



Method development and validation of apixaban in bulk and tablet dosage form by RP-HPLC

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ABSTRACT

A simple and precise accurate reverse phase high performance liquid method was developed for the determination of Apixaban in bulk and its dosage form. Chromatographic separation was performed on column, C18(150x4.6 ID) 5µm column with a mobile phase contain the gradient mixture of the solvent (water: Acetonitrile) in the ratio of 55:45(v/v) as mobile phase at the flow rate 1.0 ml/min The retention time of Apixaban was found to be 4.960 and theoretical plate count for Apixaban was 4207. The percentage of accuracy was found to be 98.40%. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantification, robustness and ruggedness. Linearity of Apixaban was found in the range of 50-150µg/ml. correlation coefficient was 0.994. The LOD and LOQ values for Apixaban were 44.18 and 58.12µg/ml respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for estimation of Apixaban in bulk and its dosage form.

Keywords: Apixaban, RP-HPLC, Method development, Validation.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1-5].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis [6-10].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the method development and validation of Apixaban in pharmaceutical dosage form.

Plan of work

- Solubility determination of apixaban in various solvents and buffers.
- Determine the absorption maxima of the drug in UV-Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for

proper resolution and retention times.

- Validate the developed method as per ICH guidelines [11-15].

METHODOLOGY

Mobile Phase

A mixture of 55 volumes of water and 45 volumes of acetonitrile were prepared. The mobile phase was sonicated for 10mins to removes gases and filtered through 0.45 μ m membrane filter for degassing of mobile phase [16-20].

Determination of Working Wavelength (λ_{max})

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately

Preparation of standard stock solution of Apixaban

Weigh accurately 5mg of Apixaban in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 μ g/ml of Apixaban is prepared by diluting 5ml of Apixaban to 10ml with mobile phase. This solution is used for recording chromatogram [21-30].

RESULTS AND DISCUSSIONS

Solubility Studies

These studies are carried out at 25 $^{\circ}$ C

Apixaban

Soluble in methanol and in water, acetonitrile very slightly soluble in phosphate buffer.

Wavelength determination

The sensitivity of the HPLC method depends upon the proper selection of wavelength. The detection wavelength was selected as the drug showed optimal absorbance at that wavelength.

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 μ g/ml solution of the drugs in acetonitrile were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 281 nm for apixaban [31-40].

Method development of apixaban

Preparation of Standard Solution

Weigh accurately 5mg of Apixaban in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 μ g/ml of Apixaban is prepared by diluting 5ml of Apixaban to 10ml with mobile phase. This solution is used for recording chromatogram [41-46].

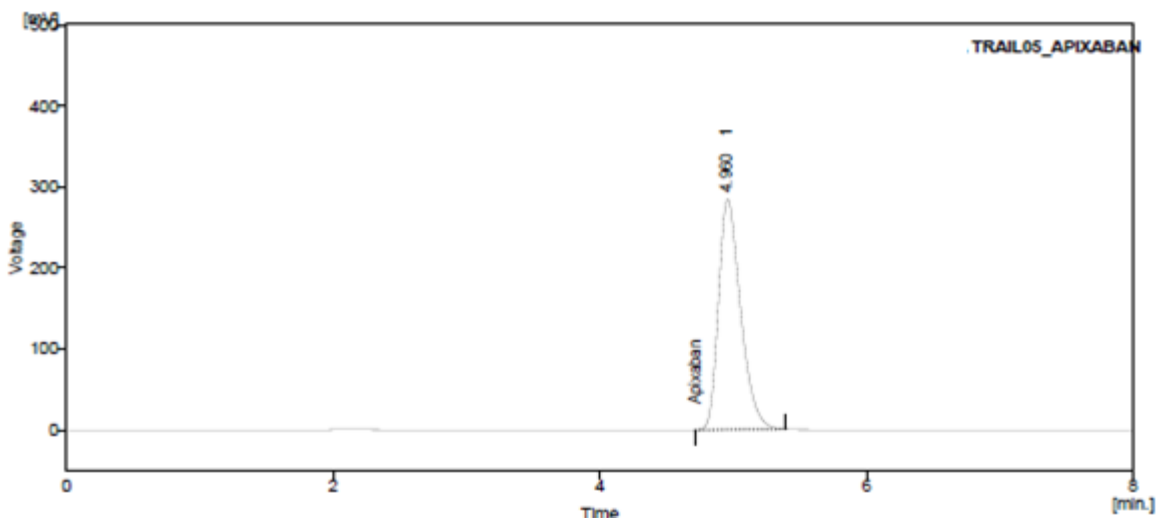


Table: Optimized chromatographic conditions

Mobile phase	HPLC Water: ACN (55:45)
Ph	5.8
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	281nm
Injection volume	20 µl

Assay

Preparation of standard solution

Preparation of mixed standard solution

Weigh accurately 5mg of Apixaban in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of apixaban is prepared by diluting 5ml of Apixaban to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

Tablets (each tablet contains 5mg of Apixaban) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 1000µg/ml were prepared by dissolving weight equivalent to 5mg of Apixaban dissolved in

sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Apixaban was made by adding 1ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Apixaban present in the formulation by using the formula given below and results shown in above table:

Where,

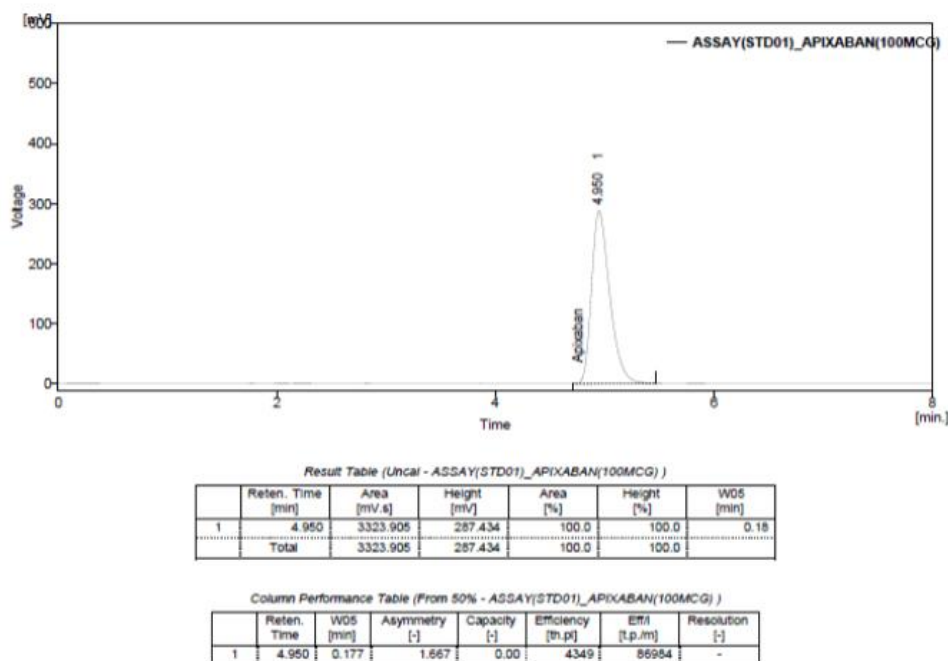
AS: Average peak area due to standard preparation

