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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FLECAINIDE ACETATE BY UV SPECTROPHOTOMETRIC METHOD

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Abstract: A simple, accurate, precise, and economical methods for "Analytical Method Development and Validation of Flecainide Acetate by UV Spectrophotometric Method" have been developed. In method-A Flecainide Acetate estimated at 295nm using dilute acetic acid. The linearity was observed in the concentration range of 2-16µg/ml with correlation coefficient of 0.9982. In method -B Flecainide Acetate estimated using methylene blue and phosphate buffer (pH 8) as an ion pair complexing agent using chloroform as an extractive solvent, the intensity of the resulting color of the organic layer was measured at 315nm. The linearity was observed in the concentration range of 200-1000µg/ml with correlation coefficient of 0.983. Validation experiments for both methods were performed to demonstrate system suitability, precision, linearity, accuracy, ruggedness, LOD, LOQ as per International Conference on Harmonization guidelines.

Keywords: Flecainide Acetate, dil. acetic acid, methylene blue, chloroform, Phosphate buffer (pH 8), UV spectrophotometric method.

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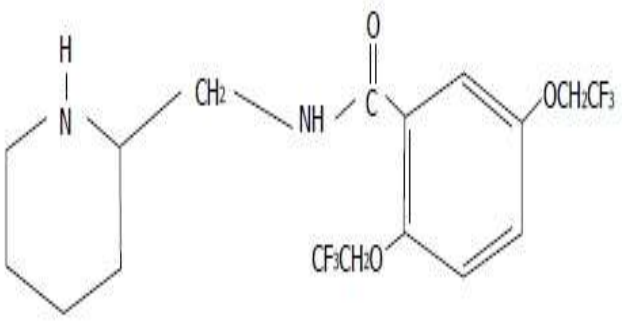
INTRODUCTION

Flecainide acetate is a class IC antiarrhythmic agent that was first synthesized in 1972. After several results of clinical trials, both in animals and humans then, the clinical efficacy of Flecainide was confirmed. Oral use of Flecainide was approved in 1984 from the Food and Drug Administration for the suppression of sustained ventricular tachycardia since study results showed about 90% efficacy without significant adverse events. Nowadays, Flecainide is recommended as one of the first line therapies for pharmacological conversion as well as maintenance of sinus rhythm in patients with atrial fibrillation and/or supraventricular tachycardias. Atrial fibrillation has been associated with significant structural changes in the atria which subsequently cause remodeling of the myocardial fibers and mitochondrial dysfunction due to oxidative stress. Several inflammatory adhesion molecules associated with oxidative stress and subsequent myocardial ischemia, such as nuclear factor kappa β (NFκB), reactive oxygen species and glycogen, impair the cellular physiology enhancing apoptotic process and cellular protein decomposition. Rapid atrial activation encountered during atrial fibrillation results in intracellular Ca^{2+} accumulation, thus promoting ischemia and cellular dysfunction. This process is facilitated by the high transient intracellular Na^{+} concentration during tachycardia, which accentuates the entry of Ca^{2+} via the Na^{+}/Ca^{2+} exchanger. Flecainide attenuates the intracellular Ca^{2+} accumulation by blocking Na^{+} channels, thus reducing oxidative stress process and further atrial remodeling.[1]Chemically Flecainide is N-(piperidin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy) benzamide. Literature survey revealed that there is no analytical method development and validations by UV spectroscopy for Flecainide acetate have been reported. [2-16] This study is useful because the drugs is administered certain types of serious (possibly fatal) irregular heartbeat. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. The aim of the present investigation is to develop and validate a simple, sensitive and reproducible and an economical UV spectrophotometric method for analysis of Flecainide acetate.



Structure of Flecainide acetate

MATERIALS AND METHODS:

Experimental:

Instrumentation:

UV-Visible spectrophotometer UV-Agilent Technologies Carry 60, spectral band width of 1nm, wavelength accuracy ±0.5nm and a pair of 1cm matched quartz cells was used to measure absorbance of the resulting solution.

Materials:

Flecainide Acetate pure drug was obtained from Indeus Life Science Pvt. Ltd, Mumbai as a gift sample with 99.10% (w/w) assay value and was used without further purification. All chemicals and reagents used were of analytical grade. Flecarite tablets were purchased from Nedmed India.com and used within self-life period. Each tablet was labeled to contain 100 mg of Flecainide acetate.

Solvent:

Dilute Acetic acid selected as solvent for developing spectral characteristics of the drug. The selection was made after assessing the solubility of drug in different solvents.

Solubility of drug:

10mg of Flecainide acetate was weighed and solubility of this sample was checked in water, anhydrous ethanol, dil. hydrochloric acid, dil. acetic acid and methanol. The drug was found to be soluble in dil. acetic acid.

METHOD A: Development of Flecainide acetate by derivative spectrophotometer method:

Identification of λ max of Flecainide Acetate:

10mg of drug was weighed and was dissolved in 10ml of dil. acetic acid (1mg/ml). 1ml of this solution was withdrawn and volume was made up to 10ml. Appropriate dilutions were made with dil. acetic acid to give concentration of 10µg/ml scanned in UV range from 200-400nm. The zero order spectrum of Flecainide acetate shown maximum drug absorption wavelength at 295nm, which could be utilized for analysis and spectrum was recorded (Fig.1).

