Pharmaceutical Sciences, March 2016, 22, 35-41
doi: 10.15171/PS.2016.07
http://journals.tbzmed.ac.ir/PHARM

Research Article

Determination of Edoxaban in Bulk and in Tablet Dosage Form by Stability Indicating High-Performance Liquid Chromatography

Pasam Satyanarayana Reddy[1*], V. Shanmukha Kumar Jagarlapudi[1], Chandra Bala Sekharan[2]
[1]*Department of Chemistry, KL University, Green Fields, Vaddeswaram, Guntur, Andhra Pradesh, India.*
[2]*Department of Biochemistry, International Medical and Technological University, Dar es Salaam, Tanzania*

Article Info

Article History:
Received: 14 December 2015
Accepted: 1 February 2016
ePublished: 30 March 2016

Keywords:
-Edoxaban
-Anticoagulant
-Stability Indicating
-HPLC
-Degradation products

ABSTRACT

Background: Edoxaban is an orally active direct factor Xa inhibitor. The aim of the present study was to develop a stability indicating HPLC method for the quantification of edoxaban in bulk and in tablet dosage form.
Methods: Edoxaban was separated on Hypersil BDS C18 column (250 x 4.6 mm, i.d. 5µm) using 0.1M K_2HPO_4: Methanol (65:35, *v/v*) as an isocratic mobile phase at a flow rate of 1.0 ml/min. Detection was performed using photodiode array detector set at 245 nm. The chromatographic conditions were optimized. The method was validated as per the guidelines given by International Conference on Harmonization guidelines.
Results: Edoxaban was eluted at 3.785 min with a total run time of 6 min. The calibration curve was found to be linear over the concentration range of 5–200 µg/ml. Limit of detection and limit of quantification for edoxaban are 0.209 µg/ml and 0.698 µg/ml, respectively. The intra- and inter-day precision values were ≤0.710% and the accuracy ranged from 99.824-100.720%. Besides, all the validation results were within acceptability criteria of general assay. The stability indicating nature of the method was established by subjecting the edoxaban to stress conditions such as acid and base hydrolyses, oxidative, photo- and thermal degradations. The degraded products formed in all stress conditions were resolved successfully from the edoxaban.
Conclusion: The developed and validated method is suitable for the determination of edoxaban in bulk and in commercial tablet dosage form.

Introduction

Edoxaban (EXN), chemically known as *N'*-(5-chloropyridin-2-yl)-*N*-[(1*S*,2*R*,4*S*)-4-(dimethylcarbamoyl)- 2- [(5-methyl-6,7-dihydro-4*H*-[1,3] thiazolo [5,4-c] pyridine-2-carbonyl)amino] cyclohexyl] oxamide, is an oral anticoagulant that acts as highly specific direct factor Xa inhibitor.[1] In 2011, Japan approved EXN for prevention of venous thromboembolisms after total hip arthroplasty with no increased risk of bleeding.[2,3] Food and Drug Administration approved EXN for the prevention of stroke and non central nervous system systemic embolism in patients having nonvalvular atrial fibrillation in 2015.[4,5]

So far EXN is not official in any pharmacopeias. The literature is poor regarding the reports on the assay of EXN. Bathala et al.,[6] analyzed EXN and its four metabolites in human plasma, urine and fecal samples, after oral administration of [^{14}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.,[7] 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. However, to the best of our knowledge, there are no reports published on the quantitative analysis of EXN in bulk and tablet dosage forms. For this reason, it is essential to establish a simple, economical, precise and accurate analytical method for the assay of EXN in bulk and tablet dosage forms.

Due to its sensitivity, repeatability and specificity, stability indicating high performance liquid chromatography with photodiode array (PDA) detector method has been widely used for the routine quality control of drugs. In the present investigation, we have developed a simple and specific stability indicating HPLC with PDA detector method for determination of EXN in tablet dosage forms. The developed method was validated in agreement with International Conference on Harmonization guidelines by assessing its linearity, system suitability, limit of detection, limit of quantitation, selectivity, accuracy, precision,

***Corresponding Author:** Pasam Satyanarayana Reddy, Tel: (+92) 48036666, E-mail: psreddyphd@gmail.com

specificity and robustness.[8,9]

Materials and Methods

Reagents and apparatus

Methanol (HPLC grade) was purchased from Merck India Limited, Mumbai, India. Analytical grade dipotassium hydrogen phosphate**,** hydrochloric acid, sodium hydroxide and hydrogen peroxide (Sdfine-Chem limited, Mumbai, India). Milli-Q-water was obtained from Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India. EXN reference drug was bought as gift sample from Lara Drugs Pvt. Ltd (Nalgonda district, Telangana).