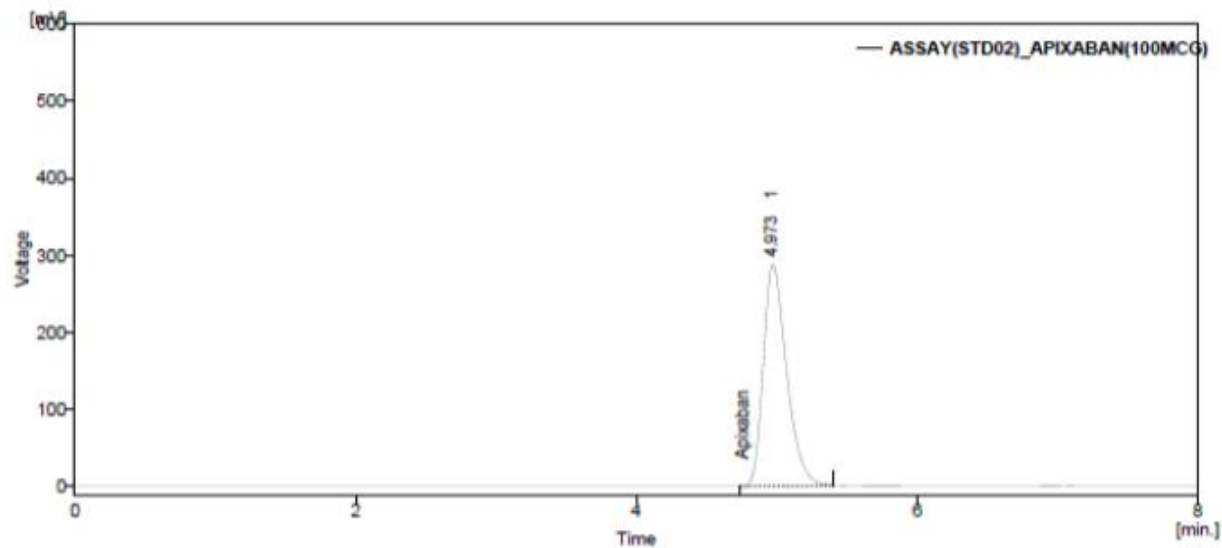
AT: Peak area due to assay preparation

WS: Weight of Apixaban in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

**Fig: Chromatogram of Assay standard preparation-1**



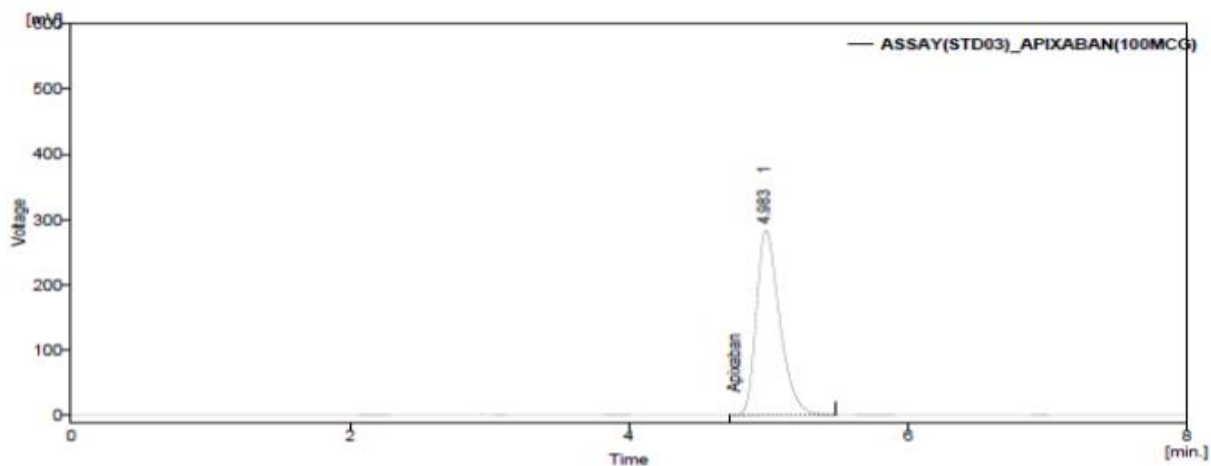
Result Table (Uncal - ASSAY(STD02)_APIXABAN(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.973	3320.771	286.916	100.0	100.0	0.18
Total		3320.771	286.916	100.0	100.0	

Column Performance Table (From 50% - ASSAY(STD02)_APIXABAN(100MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff1 [tp.m]	Resolution [-]
1	4.973	0.180	1.667	0.00	4229	84584	-

Fig: Chromatogram of Assay standard preparation-2



Result Table (Uncal - ASSAY(STD03)_APIXABAN(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.983	3293.678	283.516	100.0	100.0	0.18
Total		3293.678	283.516	100.0	100.0	

Column Performance Table (From 50% - ASSAY(STD03)_APIXABAN(100MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff1 [tp.m]	Resolution [-]
1	4.983	0.180	1.667	0.00	4246	84925	-

