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ANALYSIS OF DOFETILIDE IN BULK AND FORMULATIONS BY VALIDATED SPECTROFLUORIMETRIC METHOD

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ABSTRACT
The selected drug dofetilide do not have any native fluorescence. The structure contains tertiary amide with a oxide group in the aliphatic chain attached with the benzene ring. Hence an attempt was made to convert the compound into a fluorescence emitting moiety by treating with chromotropic acid in the presence of sulphuric acid at a temperature of 100°C. The drug was treated with 0.25 ml of sulphuric acid and 1 ml of 0.01% chromotropic acid and placed on a thermostat maintained at a temperature of 100°C for 15 minutes. The chemistry of the reaction involves the breakage of the aliphatic chain oxide group. The second step involves the attachment of the chromotropic acid resulting in the formation of fluorescence compound. The fluorescence product formed was measured in the UV Visible region. The excitation was found at 359 nm and emission at 707 nm. The linearity was found between 0.25 to 1.5 mcg/ml with a correlation coefficient of > 0.99. The percentage RSD value for the precision studies and accuracy was less than 2. The method developed was validated according to the ICH guidelines and can be applied for the routine analysis of dofetilide in bulk drugs and formulations.

INTRODUCTION
Dofetilide is an white to off white powder with a chemical composition of Methanesulfonamide, N-[4-[2-[methyl[2-[4-[(methylsulfon-yl)amino] phenoxy]ethyl] amino]ethyl]phenyl]. The molecular weight is 441.57 and formula is $C_{19}H_{27}N_3O_5S_2$. The drug was found to be soluble in 0.1N HCl, 0.1 N NaOH, Chloroform, Methanol. The molecular structure is shown in figure 1.[1]

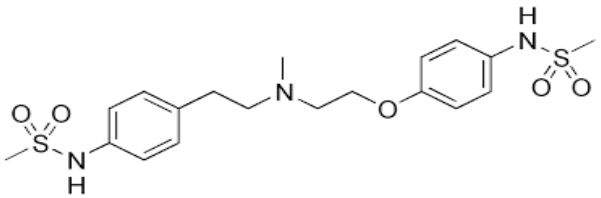

Figure 1: Structure of Dofetilide.

The formulation of dofetilide is available as capsules of 0.125 mg, 0.250 mg and 0.500 mg. Tikosyn capsules contain the following inactive ingredients: Microcrystalline cellulose, Corn starch, Colloidal silicon dioxide and Magnesium stearate The Reported literature for dofetilide includes Simultaneous Determination of Dofetilide and Amlodipine in Plasma by HPLC[2] and UV spectroscopy with HPTLC method applied for the degradation studies.[3] There are no methods reported by spectrofluorimetric technique for dofetilide. Hence an attempt was made to derive the compound into a fluorescenting moiety using chromotropic acid and sulphuric acid and measuring the excitation and emission intensity at selected wavelength.

MATERIALS AND INSTRUMENTS
The organic solvents used for the work were of AR grade. The water used for the solution preparation was procured from s.d fine chem.ltd. The Instruments used for weighing was Shimadzu digital electronics balance, pH of solution was checked with Elico Pvt. Limited, India, pH meter. The UV spectrum for the drug solution was scanned with Jasco V-600 UV/ Vis-spectrophotometer. The excitation and emission intensity of fluorescence was measured in Jasco FP-750 Spectrophotometer.

EXPERIMENTAL SECTION
Selection of solvent
The solvent selection for the work was carried out based on the solubility property of the drug. The aqueous solvents tried were water, 0.1 M Sodium hydroxide and 0.1 M Hydrochloric acid. The organic solvents used to check the solubility of the drug were methanol, chloroform, and toluene. As the drug was found to be completely soluble in methanol, it was chosen for the

solution preparation and for the dilution of the stock solution.

Preparation of standard solutions

Stock solution

A quantity of 10 mg of the pure drug dofetilide was weighed accurately into a 10 ml volumetric flask. The drug was dissolved in a minimum quantity of methanol. The volume was made up with methanol. The solution was shaken well for the uniform distribution of the drug.

Working standard solution

The stock solution was diluted by pipetting 1 ml into a 10 ml volumetric flask and diluted with methanol to get a concentration of 100 mcg/ml of dofetilide.

Volume of sulphuric acid

The sulphuric acid was selected for the development of fluorescence. The volume of the sulphuric acid was varied between 0.25 ml to 1.0 ml with heating time of 5,10 and 15 minutes. The volume of 0.25 ml was selected for the work as the intensity of fluorescence was acceptable.

