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Validated Method for the Determination of Five Membered Heterocyclic Polar Compound Imidazole in Drug Substances Using Capillary Electrophoresis and UV Detection

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Abstract

A simple and sensitive method based on capillary electrophoresis with UV detection was developed, optimized, and validated for the determination of five membered heterocyclic polar compound, imidazole in pharmaceutical drug substances. Separation was achieved on bare fused silica capillary using a simple running buffer, potassium dihydrogen phosphate at pH 4.0. Capillary temperature set at 25°C. The applied voltage was 25 kV across the capillary and the samples were injected by hydrodynamically at a pressure of 50 mbar for 5 s. Analyte was monitored at 210 nm. The achieved limit of detection value was 0.005%w/w, limit of quantification value was 0.014%w/w, and the average accuracy value was 98.4% for imidazole. The aim of present study is to develop a specific and sensitive method for the determination of imidazole to overcome the void volume and sample matrix interferences.

Keywords: Capillary electrophoresis, Heterocyclic, Imidazole, Polar compound, U.V detection

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Introduction

Imidazole and its derivatives are having biologically active and pharmalogically important heterocycles in natural products and their synthetic analogous. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. Imidazole is a planar five membered aromatic heterocyclic compound with two nitrogen atoms and is present at first and third positions, having the molecular formula C3H4N2. Imidazole is classified as aromatic compound due to the presence of sextet of π-electrons, which consists of a lone pair of electrons from the protonated nitrogen atom and one from each of the remaining four atoms of the ring as shown in Figure 1. Imidazole is highly polar compound, whose dipole is 3.61D and it is soluble in polar solvents such as water and methanol. pKa value of imidazole is 7.01. Imidazole is an amphoteric; it can serves as a base and as a weak acid. Imidazole has anti-inflammatory and analgesic effects in animals. Available acute toxicity data stands that lethaldose (LD_{50}) of imidazole for rat through oral administration is between 220 and 970 mg/kg.[1]

Most of the polar compounds are elutes at void volume or very close to void volume on regular C8 and C18 stationary phases in HPLC columns. More over tailing peak shape obtained for basic compounds in HPLC. Polar compounds retain on special columns having Amide and Hilic stationary phases, but there is limitation in the sample matrix interference, broad peak shape, and column life, whereas capillary electrophoresis technique is simple, reliable, and suitable for the determination of small polar compounds with sharp peak shapes and less solvent consumption. In the literature, some of the analytical methods reported for the separation of Imidazole and its derivatives.[2-8] UHPLC method was reported for the determination of imidazole and its derivatives in cigarette additives using Acquity UPLC BEH Hilic, 100 mm × 2.1 mm × 1.7 µm column.[2] Identification of imidazole derivatives in cigarette smoke was performed by GC-MS on a fused-silica capillary column coated with Carbowax 20M.[3] Separation of imdazoles on capillary electrophoresis using the mixture of sodium lauryl sulfate and tetrabutylammonium hydrogensulfate in phosphate-borate buffer as run electrolyte.[6] In this study, a new and simple capillary electrophoresis method has been developed, optimized, and validated for the determination of five membered heterocyclic polar compound imidazole in pharmaceutical drug substances.

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Experimental

Materials and Methods

Samples of rivaroxaban, cefprozil, and their related substances were procured in APL Research Centre – II (a unit of Aurobindo Pharma Ltd., Hyderabad.). Analytical grade (AR grade) potassium dihydrogen phosphate, orthophosphoric acid, imidazole, and nitric acid were procured from E.Merck; India. Imidazole primary standard procured from Merck, India. Highly pure milli-Q water was prepared using millipore purification system.

Imidazole stock solution was prepared at a concentration level 1000 µg/ml in diluent. Imidazole standard solution was

prepared from the above stock solution at a concentration level 5 µg/ml. Sample solutions prepared at a concentration levels 5 mg/ml and 10 mg/ml for rivaroxaban and cefprozil drug substances, respectively. Diluted aqueous nitric acid (0.05% v/v) was used as diluent for cefprozil drug substances, whereas 80:20:0.1% v/v/v acetonitrile, water, and nitric acid mixture used for rivaroxaban drug substances.

Instrumentation

Bare fused silica capillary, with 56 cm effective length, 50 µm inner diameter (P.No: G1600-61232) from Agilent technologies. Electrophoretic separations were performed on CE – G1600A system equipped with photodiode array detector with ChemStation data handling system (Agilent technologies – DE Germany).

New capillary was rinsed with 1M NaOH for 5 min and with water for 10 min. At the beginning of each working day, the capillary was rinsed with 0.1M NaOH for 5 min, water for 5 min, and with the running buffer for about 30 min. Between runs, the capillary was rinsed with running buffer at 3 bar pressure for 3 min.