Preparation of standard stock solution:

10mg of pure Flecainide Acetate was accurately weighed and transferred to 10ml of volumetric flask. Drug was dissolved in dil. acetic acid and volume was made up to 10ml. The concentration

of drug was 1mg/ml. 1ml of this solution was taken in a 10ml volumetric flask and volume was made up to the mark with dil. acetic acid. Thus Flecainide Acetate of strength 100µg/ml was obtained.

Procedure for plotting calibration curve of pure drug:

From the standard stock solution 0.2ml,0.4ml ,0.6ml, 0.8ml, 1ml, 1.2ml 1,4ml and 1.6ml dilutions were made in 10ml volumetric flask and volume was made up to the mark with dil. acetic acid to obtain concentration in range of 2-16µg/ml. The spectra were recorded, absorbance were measured at 295nm (Table 1) and calibration curve was plotted (Fig. 2).

Analysis of pharmaceutical formulation:

20 Tablets were procured from market and average weight was determined. The powder equivalent to 10mg of Flecainide Acetate was weighed accurately and dissolved in 10ml of dil. acetic acid, shaken for ten minutes and filtered. 1ml of this solution was taken in a 10ml volumetric flask and volume was made up to the mark with dil. acetic acid. Thus Flecainide Acetate of strength 100µg/ml was obtained. The solution was diluted in 10ml volumetric flask with dil. acetic acid to get a solution of 10µg/ml. Absorbance was measured at 295nm. Results are shown in the table 7.

Validation of method-A:

Method validation was performed in terms of linearity, precision and accuracy, limit of detection and limit of quantification and ruggedness as per ICH-GuidelinesQ2B (R1). [17]

Linearity:

For the method, linearity was repeated 3 times for validation. The calibration curve was constructed by plotting the response y-axis versus the theoretical concentrations of standards x-axis, by using linear regression analysis. Linearity was expressed as a correlation coefficient; r^2. The results are shown in table 1.

Precision:

The intra-day and inter-day precision of the proposed spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of Flecainide acetate and the results are reported in terms of percent relative standard deviation. Results are shown in the table 3 & 4.

Procedure for recovery studies:

20 Tablets were procured from market and average weight was determined. The powder equivalent to 10mg of Flecainide acetate was weighed accurately and taken in 3 separate 10 ml volumetric flask. To this 8mg, 10mg, 12mg pure drug was added (for 80%, 100% and 120% recovery). 10ml of dil. acetic acid was added to make up the volume, shaken for ten minutes and filtered. 1ml of this solution was taken in a 10ml volumetric flask and volume was made up to the mark with dil. acetic acid. Thus Flecainide acetate of strength 100µg /ml was obtained.1ml of this solution was diluted in 10ml volumetric flask up to the mark with dil. acetic acid and absorbance was measured at 295nm. This procedure was carried out for 3 times. Results are shown in the table 2.

Limit of detection:

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3×N/S

Where, N = Standard deviation of the response and

S = Slope of the corresponding calibration curve. Results are shown in the table 5.

Limit of quantification:

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × N/S

Where, N = Standard deviation of the response and

S = Slope of the corresponding calibration curve. Results are shown in the table 5.

Ruggedness:

Ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Flecainide acetate using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to be reproducible, since there was no significant difference between analysts. Thus, the proposed methods could be considered rugged. The results are shown in table 6.

METHOD B: Estimation of Flecainide acetate in dosage form by extractive spectrophotometric method:

Preparation of standard stock solution:

100mg of pure Flecainide Acetate was accurately weighed and transferred to 100ml of volumetric flask. Drug was dissolved in dil. acetic acid and volume was made up to 100ml.Thus Flecainide Acetate of strength 1000µg/ml was obtained.

Dye Solution:

Aqueous solution of 0.1% of Methylene blue was prepared.

Buffer:

Phosphate buffer solution (pH-8) was prepared by mixing 50ml of 0.2 M potassium dihydrogen phosphate in 200ml volumetric flask with 46.1ml of 0.2M sodium hydroxide and then added water to make up the volume.

Optimization of the Method:

In order to optimize the developed method, the effect of experimental parameters such as, dye concentration, pH of the buffer, volume of buffer and extraction solvent, on the formation Flecainide acetate-methylene blue ion-pair complex have been tested.

Effect of volume of 0.1%Methylene Blue:

The influence of the volume of 0.1%methylene bluewas studied by treating 100µg/ml of Flecainide acetate with 2 ml of buffer and varying volumes (0.2– 1.6ml) of 0.1% methylene blue. The absorbance of the Flecainide acetate-methylene blue ion-pair complex was increased with increasing volume of 0.1% methylene blue and became constant at 1.0ml, above this volume, the absorbance remained unchanged (Fig.4, Table 8). Therefore, 1ml of 0.1% methylene blue dye solution was chosen as the optimal volume for the quantification process.

Effect of pH:

At a fixed concentration of Flecainide acetate (100µg/ml), the formation of Flecainide acetate-methylene blue ion-pair complex was investigated over the pH range of 7.0-9.0 using phosphate buffer. However, the absorbance at pH 8 is slightly higher than the absorbance at other pH values. After pH 8, the absorbance decreases (Fig. 5, Table 9). Therefore, pH 8 was selected as the optimum pH for the reaction.

