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A new and stability indicating liquid chromatographic method for the determination of dabigatran in bulk drug and pharmaceutical dosage form

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ABSTRACT

The objective of the current study was to develop a validated, simple, precise, stability indicating reverse phase HPLC method for the determination of Dabigatran etexilate mesylate in bulk drug and dosage form. LC separation was achieved gradient mode on a Zorbax SB C18 (4.6x150) mm, 3.5 µm column using mobile phase containing solution A (2.72g of potassium dihydrogen phosphate in 1000 ml of water) PH 4.5 with ortho phosphoric acid solution B (acetonitrile) at flow rate 1.0 ml/min. The method employed a linear gradient elution and detection wavelength was set at 220 nm and temperature was 25°c. The retention time was 8.65 min and linearity was observed in the concentration range of 24-180 µg/ml with correlation coefficient of 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The method was successfully validated as per International Conference on Harmonization (ICH) guidelines. Dabigatran undergoes degradation under acidic, basic, oxidation, dry heat and photolytic conditions, degradation impurities did not interfere with the retention time of Dabigatran, and assay method is thus stability indicating.

KEYWORDS

Dabigatran;
Validation;
HPLC;
Stability indicating.

INTRODUCTION

Dabigatran is an oral anticoagulant from the class of the direct thrombin inhibitors, it was developed by the pharmaceutical company Boehringer Ingelheim. Dabigatran is used to prevent stokes in those with atrial fibrillation due to causes other than heart valve disease, and at least one additional risk factor for stroke and to prevent the formation of blood clots in the veins in adults who have had an operation to replace a hip or knee. The most common side effect of dabigatran is bleeding. IUPAC name of Dabigatran etexilate mesylate is N-[[2-[[[4-[[[(Hexyloxy)carbonyl] amino]iminomethyl] phenyl] amino] methyl]-1-methyl-1H-benzimidazol-5-yl]carbonyl]-N-2-pyridinyl-beta-alanine ethyl ester monomethanesulfonat. Dabigatran etexilate mesylate is available as capsule at the dose of 110 mg and 150 mg in the market under the brand name of Pradaxa, molecular formula is $C_{34}H_{41}N_7O_5 \cdot CH_4O_3S$ and having molecular weight 723.84[13,14].

A few chromatographic methods have appeared in literature for dabigatran by UPLCMS/MS assay in hu-

man plasma[1], pharmacokinetics, pharmacodynamics[2-9] and GC method for potential impurity's[10]. However no method is available for determination of dabigatran in bulk drug and pharmaceutical dosage form. In the present work we have developed a new, simple precise and stability indicating method for determination of dabigatran.

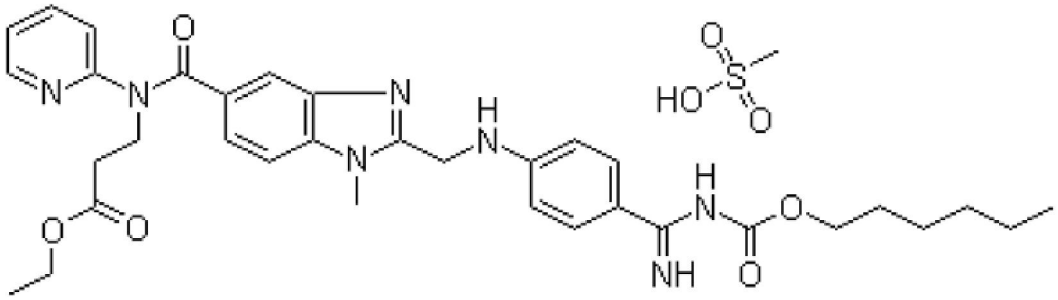


Figure 1 : Structure of dabigatran etexilate mesylate

EXPERIMENTAL

Chemicals and reagents

Dabigatran is available as tablets with brand name PRADAXA was purchased from local market, containing dabigatran 150mg. HPLC grade acetonitrile, AR grade potassium dihydrogen phosphate and Phosphoric acid were purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 0.02M potassium dihydrogen phosphate in water (2.72g of potassium dihydrogen phosphate in 1000 ml of water) PH 4.5 with ortho phosphoric acid. A, Zorbax SB C18 (4.6x150) mm 3.5 µm column and gradient mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The gradient program (T/%B) was fixed as 0/40, 5/50, 10/70, 15/70, 15.1/40, 20/40. Water: Acetonitrile (50:50) v/v used as diluent. The column oven maintained at 25°c with 1.0ml flow rate. An injection volume 20µl was used. The elution compounds were monitored at 220 nm.

Preparation of stock and standard solutions

Accurately 100mg of Dabigatran standard dissolved in 100ml diluent to get a concentration of 1000µg/ml. Further 12ml of stock solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 120µg/ml.

Preparation of tablets for assay

The formulation tablets of Pradaxa were crushed to give finely powdered material. Powder equivalent to 100mg of drug was weighed and transferred to the 100ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 12ml of this solution was taken into 20 ml flask and diluted volume with diluent to get concentration 120µg/ml.

Forced degradation studies/specificity

Forced degradation studies were performed to evaluate the stability indicating properties. All solutions for used in stress studies were prepared at an initial concentration of 1000µg/ml of Dabigatran.

