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METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF DOFETILIDE IN BULK AND PHARMACEUTICAL DOSAGE PREPARATIONS BY RP-HPLC

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ABSTRACT

A Specific, Precise, Accurate, Rugged and Robust stability indicating RP-HPLC method has been developed and validated for the estimation of Dofetilide in in bulk and pharmaceutical dosage form (Tablets) was carried out by HPLC with Phenomenex C18 (150x4.6mm ID) 1.8µm column as stationary phase by using mobile phase consisting of 20mM Potassium di hydrogen phosphate Buffer pH 6.8: Acetonitrile: Methanol(50:30:20 v/v/v) at a flow rate of 1.0mL/min and detection was carried out at 231nm. The Retention time of Dofetilide 2.3 min respectively, The method produced linear response of for Dofetilide with >0.999 correlation coefficient. The % Recoveries for Dofetilidein were obtained in between 98.0 to 102.0.

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INTRODUCTION

Dofetilide is a class III antiarrhythmic agent that is approved by the Food and Drug Administration (FDA) for the maintenance of sinus rhythm in individuals prone to the formation of atrial fibrillation and flutter, and for the chemical cardioversion to sinus rhythm from atrial fibrillation and flutter.[2]

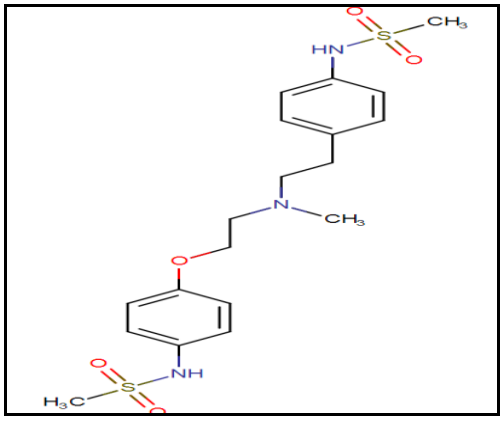


Fig No : 01 Structure of Dofetilide[4].

The mechanism of action of Dofetilide is a blockade of the cardiac ion channel carrying the rapid component of the delayed rectifier potassium current, IKr. This inhibition of potassium channels results in a prolongation of action potential duration and the effective refractory period of accessory pathways (both anterograde and retrograde conduction in the accessory pathway).

Dofetilide is an antiarrhythmic drug with Class III (cardiac action potential duration prolonging) properties and is indicated for the maintenance of normal sinus rhythm. Dofetilide increases the monophasic action potential duration in a predictable, concentration-dependent manner, primarily due to delayed repolarization. At concentrations covering several orders of magnitude, dofetilide blocks only IKr with no relevant block of the other repolarizing potassium currents (e.g., IKs, IK1). At clinically relevant concentrations, Dofetilide has no effect on sodium channels (associated with Class I effect), adrenergic alpha-receptors, or adrenergic beta-receptors.

There are no reported methods available for the analysis of individual dofetilide by HPLC method as a single drug. Two analytical methods reported with combination of dofetilide by using HPLC [9-10] and one analytical method developed by using UV-HPTLC [11]. This is only method for the determination of individual dofetilide in bulk pharmaceutical dosage forms and validated as per ICH guidelines.

EXPERIMENTAL METHODOLOGY:

Equipment's:

Shimadzu LC 2010 HPLC system with quaternary pumps, Auto sampler, UV detector and Lab solutions software is used in the current method. Analytical column Phenomenex[3] C18 (50x2.1mm ID) 1.8µm is used as stationary phase in the chromatographic separation and quantification of Dofetilide. Thermo electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic sonicator, Shimadzu analytical balance AY-220, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials:

Dofetilide was supplied by Chandra labs, prashanthi nagar, kukatpally, Hyderabad, India. Odefsey tablet containing Tykosin 0.25mg was kindly supplied by Pfizer Inc[1]. Acetonitrile, Methanol, Milli-Q water is used of HPLC-grade, Potassium di hydrogen phosphate purchased from Merck and Rankem, India.

Optimized Chromatographic conditions:

Analytical separation is carried out with Analytical column Phenomenex C18 (50x2.1mm ID) 1.8µm) by using Isocratic mode with a mixture of 20mM Potassium di hydrogen phosphate Buffer pH 6.8: Acetonitrile: Methanol (50:30:20 v/v/v). Column Oven Temperature: 30 ±0.2°C, 1.0 mL/min maintained as flow rate and 10µL maintained as sample injection volume with detection at 231nm with UV detector.

