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A Validated RP-HPLC method for related substances of Dabigatran etexilate mesylate

Pavan Kumar D*[1,2], Kirti Kumar Jain[1], Sinivasa Rao G[2]

[1]Department of Analytical Research & Development, Dr. Reddy's Laboratories Ltd., Visakhapatnam-530045, A.P., India
[2]Department of chemistry, Institute of science, GITAM (Deemed to be university), Visakhapatnam-530045, A.P., India

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ABSTRACT

The scope of this research work was to develop a reverse-phase liquid chromatographic method for the quantification of related impurities of dabigatran and to validate the method according to ICH guidelines. Chromatographic conditions were optimised with Poroshell SB C18, 150mm, 4.6mm, 2.7μm particle size column, mixer of Phosphate buffer with phosphoric acid in water and acetonitrile with percentage of 10:90 (v/v) as solvent-A and acetonitrile and buffer with a percentage of 70:30 (v/v) as solvent-B. Gradient compassion mode is employed for mobile phase delivery with a flow rate of 0.8 mL/min. Stationary phase was maintained at 35°C temperature and detection at 230 nm, with 10 μL of sample injection volume. Water and acetonitrile in the percentage 30:70(v/v) were used as a diluent. The developed RP-HPLC method was validated according to ICH guidelines. LOD and LOQ values for dabigatran its impurities were in the range from 33 to 55 ppm and 112 to 168 ppm correspondingly. Method validation results for all the parameters are meeting the ICH guidelines acceptance criteria for the parameters of robustness, ruggedness, linearity, reproducibility and recovery. The proposed method was found to be suitable for the quantitative determination of potential impurities in the bulk samples of Dabigatran etexilate mesylate API.

*Corresponding Author

Name: Pavan Kumar D
Phone: +919959595656
Email: dhpavankumar@gmail.com

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INTRODUCTION

Dabigatran, marked with brand name Pradaxa (Lin *et al.*, 2019), It is an anticoagulant drug, and it will avoid blood clots and stroke in patients with atrial fibrillation (Ankit *et al.*, 2014; Patel *et al.*, 2014). Particularly, used to avoid blood clots in surgery like hip or knee replacement and also in those with a history of prior clots. It is administrated orally (Kumar *et al.*, 2015; Delavenne *et al.*, 2012).

Common side effects include bleeding and gastritis. This drug is not suggested for pregnancy or breastfeeding women. It acts as a thrombin inhibitor (Prathap *et al.*, 2013; Eerenberg *et al.*, 2011). USFDA approved dabigatran in 2010. Dabigatran is in the List of Essential Medicines of World Health Organization (Vidushi and Meenakshi, 2017). There are several methods available for the determination of potential impurities at API stage of dabigatran. But there is no single HPLC method available for these twelve impurities of dabigatran (Desai *et al.*, 2019).

MATERIALS AND METHODS

Samples and reagents

The development samples of dabigatran and all impurities (From impurity-1 to impurity-12) were procured from Dr Reddy's Labs, PDLab R&D, and Srikakulam, India. Chemicals and reagents utilised for development are Orthophosphoric Acid (AR grade), and acetonitrile (HPLC grade) were purchased from Merck (India) Limited. Milli-Q grade purification system used for HPLC water. Chemical names of dabigatran and its impurities are tabulated in Table 1. Structures of dabigatran and its potential impurities are shown in Figure 1.

Instruments

Agilent Infinity 1260 series HPLC system and Sartorius and model MSA 225S-100-DA weighing balance is used for experimental analysis. Empower-3 software is used as data processing.

Chromatographic Conditions

Experimental analysis was performed on column Poroshell SB-18, 150mm, 4.6mm, 2.7μm particle size, a mixture of Acetonitrile and Phosphate buffer with phosphoric acid in water in the percentage of 10:90 (v/v) as solvent-A and mixture of Acetonitrile and Phosphate buffer in the percentage of 70:30(v/v) as solvent-B. Gradient method is optimised with Flow of 0.8 mL/mi. Column stationary phase has been maintained at 35°C temperature. Detection was set at l 230 nm, and the injection load was 10 μL. Water and acetonitrile in the percentage 30:70(v/v) were selected as a diluent.