Elico (Hyderabad, India) LI120 model pH meter, Kemi (Ernakulam, India) KWB 220 model water bath, Shimazdu (Tokyo, Japan) electronic weighing balance model BL 220H were used in the present investigation. The HPLC experiments were carried out on an isocratic Waters Alliance HPLC system equipped with 2695 separation modules having 2996 photodiode array detector. The chromatographic system utilizes an Empower 2 software computer program to control hardware, acquire and store data. The analytical column used is a Hypersil BDS C18 column (250 x 4.6mm, 5µm).

HPLC conditions

The mobile phase consisted of a mixture of dipotassium hydrogen phosphate (0.1M) and methanol (65:35 *v/v*) at a flow rate of 1.0 ml/min. A 0.45 mm membrane filter was used to filter the mobile phase. Prior to use, the mobile phase was degassed with a helium sparge for 15 min. Detection was performed at 245 nm using diode array detector. The column temperature was set at 30 ^{o}C and the injection volume was 10 µl.

Preparation of standard solutions

Standard stock solution of EXN (1000 µg/ml) was prepared by exactly weighing 100 mg of the same dissolved in 30 ml of mobile phase in 100ml volumetric flask and then made up to the mark with the same solvent. The stock solution was used to prepare the working standard solutions for system suitability studies (100 µg/ml), linearity (5, 10, 15, 25, 50, 75, 100, 125, 150 and 200 µg/ml) accuracy (5, 100 and 200 µg/ml), precision (5, 100 and 200 µg/ml), specificity studies (100 µg/ml) and robustness (5 and 200 µg/ml). The working standard solutions are prepared in mobile phase.

Preparation of tablet sample solution

Ten tablets (Savaysa tablets (30 mg/tablet), Daiichi Sankyo Co., Ltd, Tokyo) were exactly weighed and powdered finely. From this powder, an amount of the tablet powder equivalent to 50 mg EXN was put in a 50 ml standard flask which has 10 ml of mobile phase, shaken thoroughly and sonicated for 10 min. The same solvent was used to make up the volume to the mark and mixed well. The resulting solution was filtered using a 0.45 mm membrane filter and then appropriately diluted with the mobile phase to obtain a concentration of 200 µg/ml.

Preparation of placebo blank solution

Starch, acacia, sodium citrate, talc, magnesium stearate, hydroxyl cellulose, lactose, glucose and sodium alginate were used for the preparation of placebo blank. In preparing the placebo blank solution, 10 mg each of the aforesaid substances were weighed accurately and transferred into a 100 ml volumetric flask. About 50 ml of mobile phase was added to the flask. The contents of the flask were sonicated for 15 min. The resulting solution was filtered via a 0.45 mm membrane filter and then diluted up to the mark with mobile phase and mixed well.

Method validation

According to ICH guidelines, the developed method was validated by determining the parameters: system suitability, linearity, selectivity, limit of detection, limit of quantitation, accuracy, precision, specificity and robustness.

System suitability studies

System suitability parameters, such as peak area, retention time, tailing factor, number of theoretical plates and height equivalent to theoretical plates were measured by injecting standard solution containing 100 µg/ml of EXN into HPLC system five times. To test the system suitability, relative standard deviation of the each parameter was measured.

Selectivity

The establishment of selectivity of the method was done by evaluating the interference of the common excipients in the pharmaceutical formulation and components of the mobile phase. For this study, chromatograms of standard EXN solution (100 µg/ml), mobile phase blank, placebo blank solution and tablet sample solution were compared.

