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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF DABIGATRAN ETEXILATE

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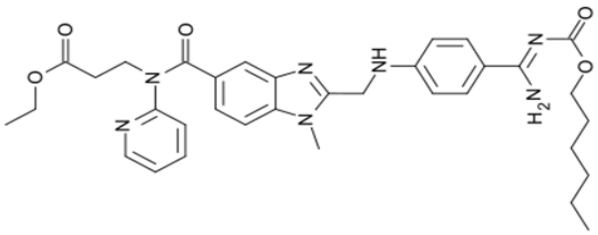
ABSTRACT

A simple, selective and rapid stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Dabigatran Etexilate has been developed and validated. Analyte was resolved on a Neosphere C_8 (150mm X 4.6 mm) column. The mobile phase consisted of Methanol: Phosphate Buffer (0.01M pH 3) in the ratio of 60:40 υ/υ and sonicated to degas. It was delivered at a flow rate of 1ml/min at ambient temperature and the retention time was about 4.4±0.05 minutes. Studies were performed on an HPLC system equipped with a PDA detector at 225nm. The drug was subjected to stress condition of hydrolysis (acid, base, neutral), oxidation, photolysis and thermal degradation. The calibration curve was linear over the concentration range of 1-5µg/ml (R=0.999). The limit of detection for Dabigatran Etexilate was found to be 0.014 µg/ml and the quantification limit was about 0.040 µg/ml. The accuracy of the method was established based on the recovery studies. The proposed method is applicable to the routine analysis of Dabigatran Etexilate.

Keywords: Dabigatran, ICH, Validation, Stability indicating method.

1. INTRODUCTION

Dabigatran etexilate is chemically Ethyl 3-{[(2-{[(4-{*N*'-hexyloxy carbonyl carbamimidoyl} phenyl) amino] methyl}-1-methyl-1*H*-benzimidazol-5-yl) carbonyl] (pyridin-2-amino) propanoate. Dabigatran Etexilate is an oral anticoagulant [1]. When ingested orally is a competitive and reversible direct thrombin inhibitor. Thrombin plays a role in the last step of blood coagulation, being composed of one active site and two secondary binding exosites. The first exosite aids active site binding through docking substrates such as fibrin, while the second binds heparin. Dabigatran thus inactivates both fibrin-bound and free thrombin through binding to the active site; proving more effective than indirect thrombin inhibitors such as unfractionated heparin.



Structure of Dabigatran Etexilate

Literature search reveals following methods reported viz., SIM HPLC [2] method for determination of Dabigatran Etexilate in capsule, Simple RP-HPLC [3] method and UPLC MS/MS [4] method for quantification of Dabigatran in plasma and its assay for therapeutic monitoring. There is a need to develop newer stability indicating method by HPLC to make it simple and economic. So we proceeded with HPLC and validated as per the ICH guidelines. The present analytical work comprises of simple, precise, rapid, sensitive and accurate method for the estimation of Dabigatran Etexilate.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Dabigatran Etexilate was provided as a gift sample by Wockhardt Pharmaceuticals, Aurangabad. It was used as such, without any further purification. Methanol (HPLC grade) purchased from S. D. fine chemical Laboratories, Mumbai, India, Water (HPLC grade).

2.2. Instruments

Jasco HPLC system comprising : Model PU 2080 Plus pump, Rheodyne sample injection port having capacity 20 µl loop, Neosphere C_8 Column, MD 2010 PDA detector, Borwin- PDA software(version 1.5), Jasco Model(V-550) UV-Visible Double beam spectrophotometer, Elga Lab water (PURELAB UHQ-II) HPLC water purification system and Shimdazu Model AY-120 balance was used for weighing purpose.