Effect of Volume of Buffer:

The influence of the volume of phosphate buffer (pH 8) on the absorbance value of the ion-pair complex was studied by treating 100µg/ml Flecainide acetate with varying volumes (0.5–3ml) of buffer. As shown in Fig. 6, Table 10, 2ml of phosphate buffer (pH 8) was sufficient to get the optimum pH value (pH 8). Therefore, 2ml was chosen as the optimum buffer volume.

Effect of Extraction Solvent:

The effect of extraction solvent was tested using different solvents such as chloroform, benzene, dichloromethane and butanol. Using chloroform as extraction solvent, the ion-pair complex showed the highest absorbance value and reproducibility. Therefore, chloroform was chosen as the best extraction solvent for extraction of Flecainide acetate-methylene blue ion-pair complex.

Selection of analytical wavelength:

The chloroform layer of selected dye was collected in 10ml volumetric flask. The resulting solution containing 100µg/ml was scanned in UV range from 200-400nm. The λmax was found to be 315nm, which could be utilized for analysis and spectrum was recorded (Fig.3).

Construction of Calibration curve:

From standard stock solution of Flecainide acetate aliquots of 200-1000µg/ml was prepared and added in 125 ml separating funnel. 1mlof methylene blue solution and 2ml of phosphate buffer pH 8 were added in to it and mixed. The aqueous solution was extracted with 5ml of chloroform shaken for 5min and funnel was allowed to stand to get two phases separated. Organic layer was collected in dry 10 ml volumetricflask and absorbance was measured at 315nm (Fig. 7 & Table 11).

Assay of Drug from pharmaceutical dosage form by Method-B:

Twenty tablets were weighed and powdered in a glass mortar. Tablet powder equivalent to 100mg of Flecainide acetate was weighed accurately and transferred into 100ml volumetric flask. The drug was dissolved in dil. acetic acid and the volume was made up to the mark. The solution was assayed as under the assay procedure described for the preparation of calibration curve for the drug. Results are listed in Table 17.

Validation of Method-B:

Method validation was performed in terms of linearity, precision and accuracy, limit of detection and limit of quantification and ruggedness as per ICH-GuidelinesQ2B (R1). [17]

Linearity:

A calibration graph was constructed by measuring the absorbance at different concentration level. It showed a linear response of absorbance in relation to the concentration of Flecainide acetate over the range of 200-1000µg/ml. For the method, linearity was repeated 3 times for validation. The calibration curve was constructed by plotting the response y-axis versus the theoretical concentrations of standards x-axis, by using linear regression analysis. Linearity was expressed as a correlation coefficient; r^2. The results are shown in table 11.

Precision:

The intra-day and inter-day precision of the proposed extractive spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for same concentrations of Flecainide acetate and the results are reported in terms of percent relative standard deviation. Results are shown in the table 13 & 14.

Procedure for recovery studies:

20 Tablets were procured from market and average weight was determined. The powder equivalent to 100mg of Flecainide acetate was weighed accurately and taken in 3 separate 100ml volumetric flask. To this 80mg, 100mg, 120mg pure drug was added (for 80%, 100% and 120% recovery). 100ml of dil. acetic acid was added to make up the volume, shaken for ten minutes and filtered. 1ml of this solution was taken in a 100ml volumetric flask and volume was made up to the mark with dil. acetic acid. Thus Flecainide acetate of strength 100µg /ml was obtained. 1ml of this solution was diluted in 100ml volumetric flask up to the mark with dil. acetic acid and absorbance was measured at 315nm. This procedure was carried out for 3 times. Results are shown in the table 12.

Limit of detection:

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3×N/S

Where, N = Standard deviation of the response and

S = Slope of the corresponding calibration curve. Results are shown in the table 15.

Limit of quantification:

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × N/S

Where, N = Standard deviation of the response and

S = Slope of the corresponding calibration curve. Results are shown in the table 15.

Ruggedness:

Ruggedness of the proposed method was evaluated by applying the developed procedure to assay of 100µg/ml of Flecainide acetate using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to be reproducible, since there was no significant difference between analyst. Thus, the proposed methods could be considered rugged. Results are shown in the table 16.

RESULTS AND DISCUSSION

Method A. Development and validation of Flecainide acetate by derivative spectrophotometer method:

Absorption Spectra:

The absorption spectra of Flecainide acetate in dil. acetic acid (10µg/ml) was measured in the range of 200-400 nm against the blank solution. The zero order spectrum of Flecainide acetate shown maximum drug absorption wavelength at 295nm (Fig. 1).

