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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FLECAINIDE ACETATE BY USING RP-HPLC METHOD

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ABSTRACT

Chromatographic separation was performed on column C18, 250mm, 5micron meter particles packing with stationary phase. The mobile consisting of phosphate buffer of pH 5 and mobile B consisting of ACN in the ratio of 55:45 v/v at a flow rate of 1ml/min. The wavelength used for the detection was 298nm with a total run time of 20min. The method was developed and tested for the linearity range of 100microgram/milli liter. From this acid degradation study was performed on 4N HCL at 80C for 6 hours. Base degradation study was performed on 4.5N NaOH at 90c for 3 hours. Oxidative degradation was performed on 25% H202 at room temperature for 36 hours and got 20% degradation.

KEYWORDS: Method development, Validation, Flecainide acetate.

INTRODUCTION

Flecainide is a medication used to prevent and treat abnormally fast heart rates. This includes ventricular and supraventricular tachycardias. Its use is only recommended in those with dangerous arrhythmias or when significant symptoms cannot be managed with other treatments. Its use does not decrease a person's risk of death. It is taken by mouth or injection into a vein.

Common side effects include dizziness, problems seeing, shortness of breath, chest pain, and tiredness. Serious side effects may include cardiac arrest, arrhythmias, and heart failure. It may be used in pregnancy, but has not been well studied in this population. Use is not recommended in those with structural heart disease or ischemic heart disease. Flecainide is a class Ic antiarrhythmic agent.] It works by decreasing the entry of sodium in heart cells, causing prolongation of the cardiac action potential.

METHOD DEVELOPMENT

Sample preparation

Weigh and transfer 30 mg of flecanide acetate to 3 ml of distilled water in a test tube and tap it with glass rod. Record the sample as soluble or insoluble. Same way it is checked in ACN, acetic acid, ethanol, methanol and hydrochloric acid

SamplejPreparation

1. The Kbr powder was kept for drying under IR-lamp.
2. A pinch of sample was taken and mixed properly with the dried kbr powder and a compressed under the compressor.
3. The kbr powder was scanned by the FT-IR.
4. Kbr sample was taken as blank.
5. Then Kbr and sample were scanned.

Preparationj ofjsolutions

Selection of wavelength for detection

Standard solutions of Flecainide acetate 100 µg /ml was prepared for the selection of wavelength and scanned between 200-400 nm in UV-visible double beam spectrophotometer at a medium scanning speed. Spectra was taken which was used for the selection of wavelength for detection and it was found that Flecainide acetate showed reasonably best response at 298 nm. So 298 nm was nominated as a wavelength for determination. (Kavathia & Misra, 2013).

Selection of stationary phase

We have tried Phenomenax C18, 250mm x 4.6 mm, 5µm particles packing and Waters C18, 250mm x 4.6 mm, 5µm for method development. In Phenomenax C18, 250mm x 4.6 mm, 5µm column peak is splited and more peak broadening. In Waters C18, 250mm x 4.6 mm, 5µm column we get good peak shape with good asymmetry and large number of theoretical plates.

Selection of Mobile Phase

The standard solution of Flecainide acetate was inserted into HPLC system and run into different composition of mobile phase A and B to find out best condition. Asymmetry, theoretical plates are important criteria for the method development. The final optimized mobile phase was determined to be pH 5 Phosphate buffer and Acetonitrile 55:45 (v/v). (Sengupta, Chatterjee, & Kanti, 2017).

Procedureto make Phosphate buffer

Phosphate Buffer pH 5 (Add 750µL Phosphoric acid in 530 ml HPLC grade Water and add 530 µL TEA in it) pH adjusted with 10% KOH.

Preparation of Mobilej Phase

Mobile Phase A:
Phosphate Buffer pH 5.
Mobile Phase B:
Acetonitrile

Preparation of Diluent

Prepare a mixture of pH 5 Phosphate buffer and ACN in the ratio of 55:45 (%V/V) and degas.

Preparation ofj standardjstockjsolutionj ofj Flecainidej Acetatej (1000j µg/ml)

Accurately weighed and transferred 10 mg of working standard into 10 ml volumetric flask. Added 5 ml of ACN and sonicated below 25ºC until dissolved. Made volume up to the mark with ACN and mixed it.

Preparation of sample solution (100j µg/ml)

Take 1 ml of stock solution and relocated it into 10 ml volumetric flask and dilute it with diluent up to the mark. Filtered it with 0.45µm PVDF filter by discarding 2 ml of filtrate.

Preparation of Blank solution:

Use Diluent as a Blank

Final OptimizedjChromatographicj Condition

Table 1: chromatographic condition.

