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# Development And Validation of Reverse Phase High Performance Liquid Chromatography Method for Quantitative Estimation of Edoxaban Using PDA Detector

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**Keywords:**

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## Abstract

**Introduction:** Edoxaban is an orally active, highly selective, direct reversible inhibitor of the serine protease factor Xa, and by increasing clotting reduces the risk of thrombus formation. The aim of the present study was to develop a simple, sensitive and accurate liquid chromatographic method for the quantification of Edoxaban.

**Material and Methods:** Quantification of analyte was achieved by utilizing Shimadzu shim-pack $C_{18}$(250 mm × 4.6 mm, 5µm) column on Shimadzu (UFLC) liquid chromatography system equipped with LC-20 AD solvent delivery system, SPD-20A photo diode array detector and 20 µl loop volume in a Rheodyne injector. The mobile phase composition was acetonitrile: water in the ratio of (50:50 % v/v) with a flow rate of 1ml/min. Detection was achieved using photo diode array set at 291nm.

**Results:** Retention time of Edoxaban was found 5.514 min with a total run time of 8 min. The calibration curve was found to be linear over the concentration range of 8-80µg/ml. The LOD and LOQ were found to be 0.283 µg/ml and 0.942 µg/ml, respectively.

**Conclusion:** The developed method was validated and the results of validation were within the prescribed limits as per International Conference on Harmonisation (ICH) guidelines.

**Introduction:** Many novel anticoagulants have been discovered and introduced into clinical practice during the past 15 years, such as DOAC (Direct oral anticoagulants also called non-vitamin-K dependent oral anticoagulants which includes direct oral factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) and the direct oral thrombin inhibitor (dabigatran etexilate).[1]

Venous thromboembolism (VTE) is the most common lifetime risk for both men and women older than 40 years of age. Prevention and treatment options include both oral and parenteral drugs. Edoxaban is one of the competent agent in belong to the oral antithrombotic category for the treatment of VTE.[2]

Edoxaban is a quickly acting, oral, selective factor Xa inhibitor and a part of the pharmacological family known as Novel Oral Anti-Coagulants (NOACs). It is monocarboxalic acid amide used for the treatment of deep vein thrombosis and pulmonary embolism.[3] Antithrombin property of edoxaban is described by inhibiting factor Xa which is an important protein in blood coagulation cascade.

Chemically name of Edoxaban 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 (Fig no. 1). The molecular formula is C24H30ClN7O4S. It has a molecular weight 548.056 g/mol. It is white to pale yellowish-white crystalline powder, slightly soluble in water and Acetonitrile and Soluble in methanol and freely soluble in DMSO.[4]

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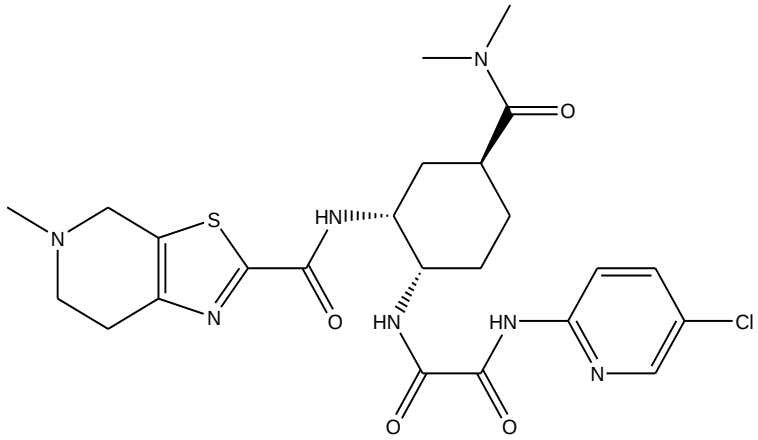


**Figure 1:** Chemical Structure of Edoxaban

Although even after development of some methods suitable analytical method for EDXN remain to be explored. Only a few methods have been reported for its estimation using HPLC is available in the literatures. Few analytical techniques for analysis of EDXN alone or in combination with other drugs have been reported. Several methods such as UV Spectrophotometric method[5,6], Stability indicating method[7,8,9,] Bioanalysis using UHPLC[10] and LC-MS/MS[11-14] approaches are among the methods that have been published.

The goal of the current work was to develop a selective, sensitive, specific and accurate RP-HPLC technique for the determination of EDXN. Because of the sensitivity, repeatability, and specificity, the stability of high-performance liquid chromatography with photodiode array (PDA) detectors has been widely used for conventional drug quality control. In the present investigation, we developed a specific and simple stable HPLC method with PDA detector for the determination of EXN in tablet dosage form. The analytical method was developed and validated according to ICH Q2 (R1) guideline. The proposed method's linearity, precision, robustness, limit of detection, limit of quantitation, and accuracy have all been evaluated in accordance with the International Council for Harmonization's criteria.[15] The present study can be employed in the routine analysis of bulk drug as well as the formulation of Edoxaban.