Detection Method

The back ground electrolyte was 5 mM of potassium dihydrogen phosphate solution, pH of the electrolyte adjusted to 4.0 using orthophosphoric acid solution. The analysis was carried out on bare fused silica capillary, having dimensions of 56 cm effective length, and 50 µm inner diameter, maintained at temperature of 25°C. Applied voltage is 25 kV with 0.5 min ramp with positive polarity. Sample injected into capillary at the anodic end and injection plug was 50 mbar for 5 s. The analyte was monitored at 210 nm. Precondition time was high flush 3 bar for 3 min and the run time was 10 min. The migration time of imidazole is about 4 min.

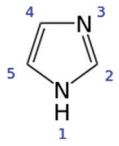

Figure 1: Imidazole

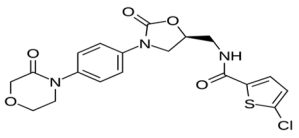

Figure 2: Rivaroxaban

Results and Discussion

Method Development and Optimization

Imidazole is highly polar compound which elutes very close to the void volume in reverse phase high-performance liquid chromatography. The objective of this work is to determine the imidazole without any interference from void volume peaks, sample matrix, and from blank at low-level concentrations using capillary electrophoresis. In recent years, much attention has been taken for using capillary electrophoresis in wide fields of chemical analysis. The proposed capillary electrophoresis method offered many advantages, including cost benefit technique, improved separation speed, less run time, and less solvent consuming rather than other chromatographic separation techniques.

Method development was initiated using 0.1% orthophosphoric acid buffer as back ground electrolyte. Analysis performed on bare fused silica capillary, 56 cm effective length and 50 µm inner diameter. In this experiment, imidazole peak having fronting due to the migration velocity of analyte is followed by background electrolyte. In another experiment background electrolyte selected as 5 mM of potassium dihydrogen phosphate solution, pH adjusted to 5.0 using potassium hydroxide solution. In this experiment, imidazole peak having tailing due to the migration of analyte is lagged by background electrolyte and sample matrix peaks closely eluted to analyte peak.

5 mM of potassium dihydrogen phosphate background electrolyte prepared and pH adjusted to 4.0 with orthophosphoric acid. In this trial, imidazole peak is symmetric and there is no interference from sample matrix.

Method development and validation experiments carried out on two drug substances, they are rivaroxaban and cefprozil.

Rivaroxaban

Rivaroxaban is used as, an oral anticoagulant, normally used in the prevention of thrombosis and thromboembolism, the formation of blood clots in the blood vessels, and their migration elsewhere in the body.[9] Structure of rivaroxaban is shown in Figure 2.

Imidazole is the by product during the cyclysation reaction between phthalimidomethyl amino alcohol (rivaroxaban intermediate-I), N,N-Carbonyl diimidazole, and resulting phthalimidomethyl oxazolidinone (rivaroxaban intermediate-II). Imidazole washed out by solvent washing. Reaction scheme is shown in Figure 3.

Cefprozil

Cefprozil is a second-generation cephalosporin type antibiotic. It can be used to treat bronchitis, ear infections, skin infections, and other bacterial infections.[10,11] Structure of cefprozil is shown in Figure 4. Catalytic amount of imidazole is used as a base in the

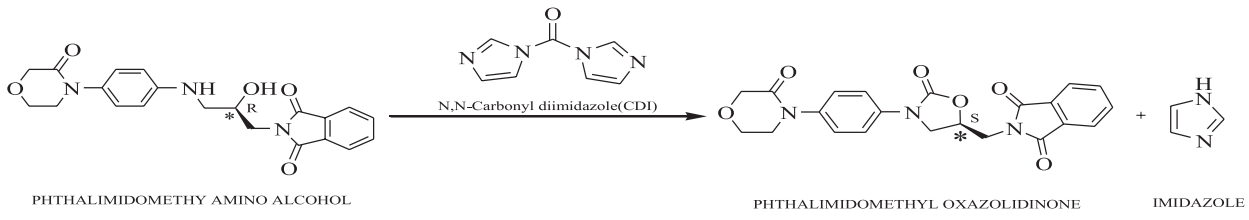

Figure 3: Schematic diagram of preparation of rivaroxaban intermediate-II

silylation of 7-Amino-3-(Z/E-propen-1-yl)-3-cephem-4-carboxylic acid with hexamethyl disilazane and trimethyl chlorosilane resulting (7-trimethylsilylamino-3-[Z/E-propen-1-yl]-3-cephem-4-carboxylic acid) trimethylsilyl ester. Imidazole does not detect in both the drug substances.

