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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND FLECAINIDE BY HPLC METHOD IN SYNTHETIC MIXTURE.

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ABSTRACT:

A new, accurate and precise High performance liquid chromatography method was developed for the estimation of Metoprolol succinate and Flecainide in synthetic mixture. The chromatographic separation was done on ODS C18 column measuring 25 cm (4.6 mm x 250 mm, 5 um) at specific temperature using Buffer: Methanol: Acetonitrile (35:15:50) and adjusted pH to 4 at the flow rate of 1 ml/min and UV detection at 222nm. The method produced linear responses in the concentration range of 100-300 µg/ml is 0.9993 and of 50-150 µg/ml is 0.9992 for Metoprolol Succinate and for Flecainide respectively. The LOD and LOQ was found to be 5.931954 and 17.97562 for Metoprolol succinate and 2.85 and 8.64 for Flecainide respectively. The repeatability (intraday and interday), accuracy and precision were found to be ≤ 2. The present work is successfully developed and validated as per ICH guidelines.

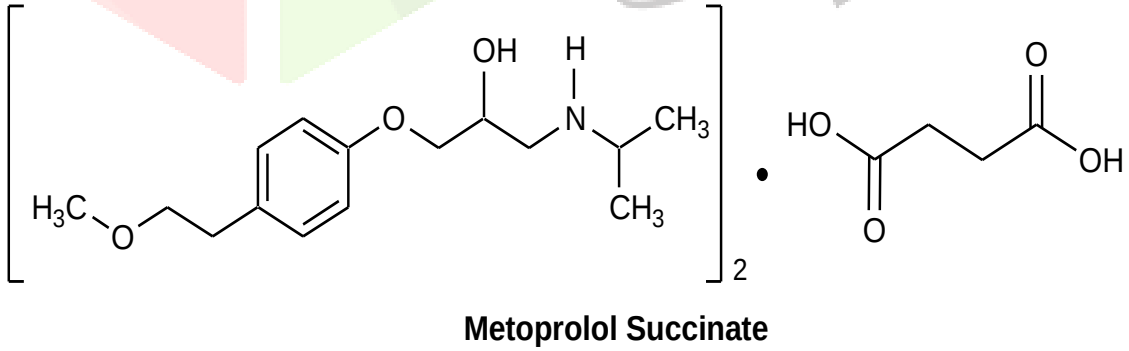
Keywords: Metoprolol succinate; Flecainide; HPLC; Method development; Method validation

INTRODUCTION:(1-4)

Metoprolol succinate and Flecainide is a beta blocker and class Ic antiarrhythmic agent respectively, which is in combination used to treat hypertension When blood pressure consistently rises to ≥140/90 mm Hg, it is considered hypertension; this threshold indicates a high enough risk of cardiovascular illness associated to hypertension to warrant medical care. greater than 140 systolic or greater than 90 diastolic pressure is called hypertension.

Metoprolol Succinate chemical name is (+)1-(isopropylamino)-3-[p-(2-ethoxyethyl) phenoxy]-2-propanol succinate. The Molecular weight is 267.3639 g/mol. The Molecular formula is $C_{34}H_{56}N_2O_{10}$. Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics. Flecainide chemical name is (RS)-N-(piperidin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy) benzamide. The Molecular weight is 414.34 g/mol.Molecular formula is $C_{17}H_{20}F_6N_2O_3$.Flecainide blocks fast inward sodium channels and slowly unbinds during diastole, prolonging the refractory period of the heart. This blockade also shortens the duration of action potentials through the Purkinje fibres. Flecainide also prevents delayed rectifier potassium channels from opening, lengthening the action potential through ventricular and atrial muscle Fibers. Finally, flecainide also blocks ryanodine receptor opening, reducing calcium release from sarcoplasmic reticulum, which reduces depolarization of cells.

While doing the literature review, we found that no other method was developed using this combination. So, we have developed and validated simultaneous estimation of Metoprolol succinate and Flecainide by HPLC method in synthetic mixture.



