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STABILITY INDICATING INNOVATIVE UPLC ANALYTICAL METHOD VALIDATION FOR ACETATE QUANTIFICATION IN FLECAINIDE ACETATE FORMULATIONS

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ABSTRACT

This scholarly inquiry delineates the conceptualization and substantiation of an economically judicious ultra-high performance liquid chromatography (UPLC) methodology, meticulously fashioned in concordance with the stringent criteria delineated by the International Conference on Harmonization (ICH), meticulously tailored for the precise quantification of acetate moieties within the compound Flecainide Acetate. The procedural instantiation leverages an intricately configured UPLC system, harnessing the unique attributes of an innovative chromatographic column and a meticulously optimized mobile phase composition. The developmental trajectory of the methodology involved a methodical optimization of chromatographic parameters, meticulously calibrated to yield an elevated echelon of separation, resolution, and acuity in the quantification of acetate entities. The method, under scrutiny, manifested a linear response within the delimited span of 50% to 200%, fortified by an exceptionally elevated correlation coefficient of unity (1.000). The method evinced commendable accuracy and precision, ascertained through a percentage recovery spectrum spanning from 100.5% to 101.7%, and a percentage relative standard deviation (%RSD) consistently residing beneath the 2% threshold, thereby adhering meticulously to the exacting standards elucidated by the ICH. Rigorous evaluations of robustness and ruggedness proffered favorable outcomes, thereby corroborating the method's inherent reliability. A salient observation of paramount import was the absence of any discernible interference from excipients, underscoring the method's discerning accuracy and precision. This novel UPLC instantiation, meticulously attuned to the tenets of the ICH, proffers a pragmatic and fiscally frugal modality for the determination of acetate content within Flecainide Acetate, positing its

pertinence for routine analyses in both bulk and pharmaceutical formulations of the aforementioned compound.

Keywords: Flecainide Acetate, Acetate, International Conference on Harmonization (ICH), Ultra Performance Liquid Chromatography (UPLC), Validation

INTRODUCTION

Flecainide Acetate (FA), chemically identified as N-(2-piperidinylmethyl)-2,5-bis(2,2,2–trifluoroethoxy) benzamide acetate with a molecular formula of $C_{19}H_{24}F_6N_2O_5$ and a molecular weight of 474.4 g/mol, is a white solid powder characterized by a melting point of 146-152°C **(Figure 1)**. Functioning as an antiarrhythmic agent (class IC), FA induces a reduction in intracardiac conduction velocity throughout the heart. This drug is employed for the management of various cardiac arrhythmias, including tachyarrhythmia, atrial fibrillation, supraventricular tachycardia, and ventricular tachycardia, achieved by blocking Na^+ current delayed rectifier K^+ current (classified as a Na^+ channel blocker drug). Additionally, Flecainide exhibits local anesthetic effects, selectively increasing anterograde and retrograde accessory pathway refractoriness **[1-4]**. The pharmacological impact of Flecainide includes the prolongation of the PR interval and widening of the QRS complex, with negligible effects on the JT interval, as it does not lengthen ventricular repolarization. Recognized in the European, British, and US pharmacopeias **[4]**.

The determination of flecainide, a potent antiarrhythmic drug, has been the focus of several chromatographic methods for its quantification in bulk powder, pharmaceutical formulations, and in conjunction with its enantiomers, metabolites, or other antiarrhythmic drugs **[3-4]**. While previous methods predominantly utilized reversed-phase high-performance liquid chromatography (RP-HPLC), this study aims to advance the analytical approach by exploring the benefits of ultra-performance liquid chromatography (UPLC). UPLC offers enhanced analytical performance with smaller particle sizes in stationary phases and higher operating pressures, enabling faster separations with improved resolution and sensitivity. The reduced particle size results in narrower peaks and better peak shapes, contributing to increased efficiency and throughput. The research objective is to devise and validate a simple UPLC method for estimating Flecainide Acetate (FA) in both bulk drug and tablet formulations. This analytical method, developed in accordance with the International Conference on Harmonization (ICH) guidelines, underwent rigorous validation for accuracy, precision,

ruggedness, and sensitivity. The emphasis on a validated analytical method aims to meet the quality assessment and analytical requirements of Flecainide Acetate in pharmaceutical formulations **[5-6]**, offering a comprehensive approach to its formulation development.