2.3. Chromatographic Conditions:

The mobile phase consisting of methanol:KH_2PO_4(0.01M pH 3) buffer was filtered through 0.45µ membrane filter, sonicated and was pumped from the solvent reservoir in the

ratio of 60:40 v/v. The flow rate of mobile phase was maintained at 1ml/min and detection wavelength was set at 225nm with a run time of 10 min. The column and the HPLC systems were kept in ambient temperature.

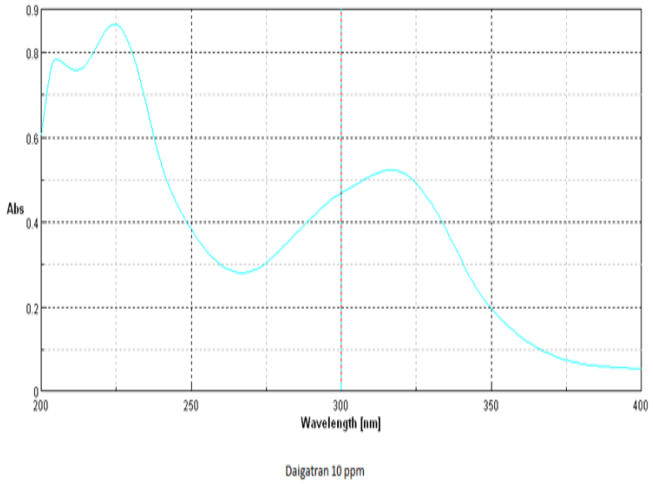


Fig. 1 : UV Specrum of Dabigatran Etexilate

2.4. Buffer Preparation:

Dissolve 1.36 gm of potassium Dihydrogen orthophosphate in1000ml of HPLC water and adjust the pH 3 with o-phosphoric acid, Filter through 0.45µm membrane filter and degas.

2.5. **Mobile Phase:**

Methanol and Buffer were mixed in the ratio of 60:40 v/v and sonicated for 15 min to degas.

2.6. **Preparation of Standard Solution of Dabigatran Etexilate:**

Standard stock solution of Dabigatran Etexilate was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000µg/ml. Further dilutions were made with mobile phase to get final (4 µg/ml) concentration.

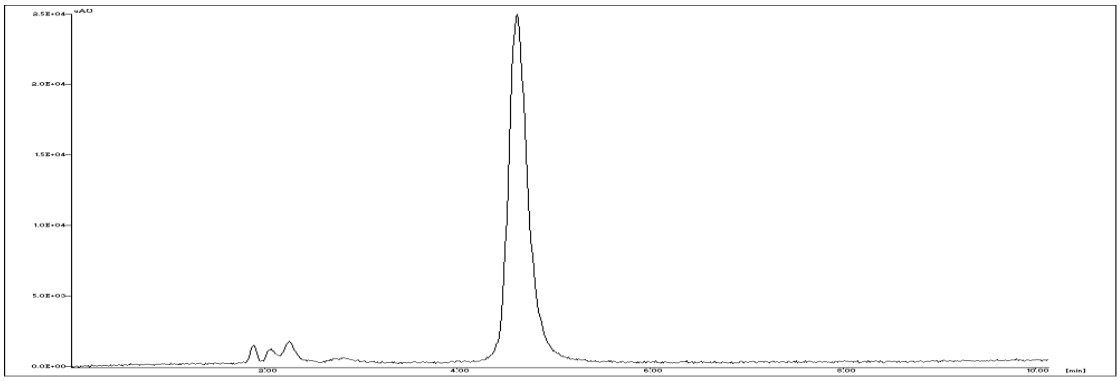


Fig. 2: Chromatogram of standard solution of Dabigatran Etexilate (4µg/ml).

2.7. Stress Degradation Studies of Bulk Drug

Stress degradation studies were carried out as per ICH Q1A(R_2) [5] to provide evidence on how the quality of drug varies with time under the influence of a environmental conditions like temperature, humidity etc.