Method Validation

The optimized method has been validated according to ICH guidelines to prove its performance characteristics, thereby verifying its suitability and reliability for the determination five membered heterocyclic polar compounds, imidazole in the drug substances.[12] The validation parameters studied in this study were specificity, sensitivity, linearity, precision, and accuracy. The results obtained from the experiments were tabulated in the next paragraphs.

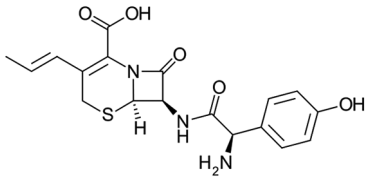

Figure 4: Cefprozil

Specificity

Specificity is the ability of the method to determine the analyte in the presence of other related substances of drug substance. For specificity determination, all the related substances of rivaroxaban and cefprozil solutions were prepared individually and injected into electropherograph as per methodology. The beauty of this capillary electrophoresis method is that there are no interference peaks from the sample matrix near the migration time of imidazole. The typical electropherograms of imidazole standard, diluents, sample, and spiked samples of rivaroxaban and cefprozil are shown in Figure 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

For determining the LOD and LOQ, signal to noise ratio method was adopted. Signal to noise ratio values were considered from the standard solution. LOD and LOQ values were predicted using

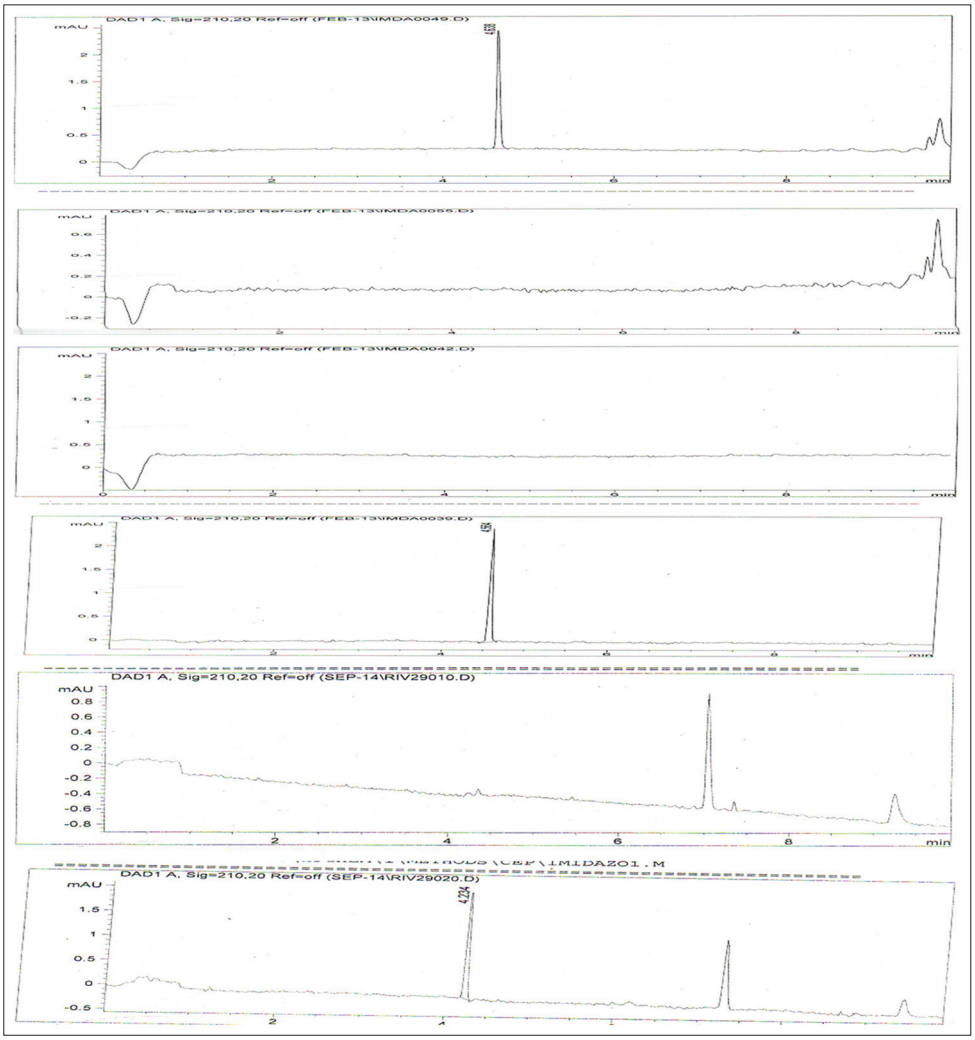

Figure 5: Electropherograms of imidazole standard, diluents, cefprozile sample, cefprozile spiked sample, rivaroxaban sample, and rivaroxaban spiked sample