Preparation of mobile phase:

20mM Phosphate Buffer Preparation:

Accurately weighed about 2.72gr of potassium di hydrogen phosphate and transferred in to 1000mL of water, mixed well. Added 2mL of triethylamine then Adjusted 6.8 with diluted Orthophosphoric acid. Buffer was filtered through 0.45µ membrane filter[5].

Mobile phase:

Mixed 20mM Potassium di hydrogen phosphate Buffer: Acetonitrile: Methanol with ratio of 50:30:20 and degassed by sonication

Preparation of diluent:
Mobile Phase used as diluent

Preparation of standard solution:

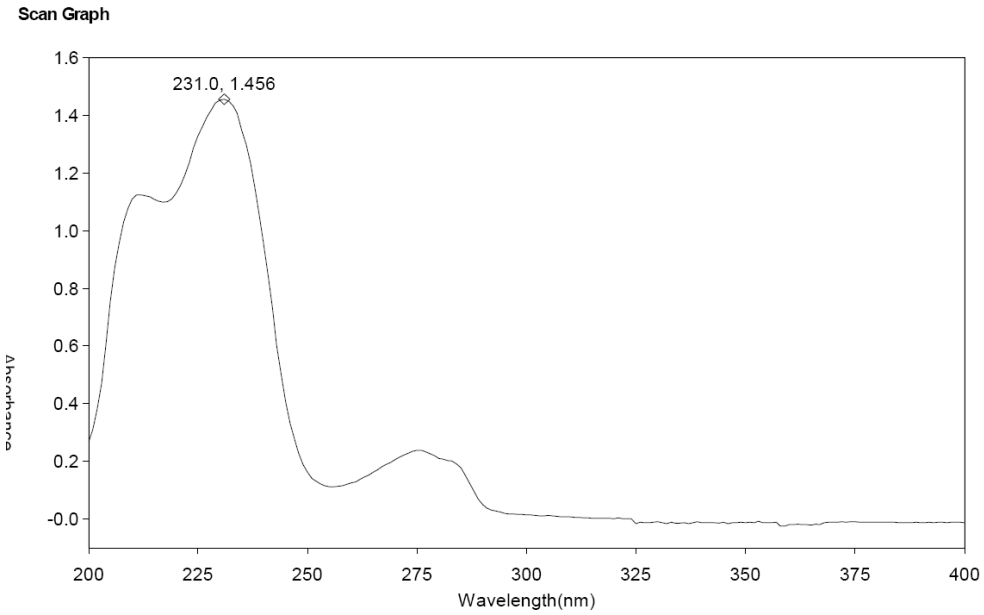
Accurately weighed about 5mg of Dofetilide transferred in to 100ml of volumetric flask and added 70mL of diluent and sonicated for 5min and diluted up to the mark with diluent (5µg/mL of Dofetilide)
Then pipetted 5ml of this solution into 50ml volumetric flask and diluted volume up to the mark with same diluent.

Preparation of Sample Solution:

(Each Capsule contains 0.25mg of Dofetilide) 10 Capsules shells were opened and powder was weighed equivalent to 2.5mg of Dofetilide sample into a 50ml clean volumetric flask added about 30mL of diluents and sonicated up to 20 min to completely dissolve and diluted up to the mark with diluent. Centrifuge this solution at 5000RPM for 10min.
Pipetted 5ml of the above supernatant solution into 50ml volumetric flask and diluted volume up to the mark with same diluent.

Selection of working wavelength (λmax):

UV spectrum of 3µg/mL of Dofetilide in methanol, spectrum was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 231 nm. At this wavelength all these drugs show good absorbance. The spectrum was shown in Figure No: 4.



Results Table - scan011,Sample001,Cycle01

nm	A	Peak Pick Method
231.00	1.456	Find 8 Peaks Above -3.0000 A
		Start Wavelength 200.0 nm
		Stop Wavelength 400.0 nm
		Sort By Wavelength
Sensitivity	Auto	

Fig No: 02 UV-VIS Spectrum shows the Isobestic point was found to be 231nm for Dofetilide.

Analytical method validation[6]:
System suitability[6]:

System suitability test was carried out on a freshly prepared standard solution was injected 5 times into the HPLC system under optimized chromatographic conditions.

