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RP-HPLC method development & validation for estimation of Flecainide acetate in bulk and tablet dosage form

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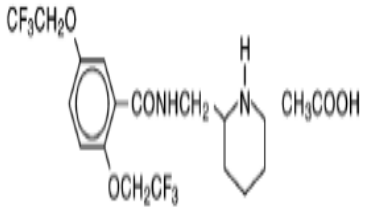
ABSTRACT

A novel stability indicating liquid chromatographic assay method was developed and validated as per ICH guide lines for the quantitative estimation of Flecainide in tablet formulation. An isocratic reverse phase LC-method was developed using C18 ODS 250cm x 4mm x 5µm particle size column and a mobile phase comprising of a mixture of buffer: acetonitrile (40:60), pH adjusted to 3.0 with Ammonium di-hydrogen phosphate. The detector set at 299nm with flow rate of 1.0ml min^{-1}. The method is linear between 5µg ml^{-1} to 25µg ml^{-1} and the limit of detection (LOD) is 0.5 µg ml^{-1}. The Accuracy of the method was found to be in the range of 99.70% to 100.26%. The mean Inter and Intraday assay Relative Standard deviation (% RSD) were less than 0.69%. The Proposed method was found to be Linear, precise and accurate for the quantitative estimation of Flecainide in tablet formulations and can be used for commercial purposes.

Keywords: Flecainide, Liquid Chromatography, Stress degradation and Method validation.

INTRODUCTION



Flecainide acetate is a class Ic antiarrhythmic agent used to prevent and treat tachy-arrhythmias (abnormal fast rhythms of the heart). It is used to treat a variety of cardiac arrhythmias including paroxysmal atrial fibrillation (episodic irregular heartbeat originating in the upper chamber of the heart), paroxysmal supraventricular tachycardia (episodic rapid but regular heartbeat originating in the atrium), and ventricular tachycardia (rapid rhythms of the lower chambers of the heart). Flecainide works by regulating the flow of sodium in the heart, causing prolongation of the cardiac action potential. Flecainide acetate is an anti-arrhythmic drug. Molecular formulae is $C_{17}H_{20}F_6N_2O_3$ molecular weight is 414.343 g/mol. Flecainide acetate is a white crystalline substance it has an aqueous solubility of 48.4 mg/ml at 37°C. Chemical name: Flecainide acetate is benzamide, N-(2-piperidinylmethyl)-2,5-bis (2,2,2trifluoroethoxy)-mono-acetate. A through literature survey has revealed the following reported methods for the estimation of Flecainide in bulk, formulation and biological fluids using different analytical techniques. The reported methods include estimation of Flecainide in biological samples using

LC-UV and LC-MS, HPLC, and Spectrophotometry for the estimation of Flecainide in bulk and pharmaceutical formulations [3-16]. In the present study attempts were made to develop a rapid, economical, precise and accurate liquid chromatographic method for the estimation of Flecainide in tablet formulations.

MATERIALS AND METHODS

Materials

All the reagents were of analytical and HPLC grade unless stated otherwise. Milli-Q-Water was used throughout the study. Hydrochloric acid, Sodium Hydroxide, Acetonitrile, sodium di-hydrogen phosphate, potassium di-hydrogen phosphate, ammonium di-hydrogen phosphate etc.(Merck, Mumbai, India) were used. Flecainide Standard was obtained as a gift sample and Flecainide tablets were purchased from Local Pharmacy.

Instrument employed

Shimadzu-1700 double beam – UV – Visible spectrophotometer with pair of 10mm matched quartz cells, Shimadzu HPLC, C18 ODS 250cmx4mmx5µm particle size column, HPLC detector is PDA, HPLC Injecting Syringe (25 ml) (HAMILTON), P^H analyzer, Ultra Sonicator etc. specification of the instruments are mentioned the data give for Electronic

Selection of solvents

The solubility of flecainide was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test for Flecainide was carried out in different polar and non-polar solvents. From the solubility studies, methanol, acetonitrile was selected solvent for proposed method**.**

Preparation of standard Stock Solution

100mg of Flecainide raw material was accurately weighed and transferred into the 25 ml volumetric flask and dissolved in minimum quantity of mobile phase and made up to 25 ml. from this dilution 20, 40, 60, 80,100 & 120 µg/mL were made in 100 mL volumetric flasks & make up with phosphate buffer of pH 3.0.