**Material and Methods:**

***Materials*:** Edoxaban was purchased from Beijing Mesochem Technology Co., Ltd, Beijing, China. High purity HPLC grade water was procured from Medicaps Pvt. Ltd., Pithampur, Dhar, India., Acetonitrile (HPLC Grade) was purchased from spectrochem Pvt. Ltd, Mumbai, India. The required chromatographic solvents and solutions were first filtered through 0.45 µm PTFE membrane disc filters were obtained from Pall Corporation (Mumbai, India) and sonicated prior to use.

***Chromatographic conditions*:** The mobile phase consisted Acetonitrile: water (50:50 % v/v) at a flow rate of 1.0 ml/min. Chromatographic separation was achieved using Shimadzu shim-pack $C_{18}$(250 mm × 4.6 mm, 5µm) column on Shimadzu (UFLC) liquid chromatography system equipped with LC-20 AD solvent delivery system, SPD-M20A photo diode array detector and 20 µl loop volume in a Rheodyne injector. The column was maintained 40°C at temperature and injection volume of 20 µl was used. Data acquisition and data processing was evaluated with Lab Solutions 5.95 version software (Shimadzu, Japan). The analysis was achieved at a wavelength of 291nm. The run time was kept 8 min.

***Method:*** *Preparation of Stock and Standard Solutions:* Stock solution (2000 µg/ml) of Edoxaban was prepared by transferring 50 mg, accurately weigh, into a 25 ml volumetric flask and adding 15 ml acetonitrile. The solution was sonicated for 15-20 minutes to dissolve Edoxaban and the solution was then diluted to make up the volume with the same solvent. From the stock solutions serial dilutions were prepared ranging 8 to 80 µg/ml using acetonitrile as solvent and Calibration curve is plotted. 20 µl of each concentration was injected in HPLC in triplicate & chromatogram was obtained. Chromatogram of standard is shown in (Fig.2)

*Preparation of Tablet sample solution:* The average of 10 Tablet (Savaysa tablets, 30 mg/tablet) was determined and grounded in a mortar. Weigh and transfer crush tablet equivalent to 50 mg into 25 ml of volumetric flask. Then add 15 ml diluent and sonicate for 30 min and makeup to 25 ml with diluents Mix well. Filter the solution with 0.45 µm membrane filters (Pall life sciences, India) and sonicated prior to use and appropriately diluted with the diluent to get the desired concentration of the sample.

**Method Optimization:**

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*System suitability:* A crucial component of the chromatographic system is system appropriateness. Resolution, capacity, tailing, theoretical plate count, relative retentions, etc. are computed and compared with a typical system specification.

**Method Validation:**

**Preparation of calibration curve:** The stock solution of 2000 µg/mL was utilized further, to prepare calibration curve ranges from 8 to 80 µg/mL by withdrawing 40, 60, 80, 140, 200, and 400 µL of stock solution, followed by analysis at λ max 291 nm in HPLC. The linearity equation was obtained by linear regression analysis method by plotting a graph between mean peak response area and concentration. LOD and LOD were determined using dilution method.

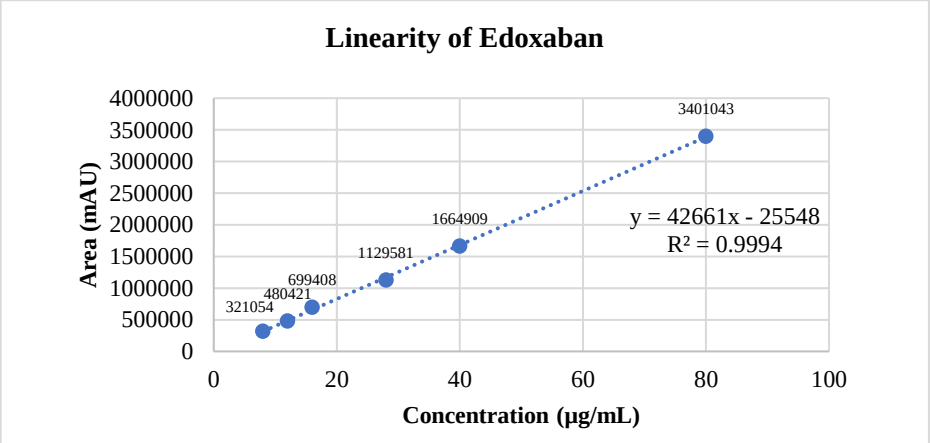



**Figure 2:** Calibration curve of Edoxaban

**Accuracy:** It is defined as the close similarity between the actual (true) value and the analytical value and is obtained by applying the test method multiple times. The accuracy of the methods is determined at three different concentrations, i.e. 80%, 100% and 120% triplicate for drugs according to ICH guidelines. From the total amount of drug found, the percent recovered were found to range from 99 to 101%.