Metoprolol Succinate

Fig. No 1: Structure of Metoprolol succinate

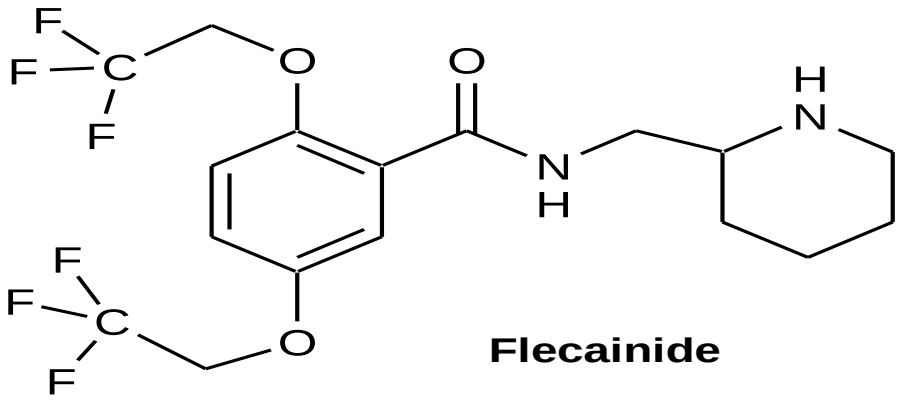


Fig. No 2: Structure of Flecainide

EXPERIMENTAL WORK:

- **Reagents and Material**:

The material and reagents used are Metoprolol succinate API, Flecainide API, Methanol HPLC, Acetonitrile HPLC, Double distilled water, Potassium Hydroxide.

- **Preparation of test solution**

Accurately weighed 100 mg of metoprolol and 50mg of Flecainide in 100ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with methanol. Concentration of Flecainide is 500 µg/ml and Metoprolol 1000 µg/ml. Take 1ml of above solution and transferred into 10 ml volumetric flask add methanol and sonicate for 10min and diluted up to the mark with methanol to give concentration for metoprolol succinate 100 µg/ml and flecainide 50 µg/ml

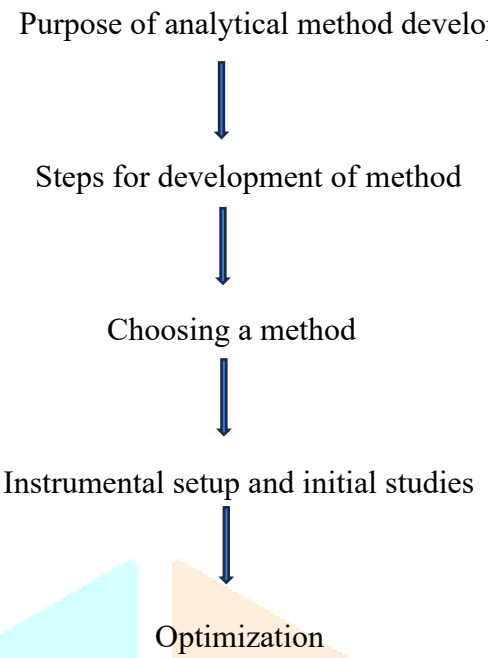
- **Preparation of buffer:**

Dissolve 5.04 g disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml. Adjust the pH with glacial acetic acid.

- **Optimization of chromatographic condition:**
  - Mobile phase- Buffer: Methanol: Acetonitrile (35:15:50 pH 4)
  - Flow Rate-1ml/min
  - Run time-15 min
  - Volume of injection-20 µl
  - Detection of wavelength-222nm

METHOD DEVELOPMENT:(5-6)

Steps for process of method development:



RESULT AND DISCUSSION:

METHOD VALIDATION:(7-9)

Method Validation is requiring when a new method has been developed and when established methods are used in different laboratories and different analysts. The performance characteristics required to validate various methods by using various guidelines such as USP, ICH, FDA, European guidelines etc. linearity, repeatability, accuracy, robustness, specificity, LOD, LOQ were studied.

1.Lineartiy:

Studies on linearity were conducted using several working standard solutions ranging from100,150,200,250, and 300 for Metoprolol succinate and 50,75,100,125, and 150 for Flecainide. After recording the absorbance at each concentration, the calibration curve was plotted to examine the linearity of the data.

Metoprolol succinate		Flecainide	
Conc.	Area	Conc.	Area
100	1021513	50	839609
150	1530024	75	1294798
200	2095365	100	1790804
250	2642352	125	2331801
300	3242356	150	2815867

Table No 1: Linearity of Metoprolol Succinate and Flecainide.