2.7.1. Alkaline hydrolysis

To 2 ml of Dabigatran Etexilate standard solution (200µg/ml) was added 2 ml of 1N NaOH and volume made up to 10 ml using methanol. Solution was kept for 4 hrs. Neutralization of the alkali treated (40µg/ml) solution was carried out by addition of 0.2 ml 1N HCl. After neutralization, 1 ml of alkali treated solution (40µg/ml) further diluted with mobile phase and volume made up to 10 ml. Final solution (4 µg/ml) was injected into HPLC system. After alkaline hydrolysis, 73.64 % Dabigatran Etexilate was recovered along with peak of degradation**.** Peak purity of Dabigatran Etexilate peak at 4.4 min was within the limits.

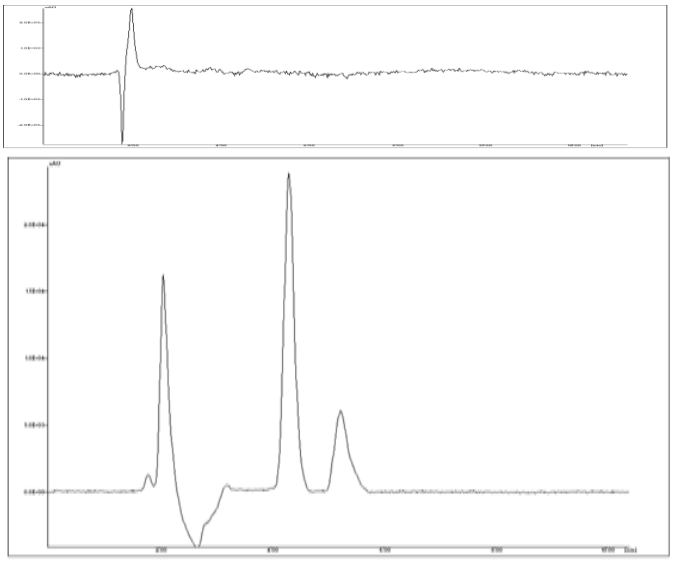


Figure 3 a): Chromatogram of blank and alkali treated solution of Dabigatran Etexilate (4µg/ ml)

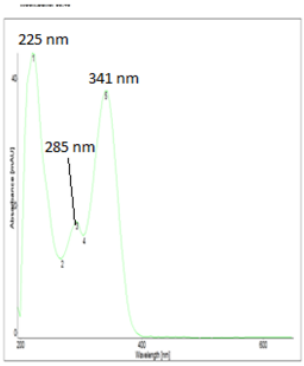


Figure 3 (b)

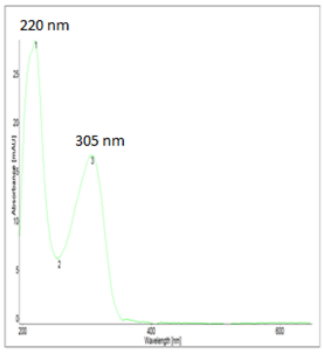


Figure 3 (c)

Figure 3 (b): UV spectrum of peak eluting at 4.4 min (c) UV spectrum of peak eluting at 4.7 min

2.7.2. Acidic hydrolysis

To 2 ml of Dabigatran Etexilate standard solution (200µg/ml) was added 2 ml of 1N HCl and volume made up to 10ml using methanol. Solution was kept overnight. Neutralization of the acid treated (40µg/ml) solution was carried out by adding 0.1 ml 1N NaOH. After neutralization, 1 ml of acid treated solution (40µg/ml) further diluted with mobile phase and volume made up to 10 ml. Final solution (4 µg/ml) was injected into HPLC system. After acid hydrolysis, 76.68 % Dabigatran Etexilate was recovered along with peak of degradation**.** The peak purity for the degradation peak at 4.4 min passes the limits.