Fig: Chromatogram of Assay standard preparation-3

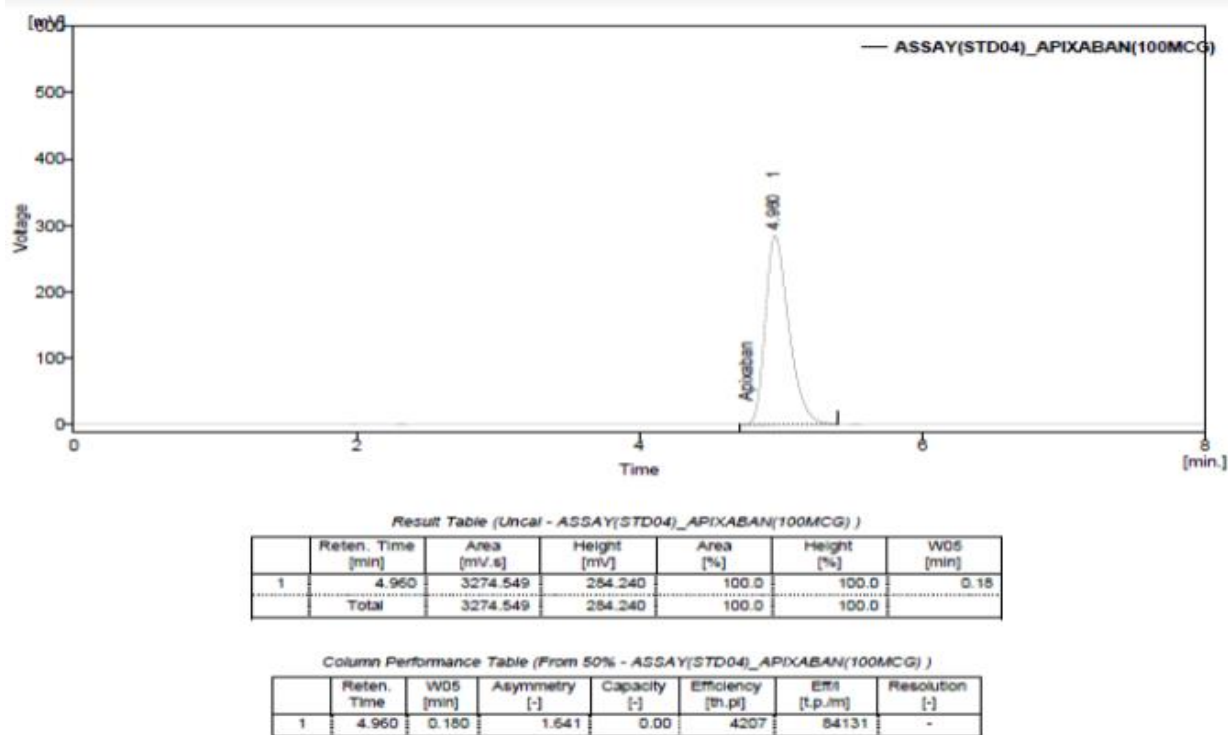


Fig: Chromatogram of Assay standard preparation-4

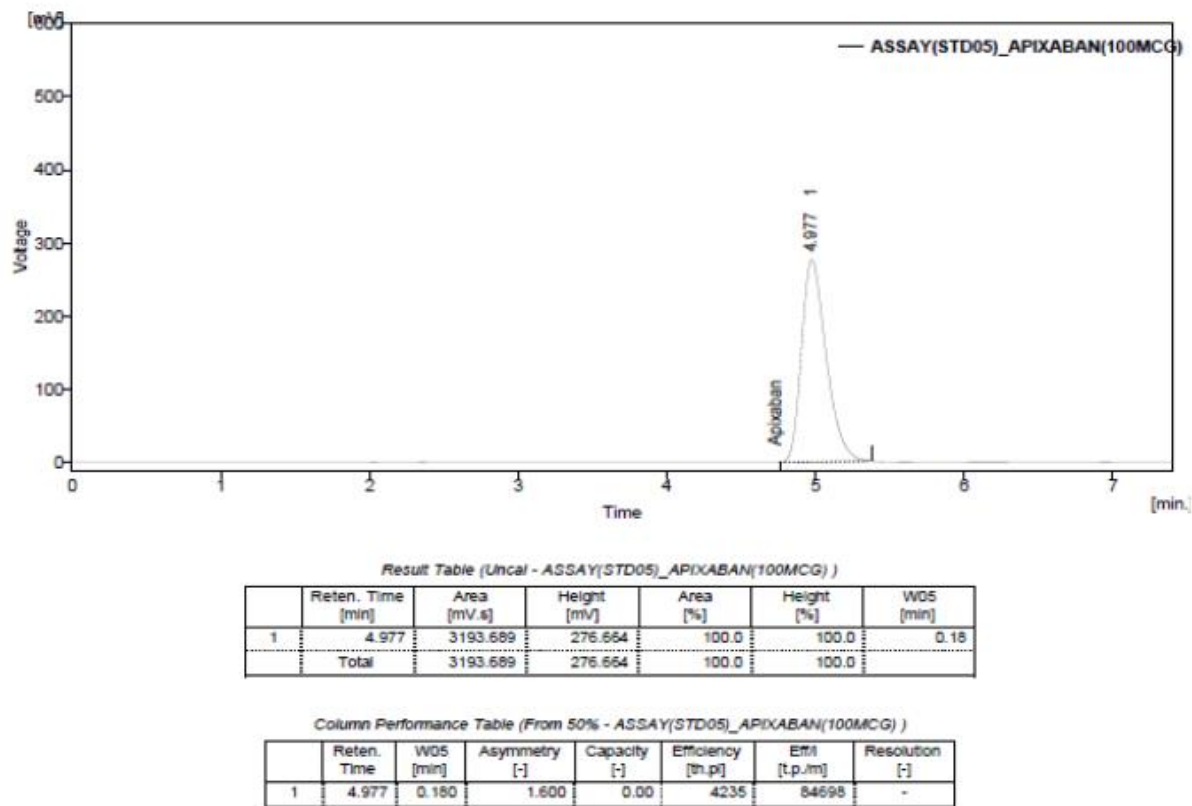


Fig: Chromatogram of Assay standard preparation-5

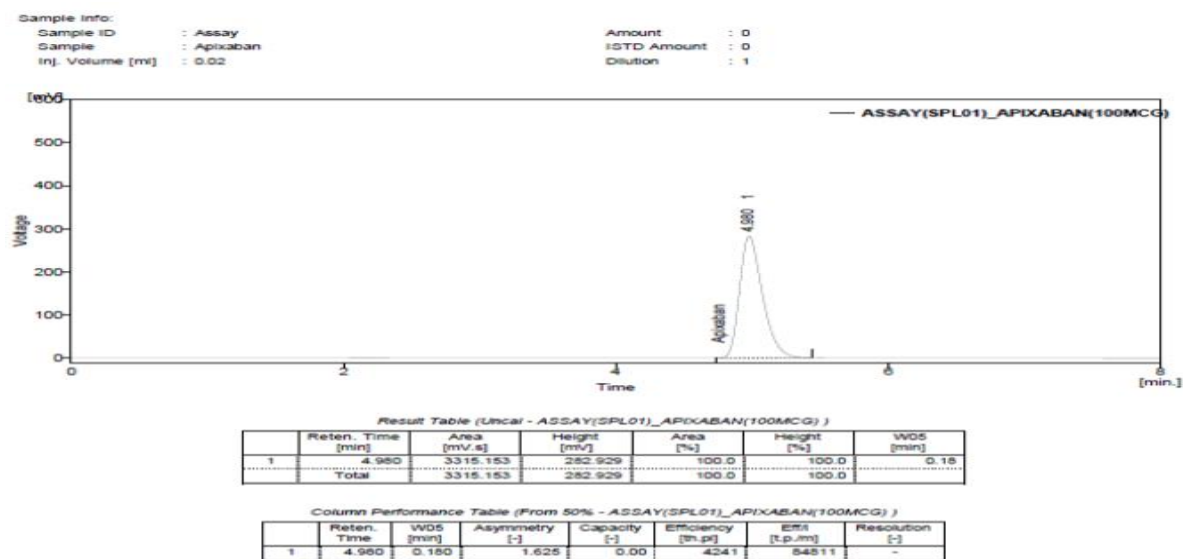


Fig: Chromatogram of Assay sample preparation-1

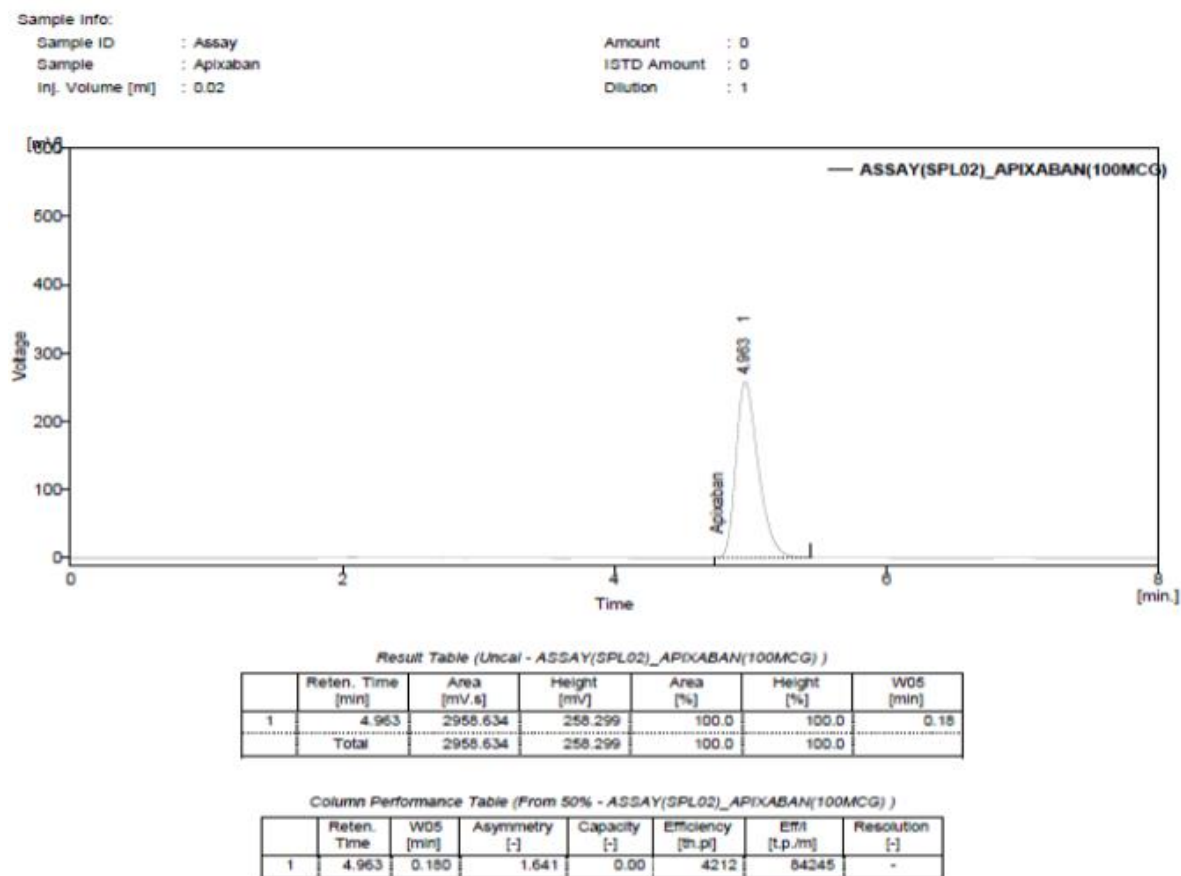


Fig: Chromatogram of Assay sample preparation-2

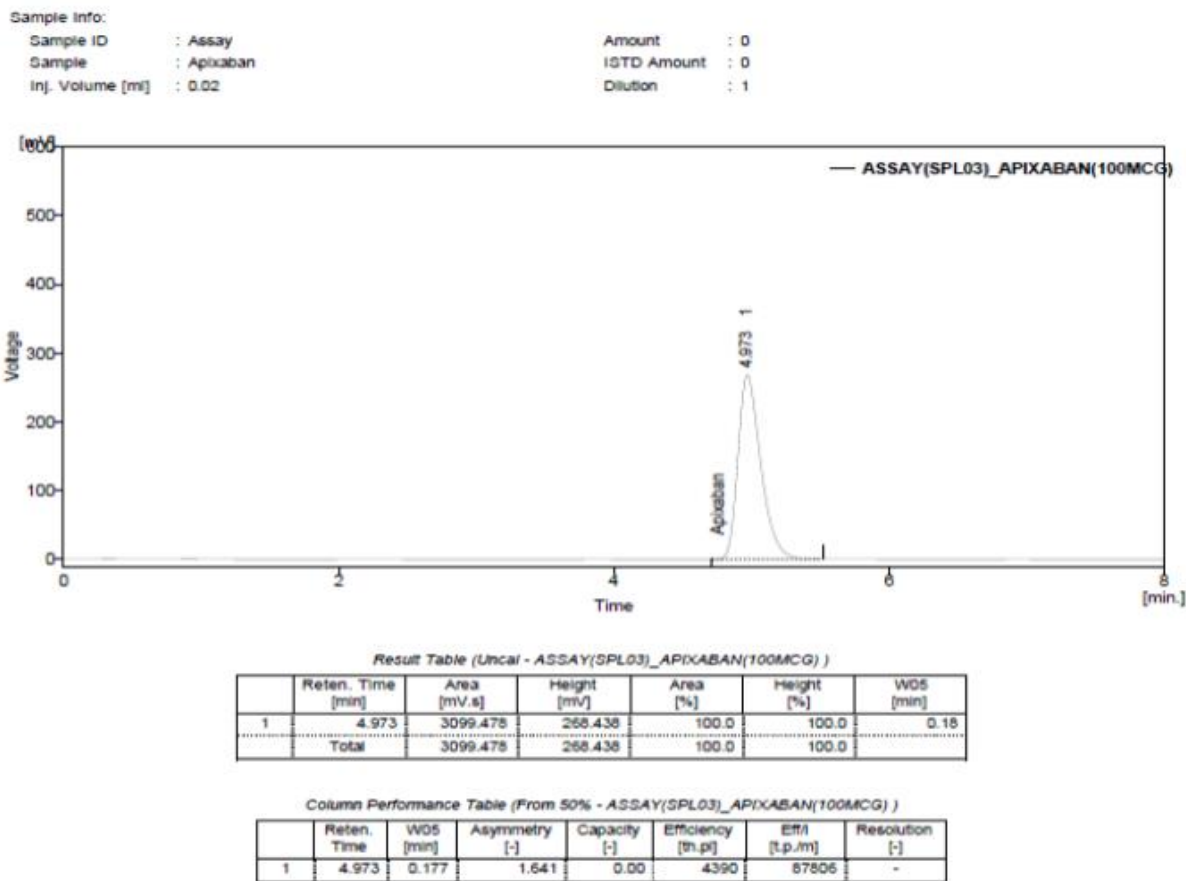


Fig: Chromatogram of Assay sample preparation-3

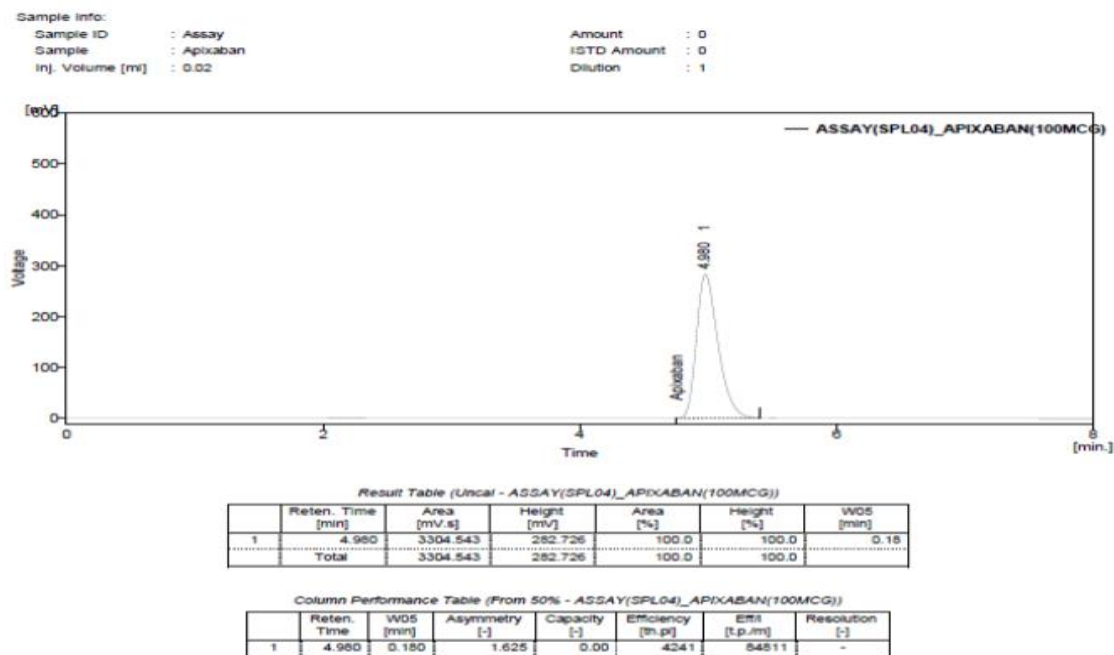
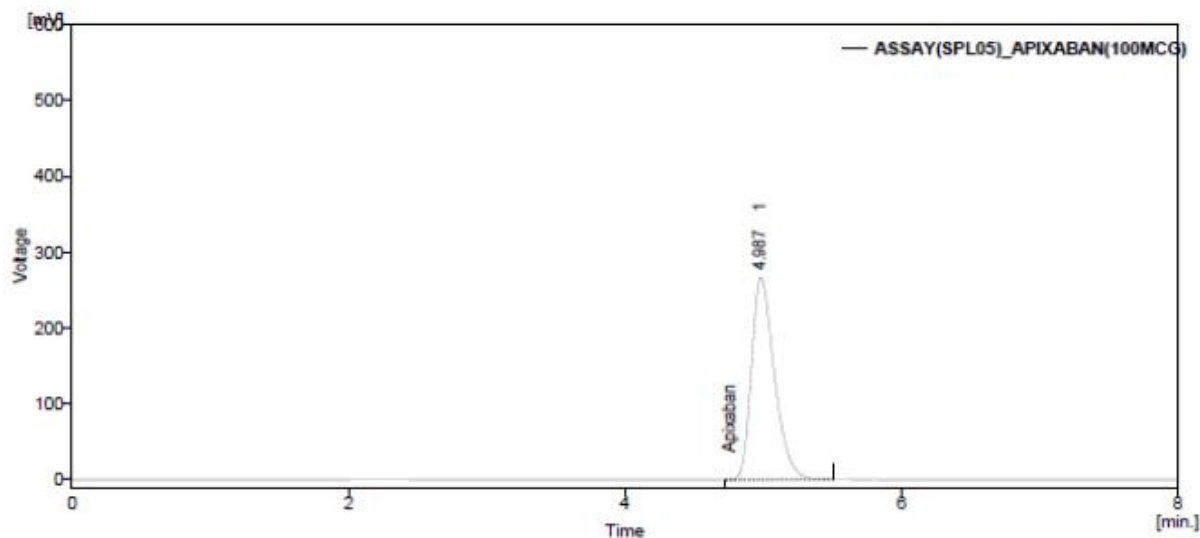


Fig: Chromatogram of Assay sample preparation-4

Sample Info:

Sample ID : Assay
 Sample : Apixaban
 Inj. Volume [ml] : 0.02

Amount : 0
 ISTD Amount : 0
 Dilution : 1



Result Table (Uncal - ASSAY(SPL05)_APIXABAN(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.987	3067.163	267.062	100.0	100.0	0.18
Total		3067.163	267.062	100.0	100.0	

Column Performance Table (From 50% - ASSAY(SPL05)_APIXABAN(100MCG))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff [t.p./m]	Resolution [-]
1	4.987	0.177	1.641	0.00	4414	86275	-

Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

APIXABAN		
	Standard Area	Sample Area
Injection-1	3323.905	3315.153
Injection-2	3320.771	2958.634
Injection-3	3293.678	3099.478
Injection-4	3274.549	3304.543
Injection-5	3193.689	3067.163
Average Area	3312.785	3288.994
Tablet average weight	10.58	
