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Original Research Article

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DOFETILIDE IN MARKETED FORMULATION

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ABSTRACT

A simple, accurate, precise, and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of dofetilide in marketed capsule formulations. Chromatographic separation was achieved on a suitable C18 column using an optimized mobile phase under isocratic conditions, with detection carried out at an appropriate UV wavelength. The method showed excellent linearity over the concentration range of 5–25 µg/mL with a regression equation Y = 240.22x + 51.731 and a correlation coefficient (r^2 = 0.9998). The developed method was validated as per ICH Q2(R1) guidelines for specificity, accuracy, precision, robustness, and system suitability. Accuracy studies demonstrated mean percentage recoveries ranging from 97.83% to 99.33%, while precision studies revealed %RSD values below 2%, indicating good repeatability and intermediate precision. Robustness testing confirmed that small deliberate variations in chromatographic conditions did not significantly affect method performance. The assay of marketed capsule formulation showed 96.00% of the labeled claim. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed that the method could effectively separate dofetilide from its degradation products, thereby establishing its stability-indicating nature. The proposed RP-HPLC method is suitable for routine quality control analysis and stability testing of dofetilide in bulk and pharmaceutical dosage forms.

Keywords: Dofetilide, RP-HPLC, Stability-indicating method, Method validation, Forced degradation, Capsule formulation.

INTRODUCTION

Dofetilide is a class III antiarrhythmic agent primarily used for the management of atrial fibrillation and atrial flutter (Falk *et al.*, 1997). It exerts its pharmacological action by selectively blocking the rapid component of the delayed rectifier potassium current, thereby prolonging the cardiac action potential duration and effective refractory period without affecting conduction velocity. Due to its narrow therapeutic index and potential to induce serious adverse effects such as QT interval prolongation and torsades de pointes, precise and reliable quantification of dofetilide in pharmaceutical dosage forms is of paramount importance.

High-performance liquid chromatography (HPLC) is widely recognized as a robust and sensitive analytical technique for the quantitative estimation of drugs in bulk and finished pharmaceutical products. In accordance with regulatory guidelines, particularly those outlined by the International Council for Harmonisation (ICH), analytical

methods employed for drug analysis must be validated for parameters such as accuracy, precision, specificity, linearity, robustness, and system suitability (Belanger *et al.,* 1997). Moreover, stability-indicating methods are essential to ensure that the analytical procedure can effectively separate the active pharmaceutical ingredient from its degradation products, impurities, and excipients, thereby providing reliable results throughout the product's shelf life.

Dofetilide is susceptible to degradation under various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic environments (Bhole *et al.,* 2019). Therefore, the development of a stability-indicating HPLC method capable of accurately estimating dofetilide in the presence of its degradation products is essential for quality control, stability studies, and regulatory compliance. Although a few analytical methods have been reported for the estimation of dofetilide, many of them lack comprehensive forced degradation studies or do not fully comply with current ICH guidelines.

In view of these considerations, the present study was undertaken to develop and validate a simple, precise, accurate, and robust stability-indicating RP-HPLC method for the estimation of dofetilide in marketed pharmaceutical formulations. The proposed method aims to achieve efficient chromatographic separation of dofetilide from its potential degradation products and to validate the method as per ICH Q2(R1) guidelines, thereby ensuring its suitability for routine quality control and stability testing.

MATERIALS AND METHODS

Materials

Dofetilide reference standard was obtained as a gift sample from a reputed pharmaceutical manufacturer and was used without further purification. Marketed dofetilide capsule formulation (label claim: 0.25 mg) was procured from a local pharmacy. HPLC-grade methanol and acetonitrile were purchased from Merck (India) and used as components of the mobile phase. Analytical reagent (AR) grade potassium dihydrogen phosphate and orthophosphoric acid were used for the preparation and pH adjustment of the buffer solution. Hydrogen peroxide (30% v/v), hydrochloric acid, and sodium hydroxide of analytical grade were employed for forced degradation studies. HPLC-grade water was prepared using a Milli-Q water purification system. All solutions were freshly prepared, filtered through a 0.45 µm membrane filter, and degassed prior to use.

Methods

Mobile Phase Selection

Initially to estimate Dofetilide number of mobile phase in different ratio were tried. Results were shown in table no 7.4 (Lindholm, 2004).

Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 25mM KH_2PO_4 and Acetonitrile in the ratio of 15:85 adjust the pH 4 with OPA. The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min.

Selection of wavelength

100 mg of Dofetilide was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase (Snyder *et al.,* 1997). From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 282.00 nm.