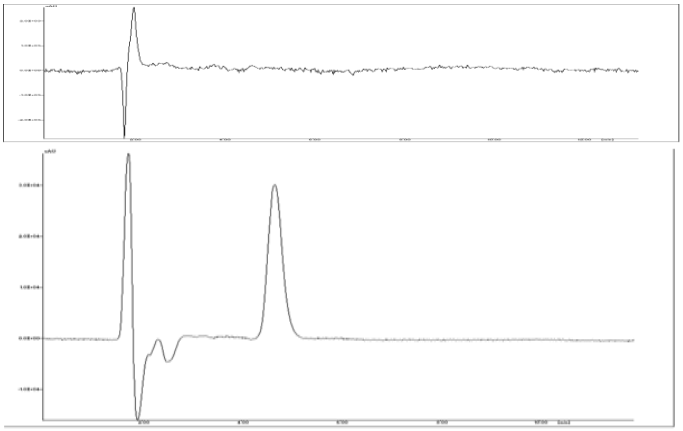


Fig. 4 (a): Chromatogram of blank and acid treated Dabigatran Etexilate (4µg/ml).

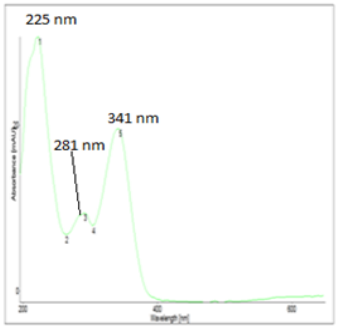


Figure 4 (b)

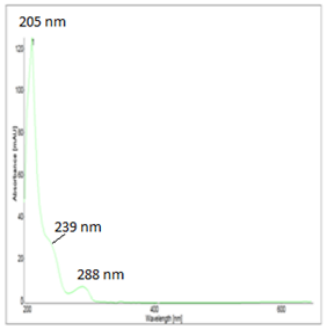


Figure 4 (c)

Figure 4 (b): UV spectrum of peak eluting at 4.4 min (c) UV spectrum of peak eluting at 1.7 min

Table 1: Summary of stress degradation study of Dabigatran Etexilate

Sr. No.	Stress Degradation Condition	% Recovery
1	Base (0.1 N NaOH), kept for 4 hrs.	73.64
2	Acid (0.1 N HCl), Kept for overnight.	76.68
3	Neutral (Refluxed at 60 C for 1 Hr.)	89.63
4	H_2O_2, 30% (Refluxed at 60 C for 1 Hr.)	87.64
5	Dry heat (80^0C for 6 hrs.)	93.60
6	Photo stability [UV, 200 watt hrs/square meter Florescence , 1200 Lux. Hrs]	91.25

2.7.3. Neutral Hydrolysis

To 2 ml of Dabigatran Etexilate standard solution (200µg/ml) was added 2 ml water and volume made up to 10ml using methanol. Solution was refluxed at 60° C for 1 hr. 1 ml of neutral stressed solution (40µg/ml) further diluted with mobile phase and volume made up to 10 ml. Final solution (4µg/ml) was injected into HPLC system. After neutral hydrolysis, 89.63% Dabigatran Etexilate was recovered with no peak of degradation**.** Peak purity of Dabigatran Etexilate peak at 4.4 min was within the limits.

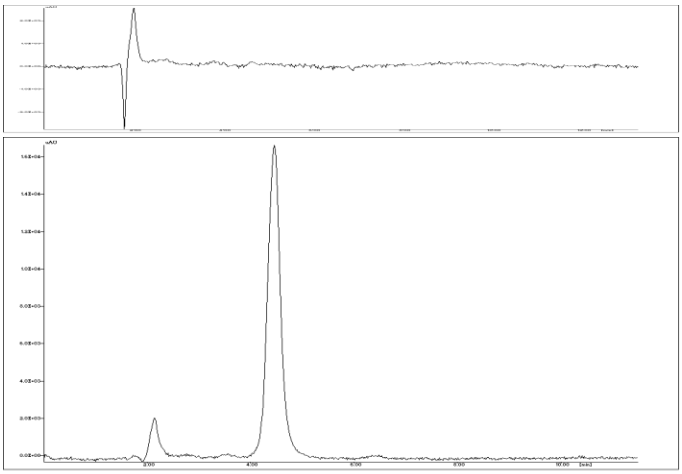


Fig. 5: Chromatogram of neutral treated Dabigatran Etexilate (4µg/ml)