Acceptance Criteria: %RSD should be ≤ 2.0 for the peak area of individual component and also evaluate theoretical plates, asymmetric factor, and resolution between peaks.

Specificity[6,7,8]:

Preparation of placebo solution:

A quantity 360mg of Placebo blend powder(equivalent to 10 Capsules powder without active ingredient) was weighed and transferred in to a 50ml clean volumetric flask added about 30mL of diluents and sonicated up to 20 min to completely dissolve and dilute up to the mark with diluent. Centrifuge this solution at 5000RPM for 10min.

Pipetted 5ml of the above solution into 50ml volumetric flask and diluted volume up to the mark with same diluent.

Blank, Three placebo preparations, standard, one sample preparations were injected in to Chromatographic system and recorded the chromatogram.

Acceptance Criteria: Blank and Placebo should not show any interference at the retention time of the main peak

Linearity[6]:

Linearity of the method was evaluated at the five equal-spaced concentration by diluting the standard stock solution to give solution over the range of 50-150% target of Dofetilide. Calibration curve was constructed at five linear concentrations of Dofetilide (2.5, 4, 5, 6& 7.5µg/mL).solutions were injected in to chromatographic system, after getting the results plotted a graph concentration versus area to evaluate correlation coefficient.

Acceptance criteria: Correlation coefficient Not less than 0.99.

Method Precision[6]:

Method precision Validation parameter investigated using the six individual sample preparations as reported above. Six sample were injected individually in to chromatographic system & calculated the % Assay of individual samples.

Acceptance Criteria: % Assay should be 95.0 to 105 & %RSD for six preparations assay should be ≤ 2.0

Accuracy & Recovery[6]:

Accuracy of the method was determined by measuring with analytes in the presence of placebo. Accuracy solutions prepared in to three level (50%, 100% and 150%). For each level thee preparations were prepared individually. 50%, 100% & 150% Solutions were prepared with different drug weights with constant weight of placebo in the manner of sample preparation. Concentrations of Dofetilide (2.5, 5 & 7.5µg/mL).

Acceptance criteria of %Recovery should be 98.0 to 102.0 & Acceptance criteria of % RSD for nine preparations recover values should be ≤ 2.0

Robustness[6]:

To determine the Robustness of the developed method, experimental conditions were deliberately changed and %RSD for Replicate injections of standard solution peak Areas, theoretical plates, tailing factor and resolution were evaluated. The mobile phase flow rate was changed to 0.8mL/min & 1.2mL/min (Actual flow rate: 1.0mL/min).

The Wavelength was changed to 229nm & 231nm (Actual Wave length: 231nm). Acceptance Criteria: System suitability should be within the acceptance criteria Acceptance Criteria: System suitability should be within the acceptance criteria

Intermediate Precision (Ruggedness)[6]:

Intermediate precision (also called within-laboratory or within-device) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similar objects over an extended period of time. The Intermediate Precision (Ruggedness) was investigated using six individual sample preparations as reported above. Six preparations were injected individually in to chromatographic system & Calculated % Assay of individual samples. Acceptance criteria of %Assay should be 95.0 to 105.0, % RSD for six preparations assay values should be ≤ 2.0 & % RSD for Method precision and Intermediate Precision assay mean values should be ≤ 2.0.

RESULT AND DISCUSSION:

System suitability[6]:

Table No-01: Results of System suitability.

Parameter	Dofetilide
Retention Time in min	2.330
Theoretical plates	26650
Tailing Factor	1.0
Resolution	---
Area	45574976
%RSD	0.2