Linearity

To determine the linearity the calibration curve was constructed with ten calibration points for EXN, with the concentrations 5, 10, 15, 25, 50, 75, 100, 125, 150 and 200 µg/ml. The peak area values were plotted against the respective concentrations of EXN to get the calibration curve. The results were subjected to regression analysis by the least squares method to calculate the slope, intercept and regression coefficient.

Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of the proposed method was expressed in terms of limit of detection (LOD) and limit of quantitation (LOQ). According to 3:1 and 10:1 signal/noise ratios, the LOD and LOQ were determined

respectively. For this, five replicate injections of dilute solutions with known concentration (5 µg/ml) were injected into the chromatographic system.

Precision & accuracy

Precision of the method was studied by repeatability and intermediate precision. Repeatability was determined by injecting five standard solutions of three different concentrations (5, 100 and 200 µg/ml) on the same day and intermediate precision was determined by injecting the same solutions for three consecutive days. Standard deviation and relative standard deviation was then calculated to represent precision. Percent recovery and percent error was calculated to represent the accuracy.

Recovery study

Recovery of the developed method was determined by standard addition technique. In this technique known quantities (50, 100 and 150%) of EXN were spiked to the tablet sample solution and then analysed by the developed method. The recovery percentage was determined.

Specificity (stress degradation studies)

Stress degradation studies was performed using different ICH recommended stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses.

Acid degradation studies

Acid degradation study was carried out in water bath at 80°C using 0.1N HCl. For this, 50 mg of EXN was taken in 50 ml volumetric flask. Five milliliter of 0.1N HCl was added in the flask and kept on a water bath at 80ºC for 1 hr. After completion of the stress the solution was cooled, neutralized using 0.1N NaOH and filled up to the mark with mobile phase.

Base degradation studies

Base degradation study was carried out in water bath at 80°C using 0.1 N NaOH. For this, 50 mg of EXN was taken in 50 ml volumetric flask. Five milliliter of 0.1N NaOH was added in the flask and kept on a water bath at 80ºC for 1 hr. After completion of the stress, the solution was allowed to cool, neutralized by using 0.1N HCl and completed up to the mark with mobile phase.

Oxidative degradation studies

Oxidative degradation study was carried out in water bath at 80°C using using 3% H_2O_2. To perform this, 50 mg of EXN was taken in 50 ml volumetric flask. Five milliliter of 3% H_2O_2 was added to it. The contents of the flask were heated in a water bath at 80 ºC for 1 hr. After completion of the stress, the solution was cooled and the volume of the flask was completed up to the mark with mobile phase.

Thermal degradation studies

Thermal degradation studies were performed in hot air oven at 105°C. For this study, 100 mg of EXN powder was taken in glass petric dish and placed in oven at 105°C for 2 hrs. After specified time, the sample was cooled, transferred to a 100 ml volumetric flask and dissolved in 30 ml of mobile phase and up to mark with mobile phase.

Photolytic degradation studies

For photolytic degradation study, 100 mg of EXN powder was taken in glass petric dish and placed in the direct sunlight for 24 hrs. After completion of the stress, the drug sample was cooled, transferred to a 100 ml volumetric flask and dissolved in 30 ml of mobile phase and up to mark with mobile phase.

After degradation, in mobile phase stress degraded samples were diluted to give a final concentration of 100 µg/ml and filtered through a 0.45 mm membrane filter before injection in the chromatographic system. The chromatograms were recorded and assay was performed by comparison with the peak area of EXN sample (100 µg/ml) without degradation.

Robustness

Deliberate variations were done in the chromatographic conditions of the proposed method to evaluate the robustness of the method. For this study, minor changes were made in mobile phase composition, flow rate, column temperature, detection wavelength and pH of mobile phase. The consequences of these changes were then measured. The assay was carried out at two different concentration levels of EXN (5 and 150 µg/ml).

Assay of EXN in tablet dosage form

For this purpose, 10 µl of the tablet sample solution (prepared as described under "Preparation of tablet sample solution") was injected into chromatographic system in triplicate. The chromatograms were recorded. The area under the peak was calculated. The concentration of EXN in the sample was calculated using either the calibration curve or regression equation.

