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DEVELOPMENT OF UV SPECTROSCOPY AND HPTLC METHOD FOR THE ANALYSIS OF DOFETILIDE AND ITS DEGRADATION PRODUCTS

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ABSTRACT

There are no reported methods till date for the analysis of dofetilide by UV spectroscopy and HPTLC method as a single drug. The aim of the present study is to develop a UV spectroscopic and HPTLC method and validate it according to ICH guidelines. The developed method was adopted for the stability testing of dofetilide. The maximum absorbance in UV spectroscopy was found at 231 nm with methanol as the solvent. The linearity was established at the concentration range of 2-18 mcg/ml with a correlation coefficient of 0.9987 .The Interday and intraday studies gave reproducible values with a % RSD of less than 1.The HPTLC method was developed with a mobile phase of Methanol: Toluene: Acetonitrile in ratio of 4:2:4. The Rf value was found to be 0.60 ± 0.02 with symmetrical peaks. The linearity range was 1000-10000 ng/spot with a correlation coefficient of 0.9961. The precision studies were found to be acceptable and % RSD value was found to be below 1. The drug was subjected to various stress conditions of acid hydrolysis, base hydrolysis, oxidation, photolysis and thermal degradation according to ICH guidelines. Successful separation of the drug peak and the degradants were found at well separated Rf value. The drug was found to be stable with acid hydrolysis, oxidation, base hydrolysis and thermal degradation at 40^{o}C upto 4 hours with degradation of less than 10% and the degradation of more than 10% was found in elevated temperature of 40^{o}C after 4 hours, 60^{o}C, 80^{o}C and photolysis. The drug solution was found to be stable at room temperature for 44 hours and in refrigeration for 7 days. The method developed has the merit of being simple, economical, sensitive and accurate for the analysis of dofetilide in bulk drugs.

KEYWORDS: Dofetilide, UV Spectroscopy, HPTLC, Degradation studies, Stability Indicating method.

INTRODUCTION

Dofetilide is a class III antiarrthmatic drug used as potassium channel blocker approved by USFDA in 1999 for the maintenance of sinus rhythm in individuals prone to atrial flutter and atrial fibrillation with a very potent dosage form of capsules of125 mcg, 250 mcg and 500 mcg capsules. Dofetilide is chemically N-[4-[2-[methyl[2-[4-[(methylsulfonyl)amino] phenoxy]ethyl] amino]ethyl].[1] The method developed by UV Spectroscopy and HPTLC were validated for linearity, range, interday and intraday precision, Limit of Quantitation and Limit of Detection according to ICH Q2R1 guidelines.[2]

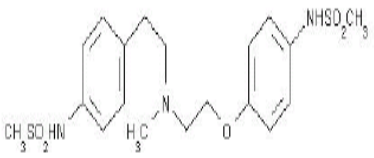


Figure:1

According to ICH guidelines - Q1A (R2) stress testing of the drug substance can help Identify the likely degradation products, which can in turn help establish intrinsic stability of the molecule. Forced degradation studies of new chemical entities or drug products are essential to help, develop or demonstrate the specificity of stability indicating methods. In general a 10% degradation of the drug is recommended.[3,4]

MATERIALS AND METHODS

The solvents used for the methods like methanol, acetonitrile and toluene were procured from Qualigens fine chemicals Ltd. (Mumbai, India). The pure drug Dofetilide was a gift sample from Par Formulations (Chennai, India). The precoated aluminium plates of TLC silica gel 60 GF_{254} 20 X 20 cm (Merck, Germany) were procured from Ponmani & Co Ltd, Coimbatore.

The Shimadzu balance (BL -220H)was used for weighing. The Jasco V630 Spectrophotometer with1 cm matched Quartz cuvettes was used for the UV Spectroscopic method. The HPTLC method was performed using Camag Linomat 5 applicator with 100

µl syringe, Camag UV chamber for spot identification, Camag TLC scanner (WINCATS software) for detection.

UV Spectroscopy Method

The stock solution for the standard drug of 1 mg/ml was prepared using methanol. The maximum absorbance for the drug solution of 10 mcg/ml was found to be at 231 nm(Fig.1). The linearity was found between the concentration range of 2-18 mcg/ml (Fig.2) for UV Spectroscopy. Methanol was used as the diluent solvent for the dilutions. The precision was determined by Intraday (repeatability) and Interday(reproducibility) precision and reported as % RSD for a statistically significant number of replicate measurements. The solution stability was checked periodically and found to be stable at room temperature for 44 hours and in refrigeration for 7 days after which there was considerable decrease in absorbance.