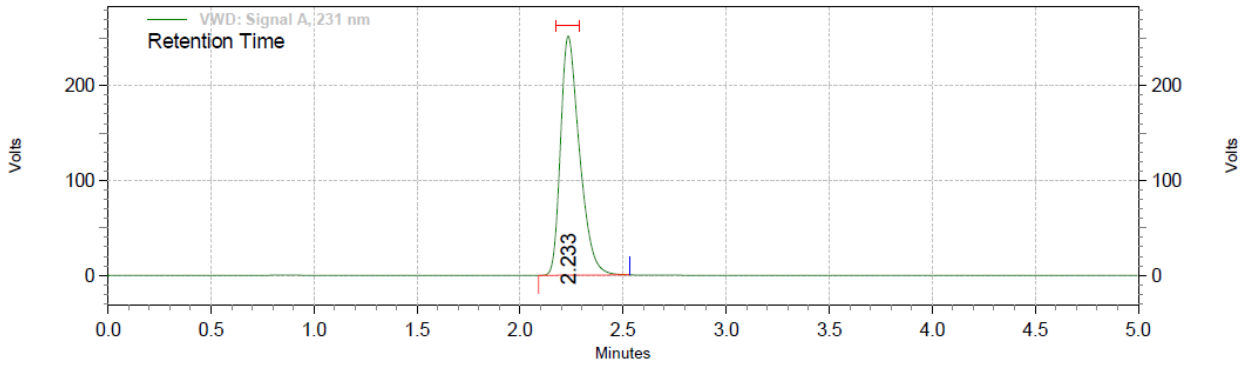


Figure No-03: Chromatogram of Standard.

From the above results of %RSD, theoretical plates, tailing factor and Resolution were obtained within acceptance criteria.

Specificity[6]:

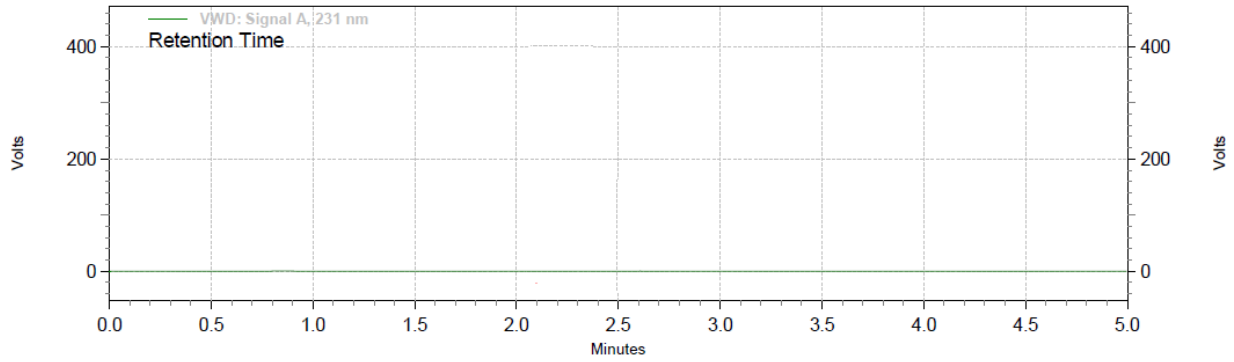


Figure 04: Placebo solution chromatogram.

From the above chromatogram placebo was not interfered at retention time of main peaks were obtained so method is specific.

Linearity[6]:

Table No-02: Results of Linearity.

Parameter	Dofetilide
Concentration range in µg/mL	2.5-7.5µg/mL
Correlation coefficient	0.9998
Intercept	1158059
Slope	9994165

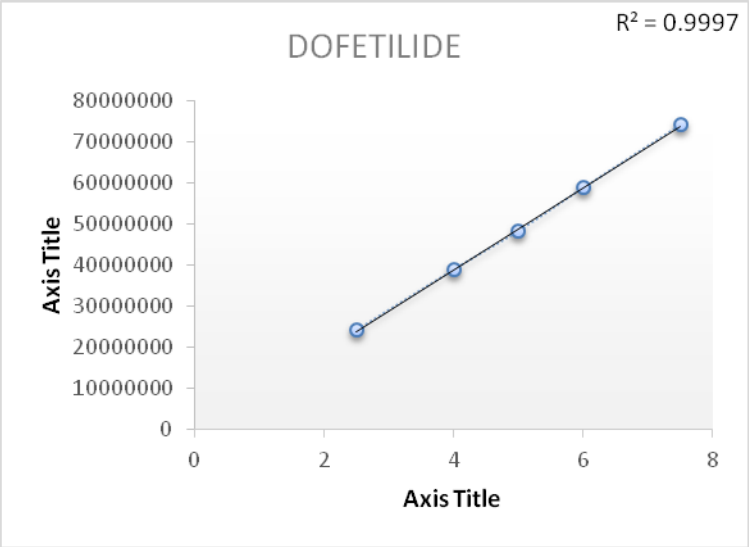


Figure No-05: Calibration curves of about Dofetilide.

From the above results correlation coefficient of three analytes were not less than 0.99 so the method is linear.