Results and Discussion

Optimization of the HPLC conditions

The HPLC conditions such as: analytical column, column temperature, mobile phase and their proportions, pH of the buffer, flow rate and detection wavelength were optimized with an aim to achieve good separation of EXN in presence of stress degradation products. The HPLC conditions were optimized based on parameters like retention time, tailing factor, peak height, peak area and number of theoretical plates.

Various HPLC analytical columns like Zorbax C8 (150 x 4.6 mm, 5 µm) and Hypersil BDS C18 (250 mm x 4.6 mm x 5 µm) were tested during method development. Based on the above said parameters Hypersil BDS C18 (250 mm x 4.6 mm x 5 µm) column

was finalized for analysis. The column maintained at a temperature of 30°C was the best value found for all parameters. For this reason, 30°C was the final temperature for the analysis.

Two buffers, 0.1M dipotassium hydrogen orthophosphate and 0.1M disodium hydrogen orthophosphate, with pH ranging from 5 to 9 were tried during method development. Good peak shape, less retention time and less tailing factor were obtained with 0.1m dipotassium hydrogen orthophosphate buffer (pH 7.0). Therefore, 0.1M dipotassium hydrogen phosphate buffer with a pH 7.0 was selected for analysis. Organic modifiers like acetonitrile and methanol were assessed. Satisfactory chromatographic separation was achieved when methanol was used. Hence, methanol was finalized as organic modifier for analysis.

Mobile phase consisting of 0.1M dipotassium hydrogen phosphate buffer (pH 7.0) with methanol in different volumetric ratios were investigated to meet the necessary system parameters. The experiments indicated that better peak area was achieved while using 0.1M dipotassium hydrogen phosphate buffer (pH 7.0) and methanol in the ratio of 65:35 *v/v*. The mobile phase with different flow rates was investigated. At the flow rate 1 ml/min, symmetric and well retained peak was obtained. Therefore, for the present study the flow rate 1 ml/min was preferred.

To determine the suitable wavelength for determination of EXN, UV spectra of the EXN in mobile phase were scanned in the range 200–400. From the UV spectra, the maximum absorption wavelength for EXN was 245 nm.

Method validation

System suitability

The system suitability results revealed %RSD of less than 1% for the parameters such as peak area, tailing factor, retention time, number of theoretical plates and height equivalent to theoretical plates. As shown in Table 1, the proposed method meets the accepted requirements (acceptance criteria: %RSD-not more 2.0%).

Table 1. Results of system suitability

Value/Parameter	Retention time(min)	Peak area	Plate count	HETP[b]	Tailing factor
Mean[a]	3.78	1987588	9982.8	2.504×10^{-5}	1.26
SD	0.0019	3906.72	77.32	1.726×10^{-7}	0.00707
%RSD	0.515	0.196	0.774	0.689	0.561

a = Average of five determinations,
b = Height equivalent to theoretical plates,
SD = standard deviation ,
%RSD = percentage relative standard deviation

Selectivity

The results of selectivity studies are shown in Figure 1. A comparison of the retention time of EXN in tablet sample solution and in the standard solution was exactly the same (Figure 1.3 & 1.4). Figure1.4 showed that there were no interferences at the retention time of EXN due to the excipients in tablet dosage forms. There were no peaks in mobile phase blank and placebo blank (Figure 1.1 & 1.2). Therefore, the proposed method was found to be selective and is suitable for the quantification of the EXN in tablet dosage form.