HPTLC method

The HPTLC method was developed with Methanol: Toluene: Acetonitrile in ratio of 4:2:4 which gave good symmetrical peaks with an Rf value of 0.60 ± 0.02 (Fig.3). The linearity was found in the concentration range of 1000-10000 ng/spot (Fig.4) with acceptable correlation coefficient. The Interday and Intraday precision studies were performed. The LOD and LOQ of dofetilide were calculated based on the regression equations of 3.3σ/S and 10σ/S respectively. The σ represents the standard deviation of the values of standard drug and S represents the slope of the calibration graph.

Forced Degradation Studies

The stability of the drug was studied at various stress conditions as indicated by the ICH guidelines Q1A (R2) for acid hydrolysis with 0.1 M methanolic Hydrochloric acid, base hydrolysis with 0.1M methanolic sodium hydroxide, 3% hydrogen peroxide for oxidation, thermal degradation at 40°C, 60°C, 80°C and photolysis by using lamp of 220-240 volts. The sampling for the solutions was done at 0, 4 and 8 hours respectively.

The amide group present in the structure undergoing hydrolysis with the change in pH of the solution and the structural rearrangement in the presence of light are studied for the degradants at separate Rf value. (Fig.5) The Rf value for the drug was found to be 0.60± 0.02. The separate peaks for the degradants products were obtained at an Rf value of 0.68, 0.77 for acid hydrolysis (Fig.6), 0.48, 0.79, 0.90 for thermal degradation (Fig.7),1.25, 1.49 for photolysis (Fig.8) and 0.43, 1.38 for oxidation (Fig.9). The drug was found to be stable with oxidation, at 40°C for 4 hours and base hydrolysis .The degradation was found to be less than 10% for oxidation, at 40°C for 4 hours, base hydrolysis and more than 10% was found in elevated temperature of 40°C more than 4 hours, 60°C, 80°C, acid hydrolysis and photolysis.