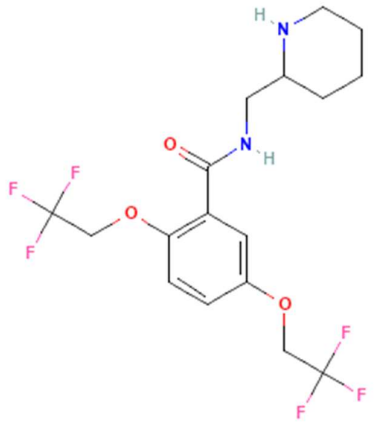

Figure 1: Chemical Structure of Flecainide Acetate

MATERIALS AND METHODS

Materials

The gift sample of Flecainide Acetate (FA) was acquired from Indeus Life Sciences Pvt. Ltd, located in Mumbai. Analytical grade solvents and reagents were procured from Merck Specialties Pvt. Ltd. in Mumbai, India. For the preparation of solutions and analyses, double-distilled water that had been filtered through a membrane filter was utilized.

Instrument

Waters Acquity UPLC HSS T3 (75X2.1)mm 1.8µm equipped with a pump, an auto sampler, a suitable detector and a suitable data acquisition system, Analytical Balance, Laboratory sonicator, Standard laboratory cleaned and dried glassware, PVDF membrane filter having 0.22µm porosity.

Methods

Method development

The analytical procedure for determination of content of Acetate in Flecainide Acetate, USP is in house developed method by UPLC. This method is applicable and specific for content of acetate test of Flecainide Acetate, USP drug substance from an identified source for release.

Selection of Chromatographic Method

The sample separation was achieved on Waters Acquity UPLC HSS T3 (1.8 µ, 75 cm X 2.1 mm i.d.) column, aided by mobile phase mixture of mobile phase A Buffer solution and mobile phase B (Acetonitrile and methanol equal ratio) was run in gradient program. The flow rate was 0.450 ml/ minute and ultra violet detector at 204nm that was filtered and degassed prior to use, Injection volume is 4.0 µl and ambient temperatures

Preparation of Mobile phase

Mobile phase A (Buffer solution): Transfer 1.0 ml of Ortho-phosphoric acid into a suitable container containing 1000 mL of water. Filter the solution through 0.22µm PVDF filter. Degas it by sonication.

Mobile Phase B: Prepare a mixture of Acetonitrile and Methanol in the ratio of

500:500 (%v/v). Mixed well and degas it by sonication.

Diluent: Water is used as a diluent.

Standard Preparation

Weight accurately about 126.0 mg of Acetic Acid into a 100 mL volumetric flask containing about 20 mL of diluent. Dilute to volume with diluent and mixed well.

Pipette 10.0 mL of above solution and transfer into 100 mL volumetric flask containing about 20 mL of diluent. Dilute to volume with diluent and mixed well.

Sample Preparation

Weight accurately about 100 mg of sample and transfer into a 100 mL volumetric flask. Add about 25 ml of diluent and sonicate for dissolve it. Dilute the volume with diluent and mixed well.

Validation

The proposed UPLC method was validated as per ICH guideline and the various parameters are given below:

Linearity

The calibration curve was established by plotting a graph of concentration versus area of standard and determining the correlation coefficient. A series of concentration ranging from about 50 to 200% of standard and injected into the UPLC. (Correlation coefficient should be not less than 0.999).

Accuracy

Accuracy is in agreement with acceptable true value and actual result observed. The percent recoveries were carried out using standard and sample at 50%, 100% and 150% level, in triplicate at each level and analyzed by UPLC.

Precision

The precision of the method was evaluated as intra-day and inter-day by carrying out six independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated. (%RSD should not be more than 2%).

Robustness

The robustness of a method is the ability of the method to remain unaffected by making slight deliberate changes in chromatographic conditions, such as change in ratio of the mobile phase and small changes in flow rate.

Equivalency with the current method

The equivalency between in-house Potentiometric method and new in house UPLC method was established by analyzing three different batches of sample.

System suitability

The system suitability was performed by analyzing five replicate injections of a standard solution. Results of peak area of standard was noted and % RSD was calculated **[7-14]**.

RESULT AND DISCUSSION

In the present investigation optimum results were achieved using he described column specification and solvent system. Flecainide Acetate did not interfere in our analysis and was used as an internal standard. Under

same experimental conditions, duration of separation was shorter and observed peak symmetry was better. Optimum retention times were achieved. The chromatogram of diluent, standard and sample were given in **Figure 2, 3 and 4**.