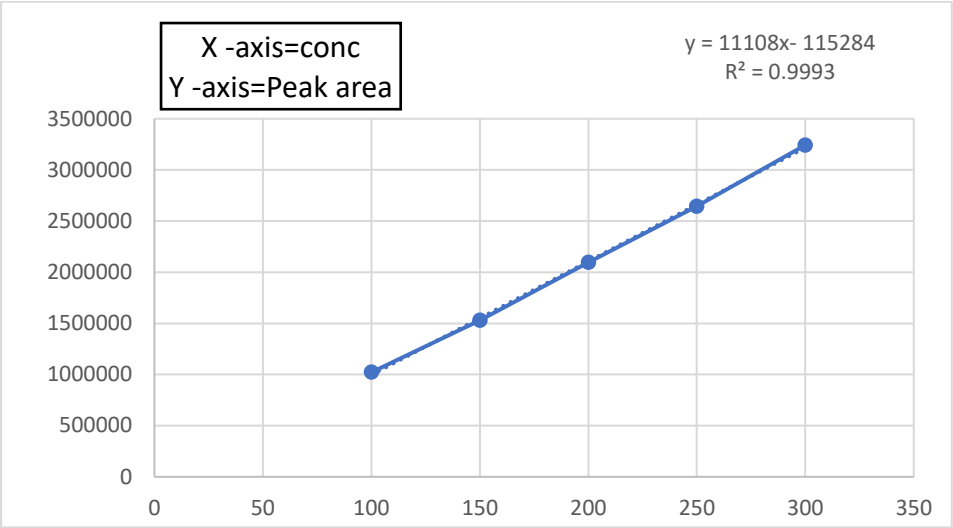


Fig. No 3: calibration curve of Metoprolol succinate

Regression Equation Data Y=mx+c	
Slope(m)	11108
Intercept(c)	- 115284
Correlation Coefficient	0.9993

Table No 2: Regression Equation Data Y=mx+c

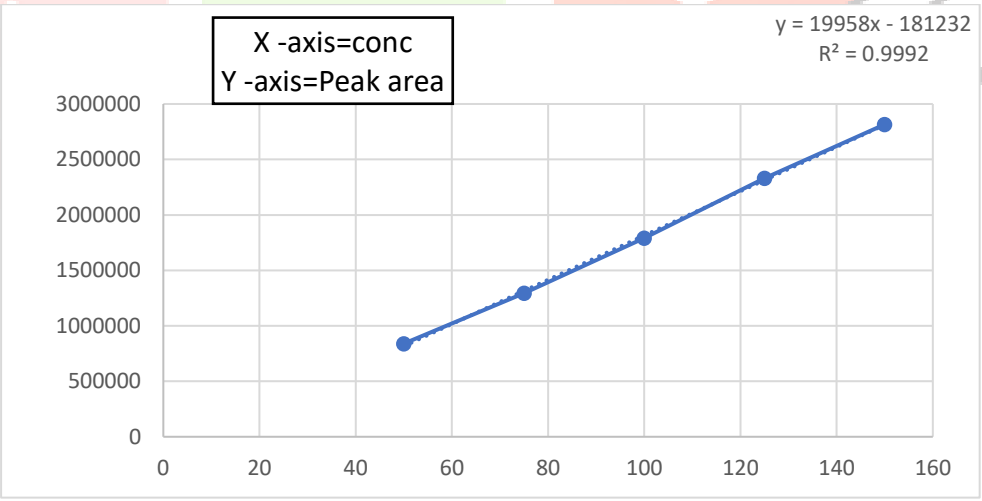


Fig. No 4: calibration curve of Flecainide

Regression Equation Data Y=mx+c	
Slope(m)	19958
Intercept(c)	-181232
Correlation Coefficient	0.9992

Table No 3: Regression Equation Data Y=mx + c

2. Repeatability:

To obtain repeatability of proposed method by examining different solution containing 100 µg/ml and 50 µg/ml for Metoprolol Succinate and Flecainide Respectively, the same solution was examined 6 times and % R.S.D value is calculated. The % R.S.D value was found to be 0.94 for Metoprolol Succinate and 0.64 for Flecainide. Since the % R.S.D value of both solutions is less than ±2, we can conclude that this method is precise.

Drug	Concentration (µg/ml)	Mean area ± SD	%R.S.D
Metoprolol succinate	100	1029685 ±9681.689	0.94
Flecainide	50	844509.3 ±5487.256	0.64

Table No 4: Result of Repeatability

2.1 Intraday:

The calculation of Intraday Precision involved analysing a solution comprising three replicates of each concentration of 100,150,250 for Metoprolol succinate and 50,100,150 for Flecainide on the same day and % R.S.D is calculated. Since the % R.S.D value of both solutions is less than ±2, we can conclude that this method is precise.