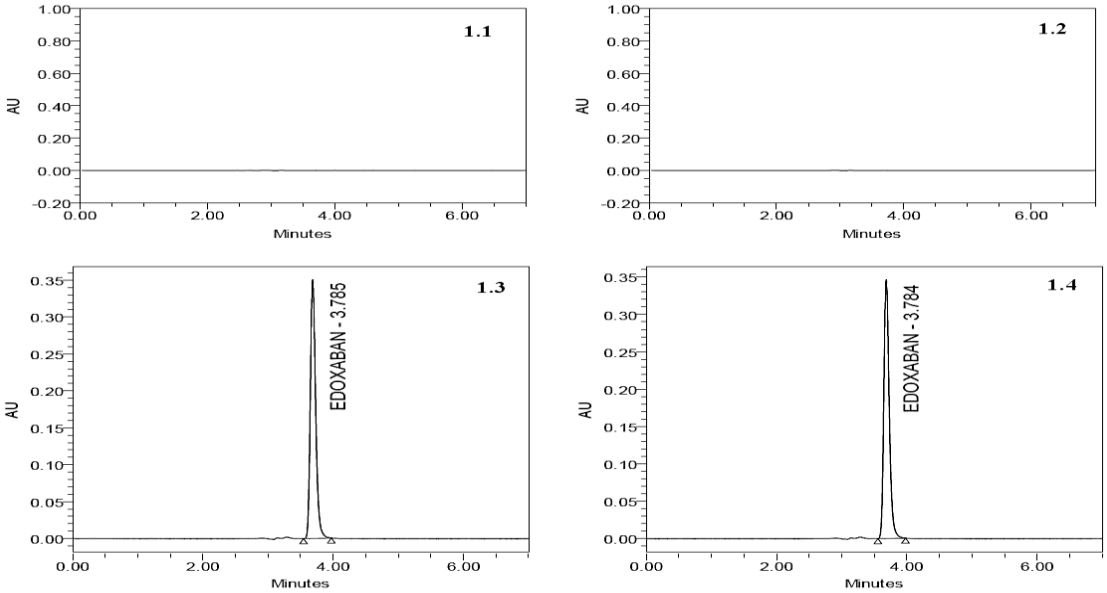

Figure 1. Chromatogram of [1.1.] Blank mobile phase [1.2.] Placebo blank [1.3.] Standard drug [1.4.] Tablet sample.

Linearity, limit of detection and limit of quantitation

The linearity was determined at ten levels over the concentration range of 5–200 µg/ml. The following equation for straight line was obtained for EXN:

$$Y = 19702X + 24446 \quad \text{Eq.(1)}$$

where Y = peak area, Slope = 19702, x = concentration of EXN in µg/ml, Intercept = 24446.

Regression coefficient (R^2) = 0.9998. The regression equation data indicated linearity of the proposed method

The LOD and LOQ values were found to be 0.209 and 0.698 µg/ml, respectively. These low values indicate that the proposed method is sensitive.

Precision and accuracy

Table 2 provides data obtained from the repeatability and intermediate precision**.** The relative standard deviation values for repeatability and intermediate precision were in the range of 0.102-0.264% and 0.157-0.710% (acceptance criteria: %RSD-not more 2.0%), respectively. The percent recovery values for accuracy were in the range of 99.824-100.031% and 99.898-100.720% (acceptance criteria: %Recovery range- 98 to 102%), respectively. The results (Table 2) indicate that the method has sufficient precision and accuracy.

Table 2. Precision and accuracy of the method

	Repeatability (intra-day)			**Intermediate (inter-day)**		
Concentration of EXN taken (µg/ml)	**5**	100	200	5	100	200
Concentration of EXN found[a] (µg/ml)	4.99	99.82	200.06	5.036	99.90	200.57
SD	0.0083	0.10	0.53	0.015	0.16	0.14
%RSD	0.167	0.102	0.264	0.305	0.157	0.710
%Recovery	99.88	99.82	100.03	100.72	99.90	100.28
% Error	0.120	0.176	0.031	0.720	0.102	0.282

a = Average of five determinations
SD = standard deviation
%RSD = percentage relative standard deviation

Recovery study

The accuracy of the proposed method was additionally established by recovery study via standard addition technique. The average recovery data (Table 3) of EXN showed results between 99.693% and 99.981% with relative standard deviation between 0.156% and 0.943%. The results of recovery study showed the method to be greatly accurate and apt for intended use.

Table 3. Recovery data of the method.

	Spiked level		
	50%	**100%**	**150%**
Concentration of EXN in tablet (µg/ml)	30	30	30
Concentration of EXN spiked (µg/ml)	15	30	45
Concentration of EXN found (µg/ml)	44.90	59.82	74.99
SD	0.019	0.094	0.071
%RSD	0.417	0.156	0.943
%Recovery	99.77	99.69	99.98
% Error	0.231	0.307	0.019

a = Average of three determinations
SD = standard deviation
%RSD = percentage relative standard deviation

Robustness

The results (Table 4) obtained from the assay of the EXN standard solutions were not affected by the varying the chromatographic conditions. The recovery values were 99.574-101.10% with relative standard deviation 0.114-0.999%. The proposed method thus remained unaffected by slight but deliberate changes in the chromatographic conditions**.** At 0.05 level of significance *P*-value was found to be less than 0.05 (Table 4), which indicates that small deliberate changes in the chromatographic conditions had no significant effect on the results.