Validation

The objective of this validation study is to demonstrate the in-house developed UPLC method is suitable for Content of Acetate in Flecainide Acetate, USP. The method validation is to be performed according to the ICH guidelines ICH Q2(R2).

Linearity

The linearity results are summarized in **Table 1**. The linearity graph is shown in **Figure 5**. The linearity data meets the acceptance criteria indicates that the method is linear within the concentration range from 50% to 200 % (62.255 µg/mL to 249.020 µg/mL) of working standard concentration.

Precision

System Precision

System precision (Repeatability of Injections) was assessed from the six replicate of a standard solution. The results are summarized in Table 2. The result meets the acceptance criteria indicates that the analytical procedure is precise with respect to the chromatographic system.

Method Precision

To evaluate the method precision, six samples were prepared and analyzed per the analytical procedure. The results are tabulated in **Table 3**. The result meets the acceptance criteria indicates that the analytical procedure is precise for its intended use.

Accuracy

To demonstrate the accuracy, Acetic acid is spiked quantitatively in to sample at 50 %, 100 % and 150 % of specification concentration of method with triplicate preparation and analyzed using the test method. The result of Acetic acid at 50 %, 100 % and 150 % are tabulated in **Table 4**. The average % recovery at each level and overall average % recovery of Acetic acid is well within the acceptance criteria indicates that the analytical test method is accurate for its intended use.

Intermediate Precision

Intermediate precision is established by doing same exercise as system and method precision by different analysts on different day. The same Lot/Batch of standard and sample were used within the laboratory. The results of Intermediate precision are tabulated in **Table 5 and Table 6**. Comparison of Precision and Intermediate Precision result is summarized in **Table 7**. The result meets the acceptance criteria and found comparable, indicates that the method is precise and rugged with respect to analyst to analyst, day to day and same equipment for its intended use.

Robustness

Variation in Flow Rate (± 0.2mL/min)

The standard and sample solution was carried out by varying the flow rate of mobile phase to 0.25 mL/min and 0.65 mL/min. in place of actual flow rate 0.45 mL/min. The system precision result of robustness is summarized in **Table 8** and the method precision result of robustness is summarized in **Table 9**.

Variation in Column Oven Temperature (±5˚C)

The standard and sample solution was carried out by varying column oven temperature to 25°C and 35°C in place of actual column oven temperature 30°C. The system precision result of robustness is summarized in **Table 10** and the method precision result of robustness is summarized in **Table 11**. The results are well within the limits defined under acceptance criteria during robustness study. The robustness results indicate that the test method is robust enough as demonstrated by altering the Flow rate, and Column oven temperature.

Equivalency with the current method

The equivalency between in-house Potentiometric method and new in house UPLC method was established by analyzing three different batches of sample and the results obtained are summarized in the **Table 12**. The results are well within the limits defined under acceptance criteria during method equivalency study between in-house method (By Potentiometric) and new in house method (By UPLC). The results indicate that the new in house method (By UPLC) results are in line with current in-house method (By Potentiometric) and new method shall be used as an alternate method for determination of Content of Acetate.

System Suitability

The system suitability is an integral part of analytical procedure. The tests are based on the concept that the equipment, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability parameters are evaluated and the results are tabulated in **Table 13**. The results for system suitability are well within the acceptance criteria; hence the given chromatography system is acceptable for its intended use.