2.7.4. Oxidation Degradation

To 2 ml of Dabigatran Etexilate standard solution (200µg/ml) was added 2 ml 30% solution of H_2O_2 and volume made up to 10ml using methanol. Solution was refluxed at 60° C for 1 hr. 1 ml of peroxide stressed solution (40µg/ml) further diluted with mobile phase and volume madse up to 10 ml. Final solution (4 µg/ml) was injected into HPLC system.

After oxidation degradation, 89.63% Dabigatran Etexilate was recovered with no peak of degradation**.** Peak purity of Dabigatran Etexilate peak at 4.4 min was within the limits.

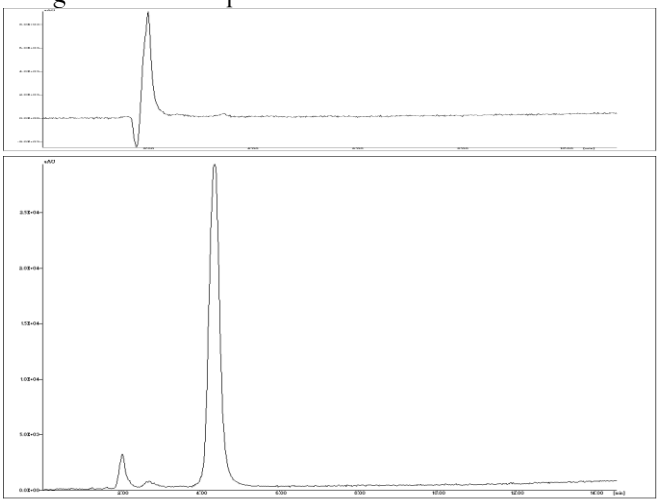


Fig.6 Chromatogram of blank and $H_2O_2$30% treated Dabigatran Etexilate (4µg/ml)

Table 2: System suitability parameter

Name	RT (Min)	Concentration (µg/ml)	Area(µV.Sec)	Asymmetry	Resolution	No of therotical plates
Dabigatran Etexilate	4.4	4	404667	1.281	2.135	7789.0

2.7.5. Degradation under dry heat

Dry heat study was performed by keeping Dabigatran in oven (80^0 C) for a period of 6 hour. A sample was withdrawn after 6 hour, weighed and dissolved in methanol to get solution of 1000 µg/ ml and further diluted with mobile phase to get (4 µg/ ml) as final concentration and was injected in HPLC system. After the dry heat degradation study, 93.6% Dabigatran Etexilate was recovered with no peaks of degradation. Peak purity of Dabigatran Etexilate peak at 4.4 min was within the limits.

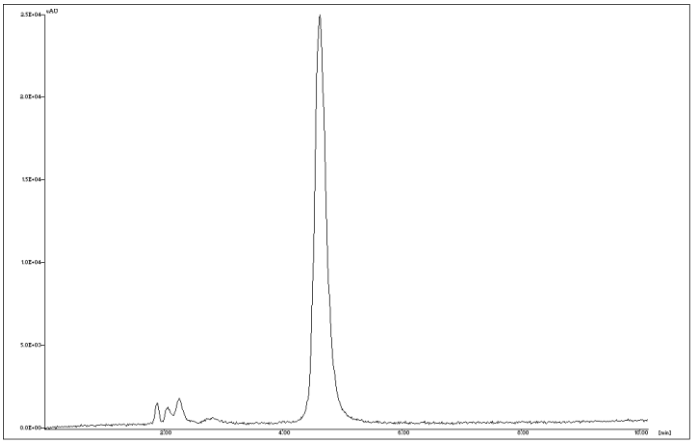


Fig 7 : Chromatogram of Dabigatran Etexilate (4µg/ml) after exposed to dry heat.

