Identification, Confirmation of different components of Edoxaban tosylate in its Bulk and Finished Dosage form by using Spectroscopic and Chromatographic techniques

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ABSTRACT

A new, simple, rapid, selective, precise, and accurate Spectroscopic method has been developed for the Identification, selection, and confirmation by Infrared Spectroscopy and UV-Visible Spectroscopy. The Identification was achieved by using IR using the KBR pellet technique. Edoxaban tosylate was detected using a UV detector at the wavelength of 292nm. The HPLC studies were carried out on Waters quaternary pump with a degasser and as an autosampler. Hypersil BDS C18 column (250 x 4.6 mm. 5µm,) was used for chromatographic separation. The mobile phase consists of a buffer pH4.5 Acetate Buffer 100%, and mobile phase B is a mixture of Acetonitrile and Methanol in the ratio of 50:50(v/v) was used. The separation achieved with gradient program [T/A- 0.01/50, 15/40, 20/25, 30/25, 35/40, 40/50]. The flow rate was maintained at 0.6mL/min with UV detection at 292 nm. The column temperature was maintained at 25ºC. LC-MS is an analytical technique that involves physical separation of target compounds (or analytes) followed by their mass-based detection. Although relatively new, its sensitivity, selectivity and accuracy have made it a technique of choice for detecting microgram or even nanogram quantities of a variety of analytes ranging from drug metabolites, pesticides and food adulterants, to natural product extracts.

Keywords: Edoxaban Tosylate, Infrared spectroscopy, UV-Vis Spectroscopy, HPLC, Wavelength, LC-MS,ICH guidelines.

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INTRODUCTION

Edoxaban, sold under the brand name **Lixiana** among others, is an anticoagulant medication and a direct factor Xa inhibitor.[1] It is taken by mouth.[1] It was developed by Daiichi Sankyo and approved in July 2011, in Japan for prevention of venous thromboembolisms following lower-limb orthopedic surgery.[2] It was also approved in the United States by the Food and Drug Administration (FDA) in January 2015, for the prevention of stroke and non–central-nervous-system systemic embolism.[3][4] It was approved for use in the European Union in June 2015.[5] It is on the World Health Organization's List of Essential Medicines.[6] In the United States, Edoxaban is indicated to treat deep vein thrombosis and pulmonary embolism following five to ten days of initial therapy with a parenteral anticoagulant.[7] It is also indicated to reduce the risk of blood clots in people with nonvalvular atrial fibrillation.[8]

In the European Union, Edoxaban is indicated for preventing blood clots in people with nonvalvular atrial fibrillation who also have at least one risk factor, such as having had a previous stroke, high blood pressure (hypertension), diabetes mellitus, congestive heart failure or being 75 years of age or older. It is also used to treat deep vein thrombosis and pulmonary embolism and to prevent either of these from reoccurring.[9] This has prompted enthusiasm for newer agents such as dabigatran, apixaban, and rivaroxaban for effective clot prevention. [10] In addition to once daily dosing, the benefits over warfarin also include significant reductions in haemorrhagic stroke and GI bleeding, and improved compliance, which is beneficial as many patients will be on lifelong therapy. [11]

Drug Chemistry

Accession Number : DBSALT001717

CAS Number : 480449-71-6

Weight Average : 720.26

Chemical Formula. : $C_{31}H_{38}ClN_7O_7S_2$

IUPAC Name : 4-methylbenzene-1-sulfonic acid; N'-(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]ethanediamide

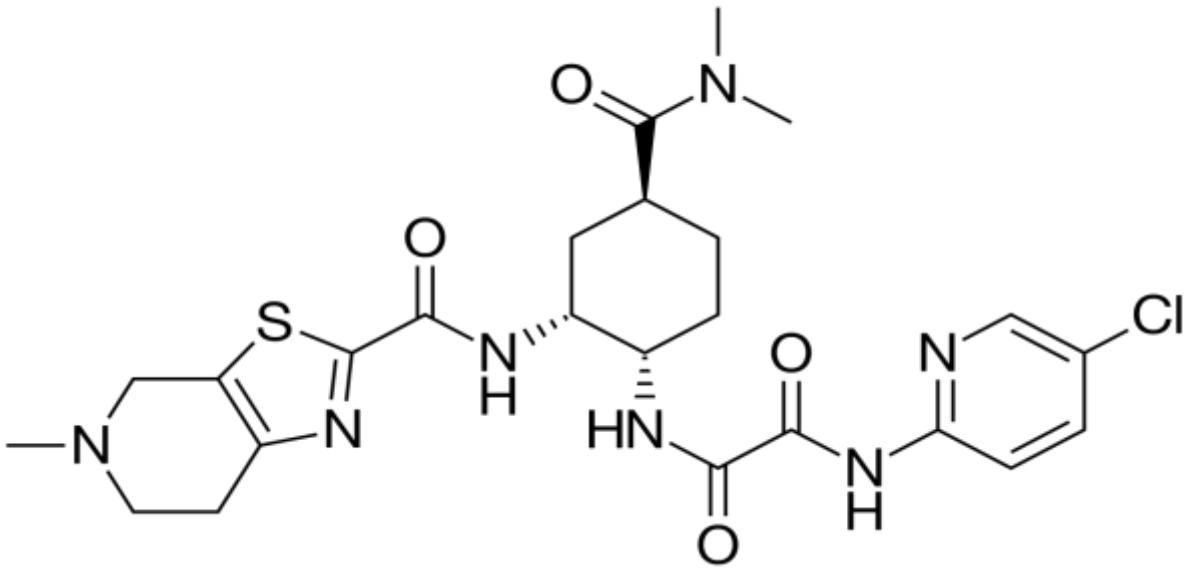


Figure 1. Chemical structure of Edoxaban Tosylate

The literature survey reveals only few methods were reported till date a few methods were reported for the Edoxaban tosylate in pharmaceutical dosage forms.

The present work describes a simple, Identification, Selection and Confirmation of wavelength of Edoxaban tosylate in its Bulk and Finished Dosage form available in market by using spectroscopic techniques according to ICH guidelines[12]

MATERIALS & METHODS

Chemicals and Reagents

HPLC-grade Acetonitrile, Methanol and water, Analytical grade Potassium Bromide were procured from Merck Chemicals. Mumbai, India.

Edoxaban Tosylate API was Purchase from MSN Labs, Hyderabad and Lixiana-60mg

(Edoxaban Tablets) from Daiichi Sankyo.

A) Identification and Confirmation of Edoxaban Tosylate in its Bulk and Finished Dosage form by Infrared Spectroscopy:

Instrumentation

The IR spectrum of Edoxaban Tosylate API and Lixiana 6mg Tablets were recorded on a PerkinElmer (spectrum-one) FT-IR spectrophotometer over the range 4000 to 400 cm-1 by pressing pallet method using KBr power dispersion.