Concentration and volume of chromotropic acid

A volume of 1 ml of working standard solution of 100 mcg/ml and 0.25 ml of sulphuric acid was selected. The strength of chromotropic acid used was varied between 0.1%, 0.01% and 0.05 %. A volume of 0.25,0.5 and 1.0ml in each strength was applied for the reaction and spectrum was recorded. Chromptropic acid of 0.01% strength and volume of 1 ml resulted in a smooth spectrum and appreciable fluorescence intensity.

Effect of reaction temperature

The temperature of heating the reaction mixture for chromotropic acid with sulphuric acid was checked at room temperature and 100^{o}C. The As the development of fluorescence was appreciable at a temperature of 100^{o}C it was selected as the suitable temperature for the work.

Effect of heating time

The time of heating of the reaction mixture in a water bath was studied at 5, 10 and 15 minutes. The heating time resulting in maximum fluorescence was found to be 15 minutes.

Selection of wavelength

The wavelength of the fluorescence derived compound was selected by scanning them in the UV visible region. The excitation wavelength was fixed and the emission measured. The excitation wavelength was found to be at 359 nm with the emission at 707 nm. The intensity of fluorescence was noted for the drug solution.

Preparation of reagent blank

The blank was prepared using 1 ml of 0.01% solution of chromotropic acid and 0.25 ml of sulphuric acid. This blank mixture was placed on the boiling water bath for 15 minutes. The solution was brought to the room temperature and volume made upto 10 ml with methanol. The 1 ml of the blank solution was diluted to 10 ml with methanol. Further 1 ml was diluted to 10 ml using methanol and the spectrum was scanned for the excitation and emission wavelength.

Fixed experimental parameters

A volume of 1.0 ml of drug solution from stock solution (1 mg/ml) was pipette into a 10 ml volumetric flask. To this1 ml of 0.01% solution of chromotropic acid and 0.25 ml of sulphuric acid were added slowly by placing the reaction mixture flask dipped in a ice bath. After the addition of the acids the reaction mixture was placed on the boiling water bath for 15 minute to develop the fluorescence. Immediately after heating the flask was cooled to room temperature using running water and the volume made upto 10 ml with methanol. The solution was diluted further 10 times with methanol to give a solution of 10 mcg/ml. The reaction mixture was finally diluted with methanol to the required concentrations for the work and the fluorescence intensity measured.

The excitation wavelength was fixed as 359 nm and emission was found at 707 nm. The reagent blank was prepared and measured following the same procedure. The spectrum of the reagent blank was recorded and shown in figure 2.

Validation of Spectrofluorimetric Method

Linearity and range

Serial dilutions of 0.25 to 1.5 ml of the standard solution of 10 mcg/ml were carried out using methanol as the solvent in separate 10 ml volumetric flask. The concentrations in the linear range were 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mcg/ml of dofetilide were prepared. The excitation was measured at a wavelength of 359 nm with the emission intensity at 707 nm. The overlay spectrum of the range of linearity has been shown in figure 3. The fluorescence intensity measured at the excitation wavelength are presented in the table 1. The emission wavelength measurements are presented in table 2. The calibration graph are shown in figure 4 and 5 for excitation and emission fluorescence intensity respectively.

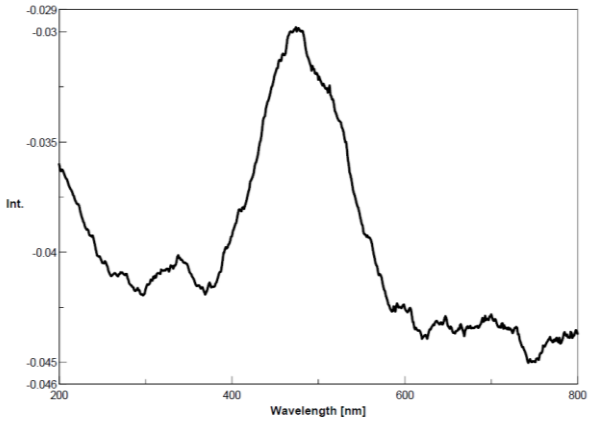

Figure 2: Reagent Blank.