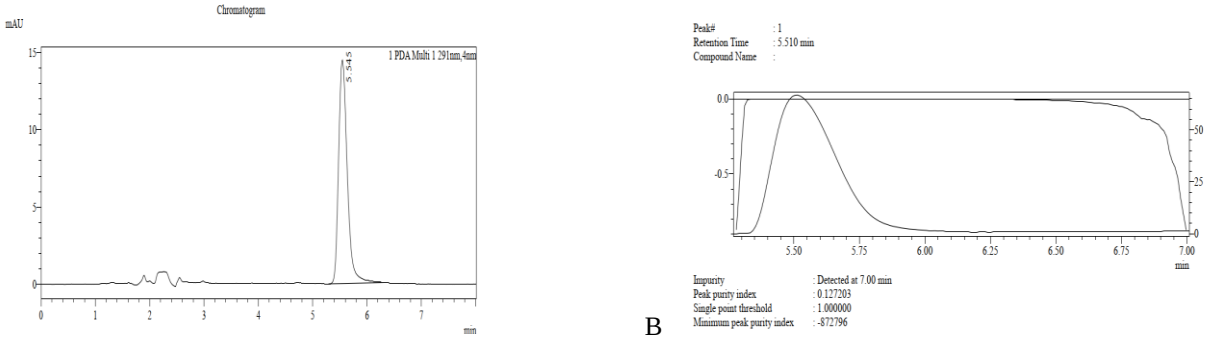
**Specificity:** The specificity of this method was determined by comparing test results from the analysis of sample solutions containing excipients with standard results from standard pharmaceutical products.

**Precision:** It was investigated to explore intra- and inter-day variations of the Edoxaban assay method. Intraday accuracy was determined by analyzing three concentrations in three repeated measurements within the linear range of the drug at three different times on the same day.

**LOD and LOQ:** Limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified to an exact value. While limit of quantification (LOQ) is the smallest amount of an analyte in a sample that can be quantified with suitable accuracy and precision.[15]

**Results and Discussion:**

The literature review revealed that Edoxaban is insoluble in water and less soluble in various organic solvents. Acetonitrile was selected as solvent of choice for Edoxaban dilution. Several trials were performed to get the suitable set of parameters for analytical method.




A B

**Figure 2: A:**RP-HPLC Chromatogram of Edoxaban, **B:** Peak Purity index

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To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of edoxaban with good peak symmetry and stable baseline was obtained with the mobile phase acetonitrile: water (50:50, %v/v) at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 291nm based on peak area. Complete resolution of the peak with clear baseline separation was obtained. The system suitability test parameters are shown in Table 1.

**Table 1:** System suitability test for the proposed HPLC method for the concentration level of 16µg/ml.

| Parameters | Edoxaban |
|---|---|
| Retention time ($R_t$), min | 5.514 ± 0.05 |
| Tailing factor | 1.3 ± 0.15 |
| Area | 702417 ± 2480 |
| Theoretical plates (N) | >3800 |

**Method validation parameters:**

*System suitability:* System suitability parameters were found to be satisfactory. All the parameters, theoretical plate count (N) >3800, resolution (Rs) >2 and tailing factor <1.5 were within acceptable value. The relative standard deviation (RSD) of peak area was found to be < 2 percentage.

*Specificity:* There were no interfering peak/s found in the chromatogram obtained in the placebo at the retention time of Edoxaban.

*Linearity:* The linear regression correlation coefficient($R^2$) of 0.9994 was obtained over the six different concentration levels ranging from 8 µg/ml to 80 µg/ml for the selected drug analyte. The average slope and intercept of linearity equations were 42705.05 and -23587.35 respectively. Regression analysis data are summarized in Table 2.