Preparation of Sample

Take a small amount of Edoxaban sample and mixed with the KBr powder. Subsequently grind the mixture for 3-5 minutes. Assemble the die-set. When assembling the die, please add the powder to the 7mm collar. Put the die together with the powder into the Qwik Handy-Press. Press the powder for 2 minutes to form a pellet. A good KBr pellet is thin and transparent. Opaque pellets give poor spectra, because little infrared beam passes through them. White spots in a pellet indicate that the powder is not ground well enough, or is not dispersed properly in the pellets. Disassemble the die set and take out the 7mm collar. Put the collar together with the pellet onto the sample holder for measuring the spectra.

B) Selection and Confirmation of Wavelength of Edoxaban Tosylate in its Bulk and Finished Dosage form (Lixiana 60mg) by UV Visible Spectroscopy:

Instrumentation

The UV spectrum of Edoxaban Tosylate API and Lixiana 6mg Tablets were recorded on a PG Instruments-T60 UV Visible spectrophotometer with UV-Win version 6.0.0 software over the range 200 to 400 nm wavelength range.

Preparation of Diluent:

Mixed Milli-Q water and Acetonitrile in the ratio of 50:50 %v/v.

Preparation of Standard(10 ppm):

Weighed and transferred 25 mg into 25ml volumetric flask. Added 15ml of diluent and sonicated to dissolved. Made up to the mark with diluent. Further diluted 1ml of the above solution to 100mL volumetric flask and made up to the mark with diluent.

Preparation of sample(Edoxaban API Sample-10ppm):

Weighed and transferred 25 mg into 25ml volumetric flask. Added 15ml of diluent and sonicated to dissolved. Made up to the mark with diluent. Further diluted 1ml of the above solution to 100mL volumetric flask and made up to the mark with diluent.

Preparation of sample(Edoxaban-Lixiana 60mg Sample):

Weighed and recorded the average weight of ten Lixiana 60mg tablets and transferred into mortar. Crushed into fine powder. Weighed 50ml volumetric flask. Added 30ml of diluent and sonicated to dissolved. Made up to the mark with diluent. Centrifuged the sample solution at 5000RPM about 5mins,Collected the supernatant solution and further diluted 0.9ml of the above solution to 100mL volumetric flask and made up to the mark with diluent.

C) Identification of Impurities of Edoxaban Tosylate in its Bulk and Finished Dosage form by High Performance Liquid Chromatography

Preparation of Buffer: Accurately weighed and transfer 1.8gms of Ammonium acetate in 1000 mL of water, adjust the pH with Dil.Orthophosphoric Acid to 4.50± 0.05, filtered and degas prior to use.

Used pH 4.5 Acetate buffer as Mobile Phase-A.

Preparation of Mobile phase-B

Mixed 500 mL of Acetonitrile and 500mL of methanol and degas.

Chromatographic conditions:

- Stationary phase : BDS Hypersil C18 (250 x 4.6 mm,5µ)
- Flow rate : 0.6mL/minute
- Column temperature : 25°C
- Selected wavelength : 292 nm
- Injection volume : 20µL
- Run time : 40minutes

Gradient Programme:

Table 01. Gradient Programme for Chromatographic condition

S.No	Flow Rate(mL/min)	Time	Mobile Phase-A(%)	Mobile Phase-B(%)
01.	0.60	0.01	50	50
02.	0.60	15	40	60
03.	0.60	20	25	75
04.	0.60	30	25	75
05.	0.60	35	40	60
06.	0.60	40	50	50

Preparation of Diluent:

Mixed Mill-Q water and Acetonitrile in the ratio of 50:50(%V/V).

Preparation of Standard(2.5 ppm):

Weighed and transferred 10 mg into 20ml volumetric flask. Added 10ml of diluent and sonicated to dissolved. Made up to the mark with diluent. Further diluted 2ml of the above solution to 100mL volumetric flask and made up to the mark with diluent.

Further diluted 5ml of the above solution to 20mL volumetric flask and made up to the mark with diluent.

Preparation of Sample solution(500ppm):

Weighed and transferred 100 mg equivalent sample powder into 200ml volumetric flask. Added 100ml of diluent and sonicated for 30mins with intermittent shaking to dissolve. Made up to the mark with diluent. Centrifuged the sample solution at 5000RPM about 5mins,Collected the supernatant solution.

D) Identification of Mass of Ionisable Impurities of Edoxaban Tosylate in its Bulk form by Liquid Chromatography-Mass Spectroscopy (LC-MS)

Instruments Used: Make: Shimadzu, Model: LC-MS 2020, Nexera UPLC, Software Used: Lab solutions

Preparation of Buffer: Accurately weighed and transfer 1.8gms of Ammonium acetate in 1000 mL of water, adjust the pH with Dil.Acetic acid to 4.50± 0.05, filtered and degas prior to use. Used pH 4.5 Acetate buffer as Mobile Phase-A.

Preparation of Mobile phase-B

Mixed 500 mL of Acetonitrile and 500mL of methanol and degas.

Chromatographic conditions:

- Stationary phase : Phenomenex Gemini C18 (250 x 4.6 mm,5µ)
- Flow rate : 0.6mL/minute
- Column temperature : 25°C
- Selected wavelength : 292 nm
- Injection volume : 20µL
- Run time : 40minutes

Gradient Programme:

Table 02. Gradient Programme for Chromatographic condition

S.No	Flow Rate(mL/min)	Time	Mobile Phase-A(%)	Mobile Phase-B(%)
01.	0.60	0.01	50	50
02.	0.60	15	40	60
03.	0.60	20	25	75
04.	0.60	30	25	75
05.	0.60	35	40	60
06.	0.60	40	50	50

Preparation of Diluent:

Mixed Mill-Q water and Acetonitrile in the ratio of 50:50(%V/V).

Used Diluent as Blank.

Preparation of Standard(2.5 ppm):

Weighed and transferred 10 mg into 20ml volumetric flask. Added 10ml of diluent and sonicated to dissolved. Made up to the mark with diluent. Further diluted 2ml of the above solution to 100mL volumetric flask and made up to the mark with diluent.

Further diluted 5ml of the above solution to 20mL volumetric flask and made up to the mark with diluent.

Preparation of Sample solution(500ppm):

Weighed and transferred 100 mg equivalent sample powder into 200ml volumetric flask. Added 100ml of diluent and sonicated for 30mins with intermittent shaking to dissolve. Made up to the mark with diluent. Centrifuged the sample solution at 5000RPM about 5mins,Collected the supernatant solution.

Results and Discussion

IR Spectroscopy:

The FT-IR spectral database is a valuable tool for the rapid, simple and cost-efficient identification of different chemicals. All spectra were recorded and evaluated using an FT-IR spectrometer. To diminish the difficulties arising from unavoidable baseline shifts and to improve the resolution of complex bands, the first derivation of the digitized original

spectrum was used. Comparison of identification results reported in the literature showed that an overall correct identification of more than 95% at the species level has not been achieved by any nongenetically method so far.The identification values obtained in this work are well within the range of those having been reported in the literature for identification. When compared with the Edoxaban tosylate API the finished dosage form(Lixiana 60mg Tablets) has shown the correlation was 0.997282.

