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Method development and validation of dabigatran in pharmaceutical dosage form by RP- HPLC method

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ABSTRACT

A simple precise, accurate method was developed and validated by reversed phase high performance liquid chromatography method used for the estimation of Dabigatran in bulk and pharmaceutical dosage form. It is reversed phase liquid chromatography. The HPLC method has been carried out by using C18 150x4.6mm 5µm column. This method has been developed by using the mobile phase consisting buffer: Acetonitrile 65:35 and the flow rate of 1ml/min by the detection of UV at 330nm. The retention time of the dabigatran is 0.999 min. The runtime is 15min. the linearity was found to be over a concentration of 25%-150% respectively. The accuracy was found to be 98.84 to 100.24%. With a correlation coefficient of0.999.The proposed method can be used for the estimation of the drug in bulk and pharmaceutical formulation. The results of analysis have been validated satisfactorily using recovery studies.

Keywords: RP- HPLC, Dabigatran, Method development.

INTRODUCTION

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. In normal phase mode, the nature of stationary phase is polar and the mobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and stationary phase. Polar compounds are retained for longer time and take more time to elute because if their higher affinity with the stationary phase**.** Reversed phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl and octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of

compounds is reversed (thus the name reverse-phase chromatography)

DRUG PROFILE

Dabigatran etexilate is an oral prodrug that is metabolized by a serum esterase to dabigatran. It is a synthetic, competitive and reversible direct thrombin inhibitor. Inhibition of thrombin disrupts the coagulation cascade and inhibits the formation of clots. Dabigatran etexilate may be used to decrease the risk of venous thromboembolic events in patients who have undergone total hip or knee replacement surgery, or to prevent stroke and systemic embolism in patients with atrial fibrillation, in whom anticoagulation therapy is indicated.

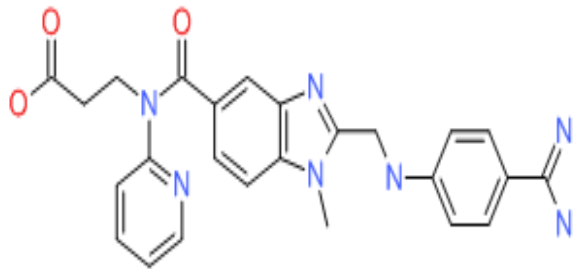

Fig no. 1 Chemical structure of Dabigatran

CAS Number	211915-06-9
Purity	≥98%
Molecular weight	627.73
Molecular formula	$C_{34}H_{41}N_7O_5$
Physical state	Solid
Solubility	Soluble in DMSO , water and ethanol
Storage	store at 4 degree centigrade
Melting point	180 ± 3 (DSC: 10 K min^-1 heating rate)

MATERIALS AND METHODS

Dabigatran pure drug (API) and Dabigatran tablets CIPLA pharmaceutical laboratories. Distilled water, Acetonitrile, Glacial Acetic Acid. All the above chemicals and solvents are from Rankem.

Instruments

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Dabigatran solutions

Methanol

Methanol is known as methyl alcohol. Methanol is easily available and in expensive compared to a Acetonitrile. Methanol is used as HPLC mobile phase for analytical and preparative analysis. As methanol mixes with water it forms adduct which has a viscosity even higher than that of water.

Acetonitrile

Acetonitrile is basically a polar solvent which is miscible with water but, never the less, has sufficient dispersive properties to elute substances from a liquid chromatography column by dispersive interactions with solute. Aceotonitrile used as HPLC mobile phase for analytical and preparative analysis.

Water

Double distilled water-HPLC grade is used as the mobile phase for analytical and preparative separations. Water for HPLC is purified and tested to ensure that it has low UV absorbance to provide most sensitive detection across all wavelengths.

