
| 15                         | 120              | 12                  | 11.86             | 98.85      |

Assay in Synthetic mixture:
Applicability of the proposed method was tested by analyzing synthetic mixture. The percentage of EXN in synthetic mixture was calculated from the calibration curve of EXN. The assay value for synthetic mixture of EXN was 100.24%. Assay result of synthetic mixture of EXN is depicted in Table 9.

| Concentration taken (μg/mL) | Label claim (mg) | Amount found (mg) | % of Label claim |
|-----------------------------|------------------|------------------|-----------------|
| 15                          | 20.20            | 20.25            | 100.24          |

The summary of validation results is depicted in Table 10.
Table 10: Summary of validation parameters

| Sr. No. | Parameters                  | Results          |
|---------|-----------------------------|------------------|
| 1       | Linearity and Range         | 5-25 µg/mL       |
|         | Regression coefficient      | 0.9999           |
| 2       | Precision (%RSD)            |                  |
|         | Repeatability               | 0.100            |
|         | Intraday Precision (n=5)    | 0.970-1.818      |
|         | Interday Precision (n=5)    | 1.354-1.592      |
| 3       | Accuracy (%Recovery)        | 98.85-102.87%    |
| 4       | Limit of Detection          | 0.654 µg/mL      |
| 5       | Limit of Quantitation       | 1.982 µg/mL      |
| 6       | Robustness (%RSD)           | 0.671-1.498      |

CONCLUSION

The UV-Visible spectrophotometric method has been developed for quantification of Edoxaban Tosylate Monohydrate in synthetic mixture. The developed method was validated as per ICH Q2 (R1) and found specific, accurate, sensitive, and precise for estimation of EXN. Developed method was applied for analysis of synthetic mixture and results were in agreement with amount of EXN spiked in placebo. Hence the proposed method can be used for the estimation of EXN and its pharmaceutical dosage form.

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