Acid degradation studies

Acid decomposition was carried out in 0.1N HCL at concentration of 1000µg/ml Dabigatran and after refluxation for 5hours at 80°c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in Figure 3(g). The results are tabulated in TABLE 3.

Alkali degradation studies

Base decomposition was carried out in 0.04N NaOH at concentration of 1000µg/ml Dabigatran and after room temperature for 15minites, the stressed sample was cooled, neutralized and diluted as per re-

quirement with diluents filtered and injected. The resulting chromatogram is shown in Figure 3(i). The results are tabulated in TABLE 3.

Oxidation

Oxidation was conducted by using 7%H2O2 solution at room temperature for 2hours, 12ml of solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 120µg/ml filtered and injected. The resulting chromatogram is shown in Figure 3(k). The results are tabulated in TABLE 3.

Temperature stress studies

1g of Dabigatran sample was taken into a petridish and kept in oven at 80°c for 7 days. 100mg of sample was taken into 100 ml flask diluted volume with diluent, further 12ml to 100ml made up with diluent. The results are tabulated in TABLE 3.

Photo stability

1g of Dabigatran was taken in to a petridish and kept in photo stability chamber 200 W.hr/m^2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 100mg of sample was taken in 100ml flask, dissolved in diluent, further 12ml in 100ml flask diluted volume with diluent. The results are tabulated in TABLE 3.

RESULTS AND DISCUSSION

HPLC method development and optimization

To develop a rugged and suitable HPLC assay method for the determination of Dabigatran, the analytical condition were selected after the consideration of different parameters such as diluents, buffer, organic solvent for mobile phase, column and other chromatographic conditions[12]. Initial trails were performed with different composition of buffer (acetate and formate) and organic phase (methanol, teterhydrofuran) with different column like C8, phenyl, cyno, amino and basic but Dabigatran peak shape was not good. Finally 0.02M potassium dihydrogen phosphate in water PH 4.5 with ortho phosphoric acid and acetonitrile with gradient and Zorbax SB C18 (4.6x150) mm 3.5 µm column was optimized. Different diluents were tried to dilute sample like water,buffer, methanol, tetrahydrofuran and mixture of water: methanol and water: teterhydro-furan,buffer:methanol and buffer:acetonitrile. Dabigatran was not dissolved, finally (water: acetonitrile) (50:50) v/v was optimized. The detection wavelength was chosen as 220nm for Dabigatran because they have better absorption and sensitivity at this wavelength (Figure 2). Hence selected method was best among the all trails by many aspects.

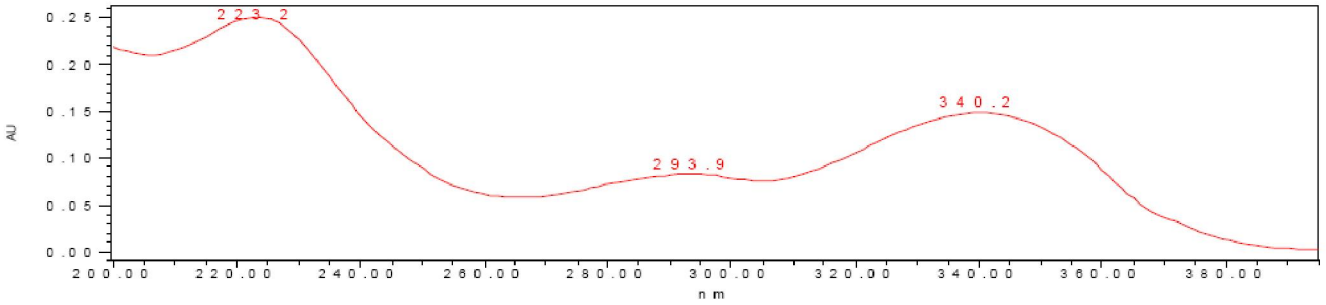


Figure 2 : Wavelength spectrum of dabigatran

METHOD VALIDATION

Specificity

A study to establish the interference, blank detection was conducted. Diluent was injected as per the test method. Solution of standard and sample were prepared as per test method and injected into the chromatographic system. The chromatograms of blank, standard and sample were shown in the Figure a, b, c.

Precision

The precision method was established by evaluating method precision and intermediate precision study. Method precision was determined by analyzing six independent assays were performed and calculated the % RSD for replicate assay determinations. Intermediate precision of the analytical method was determined by conducting method precision on another day and another analyst under same experiment condition. The result obtained for method precision and intermediate

precision are shown in TABLE 4. The percentage of RSD was calculated. The %RSD range was obtained as 0.08 and 0.16 for method precision and intermediate precision respectively (TABLE 4) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was estimated by determination of recovery for three concentrations (corresponding to 50, 100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Dabigatran were calculated, the results obtained are shown in TABLE 2. The percentage recovery was found to be 99.49-99.9% with %RSD 0.08 - 0.18 (<2.0%) indicating that the method is more accurate (TABLE 2)

LOD and LOQ

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.05µg/ml and 0.17µg/ml respectively. The %RSD value was noticed to be less than 1.0% at LOQ concentration level.

