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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF DABIGATRAN ETEXILATE IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC

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ABSTRACT

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for estimation of Dabigatran etexilate in pharmaceutical dosage forms. The HPLC method was developed using reverse phase PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size) column with mobile phases containing Methanol (70%) : water (30%). The flow rate was 1.2ml / min with UV-Visible Detector (SPD-20A) detection at λ max 230 nm and the injection volume was set at 20µl with 10min run time. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity, lod and loq. Such findings showed that the system could find practical use in its tablet dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

INTRODUCTION

Dabigatran etexilate is an oral prodrug that is hydrolyzed to the competitive and reversible direct thrombin inhibitor dabigatran. Dabigatran etexilate may be used to decrease the risk of venous thromboembolic events in patients in whom anticoagulation therapy is indicated. In contrast to warfarin, because its anticoagulant effects are predictable, lab monitoring is not necessary. Dabigatran etexilate was approved by the FDA.

METHOD DEVELOPMENT

HPLC Instrumentation & Conditions

High performance liquid chromatography SHIMADZULC 20AT reciprocating dual pump, UV-Visible Detector (SPD-20A), a reversed-phase PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)used for separation. Chromatographic data was acquired using Spin Chrome software & Hamilton injector used.

Standard and sample preparation for UV-spectrophotometer analysis

25 mg of Dabigatran Etexilate Mesylate standard was transferred into 25 ml volumetric flask, dissolve and make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and made up to the volume with mobile phase. The standard and sample stock solutions were prepared separately by dissolving standard and sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Dabigatran Etexilate Mesylate, so that the same wave number can be utilized in HPLC-UV detector for estimating the Dabigatran Etexilate Mesylate. While scanning the Dabigatran solution we observed the maxima

at 230nm. The UV spectrum has been recorded on ELICO Double Beam SL210 UV – Vis spectrophotometer.

MOBILE PHASE PREPARATION

Methanol and water were mixed in the ratio of 70:30 to make 1000ml of the mobile phase. It was filtered through 0.45 µm membrane filter and degassed.

SAMPLE & STANDARD PREPARATION FOR THE ANALYSIS

75 mg of Dabigatran Etexilate Mesylate standard was transferred into 75 ml volumetric flask, dissolved and made up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and made the required volume with mobile phase.

Table 1: Optimization of chromatographic studies

The chromatographic conditions were optimized by different means. Using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc.

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)	ACN : Water = **60 : 40**	1 ml/min	230nm	Low response	Method rejected
PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)	Methanol: Buffer = **70 : 30**	1ml/min	230 nm	Very low response	Method rejected
PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)	ACN: Buffer = **70 : 30**	1.0 ml/min	230nm	Tailing peak	Method rejected
PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)	Methanol: water= **80:20**	1.0 ml/min	230nm	Broad Peak	Method rejected
PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)	Methanol :water = 70:30	1.2ml/min	230nm	Good sharp peak	Method accepted

Table 2: SUMMARY OF OPTIMIZED CONDITIONS

Mobile phase	Methanol :water = 70:30
Wavelength	230 nm
Flow rate	1.2ml/ min.
Run time	10 min.
Column	PHENOMENEX Luna C_{18}, 5µm, 250 x 4.6mm (size)

METHOD VALIDATION

1. Accuracy: Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Dabigatran Etexilate Mesylate were taken and added to the pre-analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated.

2. Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention Times obtained by actual determination of five replicates of a fixed amount of drug. Dabigatran Etexilate Mesylate(API). The percent relative standard deviation was calculated for Dabigatran Etexilate Mesylate are presented.

3. Linearity & Range: The calibration curve showed good linearity in the range of 0 – 25 µg/ml, for Dabigatran Etexilate Mesylate (API) with correlation coefficient (r^2) of 0.998. A typical calibration curve has the regression equation of y =70244x + 34176 for Dabigatran Etexilate Mesylate.

4.Limit of Detection: The limit of detection for the drug Dabigatran Etexilate Mesylate can be calculated from the formula

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response, S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. For this method, the value was found to be 0.000451 µg/ml & area 31.76 Dabigatran Etexilate Mesylate.

5. Limit of Quantification: The limit of quantification for the drug Dabigatran Etexilate Mesylate can be calculated from the formula

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response, S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The LOQ for this method was found to be 0.00137 µg/ml & area 96.27 Dabigatran Etexilate Mesylate

6. Robustness:

Chromatographic conditions variation: To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria: The system suitability should pass as per the test method at variable conditions.

Table 3: Results of Accuracy

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	96.90	Mean= 96.38% S.D. = 0.171561 % R.S.D.= 0.1015
S_2 : 80 %	8	10	95.80	
S_3 : 80 %	8	10	96.30	
S_4 : 100 %	10	10	96.90	Mean= 96.38% S.D. = 0.045826 % R.S.D.= 0.1015
S_5 : 100 %	10	10	95.80	
S_6 : 100 %	10	10	96.30	
S_7 : 120 %	12	10	96.40	Mean= 96.49% S.D. = 0.105987 % R.S.D. = 0.01
S_8 : 120 %	12	10	96.50	
S_9 : 120 %	12	10	96.30	

Table 4: Results of Precision

HPLC Injection Replicates of Dabigatran Etexilate Mesylate	Area	Retention Time
Replicate – 1	2257741	4.63
Replicate – 2	2742277	4.65
Replicate – 3	2741481	4.61
Replicate – 4	2747443	4.62
Replicate – 5	2736501	4.68
Average	2645058	4.63
Standard Deviation	43477.658	0.008944
% RSD	0.01	0.18