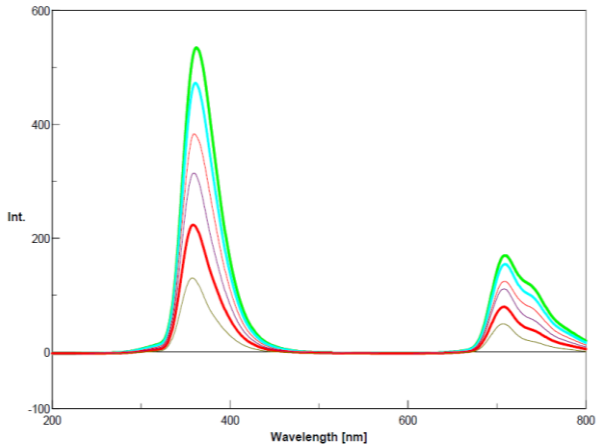

Figure 3: Linearity over lay 0.25 to 1.5 mcg/ml Dofetilide.

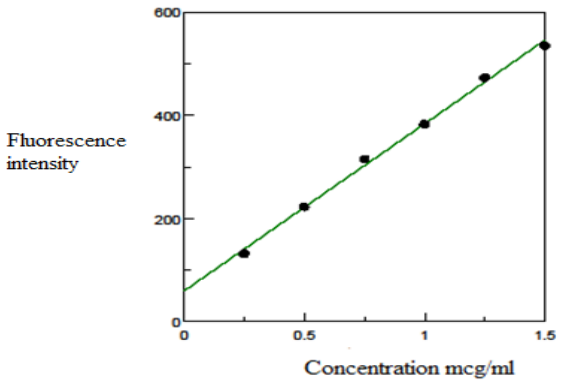

Figure 4: Calibration graph for Excitation wavelength at 359 nm.

Table 1: Calibration data for Excitation wavelength.

Sn.No.	Conc.mcg/ml	Fluorescent Intensity
1	0.25	132
2	0.5	223
3	0.75	315
4	1	383
5	1.25	473
6	1.5	535

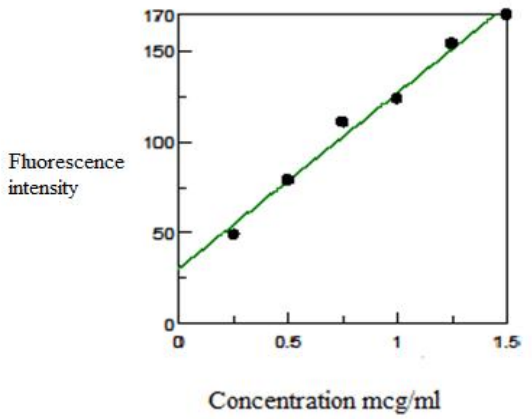

Figure 5: Calibration graph for Excitation wavelength at 707 nm.

Table 2: Calibration data for Emission wavelength at 707 nm

S.No	Conc. (mcg/ml)	Fluorescent intensity
1	0.25	49
2	0.5	79
3	0.75	111
4	1	124
5	1.25	154
6	1.5	170

Precision

The standard solution of 0.25 mcg/ml was prepared. The intraday precision was performed by repeating the procedure three times a day by six replicate measurement of the final methanol solution. The interday precision was executed on three consecutive days with six replicate measurements of the drug solution. The representative spectrum is given in figure 6 for the precision studies. The Relative standard deviation for the six determinations were calculated as given in the table 3.

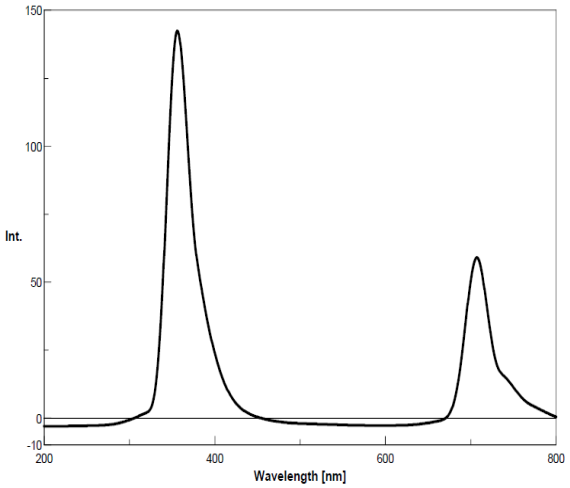

Figure 6: Precision 1.

Table 3: Intraday and interday Precision study.