Method Precision[6]:

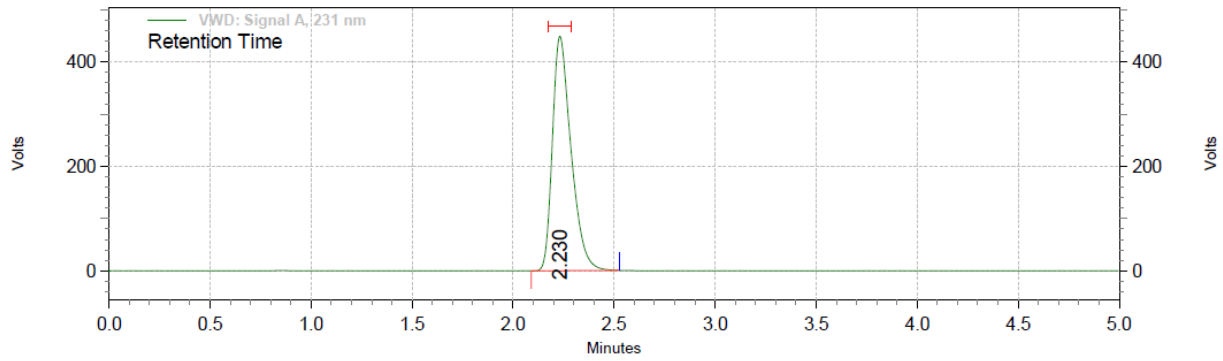


Figure 06: Test solution-01 chromatogram.

Table No-03: Method Precision results.

Parameter	Dofetilide
Method Precision_01	98.9
Method Precision_02	99.0
Method Precision_03	99.1
Method Precision_04	99.0
Method Precision_05	99.1
Method Precision_06	99.3
Average	99.1
Std Deviation	0.16
%RSD	0.2

From the above results were within the acceptance criteria, % Assay results obtained 95.0 to 105.0% and % RSD was less than 2.0 so the method is precise.

Accuracy and Recovery[6]:

Table No-04: Accuracy and Recovery results.

Parameter	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery (%)
50% Recovery	2.50	2.46	98.6
100% Recovery	5.00	5.00	100.0
150% Recovery	7.50	7.50	100.0
Mean	-	-	99.5
%RSD	-	-	0.76

From the above results %Recovery obtained within the acceptance criteria (98.0 to 102.0%), %RSD also obtained less than 2.0. so method is accurate.

Intermediate Precision[6]:

Table No-05: Results of Intermediate Precision.

Parameter	Dofetilide
Method Precision_01	98.6
Method Precision_02	99.3
Method Precision_03	99.4
Method Precision_04	98.0
Method Precision_05	98.4
Method Precision_06	99.6
Average	98.9
Std Deviation	0.64
%RSD	0.6

From the above results were within the acceptance criteria, % Assay results obtained 95.0 to 105.0% and % RSD was less than 2.0, %RSD of Day-01 & Day-02 Assay Results less than 2.0%, so method is Rugged.

Robustness[6]:
Decreased Flow rate 1.2mL/min:

Table No-09: Robustness increased Flow rate 1.2mL/min results.

Parameter	Dofetilide
Retention Time in min	1.86
Theoretical plates	26854
Tailing Factor	1.1
Resolution	---
%RSD	0.7

Increased Flow rate 0.8mL/min:

Table No-08: Robustness increased Flow rate 0.8mL/min results.

Parameter	Dofetilide
Retention Time in min	2.951
Theoretical plates	25412
Tailing Factor	1.1
Resolution	---
%RSD	0.9

From the above results system suitability results were within the acceptance criteria, so method is Robust

CONCLUSION

Method was found to simple, accurate, specific, reliable and robust and method was described for the estimation of Pharmaceutical dosage form (Capsule) consisting of Dofetilid. Active ingredient was successfully resolved and quantified using HPLC with Phenomenex C18 (150x4.6mm ID) 1.8µm column in a relatively short run time of 5 minutes with 1.0mL/min flow in Isocratic mode of the chromatographic system. The proposed method provides a good resolution between active ingredients. The developed method was validated as per described in the ICH Q2B guidelines like system suitability, specificity, linearity, method precision, accuracy and recovery, robustness and ruggedness. The proposed method has the advantages of repeatability, sensitivity and requires less expensive reagents. In forced degradation studies all main peak purity angles were founded less than peak purity threshold so this method is defined as stability indicating method.

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