Selection of λ_{max}

The solution was scanned between 200 and 400 nm range mobile phase as blank. From the UV Spectra **299nm** was selected as λ_{max} for analysis of Flecainide. Stability of the Flecainide in mobile phase was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that Flecainide in mobile phase was stable for more than 2 hours.

Method development

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample, molecular weight, and solubility. The drug selected for the present study was polar in nature. So reversed phase chromatography can be used, this reverse phase HPLC was selected for the initial separation from the knowledge of properties, C_{18} column was chosen as stationary phase.

Preparation of mobile phase

0.01M solution of buffer was prepared by using water as solvent to this organic solvent acetonitrile was added. While conducting trials various buffers was used by changing the proportional of buffer and acetonitrile along with altering the P^H to decide the final development method**.** Then it is subjected to vacuum filtration and then proper sonication should be done which is for 15 min

Sample injection

After preparation of mobile phase, sample was prepared by taking little a quantity of mobile phase of subsequent trials in a test tube ,to this a little quantity of sample was added and then it is sonicated. Before injecting the sample, pump is subjected to purging and then wavelength of 299nm was adjusted then the blank was injected initially in order to get stable base line after attaining stable baseline sample was injected with syringe.

Table 1: Optimized chromatographic conditions

.Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu with 2487 PDA)
Column	C18 ODS 250cmx4mmx5µm particle size
Mobile Phase*	(0.01M Ammonium di-hydrogen Phosphate - pH 3.0) acetonitrile: buffer (40 : 60 % v/v)
Flow rate	1 ml/min

Detection at UV wave length	299 nm
Injection volume	20µl
Column Temperature	Ambient
Retention time	2.773 min.

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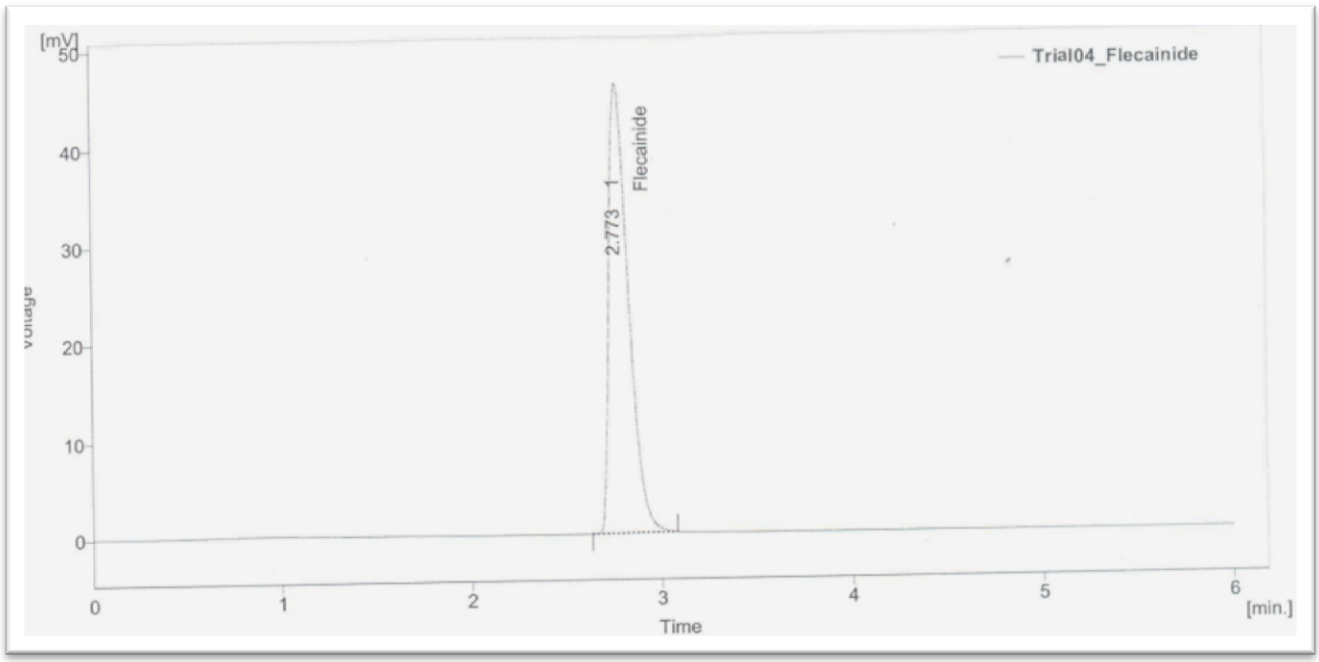