Standard and Sample Preparation:

Related substance by HPLC was performed with 0.5 mg/mL test concentration. Resolution in related substance, all the twelve impurities are spiked 0.10% concerning 0.5 mg/mL test concentration.

RESULTS AND DISCUSSION

Analytical method validation

Analytical method validation for the estimation related impurities by HPLC of Dabigatran etexilate mesylate API was performed by following Validation of Procedures of ICH guidelines.

Limit of Detection and Limit of Quantification

Limit of detection and Limit of quantification values for dabigatran and its 12 related impurities were established by preparing the known concentration solutions from their stock solutions that would give a signal of the peak to baseline noise ratio of 3:1 and 10:1 respectively. The LOQ concentrations were confirmed by verifying precision and accuracy at LOQ concentration. LOD and LOQ concentrations of impurities are as tabulated in Table 2. The % RSD of an area of all the impurities in six preparations at LOQ concentration were found within the specified limit, which confirms that the analytical procedure is precise at LOQ Concentration. The results are tabulated in Table 3. LOD and LOQ chromatograms are shown in Figures 2, 3 and 4.

The percentage recovery of each impurity ranged from 95 to 108. Recovery all the impurities are well within the acceptance limit, which confirms that the method is accurate at LOQ level.

Precision

Precision is a term used to describe data from an experiment that has been repeated several times. Degree of scattering between a sequence of measurements attained from the various sampling of the homogeneous sample under the agreed conditions is defined as the precision of an analytical procedure. Repeatability of the related substance procedure was checked by six-fold analysis by adding all the twelve impurities at LOQ concentration as well as at 0.10% level in Dabigatran test sample. The study was also performed on a different day with a different analyst for the evaluation of inter-day and intra-day variation and analyst. The % RSD of all individual impurity areas in every six preparations were found well within the set acceptance limit, which confirms that the method is precise.

Linearity

Linearity for all the impurities was carried out from the limit of quantification (LOQ) to 150% concentration of the test concentration 0.05 mg/ml. Responses for all impurities were recorded and plotted the calibration curve for each impurity concentration versus response; the correlation coefficient achieved for each impurity was more than 0.999.

Accuracy

A known amount of each impurity is added in Dabigatran test sample, and the study was conducted to define the accuracy of the procedure for the quantification of all impurities. Experiments were performed in triplicate at LOQ, 0.05%, 0.1% and 0.15% of the test concentration (0.05 mg/ml) and calculated the recovery of all the fourteen impurities. The percentage recovery of each impurity was within the acceptance limit, which confirms that the proposed method was accurate. Spiked test chromatogram, as shown in Figure 5.

Robustness

To prove the robustness of the test procedure, method conditions were altered and estimated the