Hence we can confirms that the selected API(Bulk drug) and Finished dosage form(Lixiana 60mg Tablets) both were same.

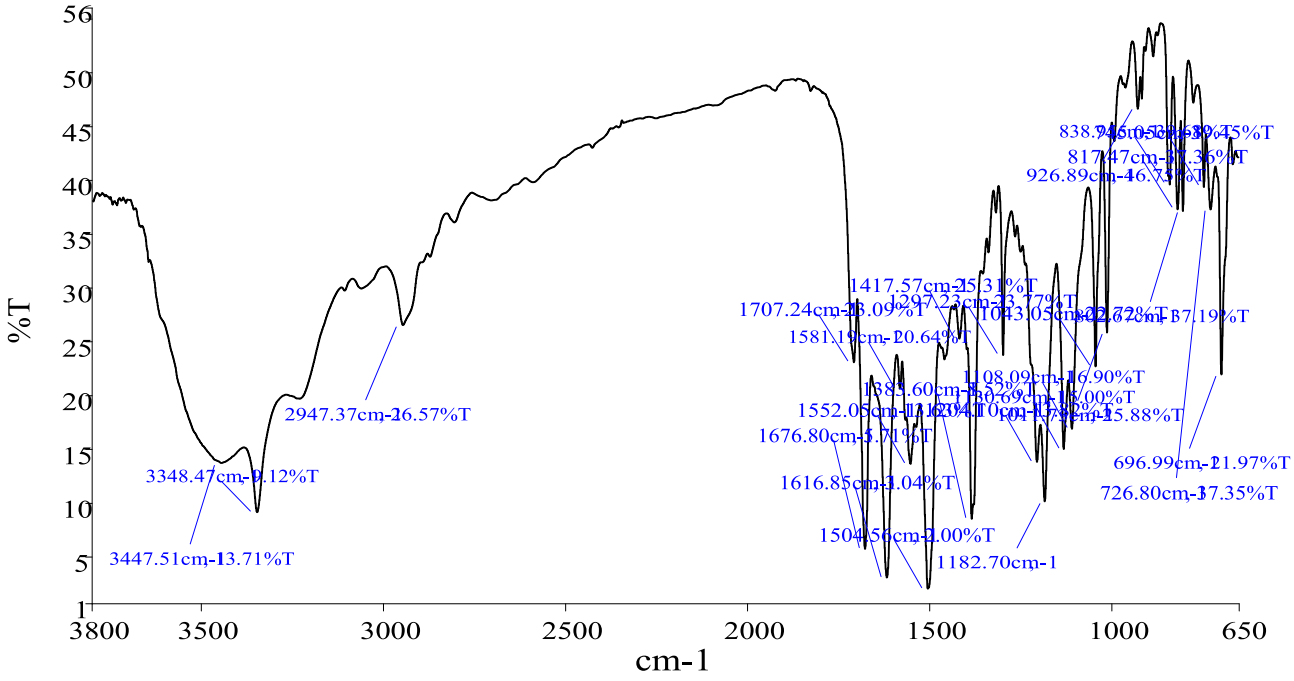
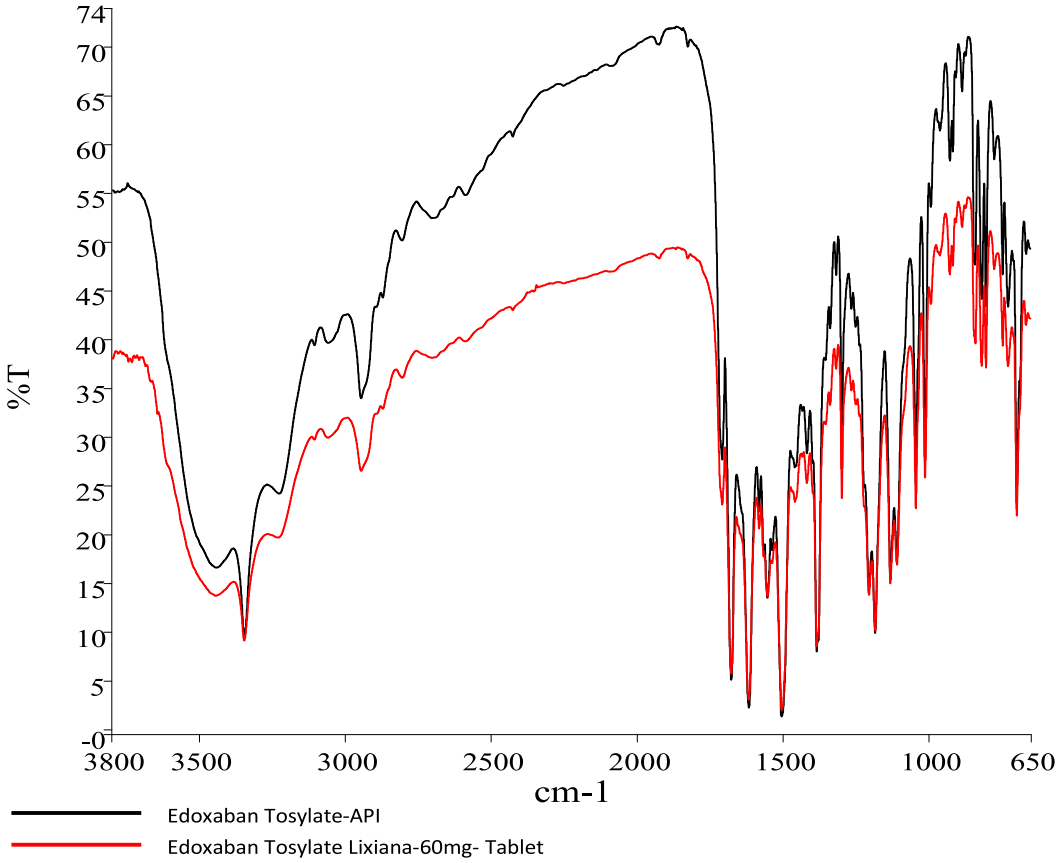


Figure 2. IR Spectrum for of Edoxaban Tosylate API(Bulk Drug)



Source Spectra			
Sample Name	Best Hit	Correlation	Pass / Fail
Edoxaban Tosylate-API	C:\pel_data\spectra\Edoxaban Tosylate Lixiana-60mg-Tablet.sp	0.997282	Pass

Compared References		
Sample Name	Correlation	Pass / Fail
C:\pel_data\spectra\Edoxaban Tosylate Lixiana-60mg- Tablet.sp	0.997282	Pass

Figure 3. IR Spectrum for of Edoxaban Tosylate Bulk Drug and Lixiana 60mg.