2.7.6. Photo-degradation studies

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hours. Sample was weighed, dissolved and diluted, 1 ml of this drug solution (40µg/ml) was diluted to 10ml with mobile phase, Final solution (4 µg/ml) was injected into HPLC system. After the photo degradation study for UV light 91.4% and Fluorescence light 91.0% Dabigatran Etexilate was recovered. After Photo-degradation study, 91.2% Dabigatran Etexilate was recoverd with no peaks of degradation. Peak purity of Dabigatran Etexilate peak at 4.4 min was within the limits.

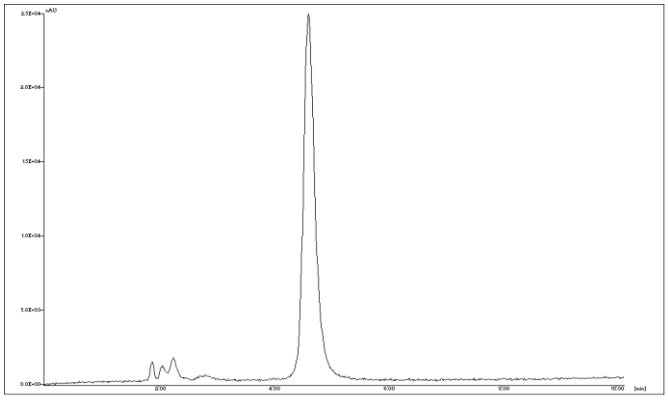


Fig 8: Chromatogram of Dabigatran Etexilate (4µg/ml) after photo degradation

3. RESULTS AND METHOD VALIDATION

The method was validated as per ICH Q2 (R1) guidelines [6].

3.1. Specificity:

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.997, indicating the non interference of any other peak of degradation product or impurity.

3.2. Linearity and Range:

Range is defined as the interval between the upper and lower levels of analyte. Range chosen was 1-5 µg/ml. Linearity was tested for the range of concentrations 1-5 µg/ml. Each sample in five replicates was analyzed and peak areas were recorded. Area was plotted against the corresponding concentrations to obtain the calibration curve. Figure 7 represents the calibration curve for Dabigatran Etexilate. The results obtained are shown in Table 3.

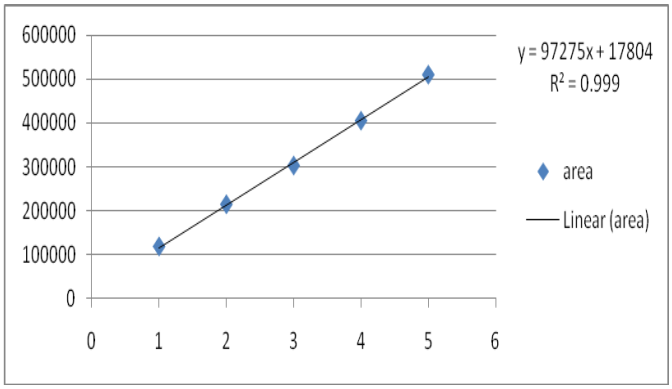


Fig 9: Calibration curve for Dabigatran Etexilate (1-5µg/ml)

Table 3: Results of linearity of Dabigatran Etexilate

Concentration (µg/ml)	Area of Dabigatran Etexilate
1	118059
2	213797
3	302423
4	404667
5	509200
Correlation coefficient (r)	**0.999**
Slope	**97295**
y-intercept	**17704**

Table 4: Recovery studies of Dabigatran Etexilate

Level (%)	Recovered Conc. (µg/ml)	Area of Dabigatran Etexilate	% Recovery
80	3.6	362153	98.09
100	4	404464	98.98
120	4.4	443612	99.65

3.3. Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard solution to sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 4 µg/ml of Dabigatran Etexilate standard solution. These solutions were injected to obtain the chromatogram. The drug concentrations were calculated by using linearity equation of Dabigatran Etexilate. The results obtained are shown in Table 4.

