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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DOFETILIDE

PATEL TR[1*], PATEL RM[1], SINHA A[2] AND PATEL KB[3]

1: Assistant Professor, Department of Pharmaceutical Chemistry and Quality Assurance,
Saraswati Institute of Pharmaceutical sciences, Dhanap, Gandhinagar-382321

2: Shri Sarvajanik Pharmacy College, Gujarat Technological University, Mehsana-384001,
Gujarat, India

3: Assistant Professor, Department of Pharmaceutical chemistry and Quality Assurance,
Nootan Pharmacy College, Sankalchand Patel University, Visnagar 384315

***Corresponding Author: Dr. Tinkal R Patel**; **E Mail:** ptinkal93@gmail.com

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ABSTRACT

A simple, rapid, accurate and economic HPTLC method has been developed for estimation of Dofetilide. In HPTLC method, chromatographic separation was carried out on TLC plate, precoated with silica gel 60F 248 using mobile phase Chloroform: Methanol: Ammonia (7:3:0.5) and scanning at 248 nm. HPTLC methods showed satisfactory linearity with correlation coefficient greater than 0.99 and linearity was demonstrated from 500-3000 ng/band for DOFE. The methods successfully developed and validate the Dofetilide in their dosage form. All method validation parameters for HPTLC lie within its acceptance criteria as per ICH Q2(R1) guidelines. Hence, the proposed methods can be used for Dofetilide in quality control analysis.

Keywords: Dofetilide, Validation, HPTLC, Quantitative determination, Methanol

➢ INTRODUCTION:

Dofetilide chemically is N-[4-[2-[methyl[2-[4-(methylsulfonyl)amino]phenoxy]ethyl]amino]ethyl]phenyl]-methanesulfonamide used as an antiarrhythmic drug with Class III (cardiac action potential duration prolonging) properties and is indicated for the maintenance of normal sinus rhythm Tikosyn (Dofetilide) Capsule approved by

FDA, 1999. It is marketed under trade name Tikosyn by Pfizer, and is available in the United States in capsule containing 0.125,025 and 0.5 mg of Dofetilide. The mechanism of action is blockade of the cardiac ion channel carrying the rapid component of the delayed rectifier potassium current, Ikr. 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 **[1, 2, 3]**.

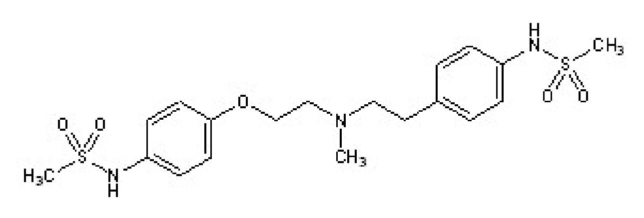

Figure 1: Structural formulas of DOFE

Literature survey revealed that it is official in USP 36 NF 31. Official and reported HPLC methods and one spectroscopic method are available, there is no any reported HPTLC method are available. This study presents HPTLC method for the determination of dofetilide in bulk and pharmaceutical formulations. Accordingly, the objective of this study was to develop and validate HPTLC method for the estimation of dofetilide in bulk and pharmaceutical formulation as per ICH guidelines **[4-9]**.

MATERIAL AND METHODS:

Chemicals, Reagents and Solutions:

Dofetilide, Lable claim: Dofetilide 500mcg, AR grade Chloroform, Methanol and Ammonia, Silica gel 60 GF254 plates were obtained from E.Merck, Mumbai India, Whatman filter paper no. 41.

Instrumentation and Chromatographic Conditions:

Pre-coated silica gel aluminium Platte 60F-254 (20 x 20 cm with 250µm thickness)(E.Merck), Mobile phase: Chloroform: Methanol: Ammonia (7:3:0.5 v/v), Chamber Saturation: 20min, Distance rum by mobile phase:70mm, Desaga – 250 µL dosing syringe (Hamilton), Desaga TLC scanner, Proquant Software, Desaga Photo chamber, Providoc with Canon power shot G5 digital camera, UV cabinet with dual wavelength UV lamp, pH meter, Ultrasonicator.

Preparation of Standard Stock Solution:

Accurately weighed quantity of 10mg DOFE was transferred into 10ml volumetric flask dissolved in HPLC grade methanol using ultra sonication and diluted up to mark to give a stock solution having concentration of 1000 µg/ml.