UV Spectroscopy

Specificity

Auto zero performed by placed diluent as blank. The UV spectrum of Blank and standard solution and Lixiana 60mg Tablets sample of Edoxaban Tosylate were recorded over the range 200 to 400 nm wavelength range.

No response was observed in Blank. Hence the method is specific.

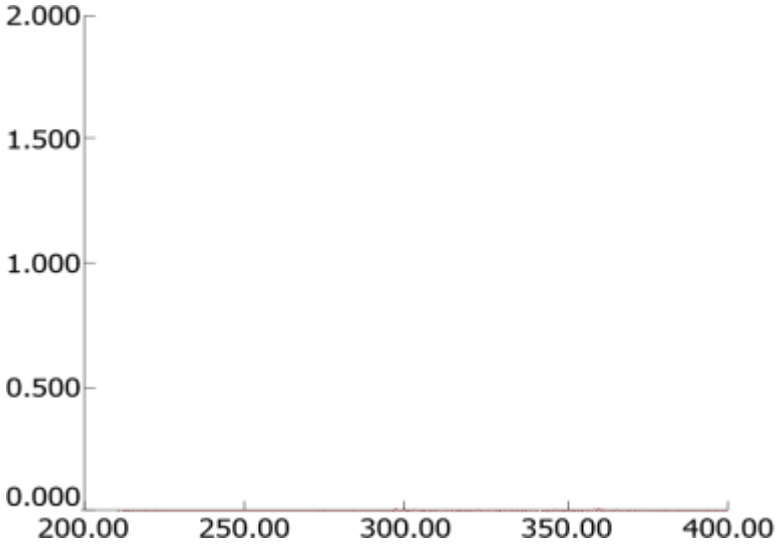


Figure 4. UV Spectrum for of Edoxaban Tosylate Blank.

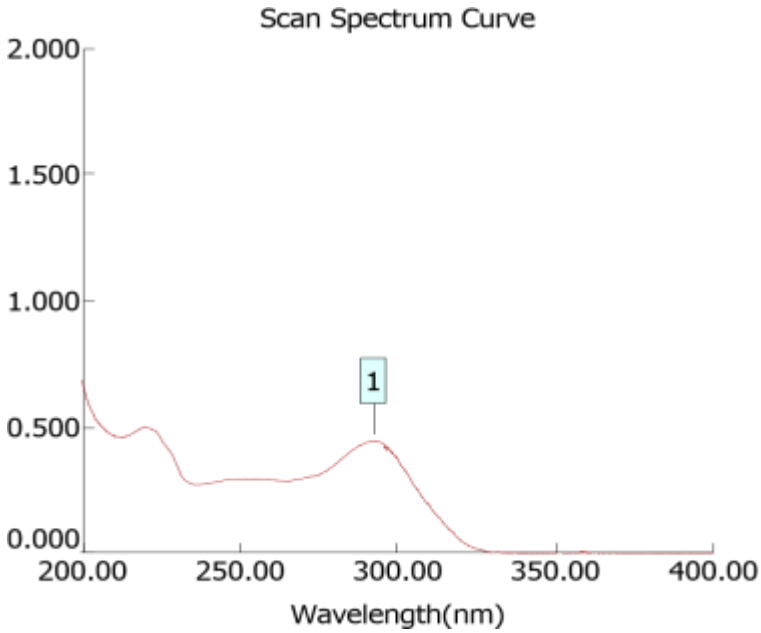


Figure 5. UV Spectrum for of Edoxaban Tosylate Bulk Drug (API)

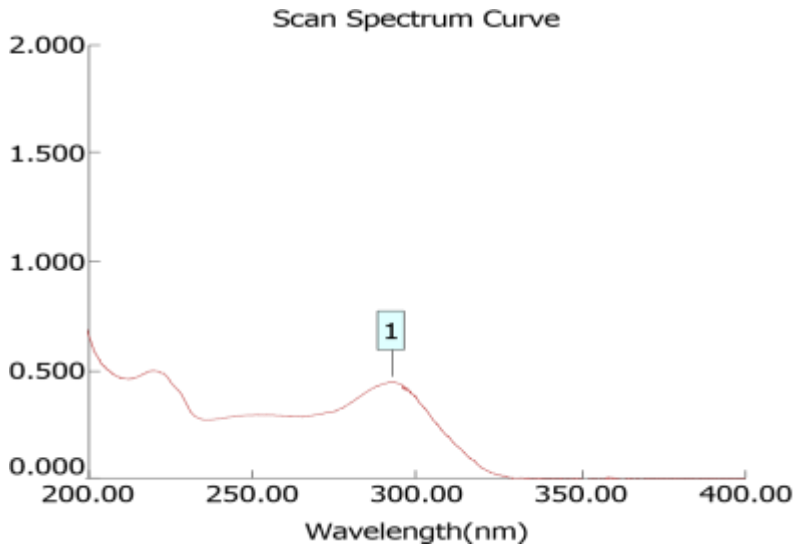


Figure 6. UV Spectrum for of Edoxaban Tosylate Lixiana 60mg Tablets

Linearity

Auto zero performed by placed diluent as blank. The Wave length of Edoxaban tosylate as conformed as 292nm.At this wavelength standard linearity graph has constructed at multiple concentrations of Edoxaban tosylate standard.

With the Standard linearity graph it is concluded that the current UV Spectroscopic method is Linear and can use for regular analysis.

Table 3. Linearity of Edoxaban Tosylate Standard

S. No	ID	Type	Conc [µg/mL]	Abs	292.00 nm
1	Standard-01	Standard	1	0.054	0.054
2	Standard-02	Standard	2	0.108	0.108
3	Standard-03	Standard	3	0.162	0.162
4	Standard-04	Standard	4	0.217	0.217
5	Standard-05	Standard	5	0.269	0.269
6	Standard-06	Standard	10	0.545	0.545

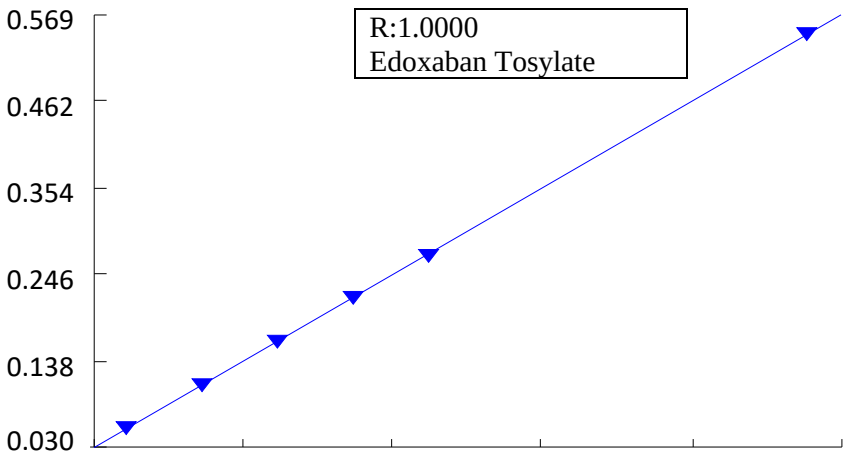


Figure 7. Linearity graph for of Edoxaban Tosylate Standard.