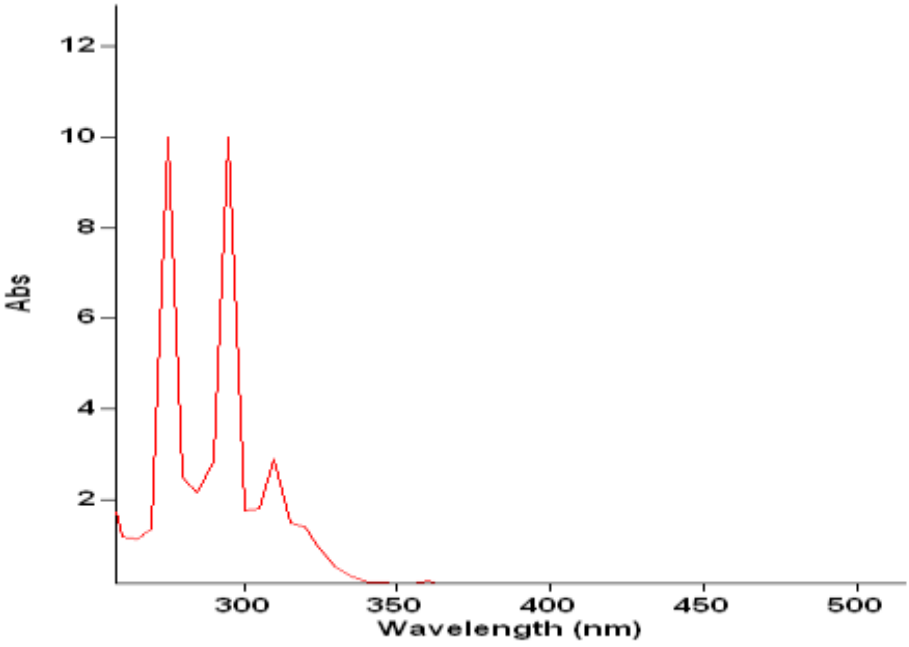


Fig. 1: Identification of λmaxof Flecainide Acetate by Method A

Linearity:

Standard calibration curve for Flecainide acetate, covering the range 2-16µg/ml, prepared by serial dilution with dil. acetic acid for pure drug. The procedure was adopted as per desired protocol, based on ICH Q2B guidelines. The calibration curve was obtained by plotting absorbance Vs analyte concentration. The slope and intercept of the calibration line was determined by linear regression (Fig. 2, Table 1)

Table 1: Linearity table of Flecainide Acetate by Method A

Sr. No.	**Concentration µg/ml**	**Absorbance**
1	02	0.1653
2	04	0.2242
3	06	0.2864
4	08	0.3411
5	10	0.3874

6	12	0.4671
7	14	0.5231
8	16	0.5820

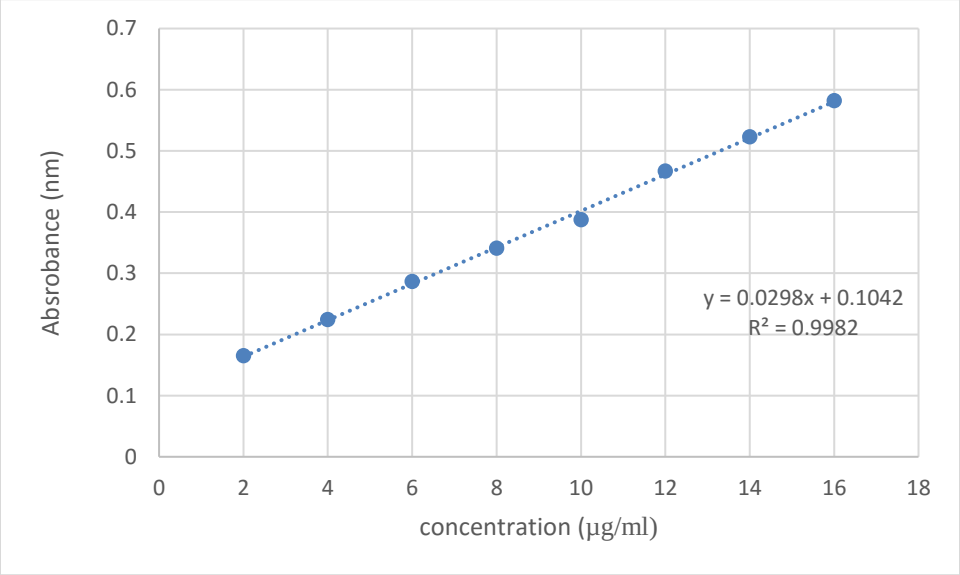


Fig.2: Linearity curve of Flecainide acetate by Method A

Accuracy:

As shown in Table 2 excellent recovery were made at each added concentration.

Table 2: Excellent recovery at each added concentration by Method A

Sr.No.	Level of % recovery	Initial amount present µg/ml	Amount of standard added µg/ml	Total amount present µg/ml	Total amount recovered µg/ml	% Recovery	Mean	Statistical analysis	
								SD	%RSD
1	80	10	8	18	18.01	100.05	99.71	0.7071	0.7091
	80	10	8	18	17.98	99.88			
	80	10	8	18	17.86	99.22			

	100	10	10	20	19.63	98.15			
2	100	10	10	20	19.94	99.70	99.25	0.9578	0.9650
	100	10	10	20	19.98	99.90			
	120	10	12	22	22.02	100.09			
3	120	10	12	22	21.96	99.81	99.76	0.6082	0.6096
	120	10	12	22	21.87	99.40			

Precision:

Precision evaluated through inter-day and intra-day of the pure drug from solvent are presented in Table 3 &4.