Table 5: Interday and Intraday precision study of Dabigatran Etexilate 3µg/ml

Replicate	Interday	Intraday
1	303471	301510
2	302589	301012
3	304301	301416
4	301584	301173
5	302610	301262
6	3022854	298190
Mean area	302901	300760
Std. Dev.	916.91	1271.52
%RSD	0.30	0.42
RSD	0.003	0.0042

Table 6: Summary of validation study

Validation parameters	Dabigatran Etexilate
Linearity Equation (r2)	Y=97275x+17804
Range	$R^2 = 0.999$ 1-5µg/ml
Precision (% RSD)	
Interday	0.30
Intraday	0.42
Accuracy	% Recovery
80	98.09
100	98.98
120	99.65
Limit of Detection	0.014µg/ml
Limit of Quantitation	0.040µg/ml
Specificity	Specific
Robustness	Robust

3.4. Precision

System precision: Precision of the system was evaluated by analyzing six independent standard preparations and % RSD value obtained was calculated to determine system precision.

Method precision: Precision of the system was evaluated by analyzing six independent sample preparations and % RSD value obtained was calculated to determine method precision. The results obtained are shown in Table 5.

3.5. Limit of detection and quantification (LOD and LOQ)

From the linearity data the limit of Detection and Quantitation was calculated, using the following formula.

LOD= 3.3 σ/ S and LOQ = 10 σ/ S

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

3.6. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, flow rate, pH ware altered and the effects on the peak area were noted.

4. DISCUSSION

The analytical method was developed by optimizing chromatographic conditions. The column used for study was Neosphere C_8, 150 x 4.6 mm. Since it gave acceptable system suitability parameter. Ambient temperature was found to be suitable for the drug analysis. The flow rate was fixed at 1 ml/min because of satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of (60:40 ʊ/ʊ) Methanol: Phosphate buffer was fixed due to good symmetrical peak. Run time was selected to be 10 min because analyte elute at around 4.4 min. The percent recovery was found to be 98.05-99.65. Both Intraday and Interday precision was found to be well within range. In specificity study all degradant impurity was resolved from the analyte peak. The analytical method was found linear over the range of 1-5µg/ml.

5. CONCLUSION

The primary target in developing this stability indicating HPLC method is to achieve the resolution between Dabigatran Etexilate and its degradation products. Forced degradation study showed the method is highly specific and no degradation products were eluted at drugs RT. The developed method was found to be specific and method was validated as per ICH. Degradation was observed for stress conditions like base, acid, oxidation except in photo degradation, dry heat. Proposed study describes new LC method for the estimation of Dabigatran Etexilate. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of Dabigatran Etexilate.

6. ACKNOWLEDGEMENT

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7. REFERENCES

1. www.en.m.wikipedia.org/wiki/Dabigatran.com (accessed on 15/5/2014).
2. Bernardi, Raquel, Pedro E et.al,. *An international journal of Analtical Sciences*, 2013; 37-41.
3. Bernardi, Raquel, Pedro E et.al, *Journal of Analytical Methods*, 2013; 95-98.
4. Xavier, Julie, Silvy et al., *Journal of Pharmaceutical and Biomedical Analysis*, 2012; 152-156.
5. Validation of Analytical Procedures: Text and Methodology Q1A (R2), ICH Harmonized Tripartite Guideline, Geneva Switzerland, 2003.
6. Validation of Analytical Procedures: Text and Methodology Q2(R1), ICH Harmonized Tripartite Guideline, Geneva Switzerland, 2003.