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.773	327.740	46.160	100.000
	Total	327.740	46.160	100.000

Figure 1: Optimized spectra of Flecainide

Method validation

The proposed RP-HPLC method was validated as per ICH guidelines.

Linearity

The calibration curve was plotted with five concentrations of the standard drug solution 5.0-100 µg/ml solution and chromatography was repeated thrice for each dilution. The linearity was evaluated by linear regression analysis, before injecting solutions; the column was equilibrated for at least 30 min with the mobile phase flowing through the system five determinations were carried out for each solution, peak area ratios were recorded for all the solutions. The correlation graph was constructed by plotting the peak area ratios obtained at the optimum wave length of detection versus the injected amounts of the respective concentrations.

Specificity

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of standard and sample solution. The parameters like retention time (Rt), resolution (Rs) and tailing factor (Tf) were calculated.

Precision

Precision study was performed to find out intra-day and inter-day variation. It was carried out by estimating the corresponding responses 3 times on the same day and on 3 different day (first, second and fifth day) for particular concentration of flecainide (100µg/ml) and the results are reported in terms of relative standard deviation (RSD). The repeatability studies were carried out by estimating response of particular concentration of flecainide (100µg/ml) for 5times.

Stability

In order to demonstrate the stability of the standard solution of MS during analysis, the solution was stored over a period of 24 hr at room temperature and then analyzed.

Robustness

As defined by the ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ±2% variation in volume of mobile phase composition, ±0.1 ml/min in flow rate of mobile phase, ±0.1 variation in pH.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug of 10 mcg i.e., from spiking standard(prepared by taking 100mcg of drug in 100ml of mobile phase so concentration is 1mg/ml that is for 10mcg 10ml is taken) was added to the pre-analyzed sample of 80 mcg,100,120 mcg respectively. After this each sample was injected for 3 times. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method and % RSD was calculated by using the following formula; the lower values of %RSD of assay indicate the method is accurate. The mean recoveries were in range of 99.8-101.20 % which shows that there is no interference from excipients.

$$\% \text{ recovery} = (b-a) / c\ (100)$$

Where,

a- The amount of drug found before the addition of standard drug

b- The amount of drug found after the addition of standard drug

c-The amount of standard drug added

Assay of Flecainide in tablets

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of flecainide was transferred to 100 ml volumetric flask containing 10ml of buffer solution and the contents of the flask were sonicated for 15 min, to ensure the complete solubility of the drug .The mixture was then made up to 100 ml with mobile phase. The resulting solution was thoroughly mixed and filtered through vacuum filter. 5 ml of this solution was added to 100 ml volumetric flask and made up to the mark with phosphate buffer of pH (3.0). This solution (20 µl) was injected three times into the column. The mean values of peak areas of five such determinations were calculated and the drug content in the tablets was quantified using the regression equation.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments Shimadzu HPLC and Agilent HPLC, analysts and columns were 0.5- 0.5, 0.6- 0.5 and 0.4- 0.3% respectively.

RESULTS AND DISCUSSION

From the optical characteristics of these proposed methods, it was found that trail method IV for Flecainide obey Accuracy, Precision and Linearity within the concentration respectively. From the results, it was found that the percentage recovery values of pure drugs from the pre-analyzed solution of formulation were 99.40 % for Flecainide, which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method. Chromatograms are attached for the trials, linearity, accuracy, precession specificity, robustness and ruggedness.