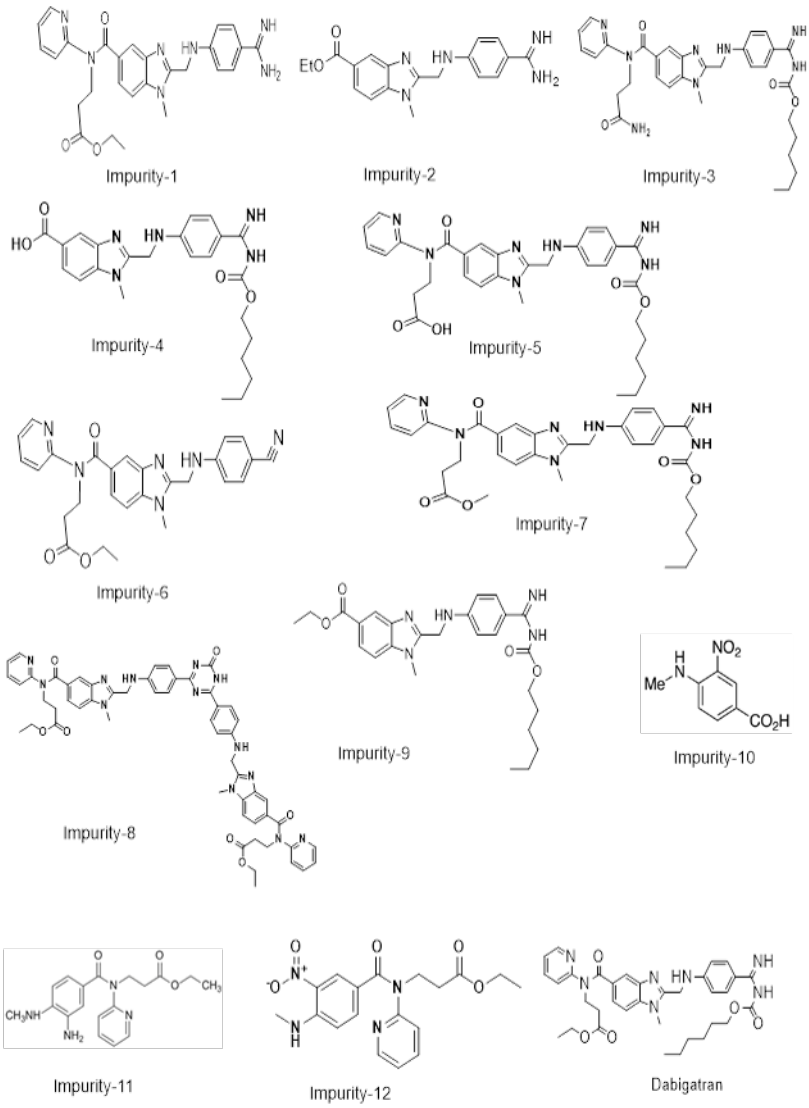

Figure 1: Structures of Dabigatran and its potential impurities

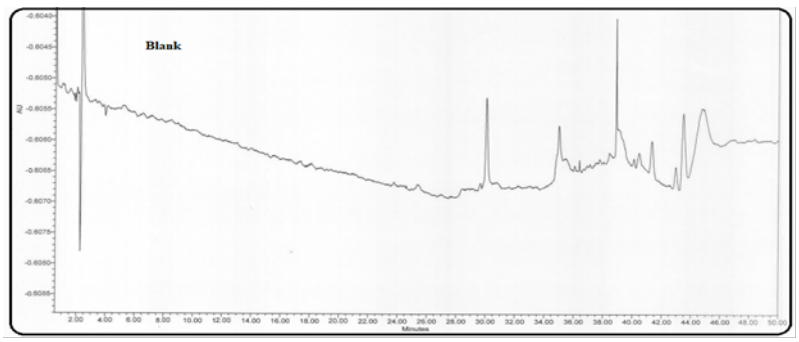

Figure 2: Typical HPLC chromatogram for Blank of Dabigatran

Table 1: Dabigatran impurities and its chemical names

S.NO	Name of the impurity	Chemical Name
1	Impurity-1	ethyl 3-(2-(((4-carbamimidoylphenyl)amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo[d]imidazole-5-carboxamido) propanoate
2	Impurity-2	ethyl 2-(((4-carbamimidoylphenyl) amino)methyl)-1-methyl-1H-benzo[d]imidazole-5-carboxylate
3	Impurity-3	hexyl ((4-(((5-((3-amino-3-oxopropyl)(pyridin-2-yl)carbamoyl) -1-methyl-1H-benzo[d]imidazol-2-yl)methyl)amino)phenyl) (aimino)methyl)carbamate
4	Impurity-4	2-(((4-(N-((hexyloxy)carbonyl) carbamimidoyl)phenyl)amino)methyl)-1-methyl-1H-benzo[d]imidazole -5-carboxylic acid
5	Impurity-5	3-(2-(((4-(N-((hexyloxy)carbonyl) carbamimidoyl)phenyl)amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo[d]imidazole-5-carboxamido)propanoic acid
6	Impurity-6	ethyl 3-(2-(((4-cyanophenyl) amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo [d]imidazole-5-carboxamido) propanoate methyl 3-(2-(((4-(N
7	Impurity-7	((hexyloxy)carbonyl)carbamimidoyl)phenyl)amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo [d] imidazole-5-carboxamido) propanoate
8	Impurity-8	diethyl 3,3'-((2,2'-((((6-oxo-1,6-dihydro-1,3,5-triazine-2,4-diyl) bis(4,1-phenylene)) bis (azanediyl)) bis(methylene))bis(1-methyl-1H-benzo[d]imidazole-2,5-diyl-5-carbonyl))bis(pyridin-2-ylazanediyl))dipropionate
9	Impurity-9	ethyl 2-(((4-(N-((hexyloxy) carbonyl) carbamimidoyl) phenyl)amino)methyl)-1-methyl-1H-benzo[d]imidazole-5-carboxylate
10	Impurity-10	4-(Methylamino)-3-nitro benzoic acid
11	Impurity-11	Ethyl-3-(3-Amino-4-(methyl amino)-N-Pyridine-2-yl)-benzamido propanoate
12	Impurity-12	Ethyl-3-(3-nitro-4-(methyl amino) benzyl)pyridine-2-yl)-amino) propionate
13	Dabigatran	Ethyl 3-(2-(((4-(N-((hexyloxy) carbonyl)carbamimidoyl) phenyl)amino)methyl)-1-methyl-N-(pyridin-2-yl)-1H-benzo[d]imidazole-5-carboxamido)propanoate