An Ultraviolet–visible spectroscopy method is to determine the Edoxaban Tosylate was validated in respect of specificity and linearity. Hence the method is adequate to quantify the presence of Edoxaban tosylate.

High Performance Liquid Chromatography

The optimized HPLC conditions several mobile phases of different compositions were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak shape, and theoretical plates. For the selection of the mobile phase primarily Buffer: acetonitrile, methanol: Buffer, acetonitrile: water, Acetonitrile : Methanol has been tested for different compositions, flow rates, and ratios. Finally, the mobile phase consisting of a 100%Buffer as mobile phase-A and a mixture of methanol and acetonitrile in the ratio of (50:50% v/v) at a flow rate of 0.6ml/min was found to be satisfactory and proper system suitability parameter results were obtained. The eluted drug peaks in good shape and well resolved.

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of an analytical method is to determine the effect of excipients and other additives that are generally present in the formulation. The test results obtained were contrasted with the results of the standard drug. Injected Blank and Standard into the chromatographic system. No Active peak response was observed in Blank Chromatogram. Hence the method is specified and Used for the Routine Analysis. A typical chromatogram of sample drug of Edoxaban was shown in Figure 9. No interfering peaks

were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method.

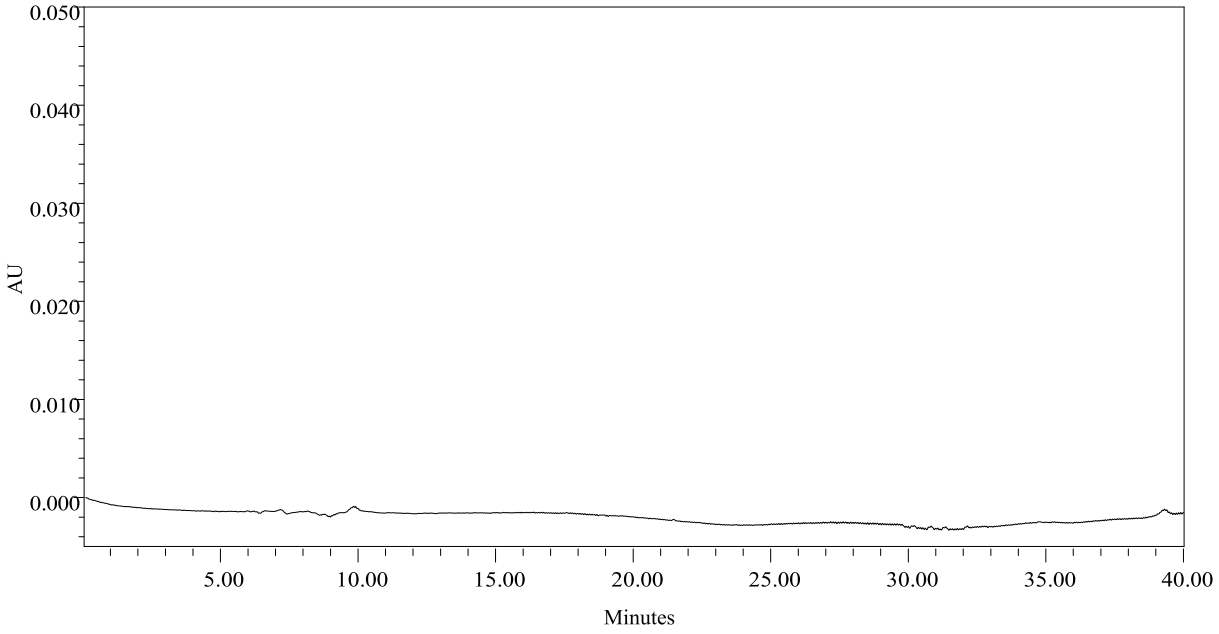


Figure 8. Typical Blank Chromatogram

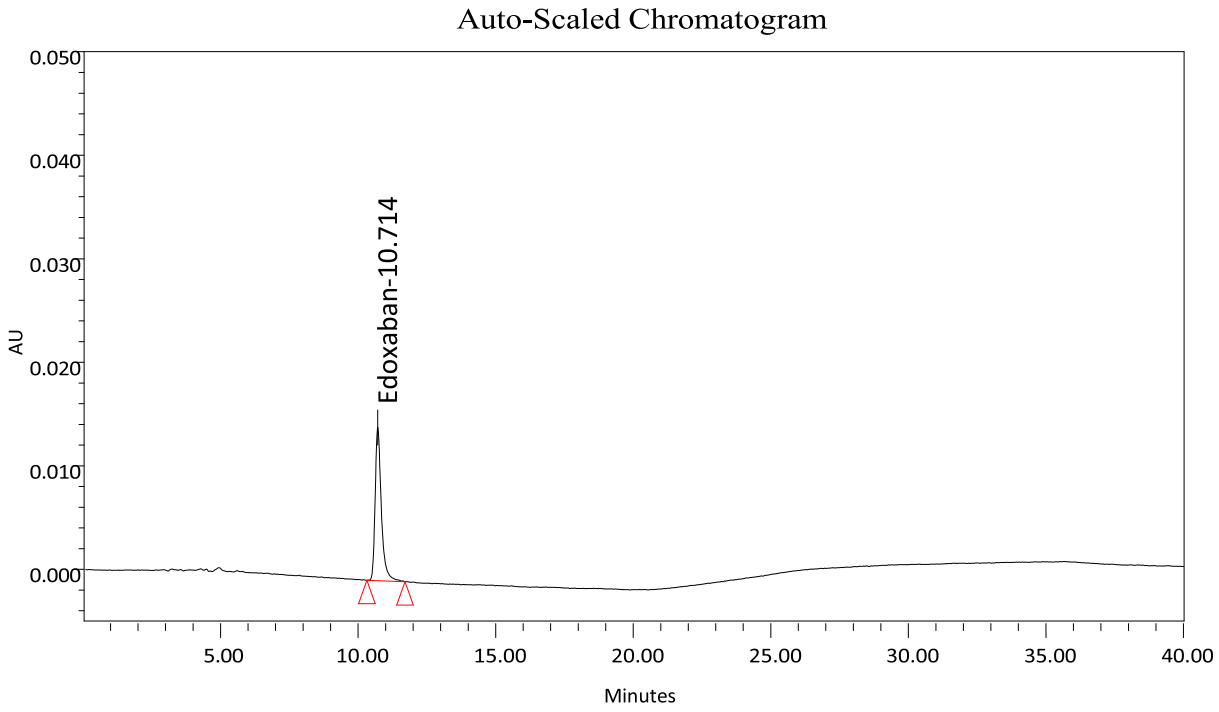


Figure 9. Typical Standard Chromatogram

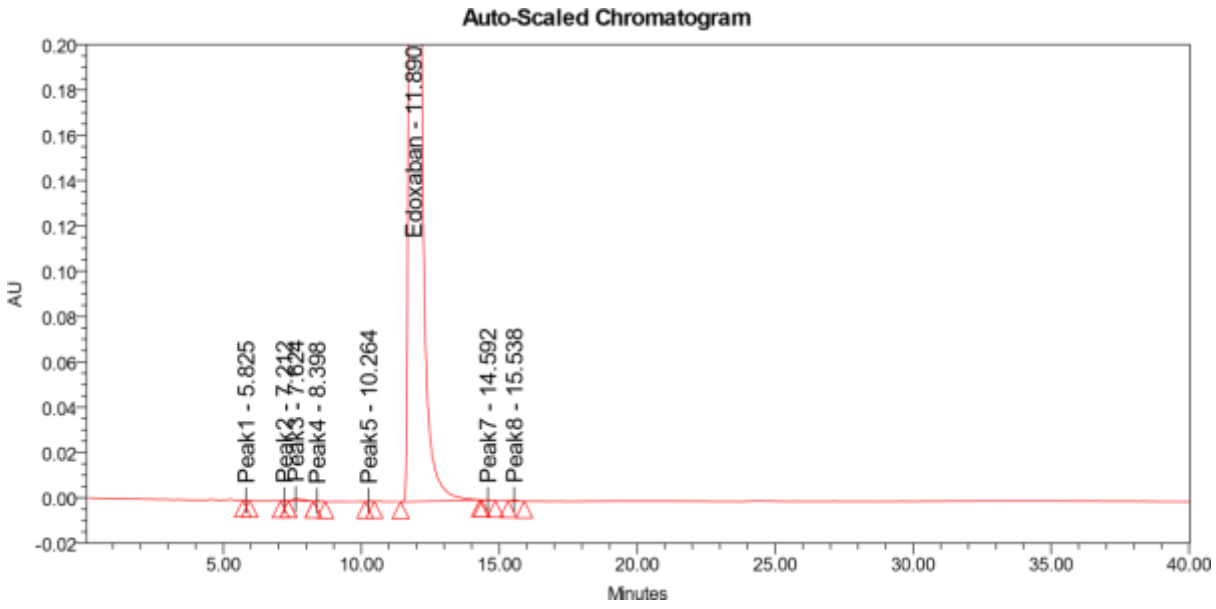


Figure 10.Typical Edoxaban API Chromatogram

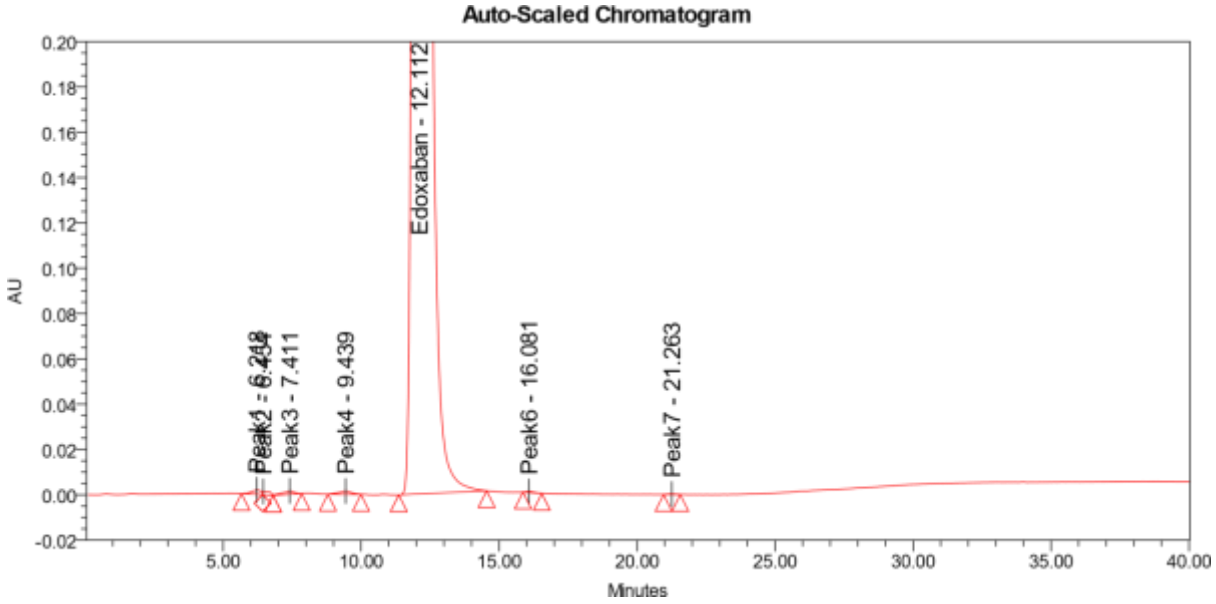


Figure 11. Typical Edoxaban Finished dosage form(Lixiana 60mgTablets) Chromatogram

Liquid Chromatography and Mass Spectroscopy

The optimized HPLC conditions ,several mobile phases of different compositions were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak

shape, and theoretical plates. The developed HPLC method was incorporated to test the Mass spectra of Ionisable compounds separated through HPLC by LC-MS. Minor changes have made in order to get the separation in LC-MS. Active Peak(Edoxaban tosylate API) RT was observed at 10.616 and the peak at RT 5.808 has shown high response when compared with the rest of the impurities. When the sample is subjected to analyse by mass the ESI+ of peak at RT 5.850 has shown 288.9 and at RT 10.700 has shown 548.0.