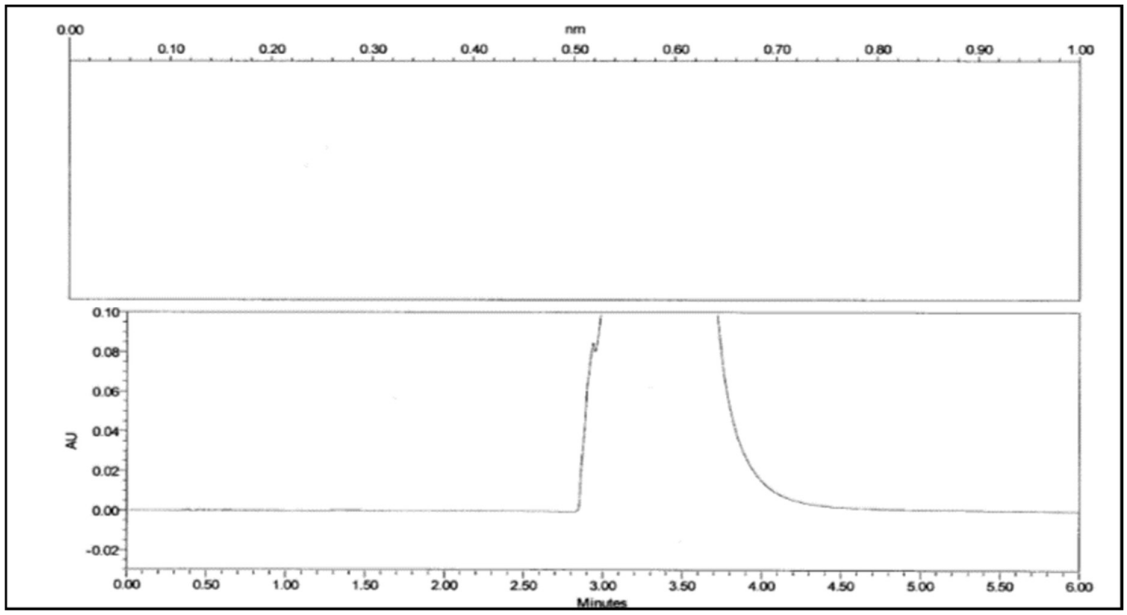

Figure 2: A typical Chromatogram of diluent

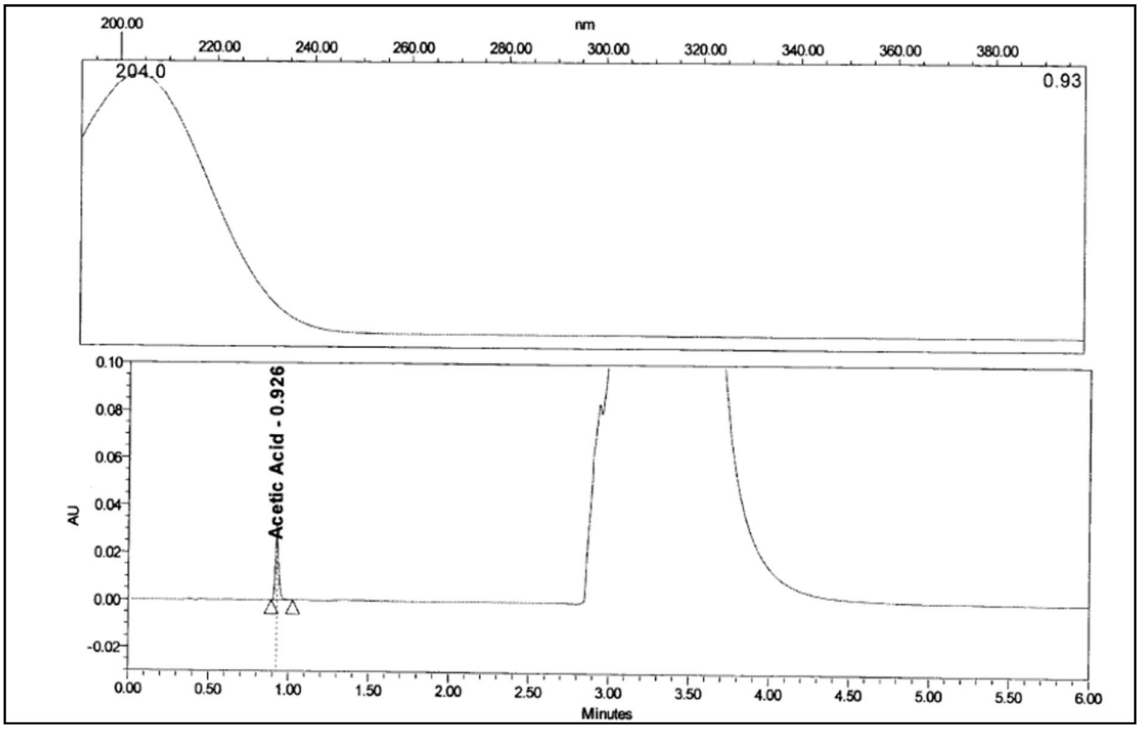

Figure 3: A typical Chromatogram of Standard

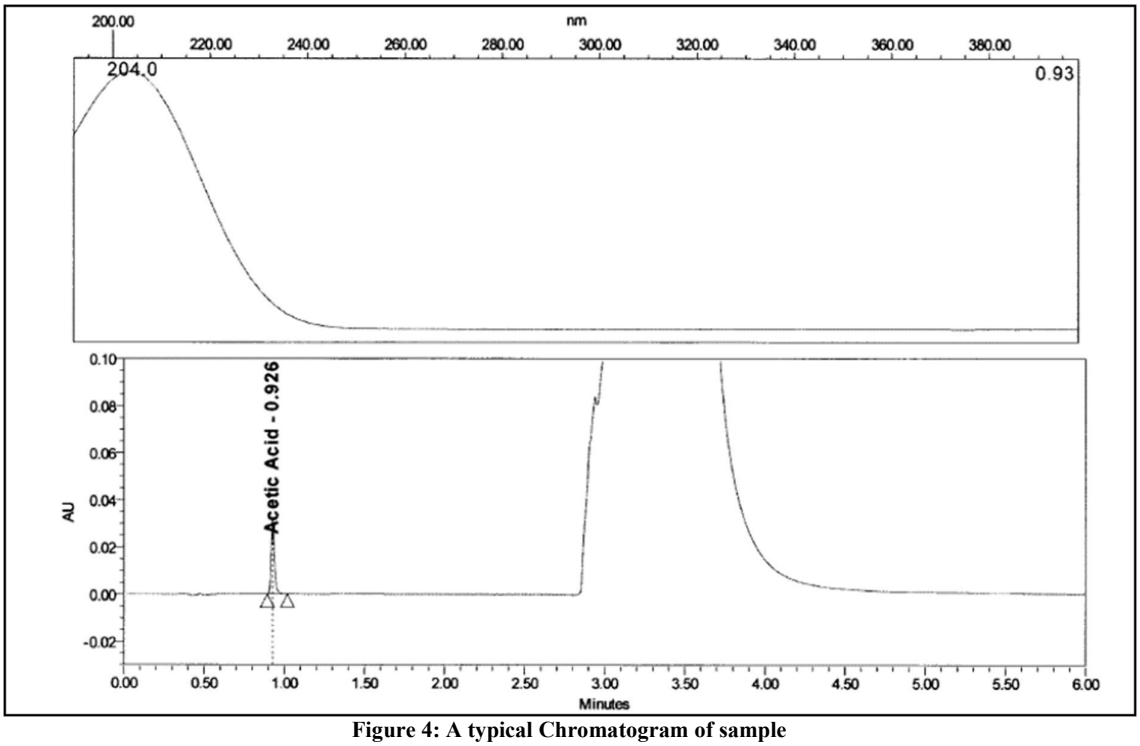

Figure 4: A typical Chromatogram of sample

Table 1: Result of Linearity

Linearity Level	Concentration (µg/mL)	Peak Area
50 %	62.255	20745
80 %	99.608	33308
100 %	124.510	41797
120 %	149.412	50095
150 %	186.765	62439
200 %	249.020	83629
Correlation Coefficient : 1.000	Y-intercept : -169.84	
Slope : 336.2117	Y-intercept bias at 100% level : -0.4	

