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Development and validation of UV- spectrophotometric method for the estimation of dabigatran etexilate mesylate (dem)

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ABSTRACT

Objective

To develop and validate a simple, accurate, precise and cost effective UV-Spectrophotometric method for the estimation of Dabigatran Etexilate Mesylate (DEM).

Method

UV spectrophotometric method was performed at 226 nm and the samples were prepared with methanol as solvent.

Results

Linearity range was found at 2-10µg/ml in UV with correlation coefficient (R) 0.999 and the regression equation is y = 0.066x + 0.012.

Conclusion

The proposed methods were simple, precise, Accurate and can be used for the analysis of drug in bulk form.

Keywords: UV-Visible Spectrophotometer, Dabigatran Etexilate Mesylate, ICH guidelines.

INTRODUCTION [1, 2]

DEM is chemically β-Alanine,N-[[2-[[[4-[[[(hexyloxy) carbonyl]amino]phenyl]amino]methyl]-1-methyl-1H-benzimidazol-5-yl]carbonyl]-N-pyridinyl-,ethyl ester,methane sulfonate. Empirical formula is $C_{34}H_{41}N_7O_5$. CH_4O_3S and molecular weight is 723.86 (mesylate salt), 627.75 (free base). It is highly soluble in methanol, slightly soluble in ethanol, and sparingly soluble in isopropanol. A saturated solution in pure water has a solubility of 1.8 mg/ml. It is a yellow-white to yellow powder. DEM is an orally available mesylate salt form of the etexilate prodrug of dabigatran and a direct thrombin inhibitor with anticoagulant activity. Thrombin, a serine protease, is responsible for the conversion of fibrinogen to fibrin in the coagulation cascade. Inhibition of thrombin consequently prevents thrombus development. Dabigatran inhibits free

thrombin, fibrin-bound thrombin and thrombin-induced platelet aggregation which results in a prolongation of a PTT (partial thrombo plastin time), ECT (Ecarin clotting time), and TT (thrombin time). It is used in embolism associated with atrial fibrillation, cardioversion of atrial fibrillation/flutter, thromboprophylaxis in orthopaedic surgery, cerebral embolism, and treatment of acute venous thromboembolism. DEM is available in the form of Capsules with the Brand name of PRADAXA (Marketed by Boehringer Ingelheim, India) with strengths 75 & 150 mg.

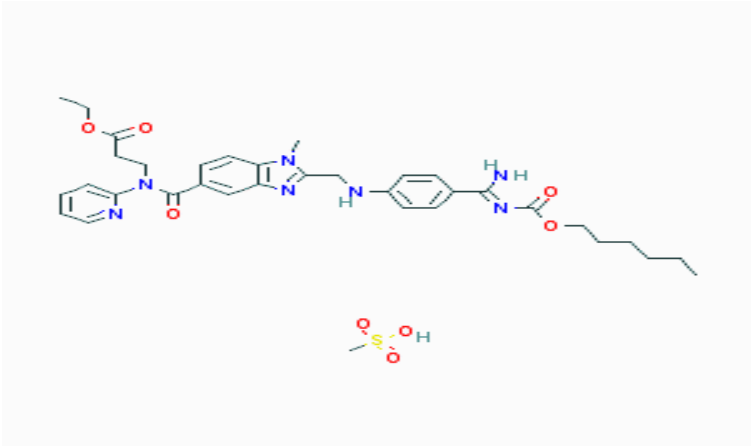

Fig. 1: Structure of DEM [3]

The literature survey reveals that various analytical methods like Spectrophotometric, HPLC were reported for the determination of DEM in formulations. UV-Spectrophotometric method was developed with acetone as solvent **[4].** Also a few LC-MS, UPLC-MS methods were reported for the quantification of Dabigatran etexilate in human plasma. A Gas Chromatographic method was reported for the quantification of residual hexylmethane sulfonate in the DEM. The literature survey reveals that there were no reported methods for UV. So far there are no published UV methods by using methanol as solvent for the determination of DEM in bulk. Hence an attempt was made to develop, a simple, precise, accurate, robust, and economical UV-Spectrophotometric method for the estimation of DEM in bulk.

MATERIALS AND METHODS

Instrumentation

Lab India – T60, UV/Vis double beam spectrophotometer with a spectral band width of 1 nm, wavelength accuracy of ± 0.3 nm and 1.0 cm matched quartz cells were used for UV determinations.