Linearity

The linearity plot was prepared with six concentration levels (24, 48, 96, 120, 144 and 180 µg/ml of Dabigatran). These concentration levels were respectively corresponding to 20, 40, 80, 100, 120 and 150 % of test solution concentration. The results obtained are shown in TABLE 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (Figure 4).

Robustness

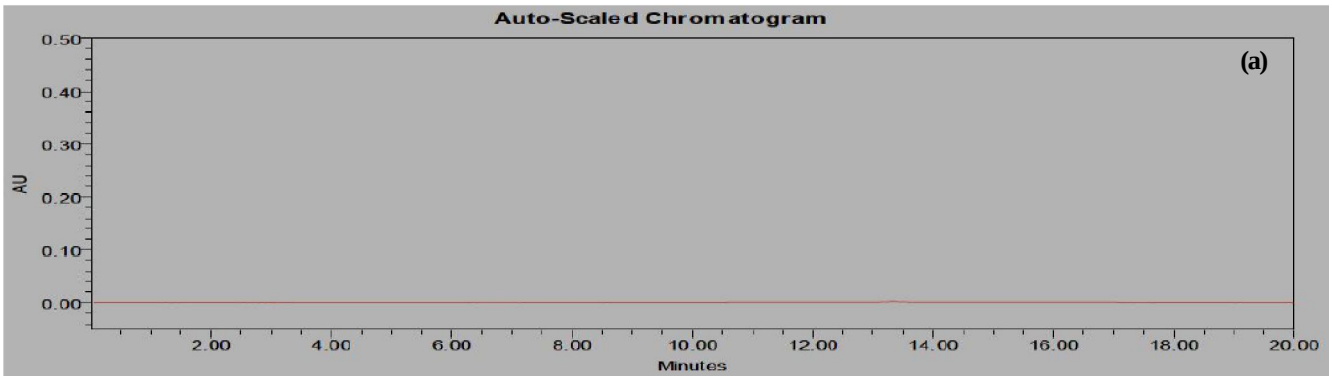
Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min), PH (±0.1units) and column temperature (±5°c). In the all above varied conditions, the components of the mobile phase were held constant. The results are tabulated in TABLE 5. Under all the deliberately varied chromatographic conditions, the reproducibility of results was observed to be reasonably good. Hence the proposed method has good robustness for the assay of Dabigatran in bulk and dosage forms

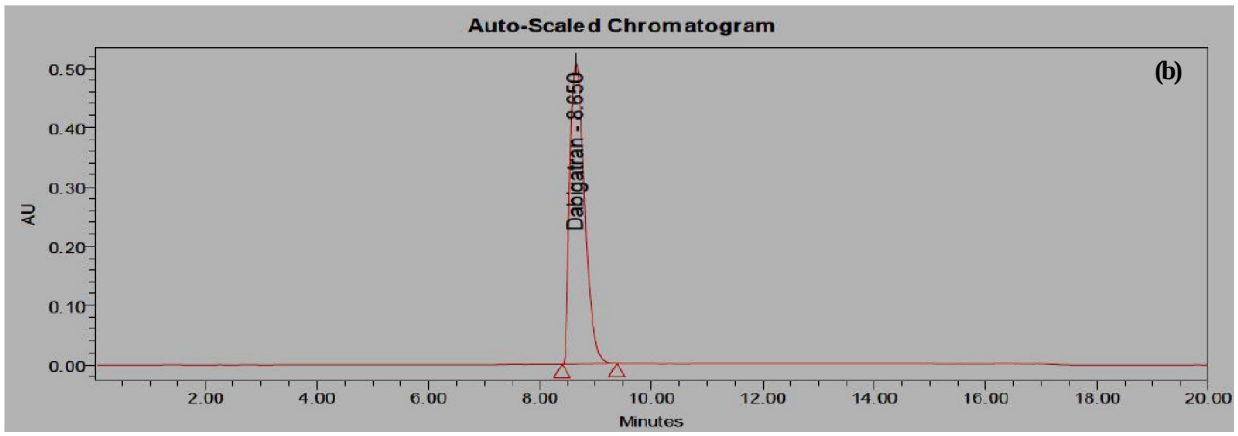
Solution stability and mobile phase stability

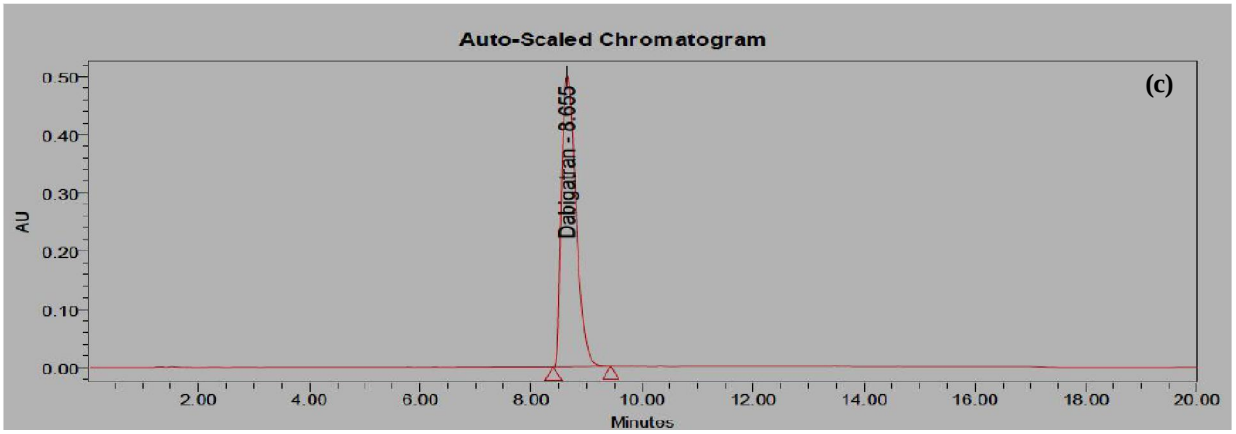
Solution stability checked for stability of standard and sample solutions. Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