Table 3: Assay of intra-day precision by Method A

Sr. No.	**Concentration of drug µg/ml**					
	6	**8**	**10**	**Mean**	**SD**	**%RSD**
1	99.68	98.42	99.42			
2	100.04	100.22	99.78	99.59	0.1501	0.1507
3	98.62	100.18	99.96			
Mean	99.44	99.60	99.74			

Table 4: Assay of inter-day precision by Method A

Sr. No.	**Concentration of drug µg/ml**					
	6	**8**	**10**	**Mean**	**SD**	**%RSD**
1 day	100.04	98.97	99.96			
2 day	99.72	99.73	99.67	99.71	0.2570	0.2578
3 day	99.83	99.58	99.99			
Mean	99.86	99.42	99.87			

Limit of detection (LOD) and limit of quantification (LOQ):

The LOD determined as the amount of drug and LOQ was determined as the lowest concentration for drug shown in Table 5.

Table 5: LOD and LOQ for drug in solvent by Method A

Sr. No.	Concentration µg/ml	Absorbance	SD	Slope	LOD µg/ml	LOQ µg/ml
1	Flecainide acetate 10 µg/ml	0.3874	0.00374	0.0298	0.41	1.25
2		0.3844				
3		0.3854				
4		0.3784				
5		0.3802				

Ruggedness:

Ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Flecainide acetate using the same instrument by two different analysts under the same optimized conditions at different days. No significant difference could be observed. Results are shown in table 6.

Table 6: Ruggedness data at 10 µg/ml by two analysts by Method A

Test concentration µg/ml	Analyst 1	Analyst 2
10	0.3861	0.3858
10	0.3841	0.3893
10	0.3891	0.3844
10	0.3875	0.3863
10	0.3869	0.3871
Mean	0.3867	0.3865

SD	0.001841	0.001812
%RSD	0.4760	0.4689

Analysis of pharmaceutical formulation:

The applicability of the developed method was checked by analyzing commercially available pharmaceutical formulation. The formulation selected was FLECARITE 100 tablet. Results are shown in table 7.

Table 7: Analysis of pharmaceutical formulation by Method A

Formulation	Labelled amount (mg)	Amount recovered (mg)	% drug recovered	Mean	SD	%RSD
FLECARITE 100	100	99.42	99.42	99.26	0.8033	0.8093
	100	100.03	100.03			
	100	98.44	98.44			

Method B. Estimation of Flecainide acetate in dosage form by extractive spectrophotometer method:

Absorption Spectra:

The absorption spectra of the ion-pair complex, which was formed between Flecainide acetate and methylene blue, was measured in the range of 200-400 nm against the blank solution (Figure 3). The ion-pair complex shown maximum absorbance at 315nm.

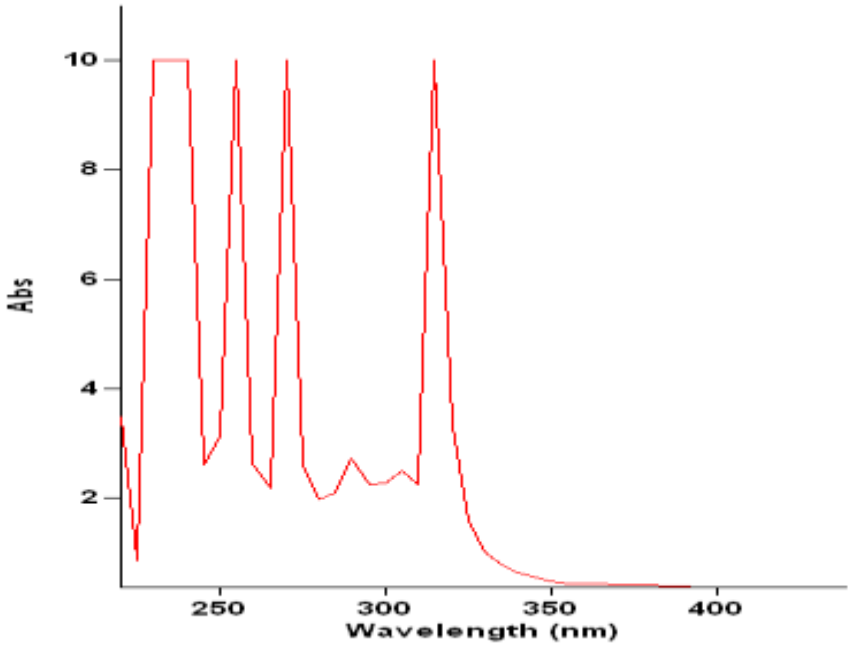


Fig.3: Identification of λmax of Flecainide Acetate by Method B

Optimization of the Method:

The reaction conditions of the method were carefully studied to achieve complete reaction, highest sensitivity and maximum absorbance. Reaction conditions for the formation of the ion-pair complex were optimized by studying preliminary experiments involving pH of buffer, type of organic solvent, volume of the dye and volume of buffer for the extraction of ion-pair complex.

Effect of volume of Methylene Blue:

The influence of the volume of 0.1% methylene blue was studied by treating 100µg/ml Flecainide acetate with 2 ml of buffer and varying volumes (0.2– 1.6ml) of 0.1% methylene blue. The absorbance of the Flecainide acetate-methylene blue ion-pair complex was increased with increasing volume of 0.1% methylene blue and became constant at 1.0ml, above this volume, the absorbance remained unchanged (Fig.4, Table 8). Therefore, 1ml of 0.1% methylene blue dye solution was chosen as the optimal volume for the quantification process.