Standard weight	5	
Sample weight	10.58	
Label amount	5	
std. purity	99.8%	
Amount found in mg	4.95	
Assay(%purity)	99.08	

Observation

The amount of Apixaban present in the taken dosage form was found to be 99.08%.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 5mg of Apixaban in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

From above stock solution 100µg/ml of Apixaban is prepared by diluting 5ml of Apixaban to 10ml with mobile phase. This solution is used for recording chromatogram.

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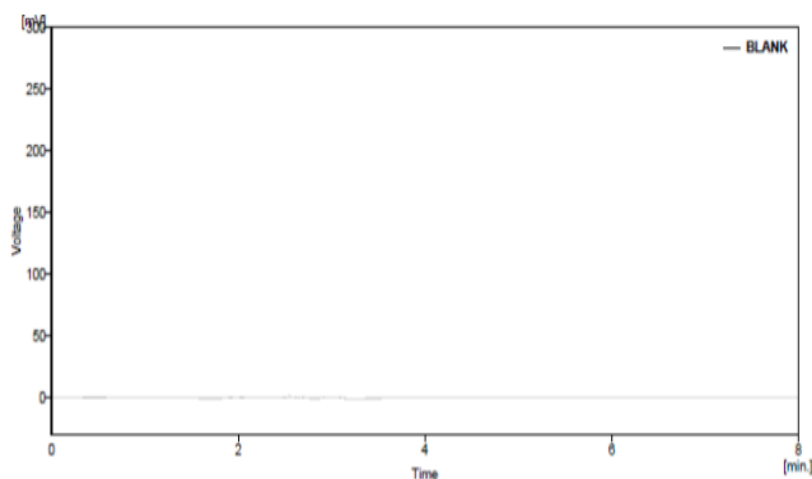


Fig: Blank chromatogram for specificity

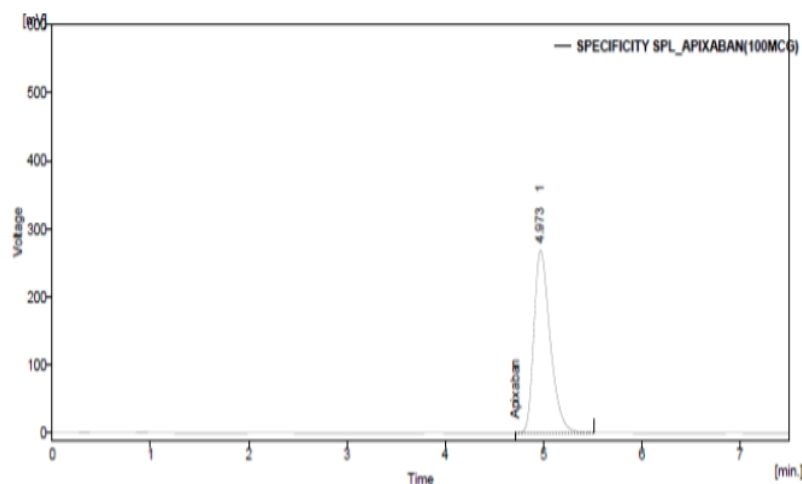


Fig: Chromatogram for specificity of apixaban sample

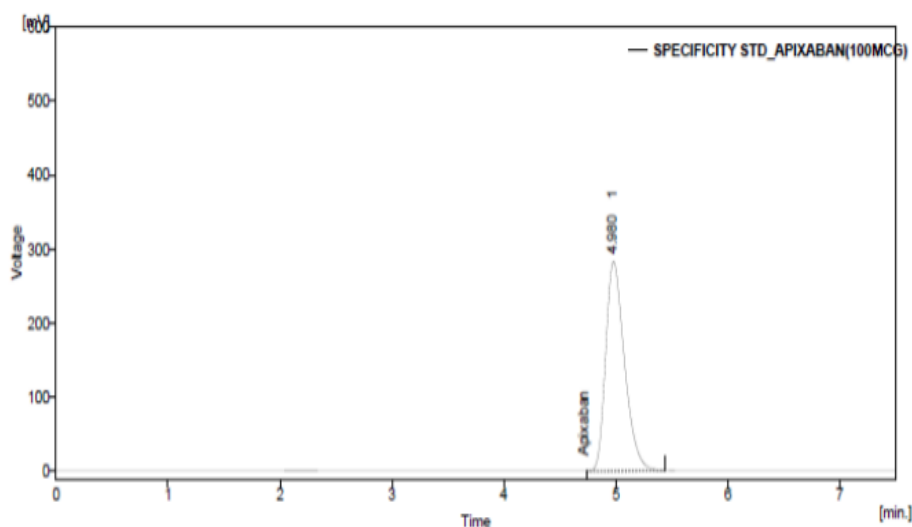


Fig: Chromatogram for specificity of apixaban standard

Observation

It is observed from the above data, diluents or excipient peaks are not interfering with the Apixaban peaks.

Linearity and range

Preparation of standard solution

Weigh accurately 5mg of Apixaban in 25 ml of volumetric flask and from this, 5ml dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Further dilutions were given in the table.

Linearity of Apixaban

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution($\mu\text{g/ml}$)
Preparation 1	2.5	10	50
Preparation 2	3.75	10	75
Preparation 3	5	10	100
Preparation 4	6.25	10	125
Preparation 5	7.5	10	150