Preparation of Working Standard Solution:

100 µg/ml of Dofetilide solution was prepared by diluting 1 ml of stock solution to 10 ml wiith methanol.

Test Sample Preparation:

About 20 Capsules were weighed and powdered; a quantity of Capsule powder equivalent of 10mg Dofetilide was weighed accurately and transferred to a 10ml volumetric flask. The Capsule powder was dissolved in methanol with aid of ultrasonication, diluted upto mark with same and filtered through a whaman filter paper (1000 µg/mL Dofetilide). From that pipette out 1 mL solution in to 10ml volumetric flask to produce a test solution having 100 µg/ mL Dofetilide was then analysed for assay determination.

- **METHOD VALIDATION:**

(A) Specificity

The specificity of the method was ascertained by analysing standard drug and sample. The spot with Dofetilide sample was confirmed by comparing the Rf and spectra of the spot with those obtained from standard. The peak purity was assessed in spectrum mode of densitometer. The peak purity of Dofetilide was assessed by comparing spectra acquired at three different positions on the spot, i.e. peak start (S), peak apex (M), and peak end (E).

(B) Linearity

The linearity response was determined by analysing six independent levels of calibration curve in the range of 50 -3000 ng/band of Dofetilide. From the working standard solution (100 µg/ml DOFE, aliquots of 5, 10, 15, 20, 25 and 30 µL were spotted on the TLC plate under nitrogen stream using AS-30 sample applicator. The plate was dried in air and developed in mobile phase in twin trough developing chamber (20 x 10cm) with stainless steel lid, previously saturated with the mobile phase for 30 min. Plate was removed from the chamber after 80 mm solvent run, dried in air and was scanned and quantified at 248 nm in remission/extinction mode with CD60 TLC scanner using ProQuant software. The calibration curve of peak area vs. Concentration was plotted and correlation co-efficient and regression line equation for Dofetilide was determined.

(C) Precision

Repeatability: Test solution analysed six times and %RSD was calculated.

Intraday and inter day Precision: Intra-Day Precision and inter day precision were determined by analysing Dofetilide (1000, 1500, 2000 ng/band) for three times on the same day and on three different days over a week. The results were reported in terms of % RSD.

(D) Accuracy

Accuracy was determined by calculating the recovery of Dofetilide by the standard addition method. Known amounts of a standard solution of Dofetilide (500, 1000, and 1500 ng/band) were added to a pre analysed sample solution of DOFE (1000 ng/band) solution was applied in triplicate and recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curve.

(E) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated using following formula:

LOD = 3.3x σ/S (1)

LOQ = 10 x σ/S (2)

Where, σ = standard deviation of response

S = slope of the calibration curve

➢ RESULT AND DISCUSSION:

- **Method Development and Optimization**

Selection of scanning wavelength

Wavelength was selected by scanning 1000 ng/band of Dofetilide under the multi-wavelength mode. A band was scanned between 200-400 nm. At 248nm, peak shape and baseline were found to be good and also degradation spot detected at this wavelength. So, 248 nm was selected for TLC densitometer scanning.

Optimization of mobile phase for HPTLC method

Various solvents like Toluene, Methanol, Chloroform, Ethyl Acetate, Ammonia were tried in different proportions obtain good spot resolution of the Dofetilide **(Table 1) (Figure 2, 3)**.

Validation of proposed method:

(a) Specificity

The spot for In sample was obtained at same Rf 0.45 dofetilide as that of spot of standard. The developed method has been found to be specific. Representative chromatogram of sample was shown in **Figure: 4 and 5**.

(b) Linearity:

Overlay chromatogram of Deferasirox was shown in **Figure 8**. The linearity of Deferasirox was found to be in the range of 500-3000 ng/band with correlation coefficient 0.99 as shown in **Figure 9**. Linearity results are shown in **Table 3**.

(c) Precision: The % RSD for intra-day and inter –day precision was found to be in the range of 0.355-1.33 and 0.236-0.759 for Dofetilide, respectively, which indicates the method is precise. Precision data are recorded in **Table 4**.

(d) Accuracy: Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The percent recovery was found in the range of 98.46 -99.80 % for Dofetilide, respectively. **Table 5** which indicates accuracy of the method.

(e) Limit of detection and limit of quantification: The limit of detection (LOD) was found to be 3.3 ng/band; while the Limit of quantification (LOQ) was found to be10 ng/band for Dofetilide respectively. LOD & LOQ data are recorded in **Table 6**.