Forced degradation studies

Stress studies on Dabigatran were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in acid (Figure 3g), base (Figure 3i) and oxidation (Figure 3k) of Dabigatran. There was no significant degradation of Dabigatran upon exposure to dry heat at 80°c for 7days and photolysis total impurity increased to 0.12% and 0.15%, which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.




Auto-Scaled Chromatogram
(b)
Dabigatran - 8.650
AU
Minutes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(c)
Dabigatran - 8.655
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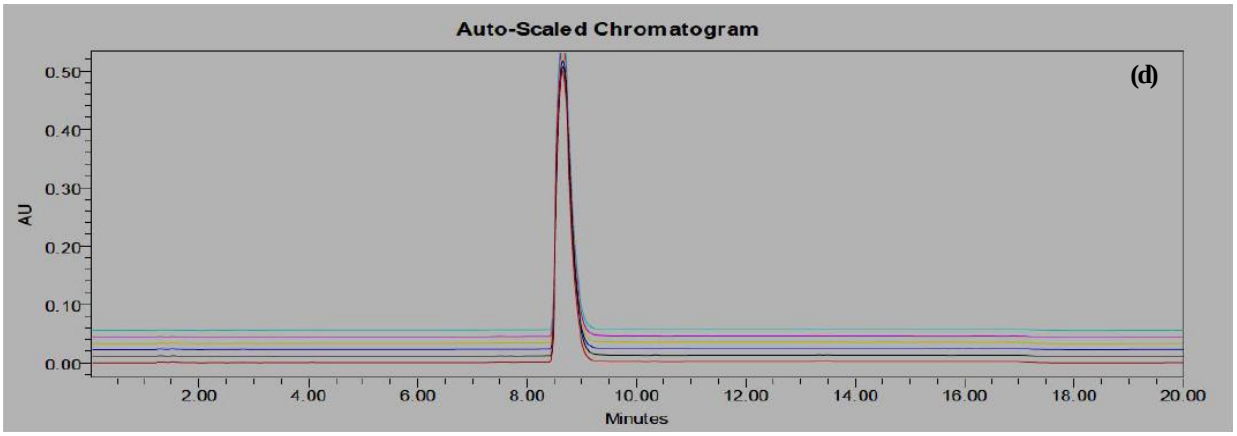
Minutes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(d)
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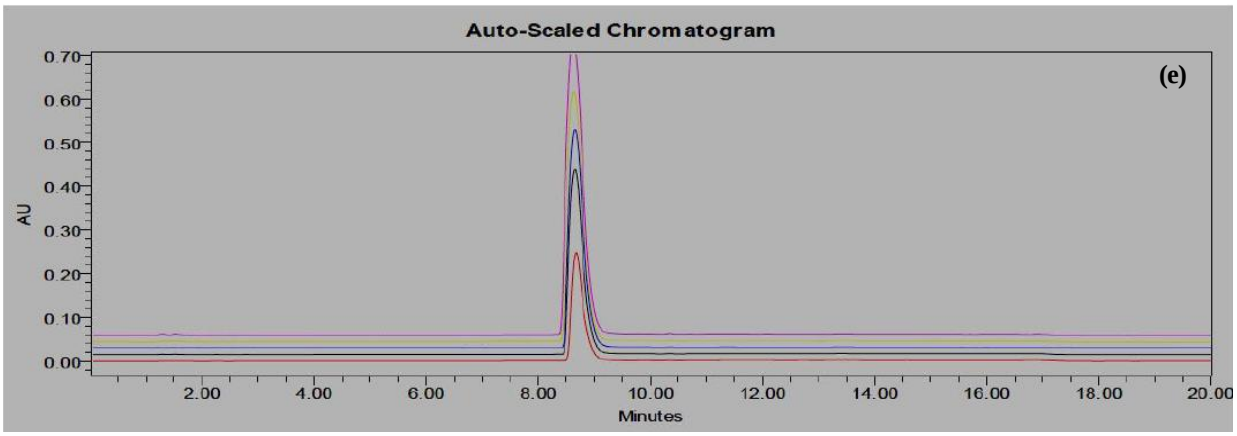
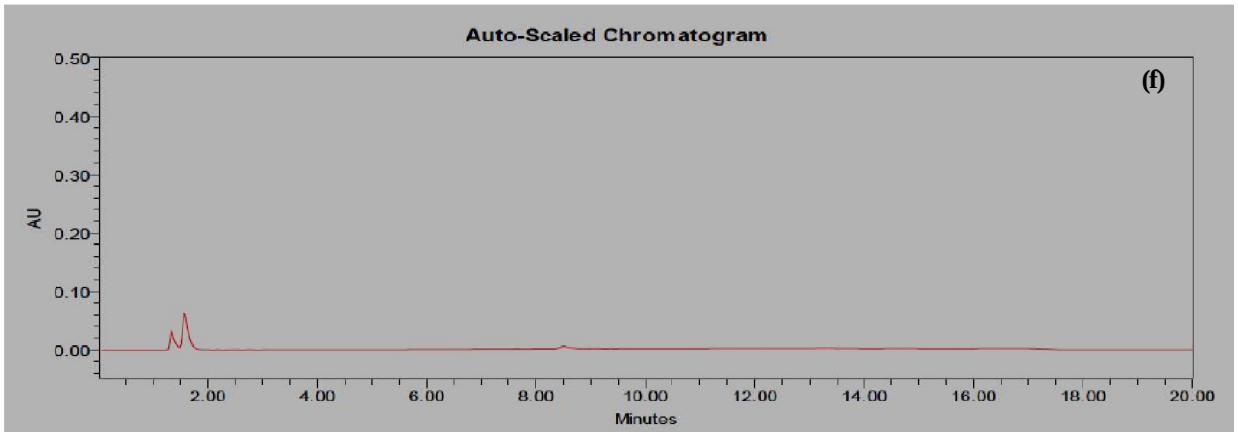
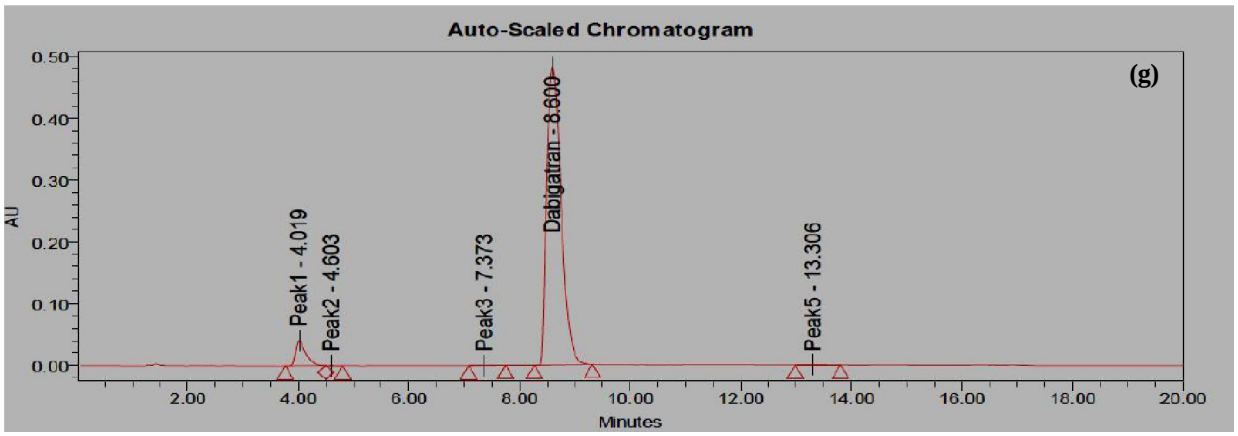
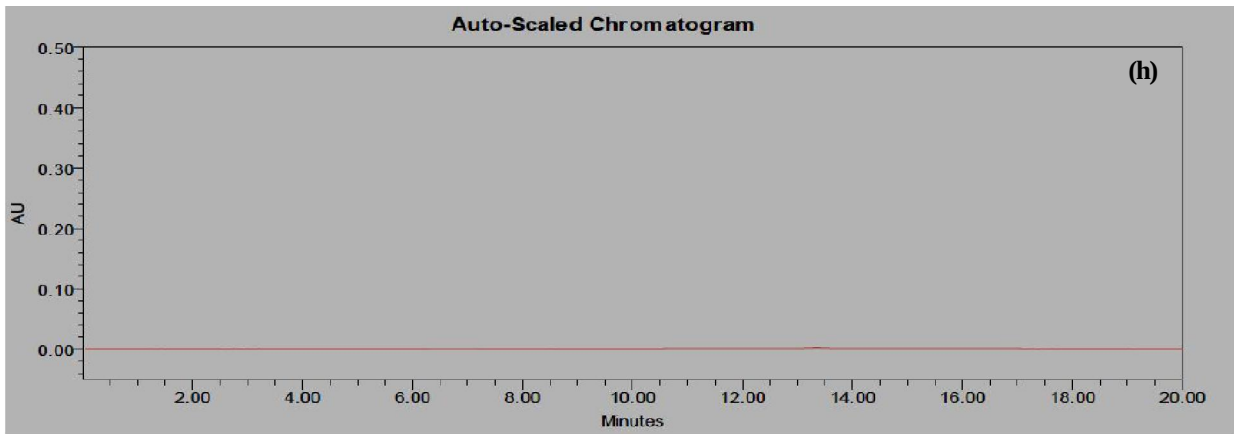
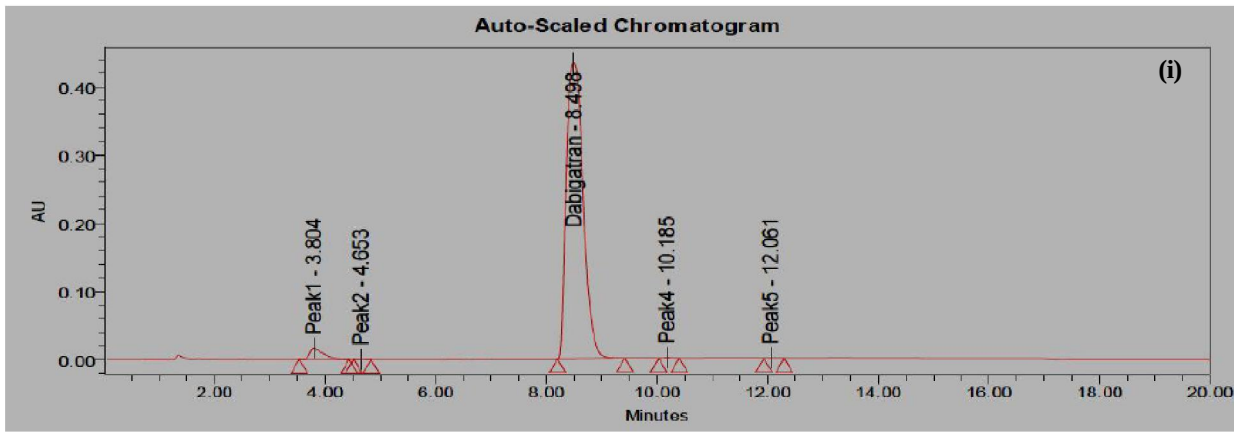