Chromatographic conditions

Mobile phase used as buffer: Acetonitrile (60:40), Flow rate 1.0 ml/min**,** column used as BDS C18 (250x5mm 4.6µ), Detection wavelength was 330nm, column Temperature 30^0C, Injection Volume was 10µL, Run time 15min. Diluent used was Acetonitrile and distilled(HPLC grade) water in 45:55 ratio

RESULTS AND DISCUSSIONS

Optimized method

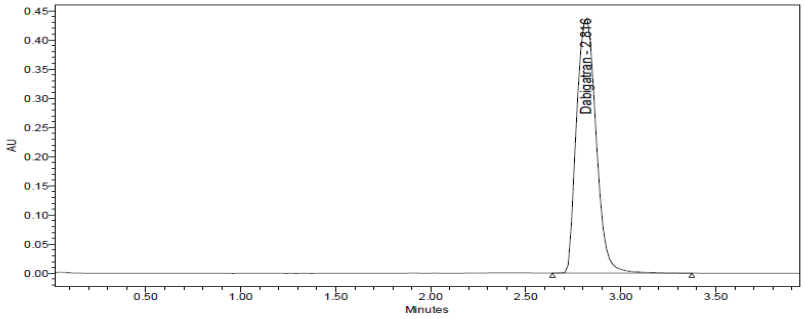

Fig 2. Optimized chromatogram of Dabigatran

Assay

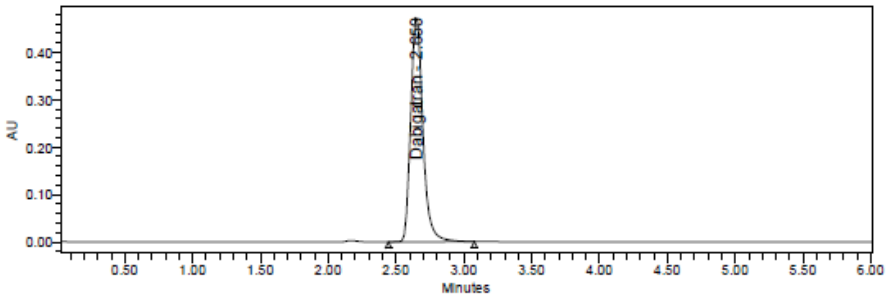

Fig 2. Assay Chromatogram of Dabigatran

Table no. 1.1 Assay data for Dabigatran

Sample No	%Assay
1	100.92
2	99.28
3.	99.89
4.	100.25
5.	100.35
6.	99.83
AVG	100.09
STDEV	0.5570
%RSD	0.56

System suitability

All the system suitability parameters were in the range and satisfactory as per ICH guidelines

Table no. 1.1 System suitability parameters for Dabigatran

S no	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Dabigatran	2.634	3204012	3505	1.50
2	Dabigatran	2.650	3249713	4443	1.25
3	Dabigatran	2.650	3187847	4444	1.25
4	Dabigatran	2.650	3207954	4087	1.33
5	Dabigatran	2.654	3173777	4558	1.27
6	Dabigatran	2.664	3267995	4684	1.21
Mean			3215216		
Std. Dev.			36387.32		
% RSD			1.13		