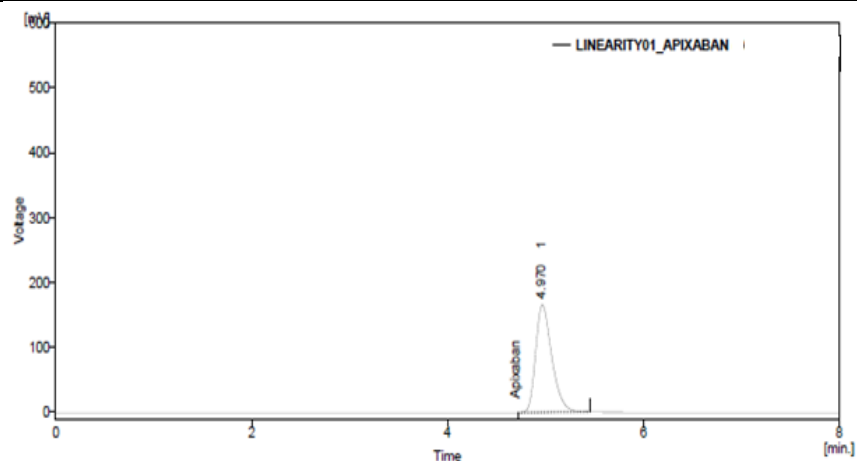


Fig: Linearity Chromatogram-1

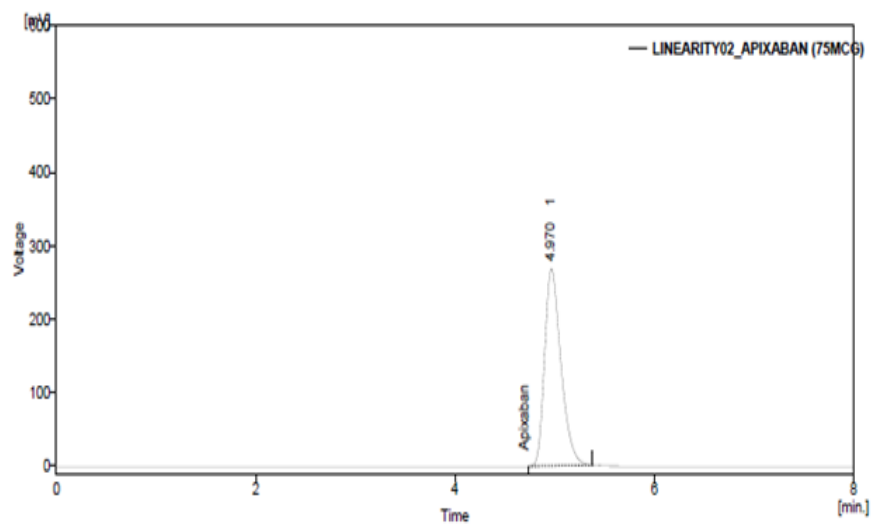


Fig: Linearity Chromatogram-2

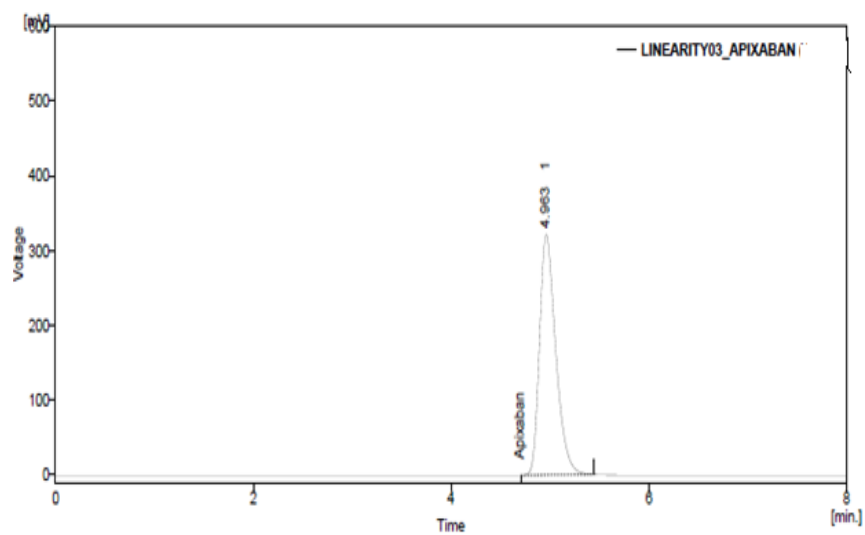


Fig: Linearity Chromatogram-3

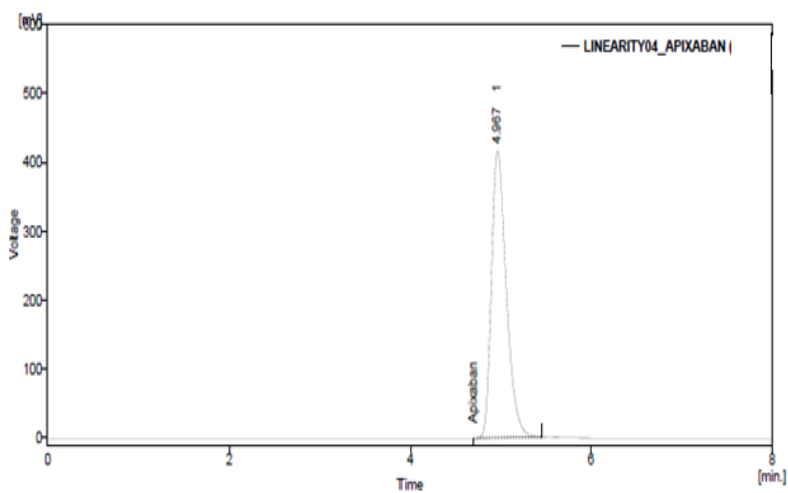


Fig: Linearity Chromatogram-4

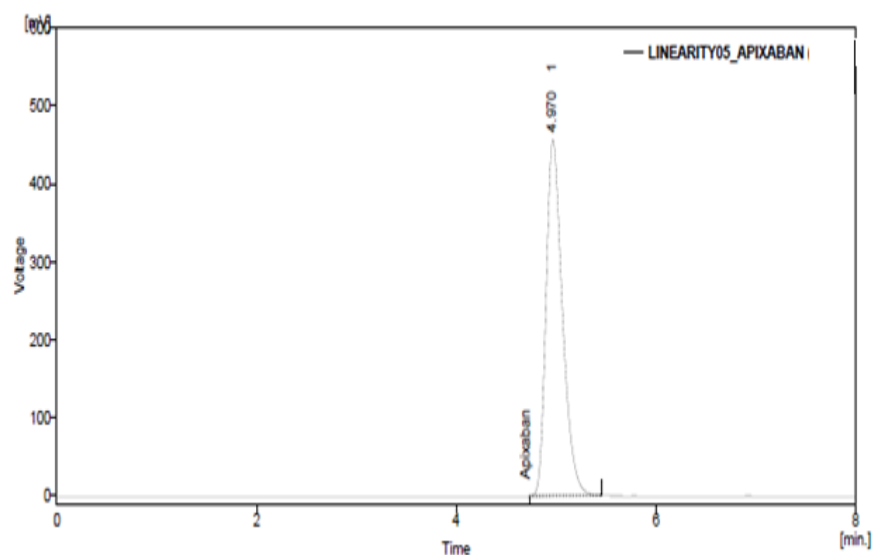
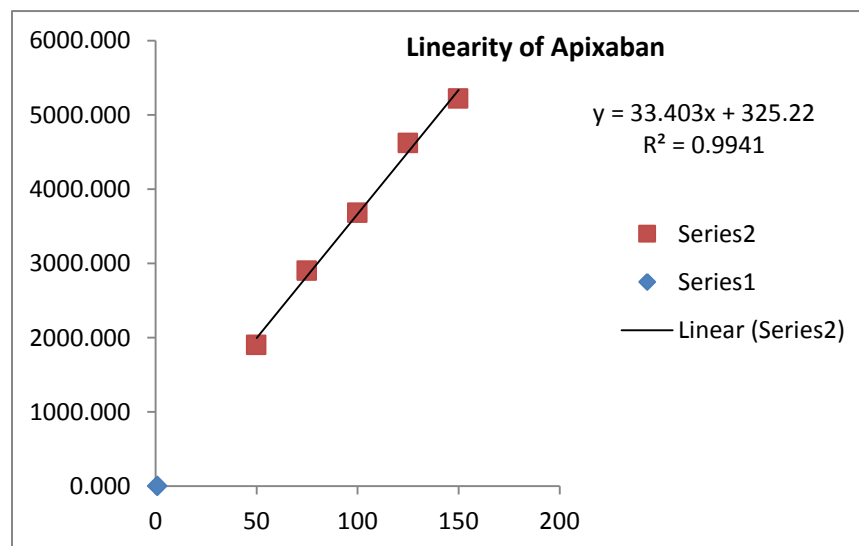


Fig: Linearity Chromatogram-5

Table: Linearity Preparations

Linearity level	Concentration (ppm)	Area	Acceptance criteria
50	50	1904.438	Squared correlation co-efficient should be not less than 0.999
75	75	3061.665	
100	100	3680.717	
125	125	4770.500	
150	150	5220.440	

$$y=33.40x+325.2, R^2 = 0.994$$



Linearity Curve for Apixaban

Acceptance criteria

The relationship between the concentration of Apixaban and area of Apixaban should be linear in the specified range and the correlation should not be less than 0.999.

Observation

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Apixaban is 0.994. The relationship between the concentration of Apixaban and area of Apixaban is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 75%, 100%, 125%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 75%, 100% & 125%.