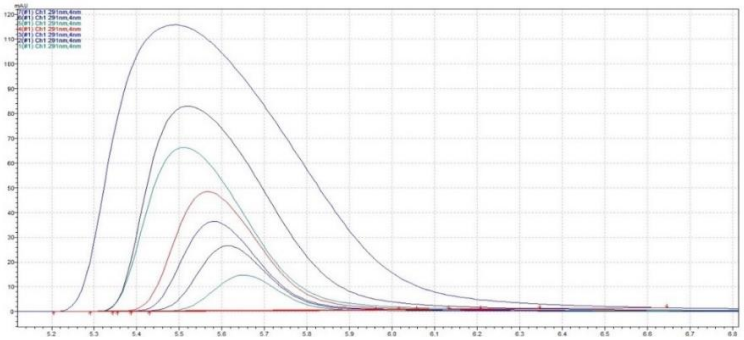

**Figure 3:** Multi chromatogram view of Edoxaban for Linearity

**Table 2:** Regression analysis of calibration graphs for EDXN for the proposed HPLC methods.

| Parameters | HPLC |
|---|---|
| Conc. Range (µg/ml) | 8-80 |
| Slope | 42705.05 |
| SD of slope | 99.54 |
| Intercept | -23587.35 |
| SD of intercept | 4024.92 |
| Correlation | 0.9994 |
| Regression equation | y= 42705.05x-23587.35 |

y = peak area; x = Concentration of analyte

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*Limit of detection (LOD) and limit of quantitation (LOQ):* The concentration of Edoxaban for determination of LOD was 0.283 µg/ml, which indicates the sensitivity of the method. Similarly, LOQ was found 0.942 µg/ml, which proves that analyte can be estimated at lower concentration.

*Accuracy and Precision:* The inter day and intraday precision values of EDXN for various concentrations ranged from 0.46% to 0.93% RSD and 0.45% to 0.97% RSD, respectively. The values for accuracy were also found within acceptable limits at the same concentrations. The data are presented in Table 3.

**Table 3.** Method validation parameters for estimation of Edoxaban.

| **Parameters** | **HPLC** |
|---|---|
| Limit of detection | 0.283 µg/ml |
| Limit of quantification | 0.942 µg/ml |
| Recovery | 98.83 to 99.63% |
| Repeatability (%RSD, n=6) | 0.5620 |
| Intermediate Precision | |
| Inter day (%RSD, n=3) | 0.46-0.93 |
| Intraday (%RSD, n=3) | 0.45-0.97 |
| Robustness (%RSD) | < 2% |

[a] %RSD = Relative standard deviation

*Robustness:* The developed method was found to be robust during robustness studies, the %RSD was found to be <2 in each case. The low values of %RSD implies that method is robust.

*Sample and standard stability:* The stability of EDXN sample solution and the standard solutions were determined. Peak area and retention time variation were found to be <1%. Also, no significant change in peak area was observed during 24 h.

The present Chromatographic method for the estimation of Edoxaban in bulk and pharmaceutical dosage forms was established and validated as per ICH guidelines. The method was developed with the intention of accurate, rapid and economic detection and quantification of the drug in bulk drug and in pharmaceutical dosage forms. The Edoxaban was detected with good chromatographic and system suitability parameter within the limits with no interfering peaks. A number of combinations of chromatographic conditions and mobile phases were tried for the development of RP-HPLC method for estimation of Edoxaban in bulk and pharmaceutical dosage forms. The best response was obtained with shimpack C18 (250mm×4.6 mm, 5µm particle size); Shimadzu LC-20AT Prominence HPLC system, equipped with SPD 20A detector and mobile phase contained Acetonitrile and water (50:50, v/v) was delivered at a flow rate of 1 mL/min. Quantitation was attained at 291 nm depends on peak area. The retention time of Edoxaban was 5.514 min. Linearity was established for Edoxaban in the range of 8-80 µg/mL with correlation coefficients ($r^2$=0.9994) and the percentage recoveries were reported between 98.83 - 99.63 % for Edoxaban for bulk drug and for marketed formulation it was found 98.25%, which shows that the method can be applied to routine analysis of the drug analyte. The RSD % values of accuracy for Edoxaban were found to be < 2 %, which indicate accuracy of the proposed method. The RSD % values of interday and intraday precision were found to be 0.46 to 0.93 % and 0.45-0.97 % for Edoxaban respectively and for ruggedness were found to be 0.43% and 0.94 % for Edoxaban respectively, which reveal that the proposed method is precise. LOD and LOQ values were found to be 0.283 µg/mL and 0.942 µg/mL. The RSD % values of robustness studies were found to be < 2 %, which indicate robustness of the proposed method. These reports prove that the method proposed is reliable and accurate for determining Edoxaban levels in bulk and in pharmaceutical dosage forms. It is easy to use, accurate, and simple. thus, it can be applied to the routine analysis for the Edoxaban concentration in bulk and in pharmaceutical dosage forms.

**Conclusions:**

In the present study, an attempt was made to develop a simple, precise, selective and sensitive validated analytical method of Edoxaban using RP-HPLC. This method is quite simple, economic, less time-consuming method up to date for the determination of Edoxaban in bulk drug and dosage form with RP-HPLC.

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