(f) Analysis of marketed formulation: Analysis of marketed formulation (Tikosyn – 500) by proposed method Applicability of the proposed method was tested by analysing the commercially available marketed formulation (Tikosyn-500). The percentage of Dofetilide was obtained from the calibration curve. Results as % Assay were shown in **Table 7**.

Table 1: Mobile phase optimization trials by HPTLC method

Sr. No.	Mobile phase	Ratio	Remarks
1	Toluene: Methanol	5:2	Tailing observed
2	Toluene:Methanol:Acetone	5:2:0.2	Tailing observed
3	Toluene:Methanol:Ammonia	4:4:2	Tailing observed
4	Methanol: Ammonia	10:0.1	Tailing observed
5	Chloroform: Methanol:Ammonia	7:3:0.5	Optimum Rf value of 0.45

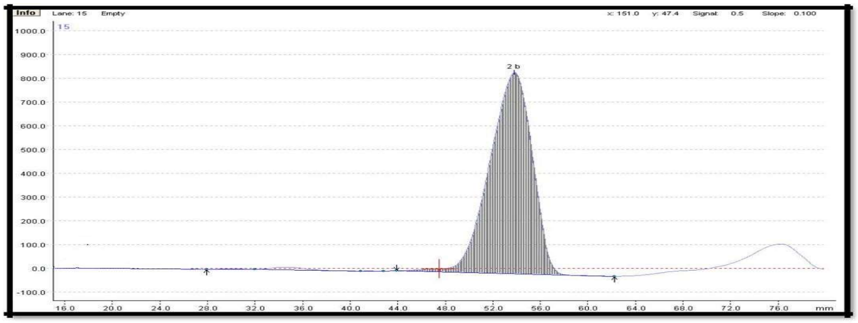

Figure 2: HPTLC Chromatogram of Standard Dofetilide

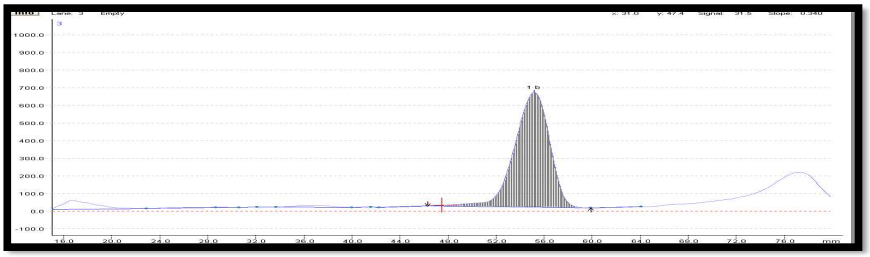

Figure 3: HPTLC Chromatogram of Test

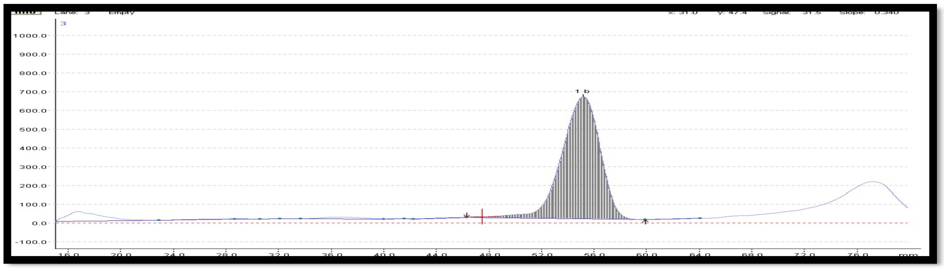

Figure 4: HPTLC Chromatogram of Test (Dofetilide 1000 ng/band)

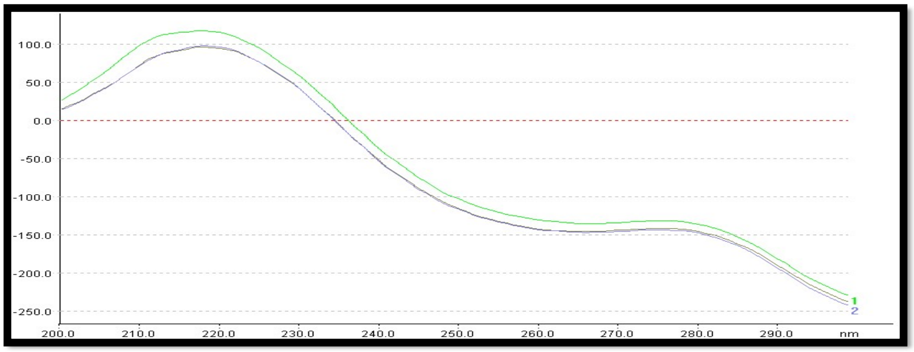

Figure 5: Peak Purity of Dofetilide test (1000 ng/band)