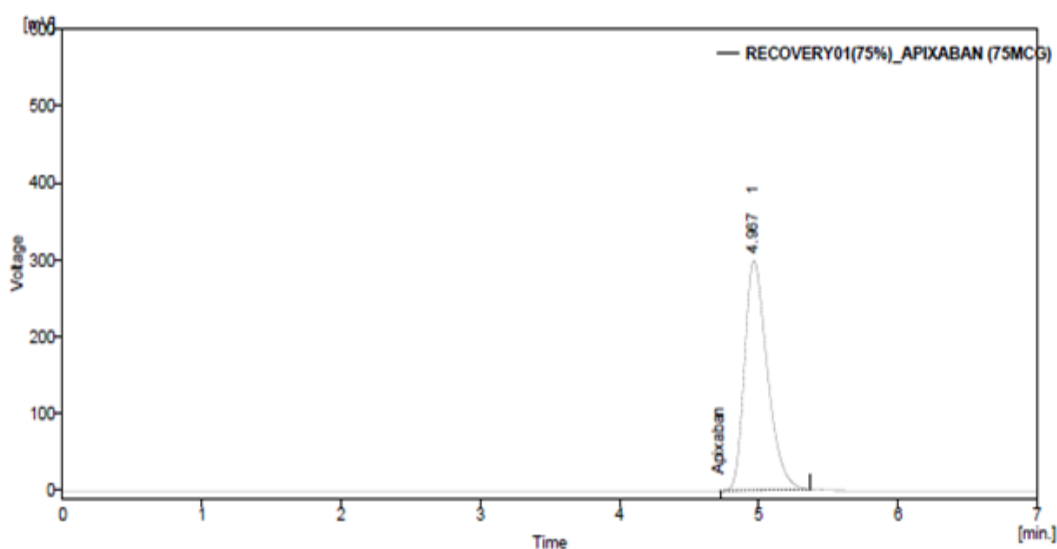


Fig: Accuracy75 %Chromatogram-1

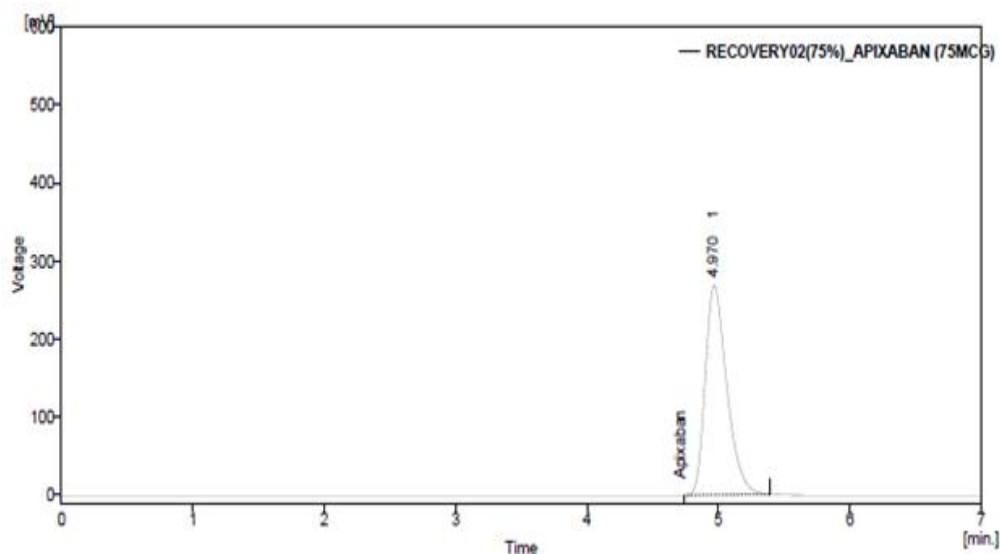


Fig: Accuracy75 %Chromatogram-2

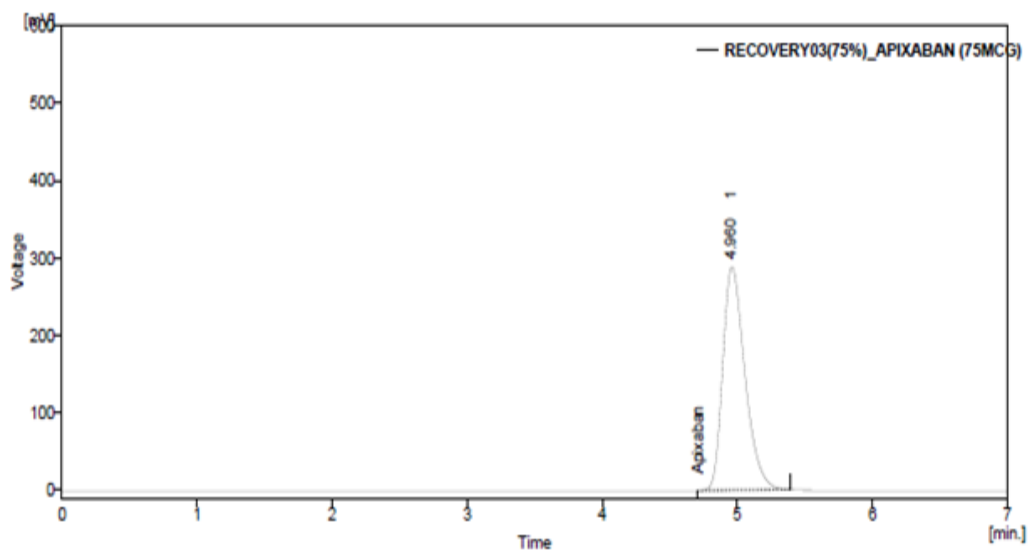


Fig: Accuracy75 %Chromatogram-3

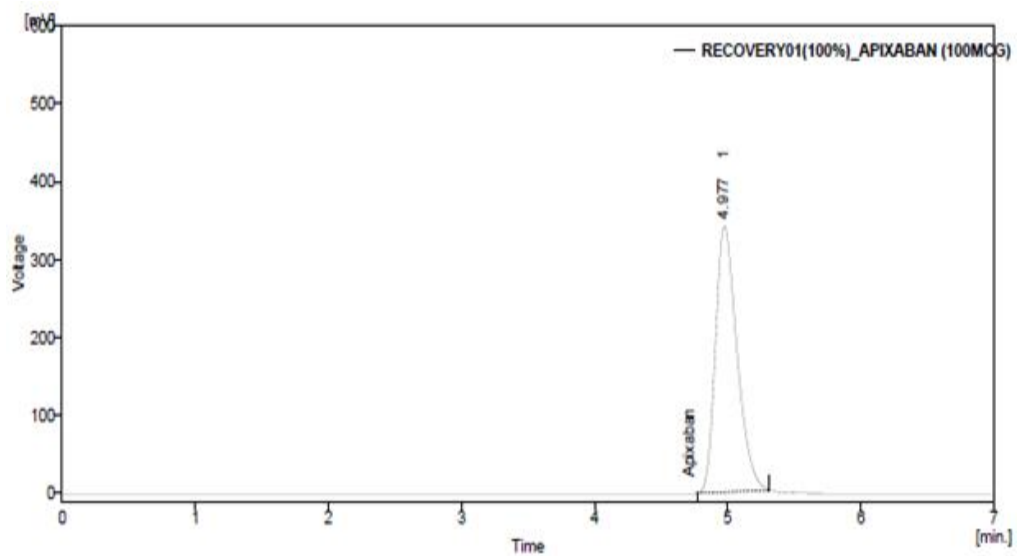


Fig: Accuracy100 %Chromatogram-1

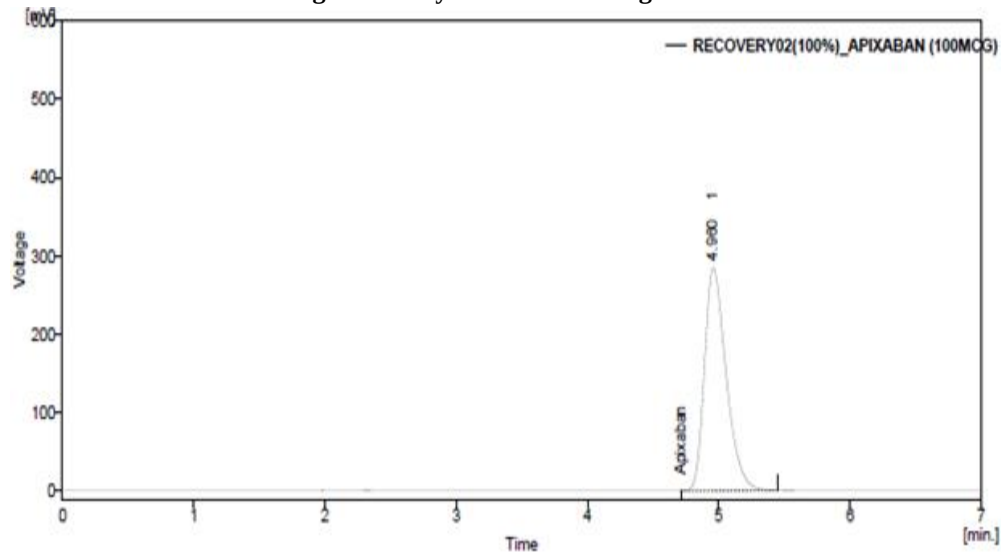


Fig: Accuracy 100 %Chromatogram-2

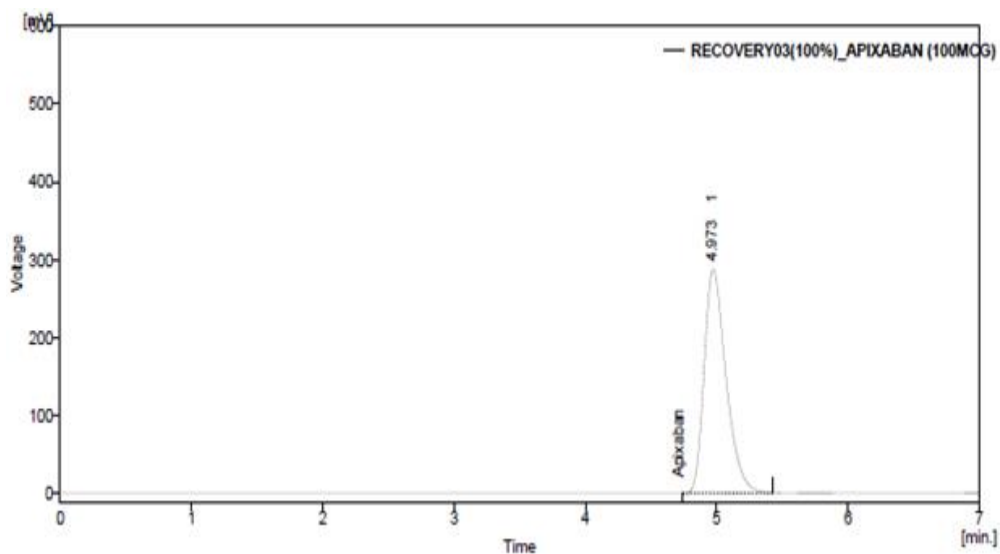


Fig: Accuracy100 %Chromatogram-3

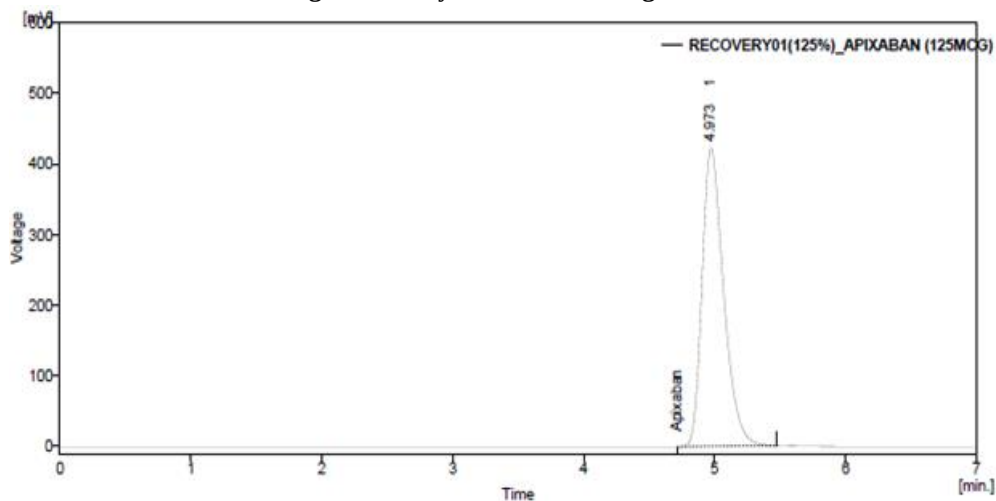


Fig: Accuracy125 %Chromatogram-1

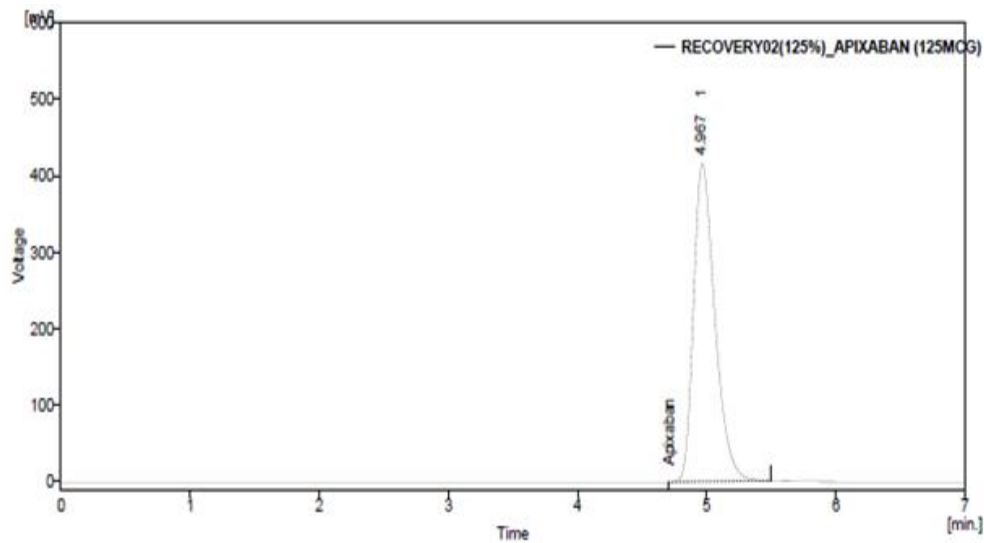


Fig: Accuracy125 %Chromatogram-2