Selection of Separation Variable

Standard drug solution of Dofetilide was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature (Weston and Brown, 1997). Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Dofetilide 10µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard Stock Solution

10mg of Dofetilide was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Dofetilide 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of the Drug

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of capsule formulation

For analysis of the capsule formulation, weight equivalent to weight 5mg of Dofetilide was transferred to 10ml volumetric flask and dissolved in mobile phase (Hong and Shah, 2008). The solution was shaking vigorously for 20mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with acetonitrile. From the above solution 1ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with methanol to get a solution containing 10µg/ml of Dofetilide. The amounts of Dofetilide in capsule formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with capsule formulation.

Validation

The following are typical analytical performance characteristics which may be tested during methods validation (ICH Q2A, 1994; ICH Q2B, 1996; ICH, 2000):

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in

the sample. The calibration plot was contracted after analysis of five different (from 5 to 25µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision

Day to Day

The statistical analysis method was carried out and the data is presented in Table.

Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different days, different analysts, different equipment etc).

The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Dofetilide was studied.

Forced Degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on Dofetilide powder and the analysis was carried out by HPLC with a U.V. detector (Dolan, 2005). 20µl of each of forced degradation samples were injected at regular intervals Shown in Table.

Acid degradation

50 mg of Dofetilide sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Dofetilide (Bakshi and Singh, 2002).

Base degradation

50 mg of Dofetilide sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Dofetilide.

Hydrolytic degradation

50 mg of Dofetilide sample was taken into a 50 ml round bottom flask, 50 ml of water was added and the contents were mixed well and

kept for constant stirring for 48 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Dofetilide

Oxidative degradation

50 mg of Dofetilide sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature.

Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Dofetilide.

Thermal degradation

50 mg of Dofetilide sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks.

Photolytic degradation

The Dofetilide was exposed to sunlight during the daytime (70,000–80,000 lux) for 2 days.

RESULTS AND DISCUSSION

The present study was undertaken to develop and validate a stability-indicating RP-HPLC method for the estimation of dofetilide in marketed capsule formulations in accordance with ICH Q2 (R1) guidelines. The discussion of the results obtained from method validation parameters is presented below.

The chromatogram of the standard dofetilide (Figure 1) showed a sharp, symmetric peak with good resolution and no interference from excipients, indicating the specificity of the developed method. The absence of additional peaks at the retention time of dofetilide confirmed that the method is selective for the analyte.

Linearity was evaluated over the concentration range of 5–25 µg/mL. The calibration curve demonstrated excellent linearity with a regression equation $Y = 240.22x + 51.731$ and a high correlation coefficient ($r^2 = 0.9998$), indicating a strong linear relationship between concentration and peak area. The response ratio values showed minimal variation across concentrations, further confirming the consistency and proportionality of detector response within the studied range.

Accuracy of the method was assessed by recovery studies at 80%, 100%, and 120% levels. The mean percentage recovery values ranged from 97.83% to 99.33%, with %RSD values less than 0.30%. These results indicate that the method is highly accurate and free from interference by formulation excipients, confirming its suitability for routine analysis.

Precision studies demonstrated excellent repeatability and intermediate precision. The intra-day precision showed a mean % label claim of 98.35% with %RSD of 0.408, while the inter-day precision showed a mean of 97.61% with %RSD of 1.112. The low %RSD values (<2%) confirm that the method is precise and reproducible under normal operating conditions.

Robustness studies were carried out by deliberately varying experimental parameters such as temperature, flow rate, and mobile phase composition. The %RSD values under all modified conditions were found to be below 1%, indicating that minor variations in chromatographic conditions do not significantly affect the analytical performance of the method. This confirms the robust nature of the developed RP-HPLC method.

The assay of the marketed capsule formulation showed 96.00% of the labeled claim, which lies within the acceptable pharmacopoeial limits. The low standard deviation and % RSD values demonstrate that the method is suitable for the quantitative estimation of dofetilide in pharmaceutical dosage forms.

Forced degradation studies revealed that dofetilide undergoes degradation under acidic, alkaline, oxidative, thermal, and photolytic conditions, with maximum degradation observed under alkaline hydrolysis. Importantly, the degraded products were well separated from the main drug peak, confirming that the method is stability-indicating and capable of accurately estimating dofetilide in the presence of its degradation products.