Table 8: Effect of volume of 0.1% Methylene Blue

Volume of 0.1% methylene blue solution (ml)	Absorbance
0.2	0.1436
0.4	0.1843
0.6	0.2146
0.8	0.2743
1.0	0.2942
1.2	0.2941
1.4	0.2941
1.6	0.2942

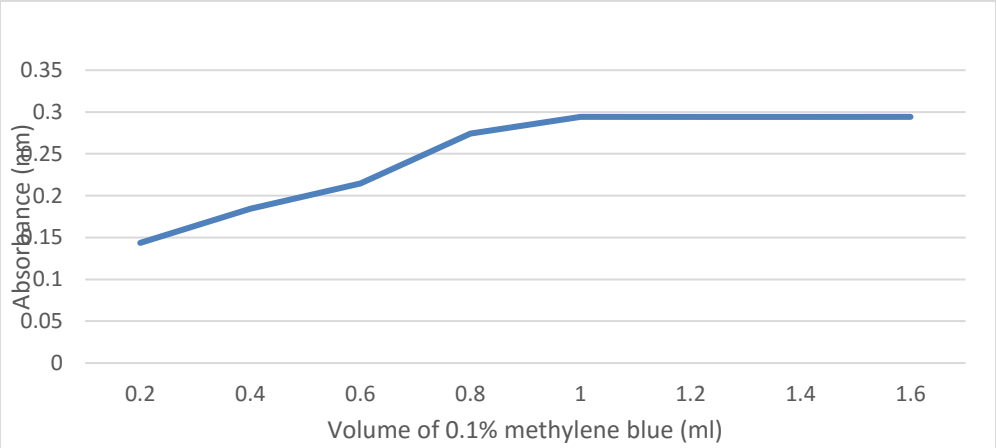


Fig. 4:Effect of volume of 0.1% Methylene Blue

Effect of pH:

At a fixed concentration of Flecainide acetate (100µg/ml), the formation of Flecainide acetate-methylene blue ion-pair complex was investigated over the pH range of 7.0-9.0 using phosphate buffer. However, the absorbance at pH 8 is slightly higher than the absorbance at other pH values. After pH 8, the absorbance decreases (Fig. 5, Table 9). Therefore, pH 8 was selected as the optimum pH for the reaction.

Table 9:Effect of pH

Phosphate buffer (pH)	Absorbance
7	0.2932
7.5	0.2930
8	0.2943
8.5	0.2912
9	0.2900

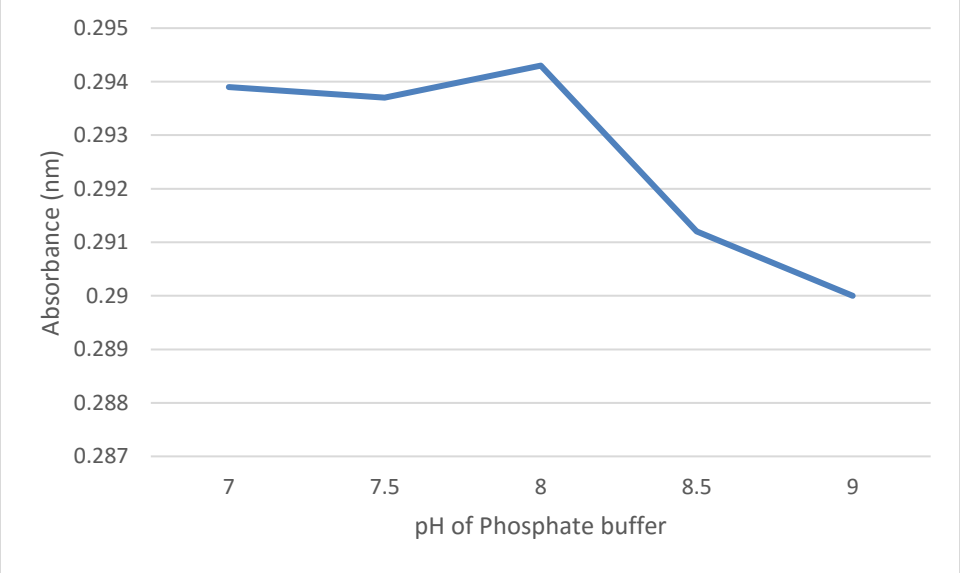


Fig. 5:Effect of pH

Effect of Volume of Buffer:

The influence of the volume of phosphate buffer (pH 8) on the absorbance value of the ion-pair complex was studied by treating 100µg/ml Flecainide acetate with varying volumes (0.5–3ml) of buffer. As shown in Fig. 6, Table 10, 2ml of phosphate buffer (pH 8) was sufficient to get the optimum pH value (pH 8). Therefore, 2ml was chosen as the optimum buffer volume.