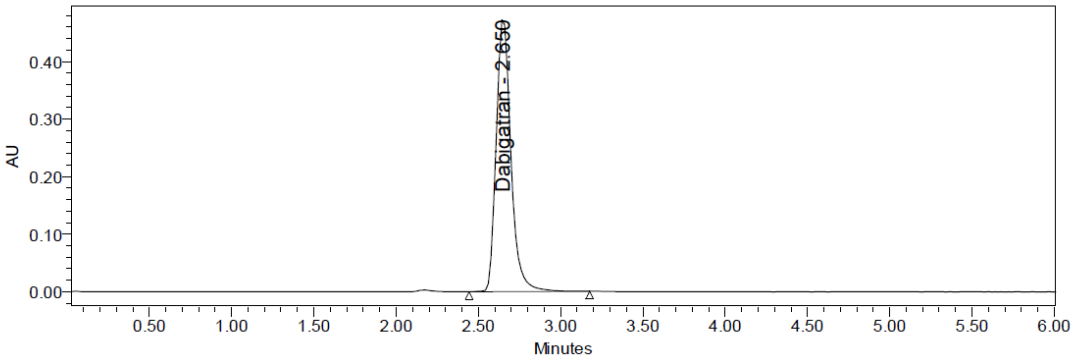

Fig 3. System suitability Chromatogram of Dabigatran

Specificity

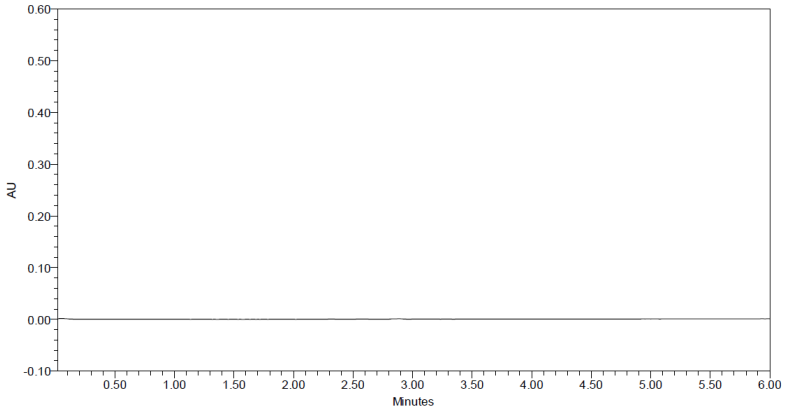

Fig4. Blank Chromatogram

Linearity

Table1.3Linearity Concentration and Responce for Dabigatran

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	37.5	762156
50	75	1628358
75	112.5	2376430

100	150	3079285
125	182.5	4014557
150	225	4710193

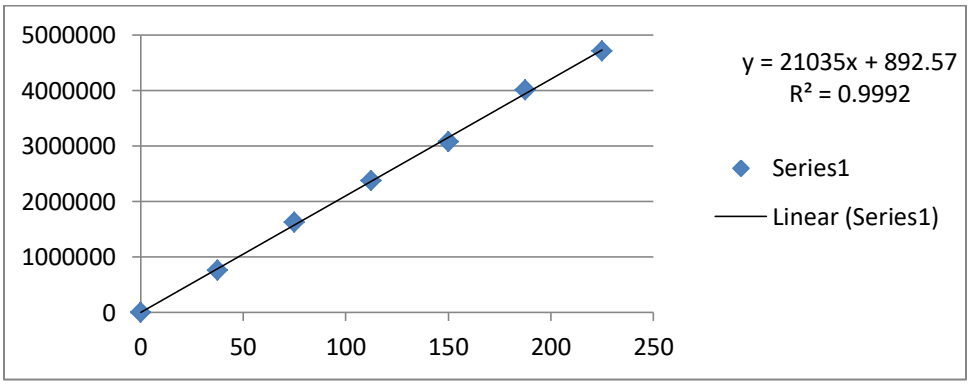

Fig5 linearity chromatogram of Dabigatran

Precision

Intermediate precisio

Table 1.4 Intermediate precision data for Dabigatran n

S.No	Peak Area
1	3173094
2	3225208
3	3243260
4	3186601
5	3223210
6	3269874
AVG	3220208
STDEV	35724.7
%RSD	1.11

Repeatability

Table 1.5 Repeatability data for Dabigatran ility:

S.No	**Peak Area**
1	3248148
2	3195254
3	3214951
4	3226458
5	3229685
6	3212904
AVG	3221233
STDEV	17927.1
%RSD	0.56

Accuracy

Table no1.6 Accuracy table for Dabigatran

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	75	74.13	98.84	99.92%
	75	75.72	100.97	
	75	75.24	100.33	
100%	150	149.23	99.49311	
	150	150.99	100.6658ö	
	150	150.14	100.0937	
150%	225	222.88	99.06	
	225	224.12	99.61	
	225	225.54	100.24	

LOD: LOD (Limit of detection)**:** Ditection limit of the Dabigatran in this method was found to be 0.012µg/ml.