Precision		Intraday precision	
Drugs	(%)	Mean area ± SD	%RSD
Metoprolol succinate	100	1030548±13857.6	1.34
	150	2109327±24143.66	1.14
	250	3266637±39333.92	1.20
Flecainide	50	842806±5812.024	0.68
	100	1801870±23182.01	1.28
	150	2833876±48573.92	1.71

Table No 5: Results of Intraday Precision

2.2 Interday:

The concentrations of 100,150,300 for Metoprolol succinate and 50,100,150 for Flecainide and three replicates each on separate days were analysed to determine the Interday Precision. % R.S.D is calculated. Since the % R.S.D value of both solutions is less than ±2, we can conclude that this method is precise.

Precision		Interday precision	
Drugs	(%)	Mean area ± SD	%RSD
Metoprolol succinate	100	1025386± 6203.72	0.61
	200	2100276± 8420.22	0.40
	300	3265000± 37625.9	1.15
Flecainide	50	846213± 5726.37	0.67
	100	1799398± 19666.6	1.09
	150	2831298±35753.3	1.26

Table No 6: Result of Interday Precision

3.Accuracy:

Accuracy of proposed method is examined by recovery studies performed at different levels of concentrations (50%, 100% and 150%) for both the drugs. The percentage recovery of Metoprolol succinate and Flecainide was found to be in a range of 98 -102%.

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	%Recovery
50	100	50	150	1530024	148.119	98.74
	100	50	150	1520000	147.217	98.14
	100	50	150	1526100	147.766	98.51
100	100	100	200	2095365	199.014	99.50
	100	100	200	2152199	204.131	102.06
	100	100	200	2095355	199.013	99.50
150	100	150	250	2642352	248.25	99.30
	100	150	250	2648200	248.78	99.51
	100	150	250	2646800	248.65	99.46

Table No 7: Result of accuracy of Metoprolol succinate

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	%Recovery
50	50	25	75	1294798	101.2154	73.9568
	50	25	75	1285863	101.3464	73.5091
	50	25	75	1285812	101.4221	73.5066
100	50	50	100	1790804	153.104	98.8093
	50	50	100	1790723	153.2349	98.8052
	50	50	100	1780850	152.5171	98.3106
150	50	75	125	2331801	198.5322	125.916
	50	75	125	2331850	198.6796	125.919
	50	75	125	2378387	198.7553	128.25

Table No 8: Result of Accuracy of Flecainide

4. Robustness:

When the changes are made in parameters like wavelength, flowrate and mobile phase and then % R.S.D value is calculated. Since the % R.S.D value of both solutions is less than ±2, we can conclude that this method is robust.

Drugs	Wavelength	Mean area ± SD	%RSD
Metoprolol Succinate	219	2113927±21352.4	1.01
	222	2093255±17892.3	0.85
	225	2114209± 21263.4	1.01
Flecainide	219	1787339±25084.7	1.40
	222	1775315±21684.7	1.22
	225	1781115±24121.3	1.35

Table No 9: Robustness study of Metoprolol Succinate and Flecainide (change in wavelength)

Drugs	Flowrate	Mean area ± SD	%RSD
Metoprolol Succinate	0.9 ml/min	2107154±20371.2	0.97
	1 ml/min	2093255±17892.3	0.85
	1.1 ml/min	2086011± 29016.5	1.39
Flecainide	0.9 ml/min	1772244±23952.69	1.35
	1 ml/min	1775315±21684.7	1.22
	1.1 ml/min	1763625±24144	1.37

Table No 10: Robustness study of Metoprolol Succinate and Flecainide (change in flow rate)

Drugs	Mobile Phase	Mean area ± SD	%RSD
Metoprolol Succinate	BMA (30:20:50)	2107155±29244.2	1.39
	BMA (35:15:50)	2093255±17892.3	0.85
	BMA (30:15:55)	2106826± 31732.8	1.51
Flecainide	BMA (30:20:50)	1769336±28893.2	1.63
	BMA (35:15:50)	1775315±21684.7	1.22
	BMA (30:15:55)	1760640±19116.2	1.09

Table No 11: Robustness study of Metoprolol Succinate and Flecainide (change in mobile phase)

5. Limit of Detection and Limit of Quantitation

The LOD and LOQ was found to be 5.931954 and 17.97562 for Metoprolol succinate and 2.85 and 8.64 for Flecainide respectively.