Table 10:Effect of Volume of Buffer

Volume of Phosphate buffer (ml)	Absorbance
0.5	0.2639
1.0	0.2640
1.5	0.2645
2.0	0.2895
2.5	0.2893
3.0	0.2893

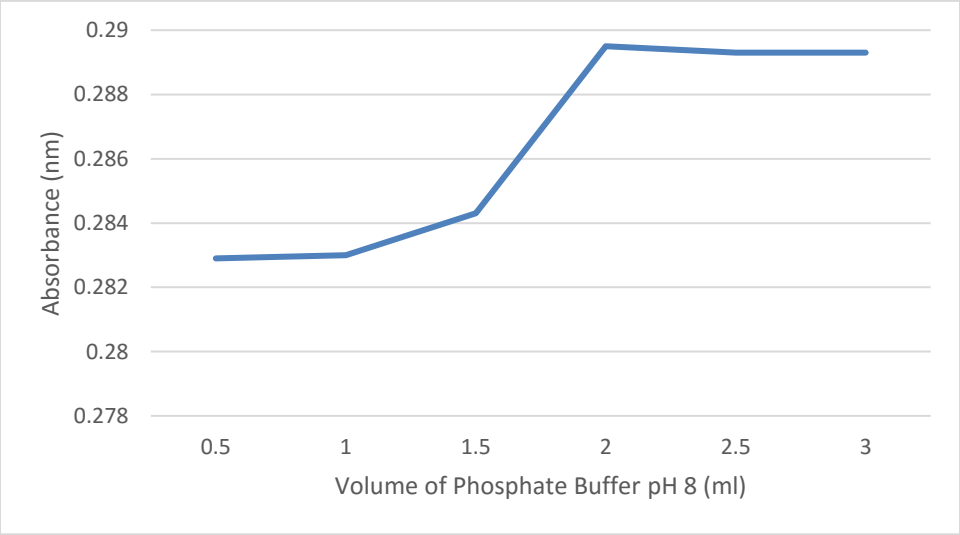


Fig. 6: Effect of Volume of Buffer

Effect of Extraction Solvent:

The effect of extraction solvent was tested using different solvents such as chloroform, benzene, dichloromethane and butanol. Using chloroform as extraction solvent, the ion-pair complex shown the highest absorbance value and reproducibility. Therefore, chloroform was chosen as the best extraction solvent for extraction of Flecainide acetate-methylene blue ion-pair complex.

Linearity:

Flecainide acetate aliquots of 200-1000µg/ml was prepared and added in 125 ml separating funnel. 1ml of methylene blue solution and 2ml of phosphate buffer pH 8 was added in to it and mixed. The aqueous solution was extracted with 5ml of chloroform shaken for 5min and funnel was allowed to stand to get two phases separated. Organic layer was collected in dry 10 ml volumetric flask and absorbance was measured at 315nm (Fig. 7 & Table 11).

Table 11: Linearity table of Flecainide Acetate by Method B

Sr. No.	Concentration µg/ml	Absorbance
1	200	0.6403
2	400	0.8993
3	600	1.3671
4	800	1.5511
5	1000	1.8362

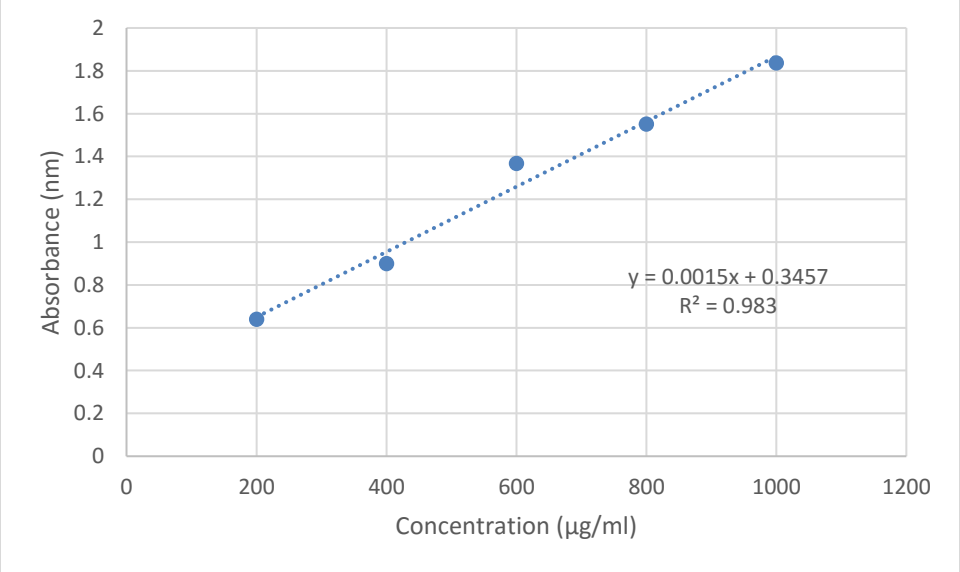


Fig. 7: Linearity curve of Flecainide acetate by Method B

Accuracy:

As shown in Table 12 excellent recovery were made at each added concentration.