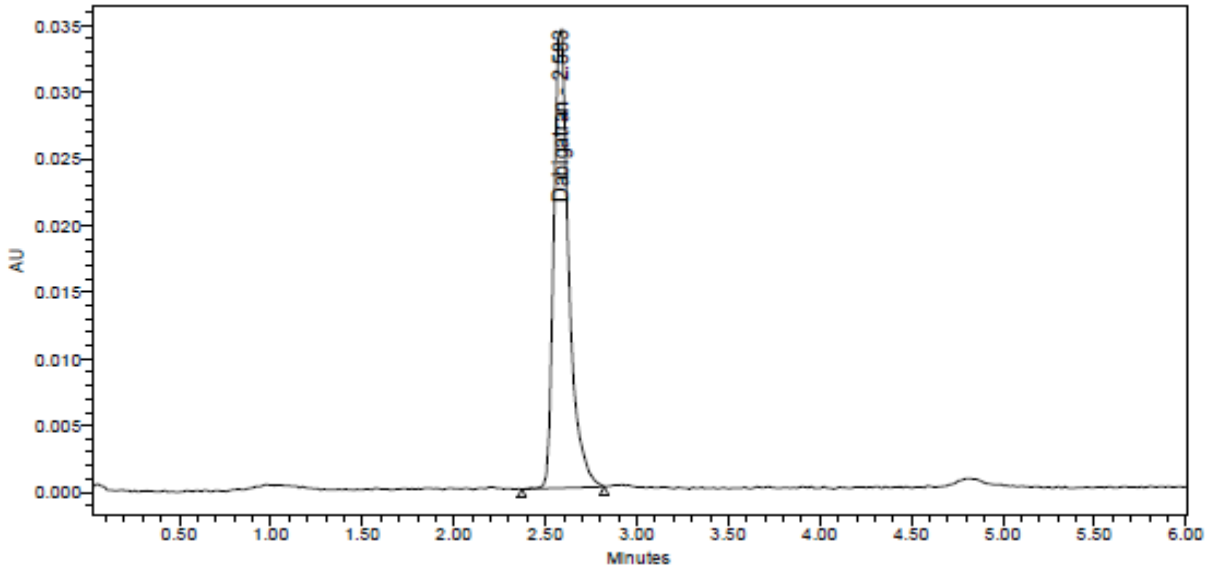

Fig 6. LOD Chromatogram of Dabigatran

LOQ (Limit of quantitation) **:** Quantification limit of the Dabigatran in this method was found to be 0.037µg/ml.

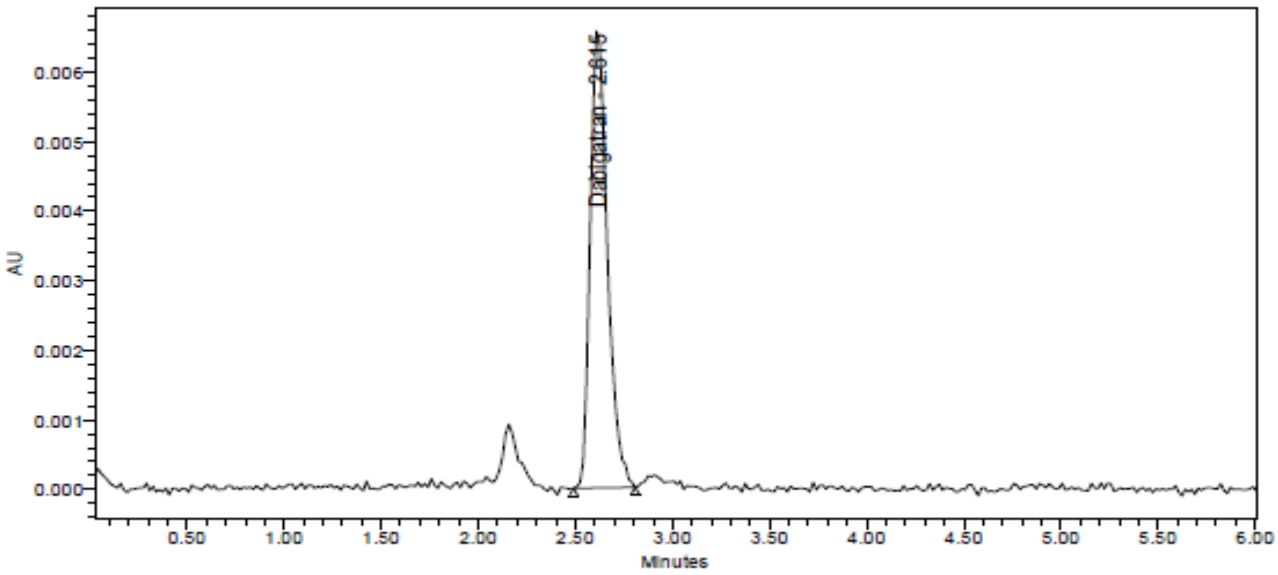

Fig 7. LOQ Chromatogram of Dabigatran

Robustness

Table no. 1.7Robustness data of Dabigatran

Parameter	%RSD
Flow Minus	1.8
Flow Plus	0.1
Mobile phase Minus	1.9
Mobile phase Plus	0.0
Temperature minus	1.08
Temperature plus	1.7

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Dabigatran etexilate in capsule dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation

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