Specificity (stress degradation studies)

The chromatograms of the samples after stress degradation treatment are shown in Figure 2. The samples submitted to degradation conditions showed significant change in the peak areas. Under acidic conditions EXN was degraded up to 6.993%. Under alkali stress DST was degraded up to 5.626%. Under oxidative stress EXN was degraded up to 7.919 %. Under dry heat and photolytic stresses, the percent of EXN degradation was 4.869% and 5.285%, respectively. From these stress studies it is therefore concluded that EXN was degraded in all the applied stress conditions. The results are summarized in Table 5. In all degraded samples, two peaks were observed in addition to the EXN peak. In all the stress conditions, the degradants peaks were well resolved from EXN peak (Figure 2.1-2.5).

Table 4. Robustness of the method.

Parameter	Concentration of EXN (µg/ml) Taken	Found[f]	*P* – value[g]	% RSD	%Recovery
Mobile phase ratio[a]	5	5.055	0.0032	0.999	101.10
	200	199.511		0.235	99.76
Mobile phase pH[b]	5	5.020	0.0022	0.458	100.40
	200	200.344		0.399	100.17
Flow rate[c]	5	5.040	0.0015	0.413	100.80
	200	199.149		0.775	99.57
Temperature[d]	5	5.034	0.0026	0.180	100.68
	200	199.539		0.379	99.77
Wavelength[e]	5	5.041	0.0022	0.114	100.82
	200	199.863		0.224	99.93

[a]0.1M K_2HPO_4: Methanol ratios (*v/v*)- 67:33, 65:35 and 63:37
[b]Mobile phase pH – 6.9, 7.0 and 7.1
[c]Flow rate (ml/min) – 0.9, 1.0 and 1.1
[d]Temperature (°C) – 29, 30 and 31
[e]Wavelength – 244 nm, 245 nm and 246 nm
[f]Mean of three values
[g]*P*-value at 0.05 level of significance