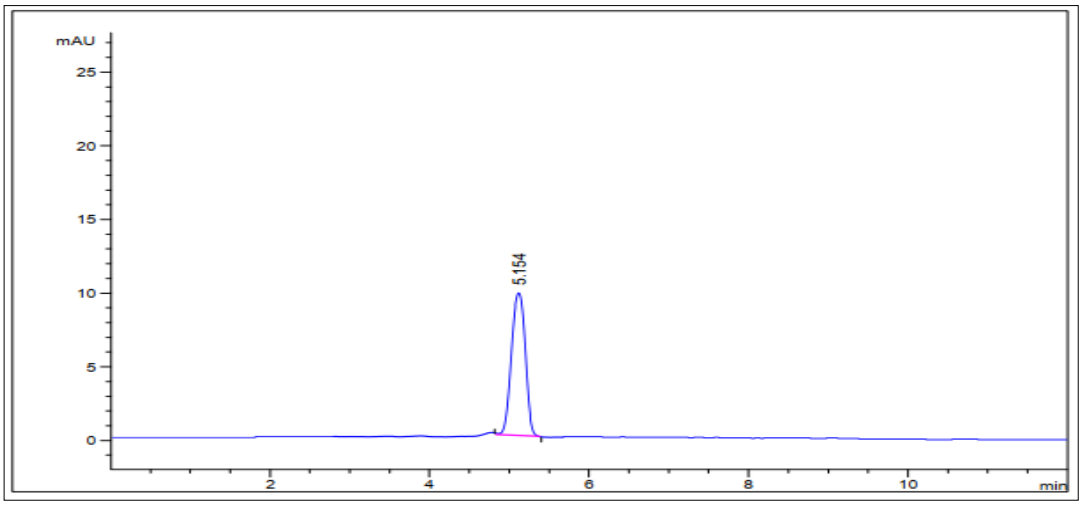

Figure 1: Chromatogram of Standard

Table 1: Calibration Curve of Dofetilide

Parameter	Value
Regression equation	Y = 240.22x + 51.731
Response (Y)	Area Under Curve (AUC)
Independent variable (X)	Concentration (µg/mL)
Slope (m)	240.22
Intercept (c)	51.731
Correlation coefficient (r^2)	0.9998

Table 2: Linearity and Response Ratio of Dofetilide

Concentration (µg/mL)	Mean AUC	Response Ratio
5	1288.339	257.668
10	2474.884	247.488
15	3676.751	245.117
20	4866.213	243.311
25	6020.518	240.821
Mean ± SD		**246.88 ± 6.51**

Table 3: Accuracy (Recovery Study) of Dofetilide

Recovery Level	Sample Conc. (mg)	Amount Added (mg)	Mean % Recovery	SD	% RSD
80%	5	4	97.83	0.294	0.299
	10	8	98.83		
	15	12	98.69		
100%	5	5	98.33	0.294	0.298
	10	10	97.93		
	15	15	99.33		
120%	5	6	98.50	0.294	0.298
	10	12	98.75		
	15	18	98.44		

Table 4: Precision Study of Dofetilide (Intra-day and Inter-day Precision)

Precision Type	Time / Day	% Label Claim
Intra-day Precision	After 1 h	99.12
	After 2 h	98.45
	After 3 h	98.25
	After 4 h	98.15
	After 5 h	98.10
	After 6 h	98.05
	Mean ± SD	**98.35 ± 0.401**
Inter-day Precision	**% RSD**	**0.408**
	Day 1	98.85
	Day 2	97.12
	Day 3	96.85
	Mean ± SD	**97.61 ± 1.085**
	% RSD	**1.112**

Table 5: Robustness Study of Dofetilide

Parameter Changed	Condition	% RSD
Temperature	−5 °C	0.895
	+5 °C	0.754
Flow rate	−10%	0.553
	+10%	0.658
Mobile phase ratio	−2%	0.754
	+2%	0.587

Table 6: Assay of Marketed Capsule Formulation

Drug	Label Claim (mg)	Amount Found (mg)	% Label Claim	SD	% RSD
Dofetilide	0.25	0.24	96.00	0.125	0.145

Table 7: Forced Degradation Studies of Dofetilide

Stress Condition	Drug Recovered (%)	Drug Decomposed (%)
Standard drug	99.90	0.00
Acidic hydrolysis	90.25	9.65
Alkaline hydrolysis	80.25	19.65
Oxidative degradation	85.65	14.25
Thermal degradation	90.12	9.78
Photolytic degradation	84.45	15.45

CONCLUSION

A simple and reliable stability-indicating RP-HPLC method was developed and validated for the estimation of dofetilide in capsule formulations as per ICH Q2(R1) guidelines. The method showed excellent linearity, accuracy, precision, and robustness, with effective separation of the drug from its degradation products under various stress conditions. Hence, the method is suitable for routine quality control and stability analysis of dofetilide in pharmaceutical dosage forms.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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