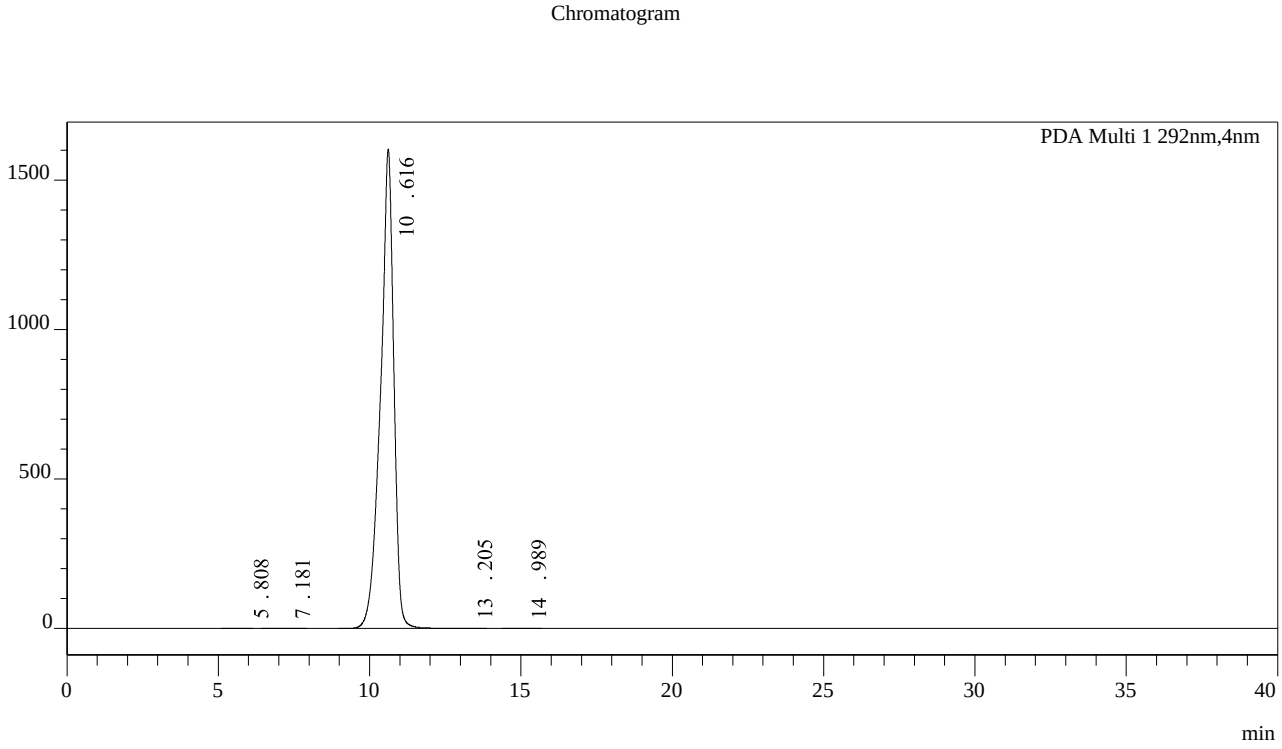


Figure 12. Typical Edoxaban API LC Chromatogram

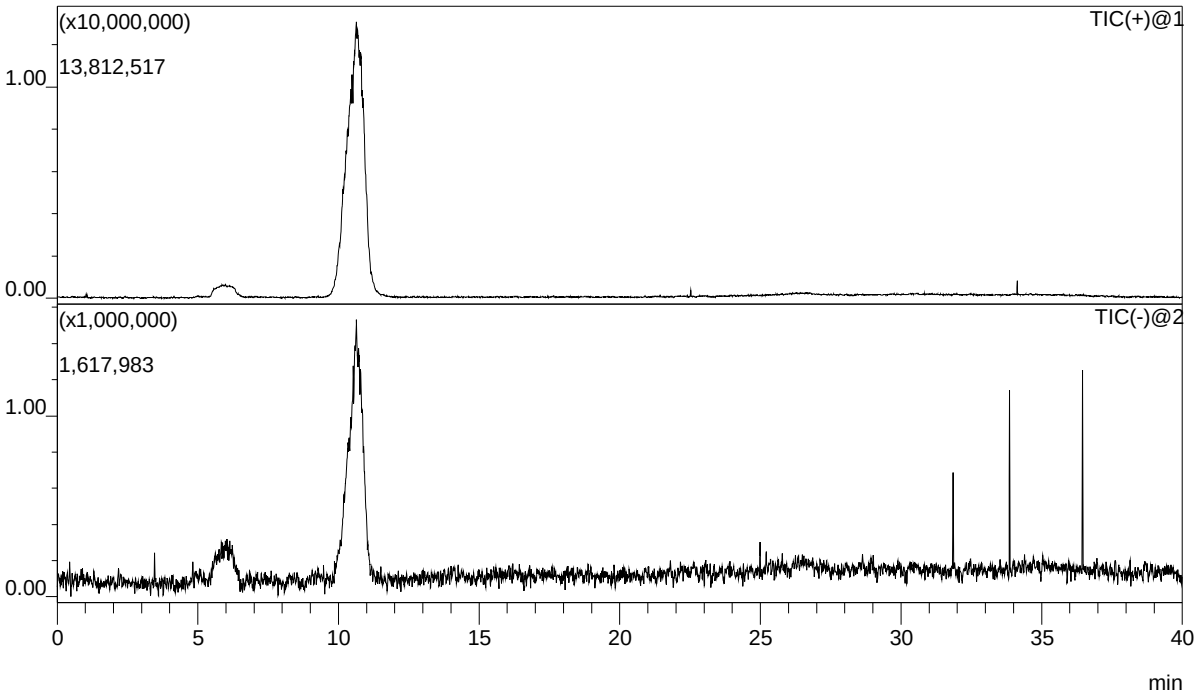


Figure 13. Typical Edoxaban API LC Chromatogram

Table 04. Typical Edoxaban API LC Peak Table

Peak#	Ret. Time	Area	Area%
1	5.808	22994	0.047
2	7.181	29072	0.059
3	10.616	48912227	99.871
4	13.205	4713	0.010
5	14.989	6488	0.013
Total		48975494	100.000