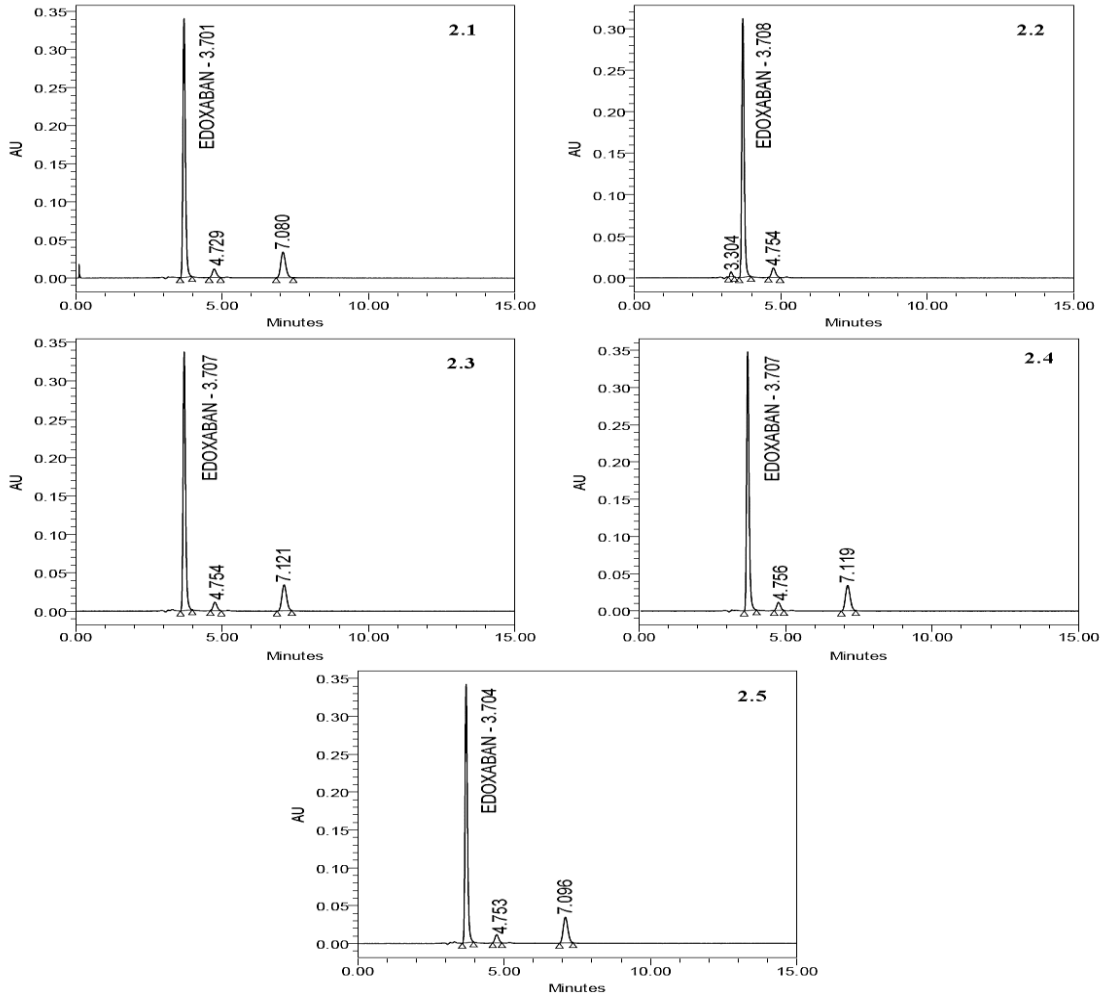

Figure 2. Chromatogram of EXN after [2.1] Acid degradation [2.2.] Base degradation [2.3.] Oxidative degradation [2.4.] Dry heat degradation [2.5.] Photolytic degradation.

Spectral homogeneity of EXN in the presence of their stress degradation products was checked. EXN passed the peak purity. In all the stress conditions, purity angle value was less than the purity threshold for EXN peak indicating that the EXN peak is spectrally homogeneous. The results are presented in Table 5.

Table 5. Stress degradation data and spectral homogeneity data.

Type of stress	Mean Peak area	% Recovered	% Degradation	Peak purity angle	Peak purity Threshold
Undegraded	1987588	-	-	-	-
Acid	1848601	93.01	6.993	0.109	0.323
Base	1875783	94.37	5.626	0.110	0.326
Hydrogen peroxide	1830206	92.08	7.919	0.110	0.327
Dry heat	1890806	95.13	4.869	0.106	0.322
Photolytic	1882540	94.72	5.285	0.104	0.322

Application of the proposed method

The aforementioned validation results indicated that the proposed method gave acceptable results. Therefore, application of the proposed method was checked by analyzing the EXN content in commercial tablet dosage forms. The results are shown in Table 6. The label claim percentage was 99.606 ± 0.149%. Good recovery with low relative standard deviation value indicates: non interference of excipients commonly present in the pharmaceutical dosage form, good accuracy and precision of the proposed method.

Table 6. Results of assay of EXN in tablets.

Concentration of EXN (mg)			SD	% RSD	%Recovery
Labeled claim	Found	Mean			
30	29.83	29.88	0.045	0.149	99.61
30	29.91				
30	29.91				

SD = standard deviation
%RSD = percentage relative standard deviation

Conclusion

A rapid and simple stability indicating HPLC method equipped with PDA detection at 245 nm has been developed for the quantification of EXN for the first time. The results of validation undertaken as per the International Conference on Harmonization guidelines reveal that the method is linear, sensitive, selective, accurate, precise, robust and stability indicating. The method is suitable for the routine analysis of EXN in either bulk or in tablet dosage forms.

Acknowledgements

The authors wish to thank the management KL University, Green Fields, Vaddeswaram, Guntur, Andhra Pradesh for providing research facilities.

Conflict of interests

The authors claims that there is no conflict of interest.

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