Table 1: Statistical data of linearity and LOD and LOQ precision

Statistical parameter	*Imidazole*
Concentration range (%w/w)	0.01441–0.07588
Slope	0.4411
Intercept	0.035
STEYX	0.069
Correlation coefficient	0.9982
Limit of detection (%w/w)	0.005
Limit of quantification (%w/w)	0.014
Precision for LOD (%R.S.D)	3.1
Precision for LOQ (%R.S.D)	4.3

LOD: Limit of detection, LOQ: Limit of quantification

Table 2: Accuracy results shows for Rivaroxaban

Amount added (%w/w)	*Amount found (%w/w)*	*% Recovery*	*Statistical analysis*	
LOQ level				
0.014	0.015	107.1	Mean	100.0
0.014	0.014	100.0	SD	7.1
0.014	0.013	92.9	%RSD	7.1
50% level				
0.025	0.024	96.0	Mean	97.3
0.025	0.025	100.0	SD	2.3
0.025	0.024	96.0	%RSD	2.4
100% level				
0.050	0.049	98.0	Mean	98.0
0.050	0.049	98.0	SD	0.0
0.050	0.049	98.0	%RSD	0.0
150% level				
0.075	0.076	101.3	Mean	100.9
0.075	0.074	98.7	SD	2.0
0.075	0.077	102.7	%RSD	2.0

LOQ: Limit of quantification

Table 3: Accuracy results show for cefprozil

Amount added (%w/w)	*Amount found (%w/w)*	*% Recovery*	*Statistical analysis*	
LOQ level				
0.022	0.021	95.5	Mean	94.0
0.022	0.020	90.9	SD	2.7
0.022	0.021	95.5	%RSD	2.8
50% level				
0.049	0.049	100.0	Mean	99.3
0.049	0.049	100.0	SD	1.2
0.049	0.048	98.0	%RSD	1.2
100% level				
0.099	0.097	98.0	Mean	97.7
0.100	0.096	96.0	SD	1.5
0.100	0.099	99.0	%RSD	1.6
150% level				
0.150	0.147	98.0	Mean	98.2
0.149	0.144	96.6	SD	1.7
0.149	0.149	100.0	%RSD	1.7

LOQ: Limit of quantification

standard concentration(S) and signal to noise ratio (SN) method using the formula 3.3 × S/SN for LOD and 10 × S/SN for LOQ. LOQ value was predicted 0.014%w/w and LOD value was predicted as 0.0047%w/w for imidazole. The LOD and LOQ solutions were prepared at about predicted concentration levels and analyzed 6 times for checking the precision and the results are tabulated in Table 1.

Linearity

The linearity of the detector was determined by preparing a series of solutions using imidazole at concentration levels from about LOQ level, to 0.076%w/w level. The data were subjected to statistical analysis using a linear regression model. The statistical evaluations such as slope, intercept, STEYX, and correlation coefficient values of linearity data are given in Table 1.

Table 4: Precision results show for revaroxaban and cefprozil

Experiment	*Rivaroxaban*	*Cefprozil*
	Imidazole	
System precision		
% RSD (*n*=6)	2.4	1.5
95% Confedence interval	±0.06	±0.04
Method precision		
Sample-1	0.052	0.097
Sample-2	0.051	0.098
Sample-3	0.050	0.101
Sample-4	0.050	0.099
Sample-5	0.054	0.097
Sample-6	0.049	0.099
Mean	0.051	0.099
% RSD	3.9	2.0
95% Confidence interval	±0.002	±0.002

Accuracy

Accuracy of the methods was performed by recovery experiments using standard addition method. The recoveries were determined by spiking imidazole at four different levels at LOQ level, 50, 100, and 150%w/w of rivaroxaban and cefprozil drug substances. These samples were prepared as per respective test procedure and analyzed in triplicate and the percentage recoveries were calculated. The percentage recovery values for analyte ranged from 97.3 to 107.1. The fully validated accuracy results are shown in Tables 2 and 3.

Precision

System precision was demonstrated by preparing the standard solution of analyte as per respective methodology and analyzing six replicate injections. Migration time reproducibility of imidazole is <0.5%. Method precision was demonstrated by preparing six sample solutions individually using a single batch of rivaroxaban and cefprozil drug substances spiked with imidazole separately at a known concentration level as per respective methodology and injected each solution and determined the content of imidazole. Achieved results such as %RSD and 95% confidence interval for six determinations and cumulative of 12 determinations are summarized in Table 4.

Conclusion

The capillary electrophoresis method was developed, optimized, and validated for the determination of five membered heterocyclic polar compound imidazole in rivaroxaban and cefprozil drug substances and the results of various validation parameters demonstrated that the method is specific, sensitive, linear, precise, and accurate.

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