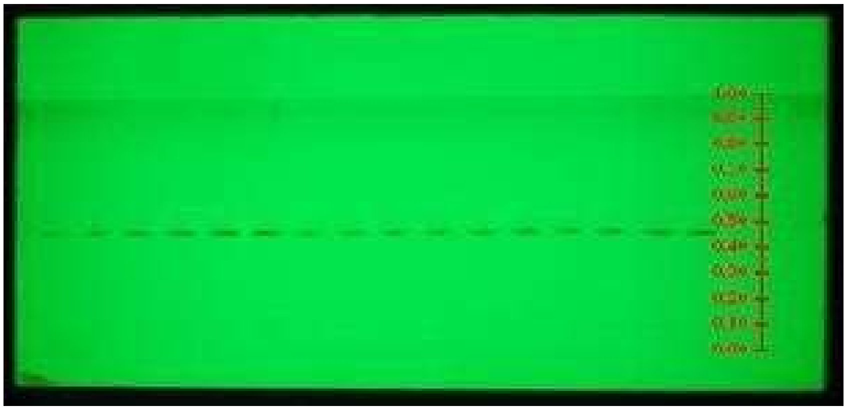

Figure 6: Vertical Chromatogram of Dofetilide (500-3000 ng/band)

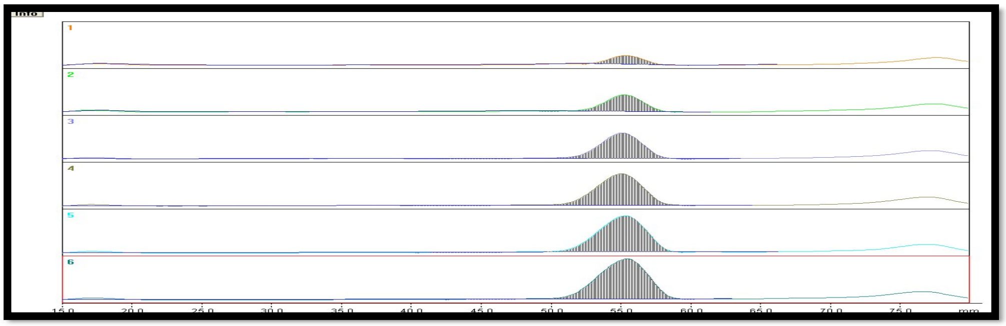

Figure 7: photo of developed of TLC Plate Linearity and test

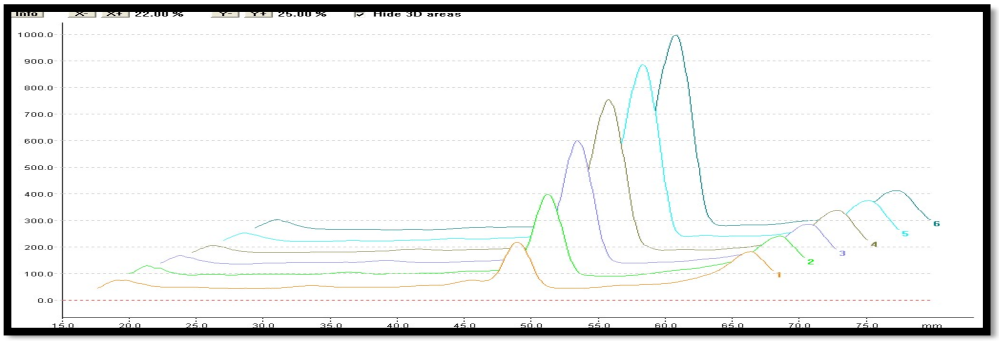

Figure 8: Overlain Chromatogram of Dofetilide (Std lane 1-6)