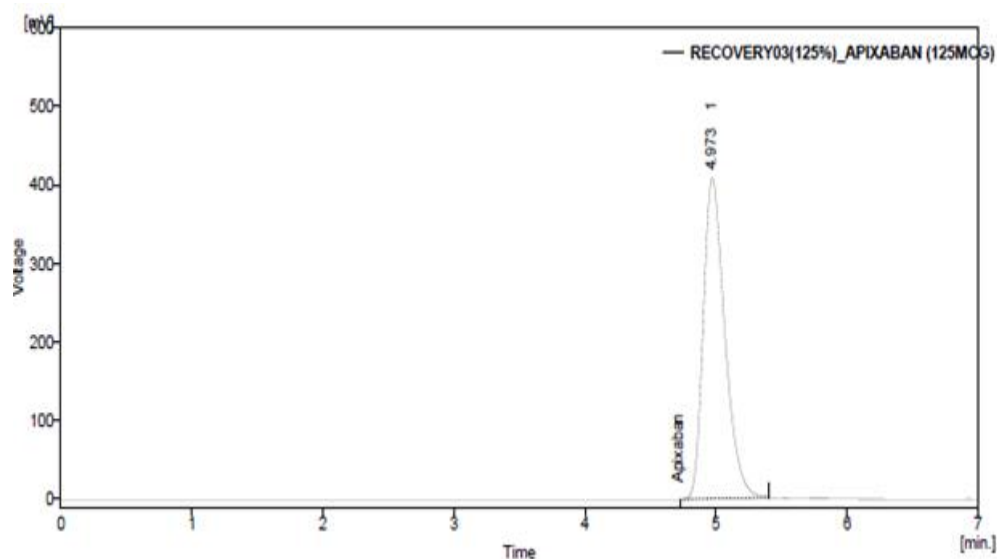


Fig: Accuracy125 %Chromatogram-3

Table: Accuracy Observation of Apixaban

Recovery level	Accuracy Apixaban				%Recovery	Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)		
75%	75	3404.393	3256.777	73.60	98.14	98.40
	75	3069.834				
	75	3296.104				
100%	100	3838.430	3483.758	98.98	98.98	
	100	3285.170				
125%	100	3327.673				
	125	4838.317	4760.862	122.63	98.10	
	125	4781.051				
	125	4663.219				

Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$

Method precision

Prepared sample preparations of Apixaban as per test method and injected 6 times in to the column