- **Limit of Detection: (Metoprolol succinate)**

$$LOD = \frac{3.3 \times \text{STANDARD DEVIATION OF Y-INTERCEPT}}{\text{SLOPE OF THE CALIBRATION CURVE}}$$

$$= \frac{3.3 \times 20070.78}{11165.56}$$

LOD = 5.931954

- **Limit Of Quantitation: (Metoprolol succinate)**

$$LOQ = \frac{10 \times \text{STANDARD DEVIATION OF Y-INTERCEPT}}{\text{SLOPE OF THE CALIBRATION CURVE}}$$

$$LOQ = \frac{10 \times 20070.7}{11165.56}$$

LOQ =17.97562

- **Limit of Detection: (Flecainide)**

$$LOD = \frac{3.3 \times \text{STANDARD DEVIATION OF Y-INTERCEPT}}{\text{SLOPE OF THE CALIBRATION CURVE}}$$

$$= \frac{3.3 \times 17268}{19977.7}$$

LOD = 2.85

- **Limit Of Quantitation: (Flecainide)**

$$LOQ = \frac{10 \times \text{STANDARD DEVIATION OF Y-INTERCEPT}}{\text{SLOPE OF THE CALIBRATION CURVE}}$$

$$LOQ = \frac{10 \times 17268}{19977.7}$$

LOQ =8.64

❖ **Specificity Metoprolol Succinate and Flecainide:**

The specificity studies conducted for Metoprolol Succinate and Flecainide aimed to confirm the ability of the analytical method to accurately detect and quantify these compounds in the presence of potential interferences. The chromatograms obtained from analyzing standard solutions of Metoprolol Succinate and Flecainide were compared to those obtained from placebo samples to assess any potential interference from matrix components. The results indicated that the peaks corresponding to Metoprolol Succinate and Flecainide were well-separated from any interfering peaks present in the placebo samples. This observation

demonstrated the specificity of the method for detecting and quantifying Metoprolol Succinate and Flecainide, ensuring reliable and accurate results in the presence of potential matrix interferences.

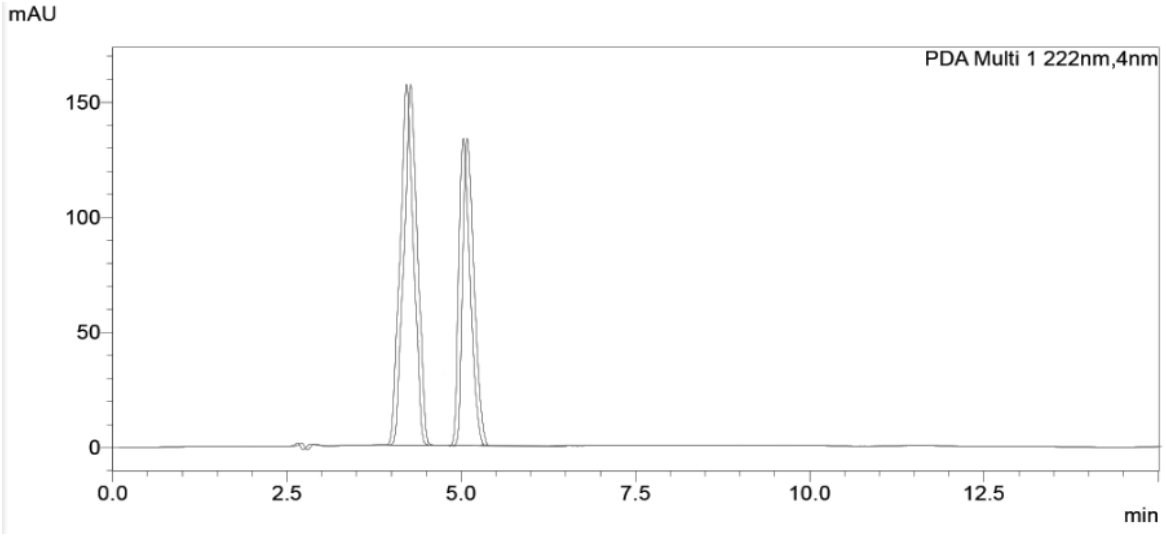


Fig. No 5: HPLC chromatogram of Metoprolol succinate and Flecainide synthetic mixture

❖ **Assay:**

- Analysis of Synthetic Mixture

The developed and validated HPLC Method was applied for determination of Flecainide and Metoprolol succinate in synthetic mixture. The sample was analysed three times. The % assay was found to be 101.45% and 101.54% for Metoprolol succinate and Flecainide, respectively.