Column	Waters C18, 250mm x 4.6 mm, 5µm
Wavelength	298 nm
Flow Rate	1.0 ml/min
Injection Volume	50µl
Run time	10 minute
Rinsing Solvent	Blank

RESULT AND DISCUSSION

As per literature review less articles is available and nowadays QbD approach is mandatory. So that method development is done on Agilent HPLC system and generate AQbD model for that.

Selectionjofj solvent:

Based on solubility study solvent used for Flecainide acetate - Acetonitrile, water, Methanol, acetic acid, anhydrous ethanol.

Table 2: Result of solubility of Flecainide Acetate

Solvent	Solubility (mg/ml)
Water (100%)	10 mg/ml
Acetonitrile (100%)	10 mg/ml
Methanol (100%)	3 mg/ml
HCl	Insoluble
Anhydrous Ethanol	10 mg/ml
Dilute Acetic Acid	2 mg/ml

Selectionjof wavelength for detection

Flecainide acetate solution was prepared using Acetonitrile and concentration was 10 µg/ml. In UV-visible spectrophotometer in range of 200 – 400 nm to determine the absorption maxima of the drug. And good response at 298 nm, so it was selected for detection.

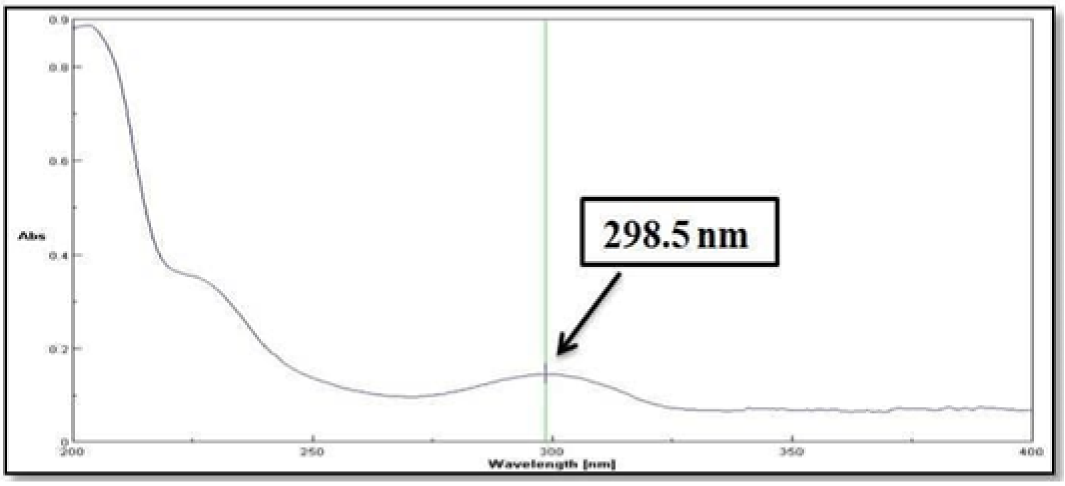

Figure 1: selection of wavelength for detection.

Selection of Stationary phase.

Different trials of columns were performed for the selection of stationary phase and determined final column was selected which showed well theoretical plates, asymmetry, elution pattern with sharp peak.

Table 3: Column trials.

Trial No:	Column	Observation
1	Phenomenax C18, 250mm X 4.6 mm, 5µm	Peak is splitted and not sharp Peak
2	Waters C18, 250mm X 4.6 mm, 5µm	Sharp peak and good asymmetry, no. of theoretical Plates

Trial – 1: Chromatogram of the sample using Phenomenex column.

Trial – 2: Chromatogram of the sample using Waters column

Optimized Conditions

- ✓ Column: Waters C18, 250mm x 4.6 mm, 5µm

- ✓ Wavelength: 298 nm
- ✓ Flow Rate: 1.0 ml/min
- ✓ Injection Volume: 50µl
- ✓ Run time: 10 minutes
- ✓ Rinsing Solvent: Blank
- ✓ Mobile phase A: phosphate buffer solution pH 5
- ✓ Mobile phase B - ACN
- ✓ Mobile phase A : B – 55 : 45 v/v
- ✓ Blank: Phosphate Buffer(pH 5) : ACN (55:45 V/V)