Table: 2 Linearity Data of Dofetilide

Sr. No.	Conc. (ng/band)	Peak Area Mean ± S.D. (n=3)	% RSD
1	500	595.6±6.574	1.10
2	1000	1287.6±6.574	0.510
3	1500	1923.73±10.57	0.549
4	2000	2727.44±12.12	0.444
5	2500	3324.79±18.20	0.548
6	3000	4135.15±20.67	0.499

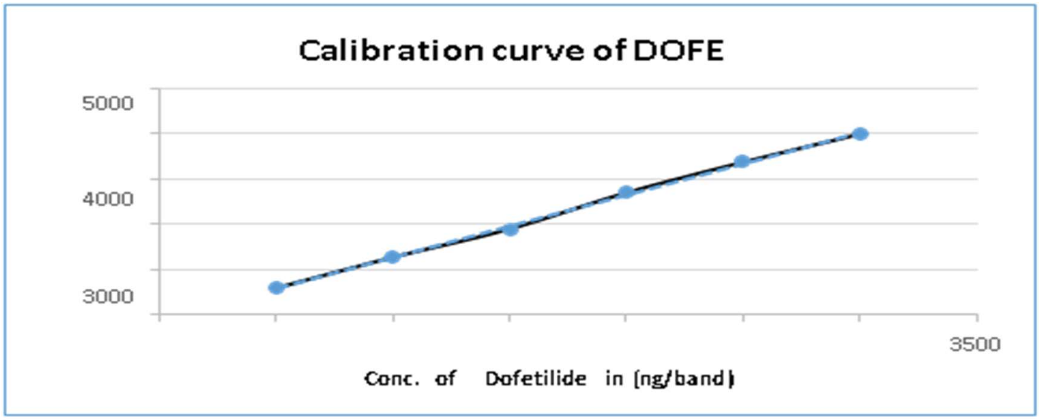

Figure 9: Calibration curve of Dofetilide

Table: 3 Linearity results of Dofetilide

Parameters	Dofetilide
Linearity Range	500 -3000 ng/band
Regression line equation	Y=1.3789x – 106.55
Co-relation coefficient	0.999

Table: 4 Results of Intraday and Interday Precision data for Dofetilide

Drug	Conc. (ng/band)	Intra-day Precision		Inter-day precision	
		Mean*± S.D	% RSD	Mean* ± S.D	% RSD
DEFE	500	597±2.12	0.355	598.3±1.414	0.236
	1000	1284±9.89	0.770	1271±2.828	0.222
	1500	1936.69±25.88	1.330	1924.6±14.60	0.759

*Average of three determinations

Table: 5 Results of Accuracy study for Dofetilide

Amount ofTest Solution (ng/band)	Amount ofStd added(ng/band)	Peak area* ± SD	Amount Recovered (ng/band)	% Recover y	% RSD
1000	500	1930±21.14	1476.9	98.46	1.095
1000	1000	2720±14.14	2049.85	102.4925	0.5199
1000	1500	3334±28.99	2495.14	99.80	0.869

*Average of three determinations

Table: 6 LOD and LOQ Data

Parameters	Dofetilide (ng/band)
LOD	3.3
LOQ	10

Table: 7 Analysis of marketed formulation of Dofetilide

Formulation	Label claim (mg/tablet)	% Assay (Mean ± S.D, n=6)
Tikosyn – 500	500	100.62

*Average of six determinations

Table: 8 Summary of validation parameters of HPTCL method for Dofetilide

Sr. No.	Parameters		Dofetilide
1	Rf		0.45
2	Linearity range		500 -3000 ng/band
3	Regression line equation		Y=1.3789x – 106.55
4	Co-relation coefficient (r^2)		0.999
5	Precision (% RSD)	Repeatability	0.43
		Intraday	0.355 -1.33
		Interday	0.236 – 0.759
6	Accuracy (% Recovery)		98.46 – 99.80%
7	% Assay		100 .6%
8	Limit off detection (ng/band)		3.3
9	Limit of Quantification (ng/band)		10

CONCLUSION

HPTLC method have been developed and validated for the determination of DOFE in capsule dosage form. The methods are found to be specific as there was no interference of any co-eluents. The proposed methods are found to be simple, accurate, precise and robust. Hence, it can be used successfully for development of method for DOFE in capsule dosage form. The developed HPTLC method for DOFE in capsule dosage form was found to be simple, rapid, precise, accurate and economical.

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