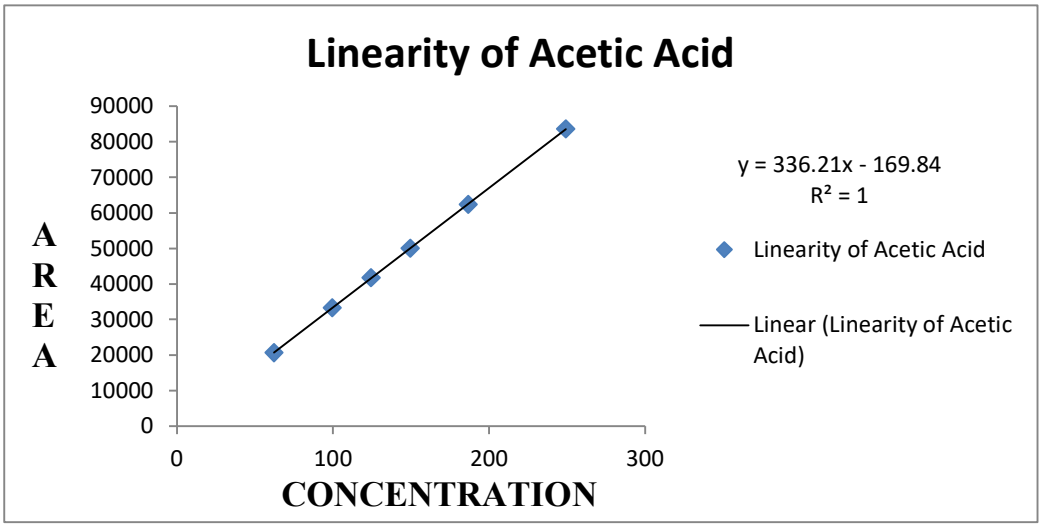

Figure 5: Linearity Graph of Acetic Acid

Table 2: Results of System Precision

Injection #	Acetic acid Peak Area
1	41846
2	41694
3	41951
4	41811
5	41933
6	41838
Average	41845
% RSD	0.2
Tailing Factor	1.3

Table 3: Results of Method Precision

Sample #	% Acetate content
1	12.7
2	12.7
3	12.7
4	12.7
5	12.7
6	12.7
Mean	12.7
% RSD	0.0

Table 4: Results of Accuracy

Accuracy Level	Set #	Sample #	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Average recovery for each set	Average % recover	% RSD
50%	1	1	63.18	64.7569	102.49	102.44	101.7	0.7
		2		64.7284	102.45			
		3		64.6913	102.39			
	2	1		63.4216	100.38	101.86		
		2		64.5576	102.18			
		3		65.1054	103.04			
	3	1		63.6479	100.74	100.94		
		2		63.7360	100.88			
		3		63.9445	101.21			
100%	1	1	126.36	127.2258	100.68	100.46	100.5	0.2
		2		126.8018	100.34			
		3		126.7733	100.32			
	2	1		126.8230	100.36	100.45		
		2		126.9376	100.45			
		3		127.0756	100.56			
	3	1		127.5631	100.95	100.73		
		2		126.7873	100.33			
		3		127.5427	100.93			
150%	1	1	189.54	190.9989	100.76	100.91	100.6	0.3
		2		191.0860	100.81			
		3		191.7567	101.16			
	2	1		190.8360	100.68	100.59		
		2		190.1429	100.31			
		3		191.0352	100.78			
	3	1		190.7132	100.61	100.32		
		2		190.1244	100.30			
		3		189.6550	100.06			
Overall average % recovery							101	N/A
Overall % RSD								0.7

Table 5: Results of Intermediate Precision (Standard Solution)

Replicate #	Peak Area
1	41919
2	41824
3	41835
4	41838
5	41695
6	41714
Average	41804
% RSD	0.2
Tailing Factor	1.3

Table 6: Results of Intermediate Precision (Sample Solution)

Sample Set #	Peak area of Acetic acid	% Acetate Content
1	42403	12.7
2	42954	12.7
3	43179	12.7
4	43533	12.7
5	42767	12.7
6	43407	12.7
Mean	N/A	12.7
% RSD	N/A	0.0

Table 7: Comparison of Precision and Intermediate Precision Results

Parameter	Precision	Intermediate Precision
Analyst	Analyst 1	Analyst 2
UPLC ID.	QC/EQ/471	QC/EQ/471
Date of Analysis	01-Oct-19	17-Oct-19
Comparison of System Precision Results		
Parameter	Precision	Intermediate Precision
Mean Area of Acetic acid	41845	41804
% RSD	0.2	0.2
Tailing Factor	1.3	1.3
Comparison of Method Precision Results		
Sample #	% Acetate content	
Parameter	Precision	Intermediate Precision
1	12.7	12.7
2	12.7	12.7
3	12.7	12.7
4	12.7	12.7
5	12.7	12.7
6	12.7	12.7
Mean	12.7	12.7
% RSD	0.0	0.0
Cumulative % RSD	0.0	

Table 8: Results of Robustness - Variation in Flow rate (Standard)

Injection #	Acetic acid Peak Area		
	Flow Rate 0.25mL/min.	Actual Flow Rate 0.45 mL/min	Flow Rate 0.65mL/min.
1	76763	41846	29115
2	76779	41694	29084
3	76730	41951	29134
4	76737	41811	29157
5	76631	41933	28995

Injection #	Acetic acid Peak Area		
	Flow Rate 0.25mL/min.	Actual Flow Rate 0.45 mL/min	Flow Rate 0.65mL/min.
6	76779	41838	29087
Mean	76737	41845	29095
% RSD	0.1	0.2	0.2
Tailing Factor	1.0	1.3	1.0

Table 9: Results of Robustness - Variation in Flow rate (Sample)