Auto-Scaled Chromatogram
(e)
AU
Minutes


Auto-Scaled Chromatogram
(f)
AU
Minutes


Auto-Scaled Chromatogram
(g)
Peak1 - 4.019
Peak2 - 4.603
Peak3 - 7.373
Dabigatran - 8.600
Peak5 - 13.306
AU
Minutes


Auto-Scaled Chromatogram
(h)
AU
Minutes


Auto-Scaled Chromatogram
(i)
Peak1 - 3.804
Peak2 - 4.653
Dabigatran - 8.498
Peak4 - 10.185
Peak5 - 12.061
AU
Minutes

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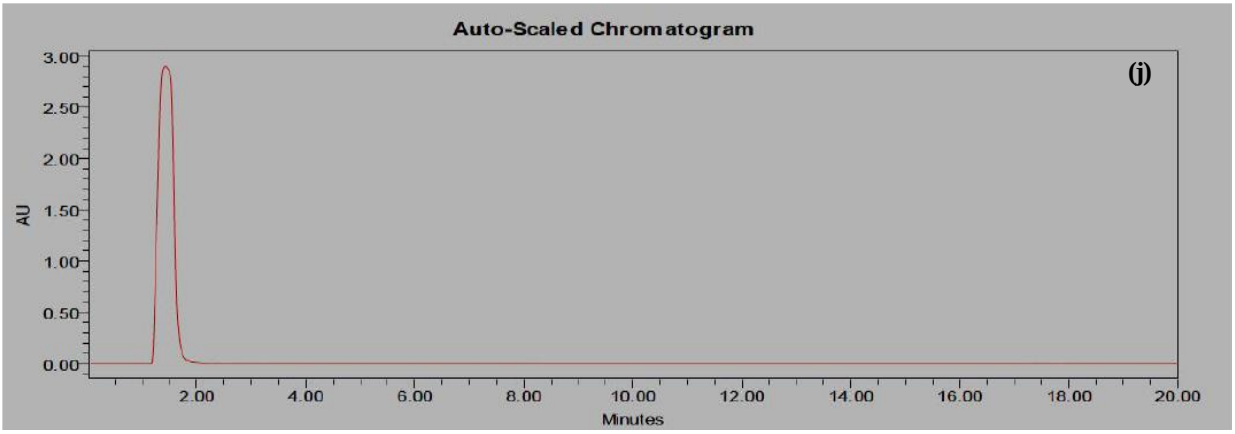
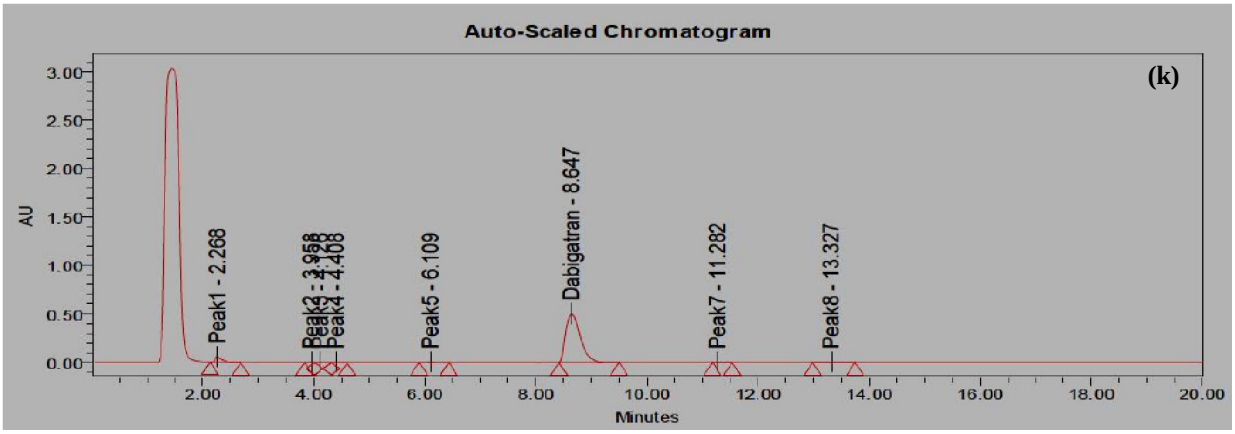
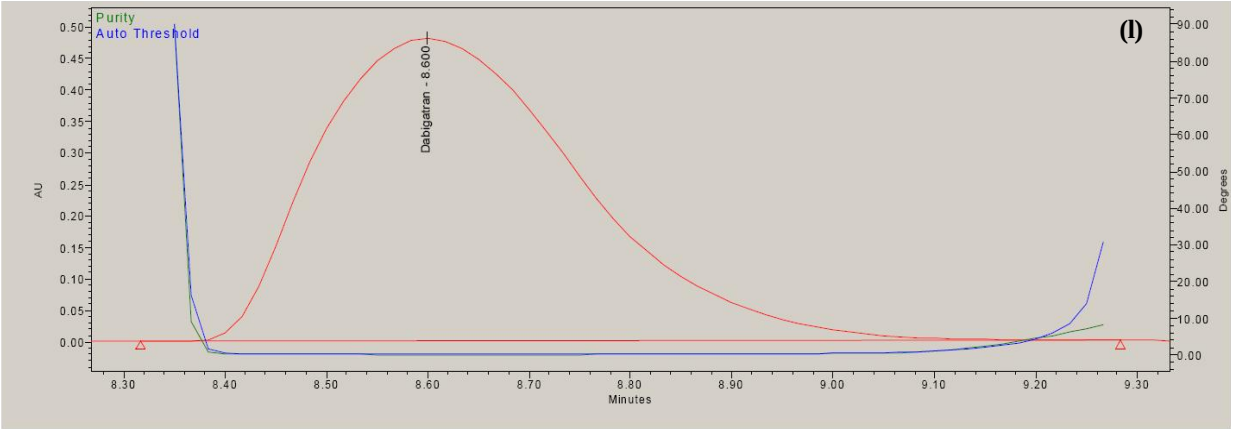