Table 5: Results of Linearity and Range

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
5	393853
10	765001
15	1113791
25	1761665

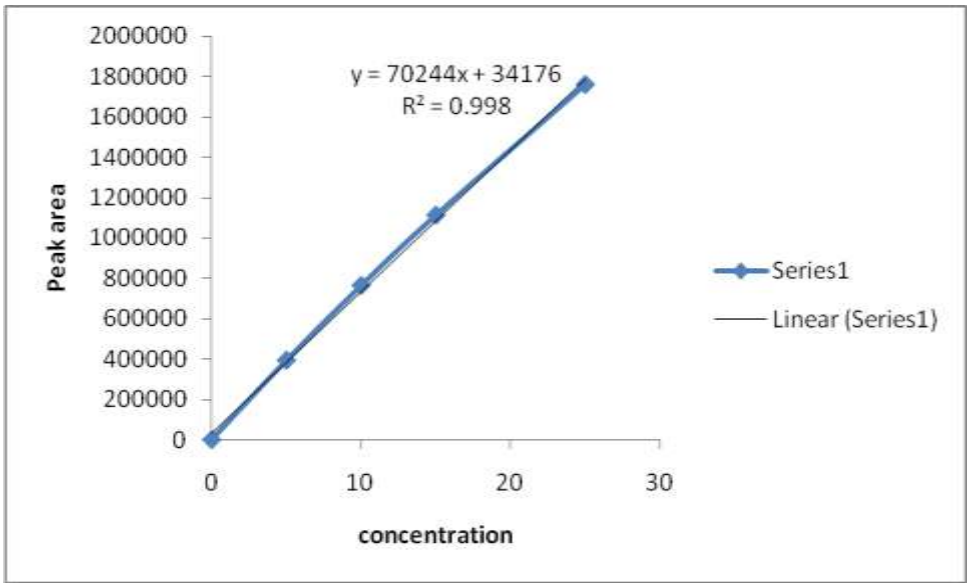

Fig. 1: Linearity Graph

Table 6: Robustness data of the proposed method

VARIATION	RETENTION TIME	AREA	% RSD
FLOW RATE 1ml/min			
1	5.47	27365014	
2	5.49	27376108	0.03
3	5.42	27365618	
4	5.48	27386816	
5	5.44	27375521	
6	5.45	27385484	
WAVELENGTH 228nm	4.62	2647443	
	4.64	2646845	
	4.62	2645978	
	4.61	2646654	
	4.61	2646850	0.02%
	4.66	2646969	
WAVELENGTH 232nm	4.65	2645841	
	4.66	2647540	
	4.65	2646812	
	4.61	2646658	0.04 %
	4.66	2648534	
	4.68	2645298	

Stability related impurity studies: Following protocol was strictly adhered to for forced degradation of Dabigatran Etexilate Mesylate Active Pharmaceutical Ingredient (API).The API (Dabigatran Etexilate Mesylate) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation

Acid hydrolysis: An accurately weighed 10mg. of pure drug was transferred to a clean and dried 10ml volumetric flask. To which 0.1 N Hydrochloric acids was added, volume made up to the mark and kept aside for 24 hrs., from that 0.2 ml was taken in to a 10 ml volumetric flask and volume made the up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

Basic hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean and dried 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added and volume made up to the mark and kept aside for 24 hrs., from that 0.2 ml was taken in to a 10 ml volumetric flask and volume made up to the mark with mobile phase, then injected into the HPLC system against a blank of 0.1NaOH (after all optimized conditions).

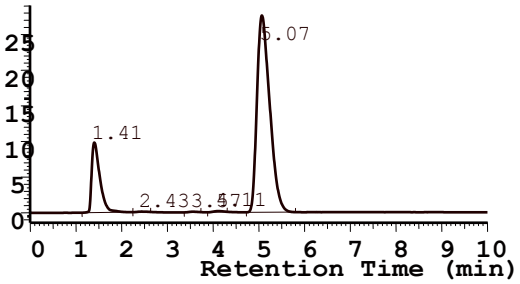

Table 7: Results of forced degradation studies of Dabigatran Etexilate Mesylate API

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 N HCl)	24Hrs.	98.36	---------	98.36
Basic Hydrolysis (0.1N NaOH)	24Hrs.	98.32	----------	98.32
Oxidation (3% H_2O_2)	24 Hrs	97.31	----------	97.31

RESULTS AND DISCUSSION

To develop a precise, linear, specific and suitable stability indicating RP-HPLC method for analysis of Dabigatran Etexilate Mesylate, different chromatographic conditions were applied and the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. Mobile phase and diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (acetonitrile, methanol, water and phosphate buffer).

The drug was found to be highly soluble in methanol. Drug was insoluble in water and acetonitrile. Using these solvents with appropriate composition newer method can be developed and validated. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Dabigatran Etexilate Mesylate it is has observed λ max 230 nm. Further, a flow rate of 1.2 ml/min and an injection volume of 20 µl were found to be the best analysis. The result showed the developed method is yet another suitable method for assay and stability studies which can help in the analysis of Dabigatran Etexilate Mesylate in different formulations. The method can also be used for get stability related impurities in Dabigatran Etexilate Mesylate. There are four impurity peaks has been seen in acidic condition, two in basic.

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Dabigatran Etexilate Mesylate API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, impurity studies which can help in the analysis of Dabigatran Etexilate Mesylate in different formulations

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