change in the resolution of dabigatran and impurity-D. Experiments are performed by changing the Flow by ± 10% and column temperature by $\pm 5^0$C. Resolution between dabigatran and impurity-D has established the robustness of the method. Data is evaluated in the Table 4.

Stability of Solution

Dabigatran test sample solution stability was established by spiking all the impurities at 0.1% level to Dabigatran Drug Substance. All solutions which are prepared in the volumetric flask were tightly capped and kept at ambient temperature for 48 hours—estimated the level of all the impurities initially, after 24 hours and after 48 hours. Results were indicated the sample solution is stable up to 48 hours.

Mobile Phase solution stability was also established for Dabigatran related impurities with new sample solutions and holding the mobile phase for 48 hours. Freshly prepared sample solutions were analysed initially, after 24 hours and after 48 hours with

Table 2: LOD and LOQ results

Name of the impurity	LOD (ppm)	LOQ (ppm)
Impurity-1	55.4	168
Impurity-2	35.4	107
Impurity-3	44.5	135
Impurity-4	50.9	154
Impurity-5	37.0	112
Impurity-6	42.7	130
Impurity-7	43.1	130
Impurity-8	43.0	130
Impurity-9	38.6	117
Impurity-10	42.3	128
Dabigatran	43.6	132
Impurity-11	33.5	102
Impurity-12	47.0	142

Table 3: Validation Data

Impurity Name	% RSD (n=6)	% RSD (n=12)	Correlation coeffi-cient	LOQ	50%	100%	150%
Impurity-1	3.6	2.8	0.9965	102.7	101.4	102.9	107.2
Impurity-2	4.3	2.7	0.9994	102.6	104.2	101.7	104.4
Impurity-3	2.3	2.8	0.9958	98.9	96.5	106.6	104.8
Impurity-4	4.3	3.8	0.9984	97.4	98.5	100.3	102.0
Impurity-5	4.6	2.9	0.9969	102.3	100.7	105.9	106.1
Impurity-6	2.2	2.5	0.9995	105.9	104.4	102.9	105.6
Impurity-7	3.5	1.5	0.9998	104.8	102.1	98.1	108.5
Impurity-8	3.8	3.0	0.9982	103.4	99.0	100.8	112.0
Impurity-9	3.8	3.5	0.9952	108.0	101.4	105.9	104.6
Impurity-10	2.6	1.3	0.9990	104.3	101.2	103.3	109.4
Dabigatran	2.5	1.1	0.9992	105.9	NA	NA	97.3
Impurity-11	2.2	1.2	0.9981	102.7	101.0	100.6	104.1
Impurity-12	3.5	4.0	0.9966	107.1	101.3	100.4	108.4