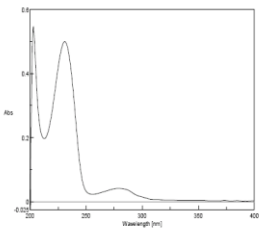


Fig.1 - Representative Spectrum of Dofetilide

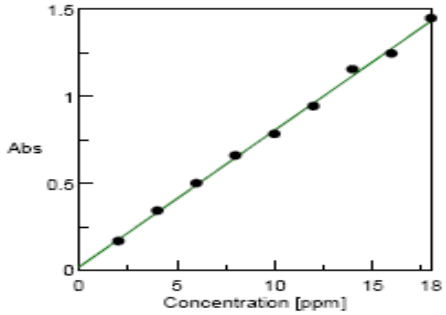


Fig.2- UV Spectroscopy Calibration Graph 2 To 18 mcg

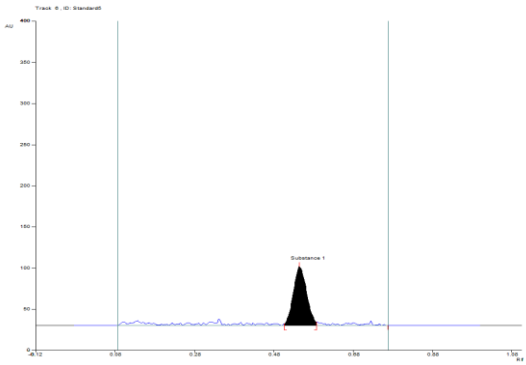


Fig.3 - Dofetilide Representative Chromatogram

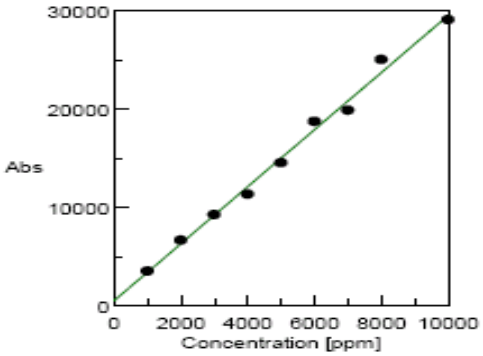
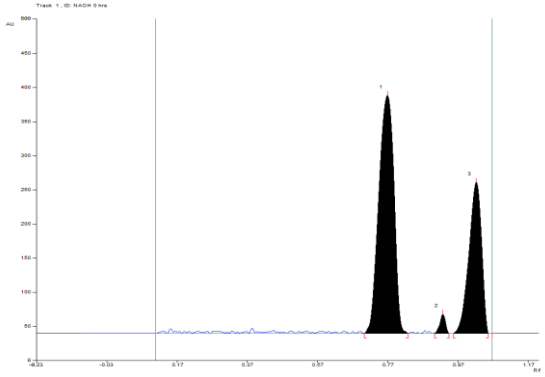
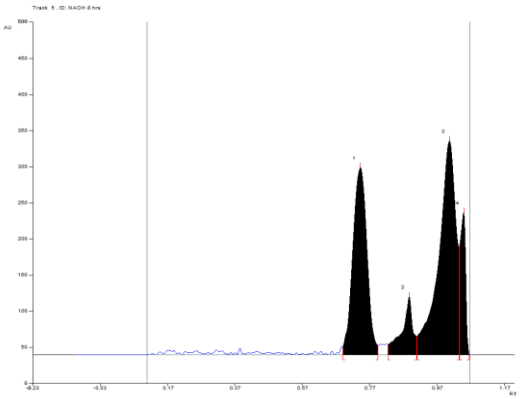