Drug	Conc. (µg/ml)	Amount found (µg/ml)	% Assay Mean ± SD (n=3)	% RSD
Metoprolol succinate	100	102.34	101.45%	0.88
		100.553		
		101.463		
Flecainide	50	50.3081	101.54%	1.46
		50.383		
		51.6312		

Table no 12: Data of determination of Metoprolol succinate and Flecainide in synthetic mixture

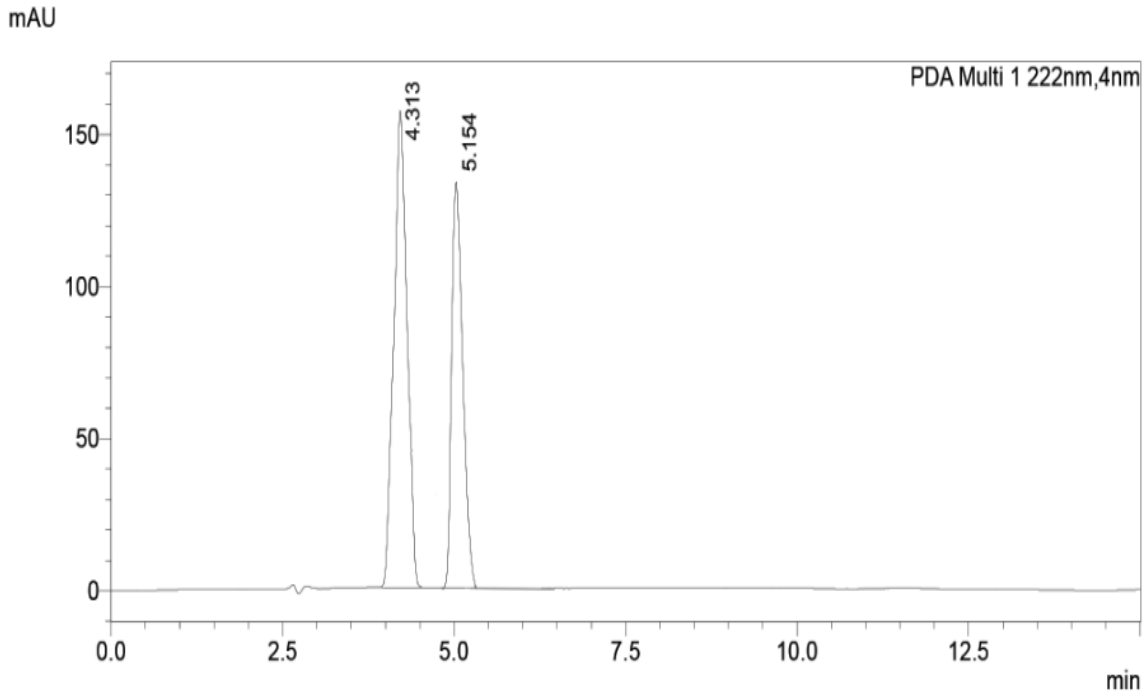


Fig.No 6: HPLC Chromatogram of Flecainide and Metoprolol from synthetic.

CONCLUSION:

Metoprolol succinate and Flecainide in a synthetic mixture was identified and quantified using high performance liquid chromatography. The proposed method's selectivity, linearity, sensitivity, precision, and accuracy were confirmed by statistical data, and the method was successfully validated in accordance with ICH requirements. According to the results above, every metric has been examined and determined to meet the pre-established acceptance requirements. Thus, we draw the conclusion that the established method is appropriate as well

Precise.

ABBREVATION:

R2 -Correlation coefficient

µL - Microlitre

mL – Millilitre

µg - Microgram

Mg - Milligram

G–Gram

S.D. - Standard deviation

%RSD - Relative standard deviation

LOD – Limit of Detection

LOQ – Limit of Quantification

HPLC – High Performance Liquid Chromatography

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