Table 4: System Suitability Results

Parameter	Resolution Between Dabigatran & DBG3A methoxy impurity
System Suitability	2.4
Robustness	
Flow Variation (-10%)	4.1
Flow Variation (+10%)	2.2
Temperature Variation (30^0 C)	2.3
Temperature Variation (40^0 C)	3.3

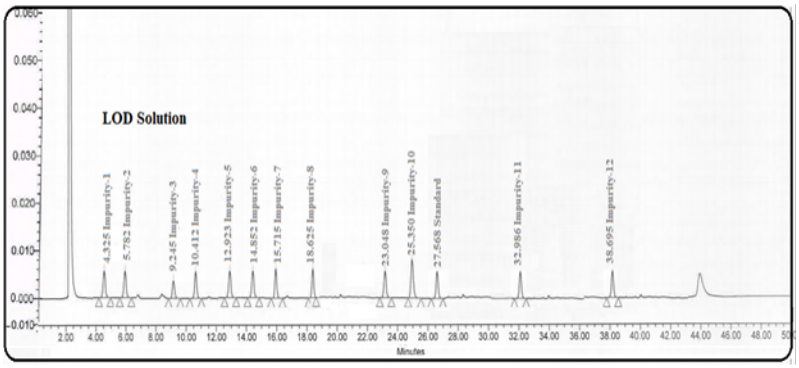

Figure 3: Typical HPLC chromatogram for LOD solution

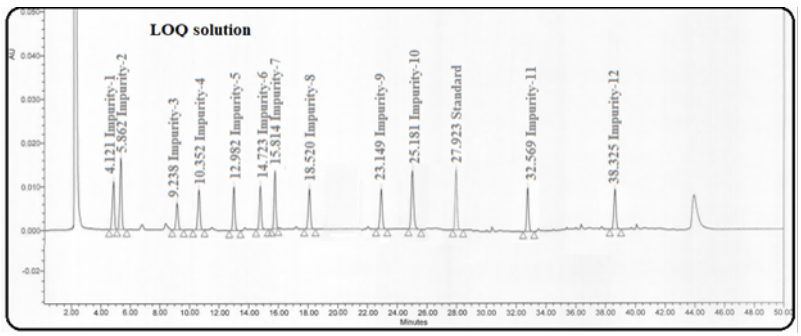

Figure 4: Typical HPLC chromatogram for LOQ solution

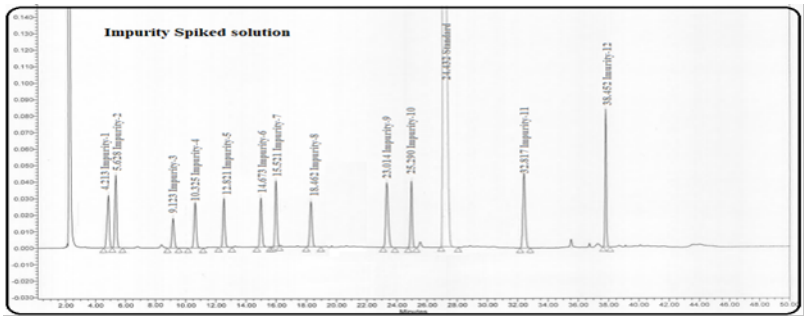

Figure 5: Typical HPLC chromatogram for impurities spiked sample of dabigatran

the initially prepared mobile phase. Results were indicated the mobile phase solution is stable up to 48hours.

CONCLUSION

Developed quantitative related substance method for dabigatran is specific, linear, accurate, and precise. The analytical procedure was fully validated and found that the data generated in all the method validated parameters tested are found satisfactory. Newly developed HPLC method can be used to determine the related substance of regular Dabigatran commercial samples.

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Conflict Of Interests

The authors declare that no conflict of interest for this study.

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