ESI+

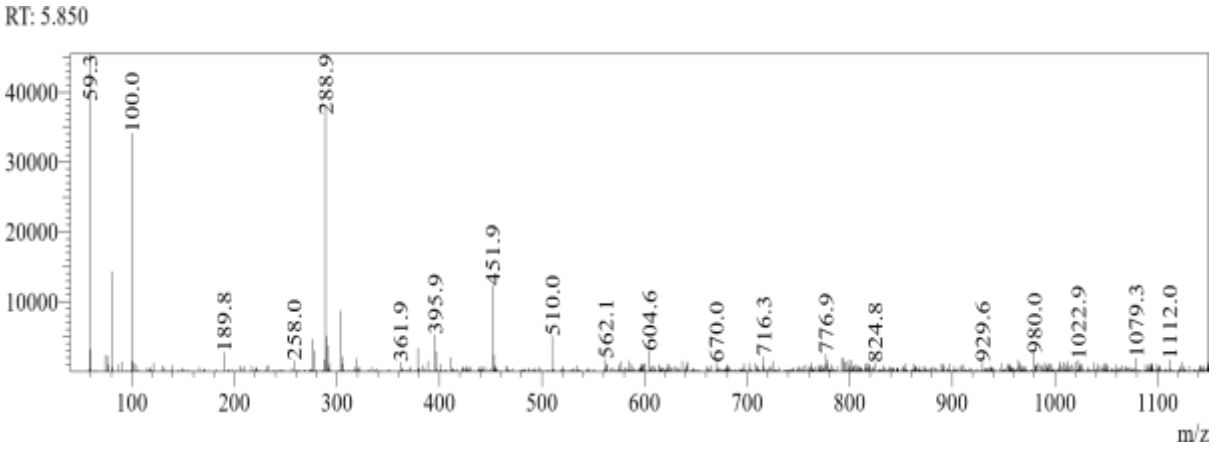


Figure 14. Typical Edoxaban API Mass Spectra-01

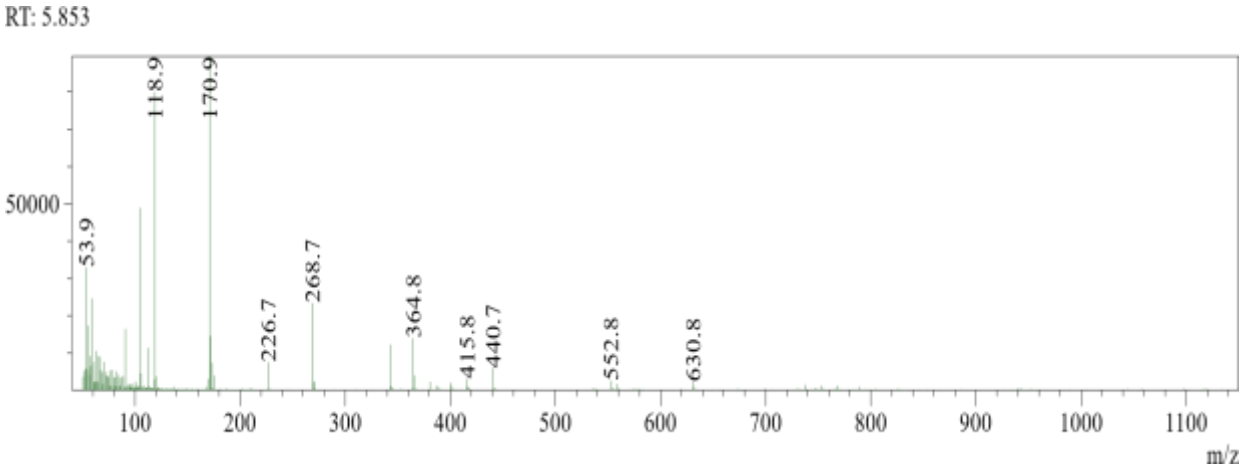


Figure 15. Typical Edoxaban API Mass Spectra-02

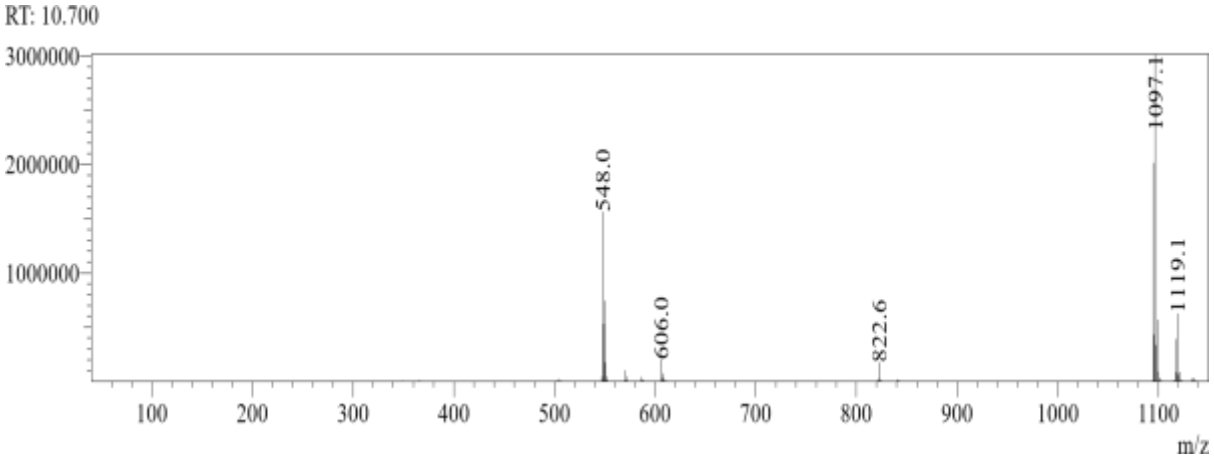


Figure 16. Typical Edoxaban API Mass Spectra-03

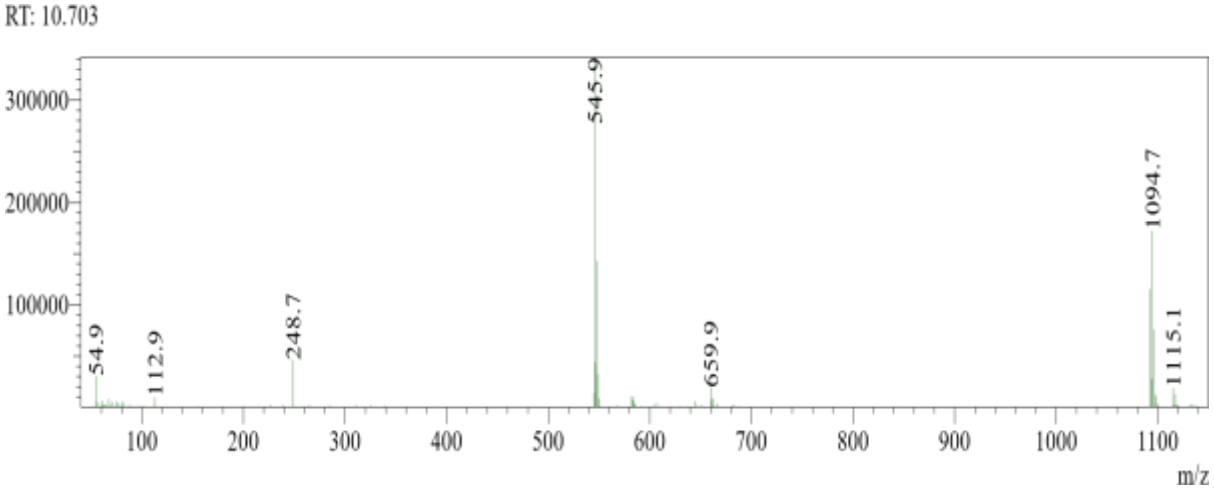


Figure 17. Typical Edoxaban API Mass Spectra-04

Conclusion

The above IR Spectroscopic method was used to Identified procured API and Finished products were same. As well as UV-Visible Spectroscopy was used to develop the Specified and linear method to determine the fixed wavelength for the regular analysis. The proposed Study describes a new HPLC method for the estimation of Edoxaban bulk and in its tablet formulation. The method was found to be simple, Specific and method is free from the interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of Edoxaban in regular quality testing laboratories. The separated impurities were subjected to analyse by LC-MS and found the ionisable compound RT and Mass.

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