Table 2: Linearity

Linearity	
Concentration mcg	**Area of the peak**

20	114.714
40	232.316
60	326.214
80	427.313
100	550.506
120	641.868

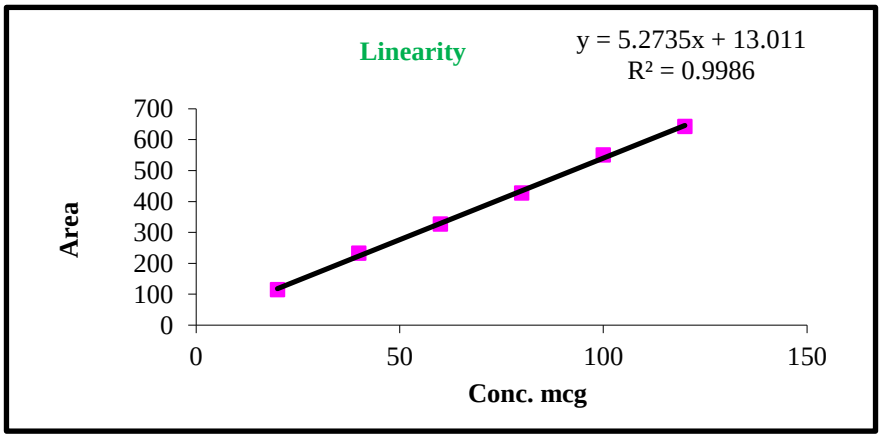

Figure 2: Linearity curve of Flecainide

Table 3: Precision

Flecainide Precision (100 mcg)		
S. No	**RT**	**Area**
1	2.753	571.164
2	2.75	570.437
3	2.757	570.945
4	2.76	570.295
5	2.757	571.338
Average	2.7554	570.8358
St.dev.	0.003911521	0.453693399
% RSD	0.141958389	0.079478792

Table 4: Accuracy

Spikiking Standard Concentration 100mcg	
Spiking Standard Areas	
Standard 1	553.617
Standard 2	553.452
Standard 3	553.494

Average	553.521
Standard value	0.070014284
% RSD	0.015492

Flecainide 130mcg

Sample 120+10	130 mcg
Spiking Standard Area	553.521
Sample 1	712.762
Sample 2	715.771
Sample 3	716.803
Average	715.112
Mcg Recovery	129.1932917
% Recovery	99.38%

Flecainide 90mcg

Sample 80+10=	90 mcg
Spiking Standard Area	553.521
Sample 1	496.127
Sample 2	498.198
Sample 3	495.608
Average	496.6443333
Mcg Recovery	89.7245693
% Recovery	99.69%

Flecainide 110mcg

Sample 100+10=	110 mcg
Spiking Standard Area	553.521
Sample 1	603.352
Sample 2	605.729
Sample 3	606.318
Average	605.133
Mcg Recovery	109.3243075
% Recovery	99.39%

Assay

Separately inject equal volumes of diluents, standard preparation and sample preparation into chromatograph, record the chromatograms, and measure the pea area response for the major peaks calculate the amount content of Flecainide in the portion of Flecainide tablets by the formula.

Sample peak area x Dilution factor of standard x Average weight of tablets

Standard peak area x dilution factor of sample

Table 5: Assay

Flecainide Assay	
Standard Areas	
Standard 1	594.328
Standard 2	595.446
Standard 3	596.406
Average	595.926
Sample Areas	
Sample 1	571.164
Sample 2	570.16
Average	570.662
Standard Area	595.926
Sample Area	570.662
Standard Weight	25.2mg
Sample Weight	72.8mg
Label Claim	50mg
Standard purity	99.83%
Average Weight	150.2mg
Content of Flecainide	99.40%

CONCLUSION

An attempt has been made to estimate Flecainide by RP-HPLC. Even though a number of RP-HPLC methods have been reported earlier for Flecainide individually and with other combinations, an effort has been made to identify a common mobile phase to come up with the isocratic elution of drug individually. The present drug of Flecainide was marketed as formulation. Flecainide – 150mg /tablet. The formulation was diluted in the linearity range and peak areas were determined, the concentrations Flecainide were then determined by comparing the peak areas of sample with that of standard peak areas of that can be identified by their retention times for Flecainide. The results obtained from HPLC method were reproducible and encouraging. The values percentage deviation was satisfactorily low and recovery close to 100 % indicating reproducibility and accuracy of method. The proposed method were proved to be superior to most of the reported method and this can be used as alternative method for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms. Thus the purpose of the present investigation was successfully achieved.

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