Fig.4 - HPTLC Calibration Graph - 1000 to 10000 ng/Spot

Fig.5 -Base Hydrolysis

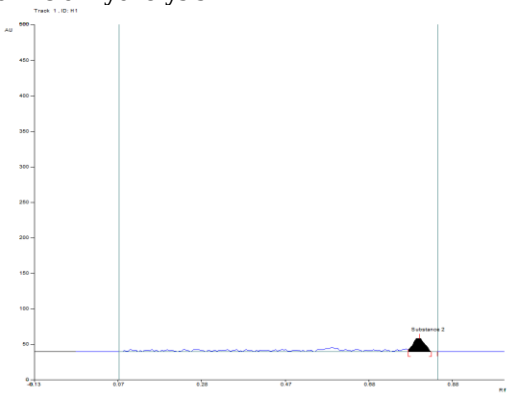


Base 0 hours

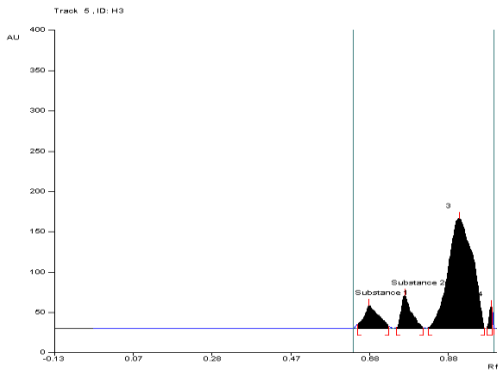


Base 8 hours

Fig.6- Acid Hydrolysis

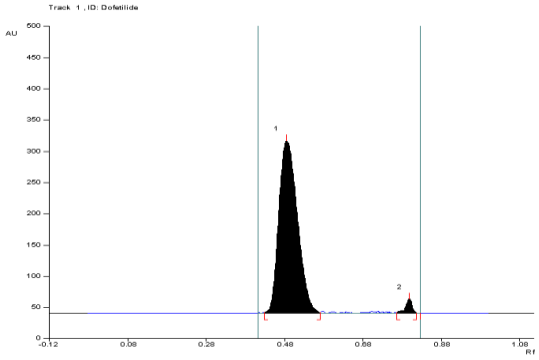


Acid 0 hours

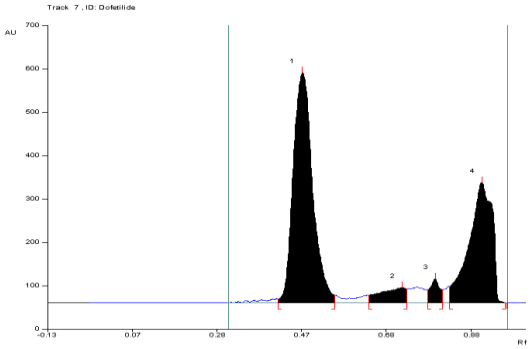


Acid 8 hours

Fig.7 - Thermal Degradation
Thermal 4 hours- 40°C

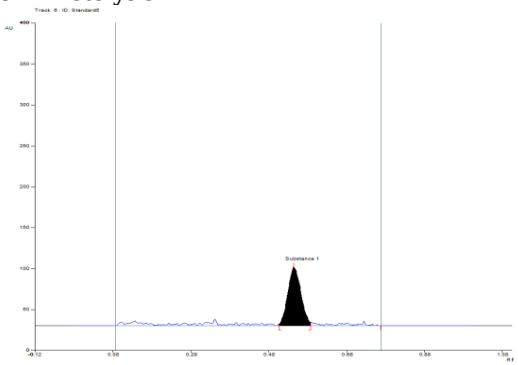


Thermal 4 hours- 40°C

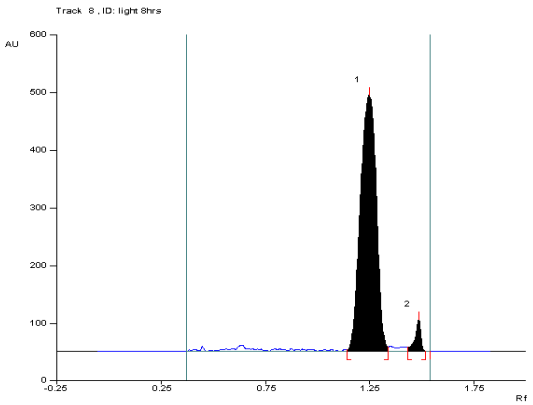


Thermal 8 hours - 40°C

Fig.8 – Photolysis

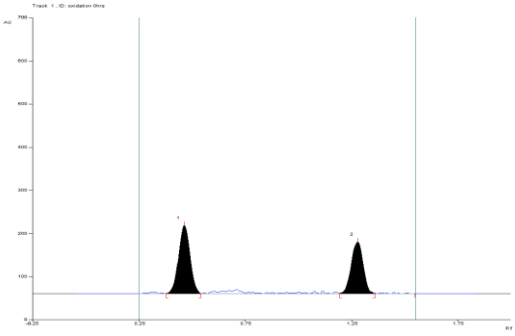


Light 0 hours

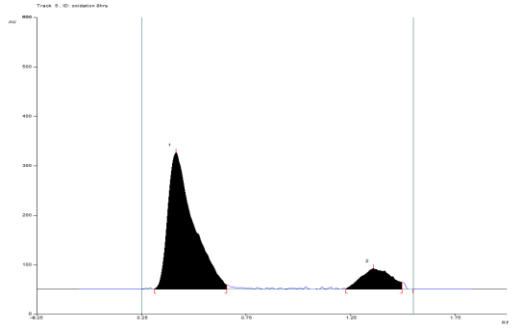


Light 8 hours

Fig.9 - Oxidation



0 hours



Oxidation 8 hours

RESULTS AND DISCUSSIONS

The UV spectroscopic method was developed for the pure drug dofetilide using methanol as the solvent and the λmax was found at 231 nm. The linearity range was established between 2-18 mcg with a correlation coefficient of 0.9987. The Interday and Intraday studies gave reproducible values with a % RSD of less than 1.

The HPTLC method was performed with various solvent system using methanol, toluene and acetonitrile of different ratios based on the solubility of the drug and the peak symmetry. The λmax selected for the drug was at 233 nm after scanning the developed spot in the Win Cats software. The mobile phase consisting of Methanol: Toluene: Acetonitrile in the ratio of 4:2:4 was selected for the work which gave good symmetrical peaks with an Rf value of 0.60 ± 0.02. The linearity was found in the concentration range of 1000-10000 ng/spot with acceptable correlation coefficient. The Interday and Intraday precision studies were performed and the % RSD value was found to be below 1. The LOD and LOQ of dofetilide were calculated statistically based on the slope of the calibration curve and standard deviation value.

The stability of the drug was studied under various stress conditions of acid, base, oxidation, temperature and light according to the ICH guidelines to identify the potential degradants which could affect the potency of the drug and thereby the pharmacological activity of the drug.

CONCLUSIONS

The UV spectroscopic method developed for its linearity, range, precision studies, LOD and LOQ can be used for the routine quality control of the drug dofetilide in bulk drugs. The HPTLC method developed for its linearity, range, precision, LOD, LOQ and its application for the degradation of the drug under various stress conditions indicates the storage conditions for the drug and drug product during its shelf life.

ACKNOWLEDGEMENTS

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Table 1: Summary of the Method Developed and Validation Parameters

Sl. No	Parameters	UV Spectroscopy	HPTLC
1	Detection wavelength(nm)	231	233
2	Beer's law limits	2-18 µg/ml	1000-10000 ng/spot
3	Regression equation (y = mx+c)	0.078625*X+0.01911	2.90372*X+509.98
4	Correlation coefficient (r2)	0.9987	0.9961
5	LOQ	0.1791 µg/ml	187.34 ng
6	LOD	0.0591µg/ml	61.82 ng
7	Solution stability		
	Room Temperature	44 hours	
	Refrigeration	7 days	

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