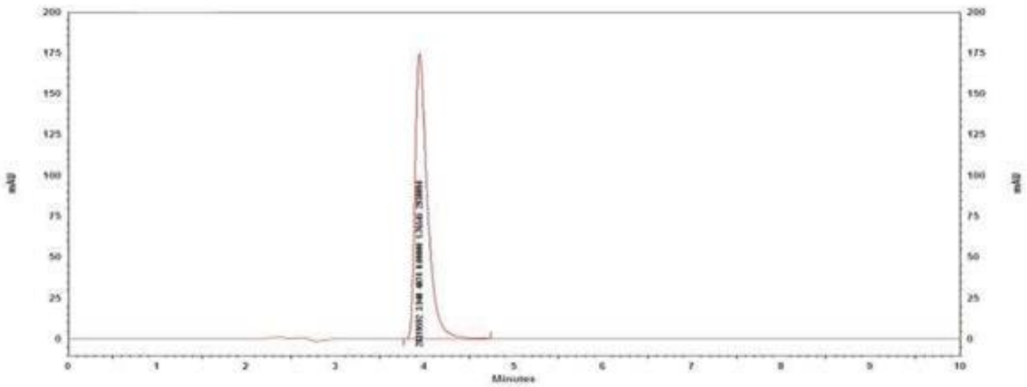

Figure 2: Chromatogram of Flecainide acetate solution. (100 µg/ml).

Method Validation

System suitability

Table 4: System suitability parameter.

No. of injection	Rt	Area	Tailing factor	NTP
1	4.023	28319590	1.591	3464
2	4.020	28319588	1.589	3460
3	4.101	28319580	1.592	3469
4	4.073	28319575	1.590	3459
5	4.111	28319598	1.590	3470

MEAN ± S.D	4.0656 ± 0.0426	28319586 ± 8.9554	1.5904 ± 0.0011	3464.4 ± 5.0299
% R.S.D	0.0942	0.0691	0.0691	0.1451
Acceptance	RSD NMT	RSD NMT 2.0	NMT 2.0	≥2000

criteria	2.0			
Interference	Pass	Pass	Pass	Pass

Acceptance Criteria

- The relative standard deviation for analyte peak of five standard injections should
- NMT 2.0%.
- The tailing factor for the analyte peak should not more than 2.
- The NTP for the analyte peak should be at least 2000 in standard solution.

CONCLUSION

The system suitability parameter pass the all the limits, it is suitable for use.

Linearity and Range (n = 5)

For, establishment of linearity calibration curve was found at 5 levels in concentration rage 100 – 300 µg/ml for Flecainide Acetate. The injections were analyzed in triplicate. It was subjected to least square regression analysis to calculate correlation coffecient and linearity equation.

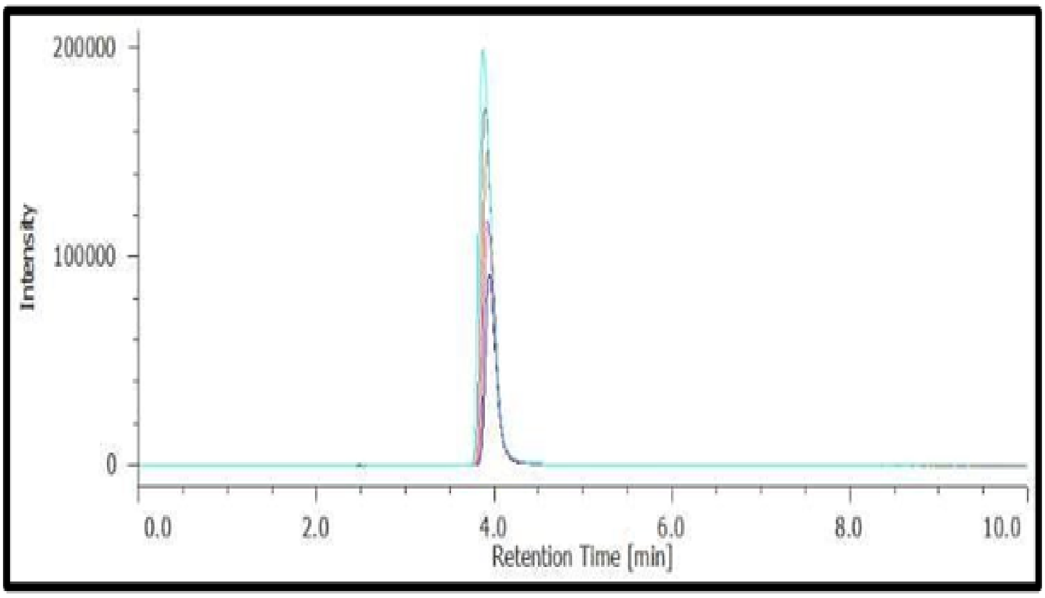

Figure 3: Linearity overlay chromatogram for flecainied acetate at 298 nm.