Chemicals and Solvents

DEM was obtained as a gift sample from MSN Laboratories, Hyderabad, India and was used without further purification. All chemicals and reagents used were of analytical grade in UV method. AR grade Methanol is procured from Merck Pharmaceuticals Private Ltd., Mumbai, India.

Selection of Solvent and Detection wavelength

Drug solution of 100µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like water, hexane, ethanol, cyclohexane and methanol. It was observed that the drug showed maximum absorbance in methanol at 226 nm, hence methanol was used as solvent and 226 nm was used as detection wavelength for DEM for further study.

Preparation of standard stock solution

Accurately weighed 100mg of the DEM was dissolved in 100ml of methanol to get 1000µg/ml stock solution. Working standard solutions were further diluted to get a concentration range of 2-10µg/ml.

Method Validation [5-10]

The proposed methods were validated for following parameters: System suitability, Linearity,

Accuracy, Precision, robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Linearity

The calibration curve was obtained with five concentrations of the standard solution (2–10 µg/ml for UV method). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Accuracy

The accuracy of the method was evaluated by recovery study of DEM at three concentration levels (50%, 100% and 150 %). A study was carried out in triplicate at 2,4 and 6 µg/ml in UV. A fixed amount of pre-analysed sample and standard drug was added and recovery was studied for the quantification of DEM. The percentage recovery and mean % recovery were calculated.

Precision

The precision was determined for DEM in terms of intraday and interday. For intraday precision evaluation, standard solution (6 µg/ml for UV) was prepared from stock solution and calculate the absorbances six times (n=6) at two different times in a day. The interday precision was studied by injecting the same concentration of standard solutions into the system six times on consecutive days. The standard deviation and the relative standard deviation were reported for precision. RSD for peak areas should be NMT 2%, which indicate the precision of the developed methods.

Robustness

Robustness of the method were determined by altering slight changes in the detection of wavelength. It was observed that there were no marked changes in spectrum obtained, which demonstrated that the developed UV methods were robust.

Limit of Detection and Limit of Quantitation

The LOD and LOQ were determined on the basis of response and slope of the regression equation.

RESULTS AND DISCUSSION

The spectrophotometric methods involve simple instrumentation compared with other instrumental techniques. The absorption spectra of the DEM was shown in Fig 1. The λ_{max} was found as 226 nm. Calibration curve was constructed in the range of expected concentrations (2–10 µg/ml). The linearity results and calibration curve were shown in table no. 1 and Fig 2. The beer's law is obeyed over this concentration range. The representative equation analysis was y=0.066x+0.012, with a correlation coefficient of 0.999. The accuracy results were shown in table 3. The intraday and interday precision were shown in table no.4 and 5. The robustness results were shown in table no.6. The LOD and LOQ values were found as 0.199 and 0.66 µg/ml, respectively.

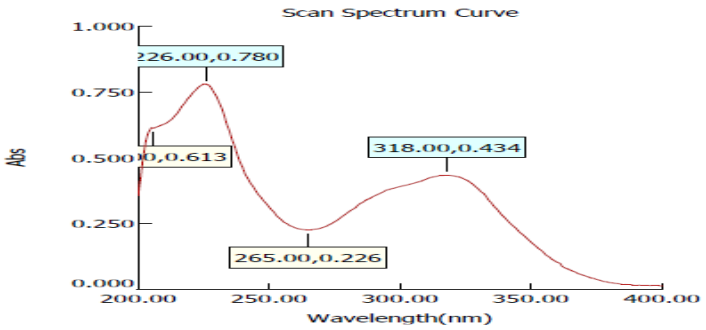

Fig. 1: UV spectrum of DEM

Linearity

The results indicate that an excellent correlation exists between the absorbance and concentration of drug.