Auto-Scaled Chromatogram
(j)
AU
Minutes


Auto-Scaled Chromatogram
(k)
Peak1 - 2.268
Peak5 - 6.109
Dabigatran - 8.647
Peak7 - 11.282
Peak8 - 13.327
AU
Minutes


Purity
Auto Threshold
(l)
Dabigatran - 8.600
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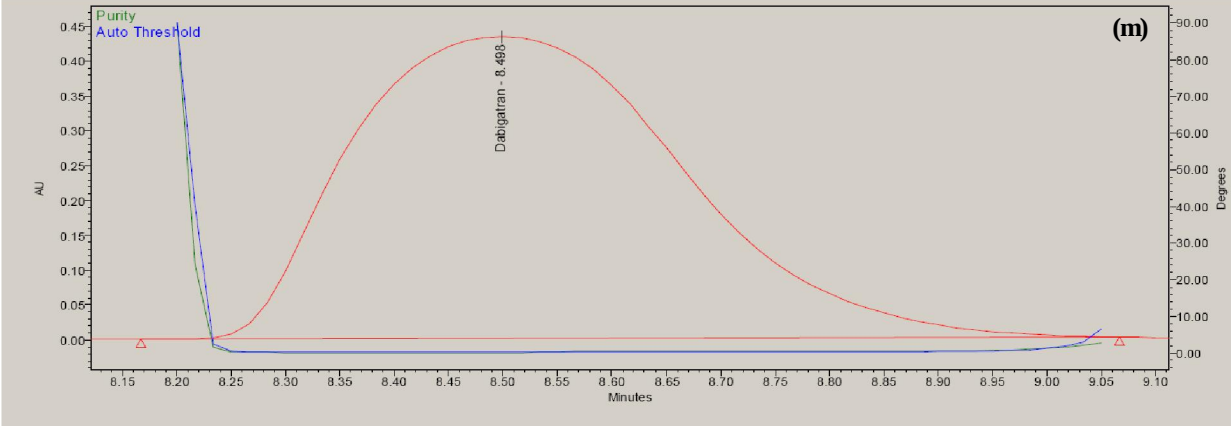
Degrees
Minutes