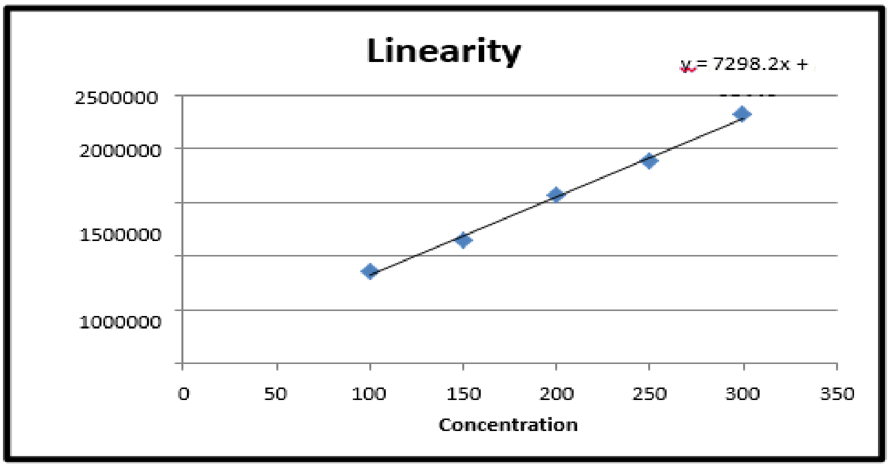

Figure 4: Linearity graph for Flecainide Acetate.

Table 5: Result table of linearity and range.

Parameter	**Flecanide aceatate**
Range	100-300µg/mL
Linearity equation	y = = 7298.2x + 95443
Regression coefficient	0.9961

Acceptance Criteria

The correlation coefficient (R^2) value must lie between 0.99 to 1.00 for Flecainide acetate.

CONCLUSION

The correlation coefficient (R^2) value for each component is well within the limit of approval norms that means the areas achieved are directly proportional to the conc. of analyte in sample. This technique considered to be linear in the range 50% to 150% of standard concentration.

Precision

Repeatability

Intraday Precision

Table 6: Result of Intraday for Flecainide acetate.

FCD conc. (µg/ml)	Peak Area (mV.s)			Mean	S.D	% RSD
100	855622	855800	856520	855980.1	475.48	0.25
200	1570042	1578512	1575423	1574559.2	428.63	0.27
300	2316952	2317563	2317096	2317203.6	319.41	0.22
Average						0.24

Accuracy (recovery n=3)

The result show that the % recovery for Flecainide Acetate was found to be in the range of 100.67 to 100.88 %

Table 7: determination of accuracy of Flecainide Acetate.

Level (%)	Amount Flecainide taken (µg/ml)	Amount of Flecainide added (µg/ml)	Total amount of ecainide (µg/ml)	Mean %Recovery ± SD	%RSD
50	100	50	150	100.67 ± 0.86	0.85
100	100	100	200	100.94 ±0.33	0.33
150	100	150	250	100.88 ±0.51	0.50

Acceptance criteria

% recovery at each level should be 98.0 % to 102.0 %.

Conclusion

Individual recovery at each level meets the established acceptance criteria. This method is accurate and pass all criteria.

Robustness

Table 8: Results of Robustness.

Factor	Retention time (min)	Peak area(mv.sec)
A. pH		
4.9	3.90	28301254
5.1	4.21	28395212
MEAN ± SD	4.055 ± 2.61	28348233 ±6643.3389
% RSD	0.64	0.23
B. FLOW RATE		
0.9ml/min	4.24	28487596
1.1ml/min	3.87	28354851
MEAN ± SD	4.055 ± 2.74	28421223.5 ± 93864.8

% RSD	0.64	0.33
C. BUFFER RATIO		
54:46	3.95	28350087
56:44	4.19	28395142
MEAN ± SD	4.07 ± 1.69	28372614.5 ± 31858.6
% RSD	0.41	0.11

Acceptance criteria

%RSD should not more than 2.0%.

Conclusion

% RSD found to be within 2.0% shows that the method is robust.

CONCULSION

QbD approach was established for the development of a new analytical method to generate the impurities of a drug substance by central composite design in Design Expert® software version 11.0. The experimental design defines the main components such as strength, temperature and time. Chromatographic separation on a Column: Waters C18, 250mm x 4.6 mm, 5µm particles packing stationary phase. The mobile phase A consisted of phosphate buffer pH 5 and mobile B consisted ACN (55:45 V/V) at a flow rate of 1.0 mL/min. The wavelength used for the detection was 298 nm with a total run time of 20 minutes. The method was developed and tested for the linearity range of 100 µg/mL to 300 µg/mL.

Aj Designj spacej in j whichj thej methodj wasj robust j couldj bej generatedj successfully. j Fromj thisj Acidj degradationj studyj wasj performedj onj 4 j Nj HClj at j 80℃j forj 6 j hoursj andj got j 12.47 j % j degradation. j Basej degradationj studyj wasj performedj onj 4.5 j Nj NaOHj atj 90℃j forj 3j hoursj andj gotj 10.85 j %j degradation. j Oxidativej degradationj wasj performedj onj 25%j H_2O_2jatj roomj temperaturej forj 36 j hoursj andj gotj 20 %jdegradation.

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