Precision	Fluorescent Intensity*	%RSD
Intraday	139	0.84
Interday		
DAY 1	136	0.46
DAY 2	141	0.90
DAY 3	132	0.97

***Mean of six readings**

Accuracy

The capsules powder of dofetilide was prepared as admixture by weighing the inactive ingredients specified in the preparation for the marketed formulation available. The mixture was triturated well in a mortar to get a homogeneous mixture. The admixture powder equivalent to 10 mg of the drug was weighed in the volumetric flask. The standard drug substance was added to the formulation at 50% and 100% level. The fluorescence was developed with chromotropic acid and sulphuric

acid. The volume was made up with methanol and filtered using whatmann filter paper. Aliquot volume of the reaction mixture was diluted with methanol to give a concentration of 1 mcg/ml. The measurements of each sample were carried out six times. The excitation and emission was measured and values were noted. The representative spectrum recording is given in figure 7. The % recovery and % RSD for the measurements were calculated and given in the table 4.

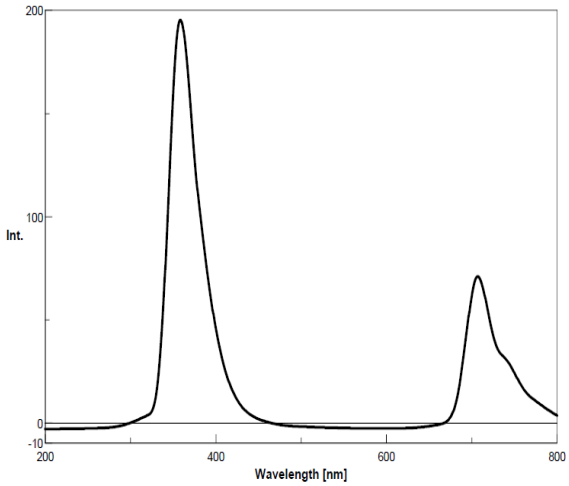

Figure 7: Accuracy.

Table 4: Recovery studies of Dofetilide.

Level of accuracy	Label Claim	Amount of Sample Powder Added	Amount of Standard Drug Added (50%Level)	Total Amount Found*	% Recovery*	% RSD*
50 %	125 mcg	100 mcg	50 mcg	51.5	103.33	0.98
100 %	125 mcg	100 mcg	100 mcg	101.8	100.55	0.63

***Mean of six readings**

Specificity

The method developed was checked for its specificity by analyzing the mixture of excipients present in the capsule admixture such as starch, cellulose and magnesium stearate with the similar procedure used for the pure drug and no specific fluorescence were found at the wavelength used for the drug.

Robustness and Ruggedness

The robustness of the method was evaluated by slight variations in volume of the reagents added for converting the compound into a fluorescence moiety. The results were found to be not having notable variation from the values recorded with the fixed parameters. The ruggedness was studied by measurements by two different analysts on three consecutive days. The results were obtained similarly.

Limit of detection and limit of Quantification

On the basis of the slope of the curve(S) and standard deviation (σ), the LOD and LOQ values were calculated by using the formula. The limit of detection was calculated with 3.3σ/S and the limit of Quantitation was calculated using 10σ/S. The limit of detection was found to be 0.12 mcg/ml and limit of quantification was found to be 0.25 mcg/ml.

Stability studies

The fresh solution of dofetilide was prepared using methanol. The fluorescence was developed by adopting the procedure and diluted with methanol to obtain a solution of concentration of 1 mcg/ml. The stability of the fluorescence in the solution form was checked periodically for every one hour. The intensity of fluorescence was noted and found to be stable till three hours. There was decrease in the intensity of fluorescence after three hours.

RESULTS AND DISCUSSION

The drugs dofetilide selected for the work belongs to the classification of Antiarrhythmic drugs. They have a very potent dosage prescribed for the treatment of less than 10 mg. Hence it is necessary to develop a simple, reliable and cost effective method for the analysis in the bulk form and formulations.

An attempt was made to develop a simple and sensitive method to analyze the drug by spectrofluorimetry. The reaction was carried in the sequence from weak acid to strong acid (acetic acid and sulphuric acid) to break the aliphatic chain oxide bond and form a derived fluorescent moiety with chromotropic acid. The rate of the reaction was increased by increasing the temperature from room temperature at 25°C (Room temperature) till 100°C.

The method developed was validated for the various parameters of linearity, range, precision, robustness, ruggedness, limit of detection and limit of Quantitation according to ICH guidelines.[4] The method developed can be applied for the analysis of dofetilide in the bulk drugs and formulation.

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