Purity
Auto Threshold
(m)
Dabigatran - 8.498
AU
Degrees
Minutes

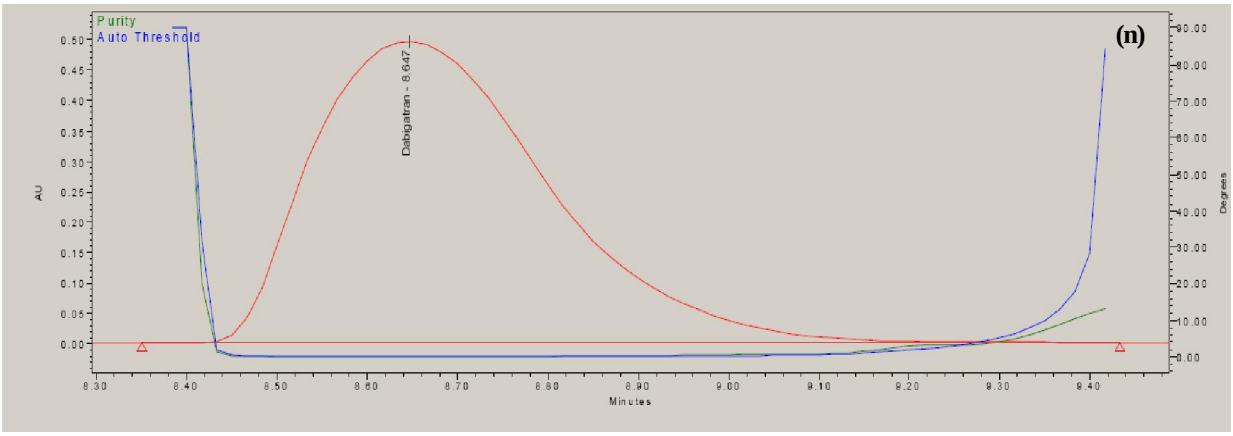


Figure 3 : Typical chromatograms of (a) blank (b) standard (c) sample (d) precision injections (e) linearity injections (f) acid blank (g) acid sample (h) base blank (i) base sample (j) peroxide blank (k) peroxide sample (l) purity plot of acid (m) purity plot of base (n) purity plot of peroxide

TABLE 1 : Results for linearity of dabigatran

Linearity level	% Level	Area
1	20	1779420
2	40	3558980
3	80	7231375
4	100	9100456
5	120	10951106
6	150	13813908
Correlation co-efficient		0.9999
	intercept	-125791
	slope	92529.4

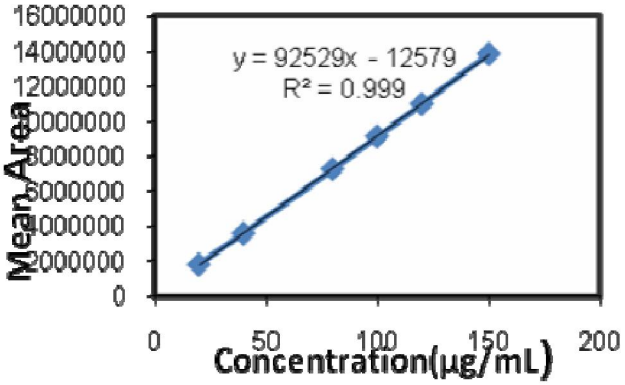


Figure 4 : Linearity of dabigatran

TABLE 2 : Recoveries study for dabigatran

Accuracy (Recovery) study							
Accuracy Level	Set No	Amount Added (µg/ml)	Amount Found (µg/ml)	Recovery (%)	Average recovery	Std Dev.	% RSD
	1	60.02	59.8	99.63			
50%	2	60.06	59.75	99.48	99.49	0.14	0.14
	3	60.04	59.65	99.35			
	1	120.0	120.12	100.1			
100%	2	120.06	119.76	99.75	99.9	0.18	0.18
	3	120.03	119.86	99.86			
	1	180.04	180.15	100.06			
150%	2	180.1	180.02	99.96	99.98	0.08	0.08
	3	180.06	179.9	99.91			

TABLE 3 : Forced degradation results for dabigatran

Stress condition	Drug recovered (%)	Drug decomposed (%)
Standard drug	100	
Acid degradation	86.85	13.15
Alkali degradation	83.75	16.25
Oxidation degradation	85.34	14.66
Thermal degradation	99.78	0.12
Photolytic degradation	99.85	0.15

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TABLE 4 : Precision results for dabigatran

Study	Set no	Assay (%)	Mean assay (%)	Stdev	RSD %
Method precision	1	100.12	100.02	0.08	0.08
	2	100.05			
	3	99.95			
	4	99.9			
	5	100.02			
	6	100.06			
Intermediate precision	1	99.85	99.93	0.26	0.26
	2	100.2			
	3	100.3			
	4	99.8			
	5	99.75			
	6	99.65			

TABLE 5 : Robustness results for dabigatran

Robust conditions	variation	Retention time (min)	USP Tailing	USP Plate count
Flow	0.9ml	9.72	1.37	9546
	1.0ml	8.65	1.35	9689
	1.1ml	7.93	1.27	9753
Temperature	20°c	8.8	1.38	9245
	25°c	8.65	1.35	9689
	30°c	8.52	1.28	9750
PH	4.6	8.8	1.39	9354
	4.5	8.65	1.35	9689
	4.4	8.55	1.32	9750

CONCLUSIONS

A validated RP-HPLC method has been developed for determination of Dabigatran in presence of degradation impurities. The proposed method was found to be a new, simple, precise, linear, accurate and specific. Degradation impurities did not interfere with the retention time of Dabigatran, and assay method is thus stability indicating.

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