Injection #	% Acetate content		
	Flow Rate 0.25mL/min.	Actual Flow Rate 0.45 mL/min	Flow Rate 0.65mL/min.
1	12.7	12.7	12.8
2	12.7	12.7	12.7
3	12.7	12.7	12.7
4	12.7	12.7	12.7
5	12.7	12.7	12.7
6	12.7	12.7	12.7
Mean	12.7	12.7	12.7
% RSD	0.0	0.0	0.3
% Difference	0.0	N/A	0.0

Table 10: Results of Robustness -Variation in Column oven Temperature (Standard)

Injection #	Acetic acid Peak Area		
	Column oven Temperature: 25°C	Actual Column oven Temperature: 30°C	Column oven Temperature: 35°C
1	42190	41846	42285
2	42161	41694	42275
3	42279	41951	42286
4	42223	41811	42305
5	42155	41933	42277
6	42075	41838	42205
Mean	42180	41845	42272
% RSD	0.2	0.2	0.1
Tailing Factor	1.0	1.3	1.0

Table 11: Results of Robustness - Variation in Column oven Temperature (Sample)

Injection #	% Acetate content		
	Column oven Temperature: 25°C	Actual Column oven Temperature: 30°C	Column oven Temperature: 35°C
1	12.8	12.7	12.7
2	12.7	12.7	12.7
3	12.7	12.7	12.7
4	12.8	12.7	12.7
5	12.7	12.7	12.7
6	12.7	12.7	12.7
Mean	12.7	12.7	12.7
% RSD	0.4	0.0	0.0
% Difference	0.0	N/A	0.0

Table 12: Results of Method Equivalency

Batch No	% Content of Acetate by UPLC	% Content of Acetate by Potentiometer	% Difference
00923	12.74	12.64	0.8
00922	12.72	12.69	0.2
00924	12.74	12.64	0.8

Table 13: Overall Summary of System Suitability Results

Parameters		% RSD	Tailing factor
Specificity/Precision/ Robustness	Initial	0.2	1.3
	BKT-1	0.2	1.3
Stability of Analyte in solution/ Accuracy	Initial	0.1	1.3
	BKT-1	0.2	1.0
	BKT-2	0.2	1.3
	BKT-3	0.3	1.3
	BKT-4	0.3	1.3
	BKT-5	0.3	1.3
	BKT-6	0.3	1.0
	BKT-7	0.3	1.0
	BKT-8	0.4	1.0
	BKT-9	0.4	1.2
	BKT-10	0.4	1.0
	BKT-11	0.4	1.3
	BKT-12	0.4	1.3
	BKT-13	0.4	1.3
	BKT-14	0.4	1.3
	BKT-15	0.4	1.3
	BKT-16	0.5	1.1
	BKT-17	0.4	1.3
	BKT-18	0.5	1.3
	BKT-19	0.4	1.3
	BKT-20	0.5	1.3
Linearity/ Intermediate Precision	Initial	0.2	1.3
	BKT-1	0.2	1.2
	BKT-2	0.2	1.2
Equivalency with the current method	Initial	0.1	1.2
	BKT-1	0.1	1.2
	Minimum	0.1	1.0
	Maximum	0.5	1.3
	Average	0.3	1.2

CONCLUSION

The analytical procedure is suitable for its intended use and it meets the acceptance criteria for selectivity with no interference is observed at the retention time of principle peak in the chromatograms obtained from diluent. The results indicate that the method is specific for its intended use. The standard and sample solution is stable up to 72 hours at room temperature. The method is linear within the concentration range from 50 % to 200 % of working concentration. The result meets the acceptance criteria; indicates that the analytical procedure is precise for its intended use. The test method is accurate within the range of method. The result meets the acceptance criteria and found comparable, indicates that the method is precise and rugged with respect to analyst to analyst, day to day and same equipment for its intended use. The robustness result indicates that the test method is robust enough as demonstrated by altering the Flow rate and Column oven temperature. The method is equivalent with the In-house Potentiometric method.

The data for each validation characteristic described in this report meets the acceptance criteria with respect to Specificity, Stability of analyte in solution, Linearity, Precision,

Accuracy, Intermediate Precision, Robustness and Equivalency with the current method.

The Validation data reveals that the analytical procedure is suitable for Content of Acetate in Flecainide Acetate, USP.

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Conflict of Interest

There is no conflict of interest.

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