Table 1: Results for linearity

CONCENTRATION	ABSORBANCE
2	0.206
4	0.307
6	0.429
8	0.550
10	0.658
Correlation coefficient	0.999
Slope(m)	0.066
Intercept(c)	0.012

Table 2: Results for regression analysis of DEM

S. NO	Parameters	Results
1	Regression equation (Y)	Y = 0.066x + 0.012
2	Correlation coefficient (R)	0.999
3	Slope (m)	0.066
4	Y – intercept (c)	0.012
5	Range	2-10 µg/mL
6	Limit of detection (LOD)	0.199 µg/mL
7	Limit of quantitation (LOQ)	0.66 µg/mL

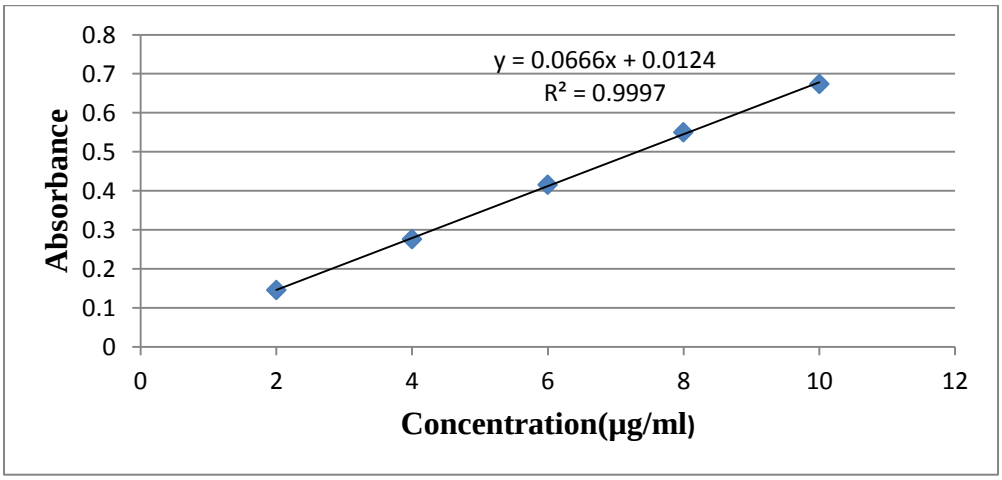

Fig. 2: Calibration curve of DEM

Accuracy

The results represent the high percent recovery values indicating that the proposed method is accurate.

Table 3: Accuracy results for DEM

S.No	Concentration Level (%)	Amount added(µg/ml)		Amount found(µg/ml)	%Recovery	Statistical parameters
		Std drug	Sample			
1		2	4	5.98	99.66	Mean=100. 21
2	50	2	4	6.01	100.16	SD=0.587
3		2	4	6.05	100.83	%RSD=0.59

4		4	4	8.01	100.12	Mean=100.24
5	100	4	4	7.99	99.87	SD=0.453
6		4	4	8.06	100.75	%RSD=0.45
7		6	4	12.01	100.08	Mean=99.97
8	150	6	4	12.01	100.08	SD=0.19
9		6	4	11.97	99.75	%RSD=0.19

Precision

The % RSD for Intraday precision and interday precision for DEM were found to be 0.07 and 0.08 which indicates the method is precise.

Table 4: Intraday precision for DEM

S. No	Absorbance	%Assay	Statistical parameter
1	0.421	99.81	
2	0.424	99.85	Mean=99.78
3	0.428	99.79	SD=0.069
4	0.426	99.80	%RSD=0.07
5	0.421	99.65	
6	0.425	99.81	

Table 5: Interday precision for DEM

	S. No	Absorbance	%Assay	Statistical parameter
1		0.420	100.02	
2		0.427	99.93	Mean=99.92
3		0.429	99.89	SD=0.077
4		0.422	99.85	%RSD=0.08
5		0.425	100.01	
6		0.425	99.84	

Robustness

All the experimental values for robustness obtained fall into the acceptance criteria.

Table 6: Robustness results for DEM

Concentration (µg/mL)	S. No	225 nm	226 nm	227 nm
6.0	1	0.427	0.429	0.426
	2	0.421	0.428	0.424
	3	0.424	0.429	0.425
	4	0.426	0.427	0.427
	5	0.428	0.428	0.427
	6	0.428	0.429	0.426
	Mean	0.425	0.428	0.425
	SD	0.002	0.0008	0.0011
	%RSD	0.64	0.19	0.27

CONCLUSION

The proposed UV and HPLC methods were simple, rapid, accurate, precise and sensitive for the quantification of DEM and moreover the developed RP-HPC method is LC-MS compatible which can be efficiently used for further LC-MS analysis. The methods rely on the use of simple working procedure,

and hence this method can be routinely employed in quality control for analysis of DEM from bulk drug.

Acknowledgement

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