Table 12: Excellent recovery at each added concentration by Method B

Sr.No.	Level of % recovery	Initial amount present µg/ml	Amount of standard added µg/ml	Total amount present µg/ml	Total amount recovered µg/ml	% Recovery	Mean	Statistical analysis	
								SD	%RSD
1	80	100	80	180	179.82	99.90	99.97	0.0809	0.0809
	80	100	80	180	179.93	99.96			
	80	100	80	180	180.12	100.06			
2	100	100	100	200	200.01	100.005	100.005	0.035	0.0349
	100	100	100	200	200.08	100.04			
	100	100	100	200	199.95	99.91			
3	120	100	120	220	219.88	99.94	99.98	0.04527	0.04528
	120	100	120	220	219.96	99.98			
	120	100	120	220	220.08	100.03			

Precision:

The intra-day and inter-day precision of the proposed extractive spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for same concentrations of Flecainide acetate and the results are reported in terms of percent relative standard deviation. Results are shown in the table 13 & 14.

Table 13: Intra-day precision by Method B

Sr. No.	Concentration of drug µg/ml			Mean	SD	%RSD
	200	200	200			
1	0.6396	0.6388	0.6373	0.6404	0.000244	0.03824
2	0.6398	0.6416	0.6420			
3	0.6412	0.6415	0.6426			
Mean	0.6402	0.6406	0.6406			

Table 14: Inter-day precision by Method B

Sr. No.	Concentration of drug µg/ml					
	200	**200**	**200**	**Mean**	**SD**	**%RSD**
1 day	0.6423	0.6378	0.6418	0.6404	0.001272	0.1987
2 day	0.6385	0.6416	0.6422			
3 day	0.6428	0.6376	0.6398			
Mean	0.6412	0.6390	0.6412			

Limit of detection (LOD) and limit of quantification (LOQ):

The LOD determined as the amount of drug and LOQ was determined as the lowest concentration for drug shown in Table 15.

Table 15: LOD and LOQ for drug in solvent by Method B

Sr. No.	Concentration µg/ml	Absorbance	SD	Slope	LOD µg/ml	LOQ µg/ml
1	Flecainide acetate 100 µg/ml	0.5623	0.00101	0.0015	2.22	6.73
2		0.5642				
3		0.5615				
4		0.5630				
5		0.5632				

Ruggedness:

The ruggedness of the proposed method was evaluated by applying the developed procedure to assay of 100µg/ml of Flecainide acetate using the same instrument by two different analysts under the same optimized conditions at different days. No significant difference could be observed. The results are shown in table 16.

Table 16: Ruggedness data at 100 µg/ml by two analysts by Method B

Test concentration µg/ml	Analyst 1	Analyst 2
100	0.3195	0.3182
100	0.3210	0.3220
100	0.3186	0.3180
100	0.3209	0.3199
100	0.3184	0.3217
Mean	0.3196	0.3199
SD	0.001234	0.001880
%RSD	0.3861	0.5879

Analysis of pharmaceutical formulation:

The applicability of the developed method was checked by analyzing commercially available pharmaceutical formulation. The formulation selected was FLECARITE 100 tablet. Results are shown in table 17.

Table 17: Analysis of pharmaceutical formulation by Method B

Formulation	Labelled amount (mg)	Amount recovered (mg)	% drug recovered	Mean	SD	%RSD
FLECARITE 100	100	98.89	98.89	99.16	0.4392	0.4429
	100	98.93	98.93			
	100	99.67	99.67			

The proposed methods are simple, accurate and precise andeconomical. For both methods validation parameters have been performed such as Limit of detection (LOD), Limit of quantitation (LOQ), accuracy, precision and ruggedness as per International conference of Harmonization (ICH) guidelines. In Method-A linearity range was found to be 2-16µg/ml for Flecainide acetate and in Method-B Flecainide acetate is found to yield a clear blue colored complex with methylene blue which was extractable with chloroform having the absorption maxima of 315nm. The colored product was due to the ion pair complex formation of the drug with the dye in the presence of phosphate buffer of pH 8.0. The linearity range was found to be 200-1000µg/ml for Flecainide acetate.The optical characteristics such as Beer's law limit, the regression analysis using the method of least square was made for the slope (m), intercept (c). Correlation coefficient (r) were also determined for the proposed methods and results are presented in table 18. Marketed formulation containing drug was successfully analyzed by the proposed methods. The Recovery studies were performed by adding a fixed amount of the drug to the pre analyzed formulation. Interference studies reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed methods.

Table 18: Linearity regression data for Flecainide acetate by Method A & B

Sr. No.	Parameters	Results of Method-A	Results of Method-B
1	Absorption maxima (nm)	295	315
2	Linearity range (µg/ml)	2-16	200-1000
3	Standard regression equation	Y=0.0298x + 0.1042	Y=0.0015x + 0.3457
4	Correlation coefficient (r^2)	0.9982	0.983
5	Accuracy (% recovery)	99.25 – 99.76	99.97 – 100.005
6	Precision	99.59 – 99.71	0.6404
7	LOD & LOQ	0.41 & 1.25	2.22 & 6.73

CONCLUSION:

The proposed derivative spectrophotometer method and extractive spectrophotometric method is found to be accurate, precise, economic and rapid for estimation of Flecainide acetate. The extractive spectrophotometric method was successfully validated and applied to marketed formulation and excellent results were obtained with the dye.

It satisfactorily eliminates interference from excipients. Hence it was concluded that the methods were successfully applied in Quality Control laboratories for routine analysis of drug in marketed formulations.

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