Acceptance criteria

The %RSD for assay of six replicate preparations should not be more than 2.0

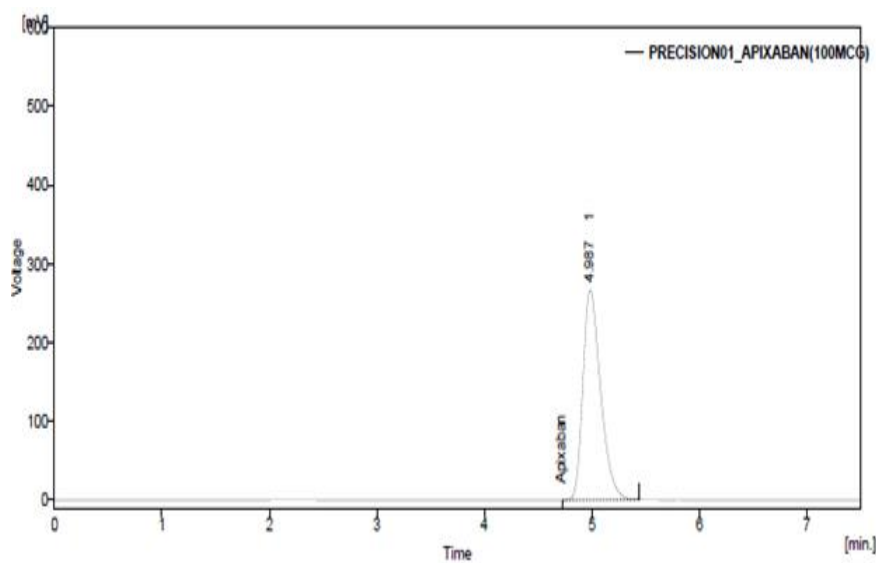


Fig: SystemPrecisionChromatogram-1

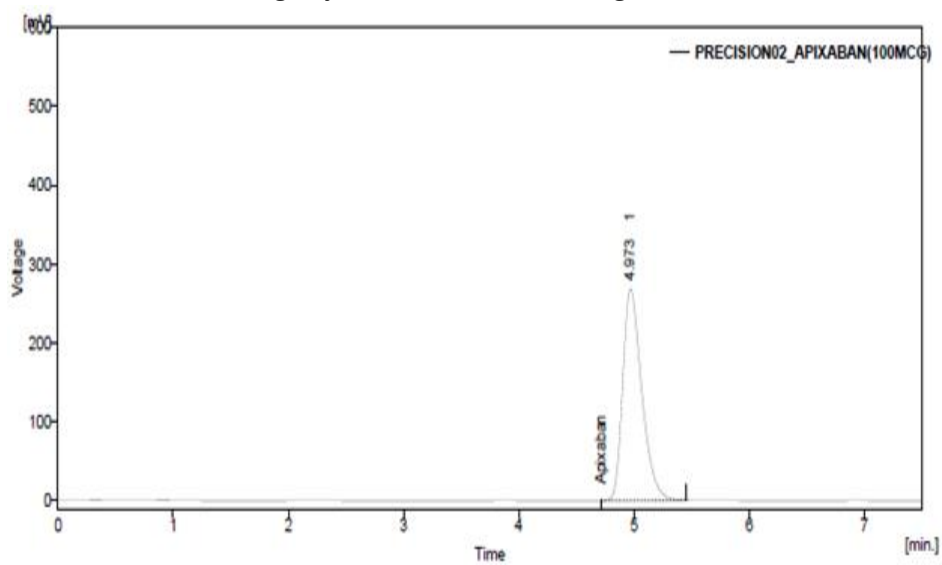


Fig: SystemPrecisionChromatogram-2

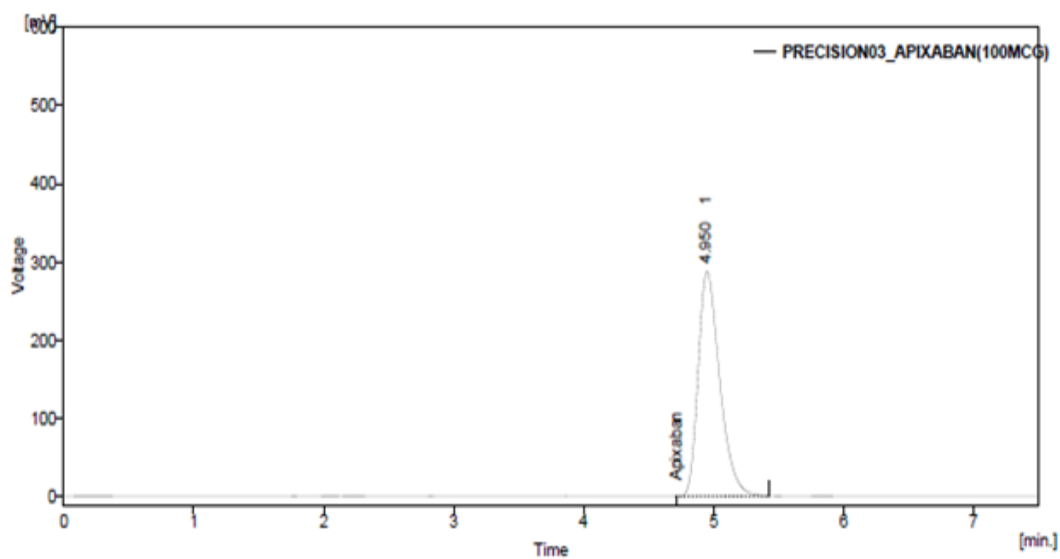


Fig: System PrecisionChromatogram-3

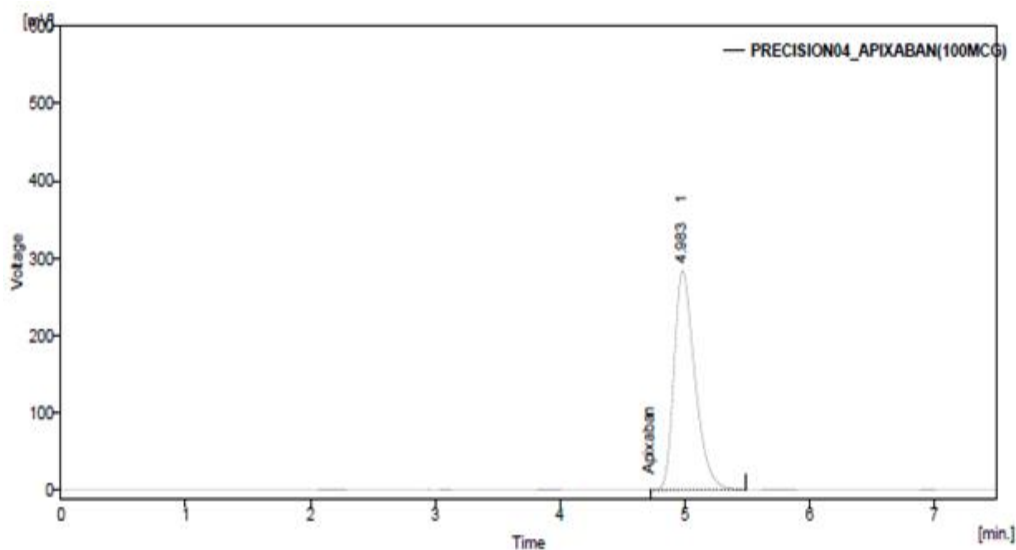


Fig: System PrecisionChromatogram-4

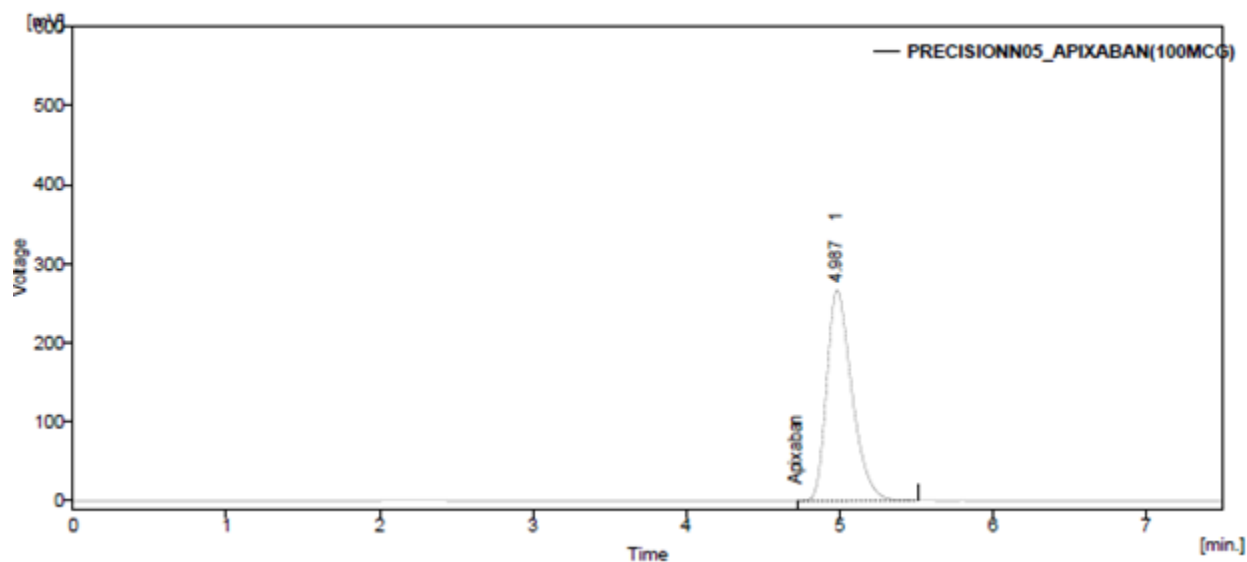


Fig: System PrecisionChromatogram-5

Table: Observations of System Precision

Injection No.	R.T	AREA
1	4.987	3059.295
2	4.973	3091.558
3	4.950	3314.065
4	4.983	3293.678
5	4.987	3067.163
6	4.980	3315.153
Average	4.9767	3290.152
Standard deviation	0.0141	23.203
% RSD	0.28	0.70

Observation

For system precision, six samples of Apixaban tablets formulation of a same batch were analyzed as per developed analytical procedure. The % RSD of method precision was found to be 0.70 for Apixaban. Therefore, the HPLC method for the determination of assay for Apixaban in formulation was found to be precise and they are in limits.

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

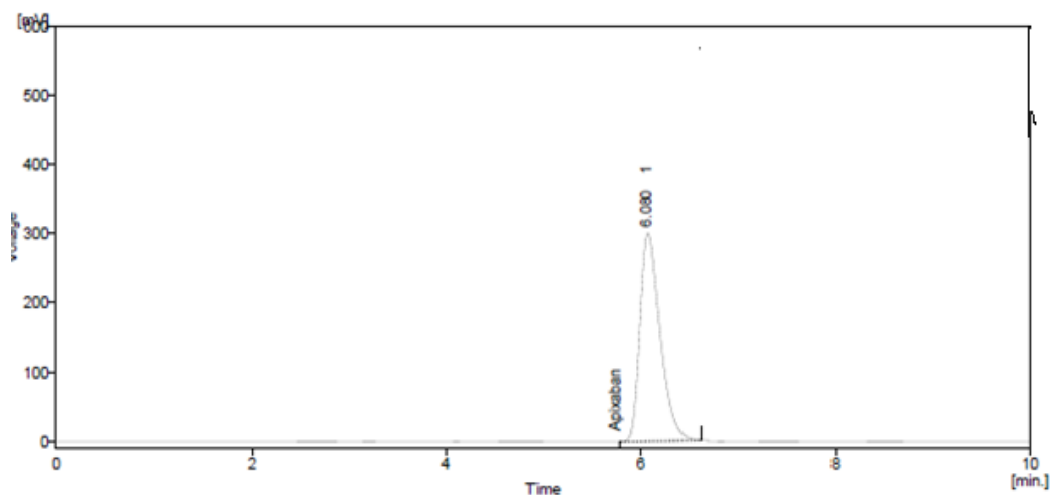


Fig: Low Flow Rate: (0.8ml/Min)

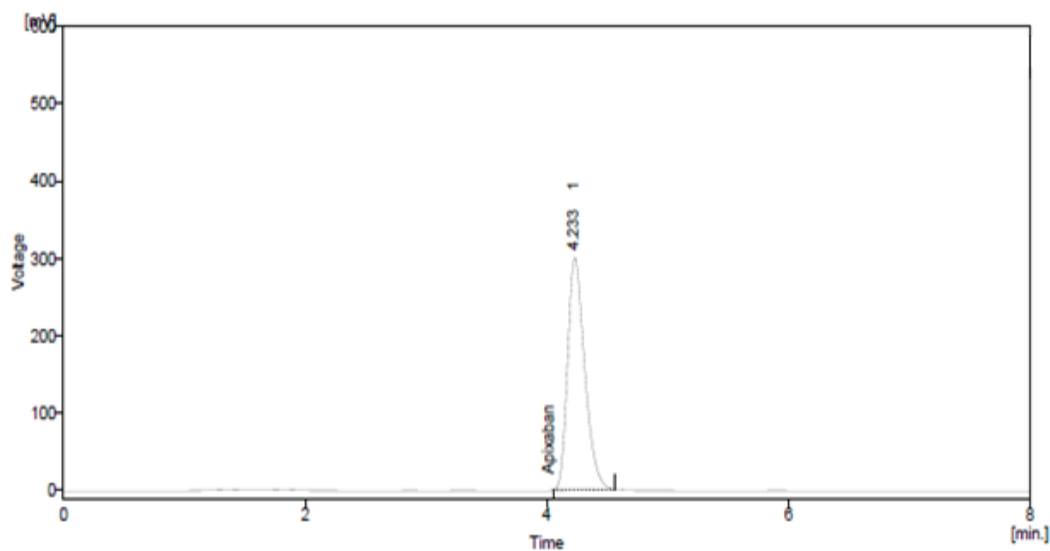


Fig: High Flow Rate: (1.2ml/Min)

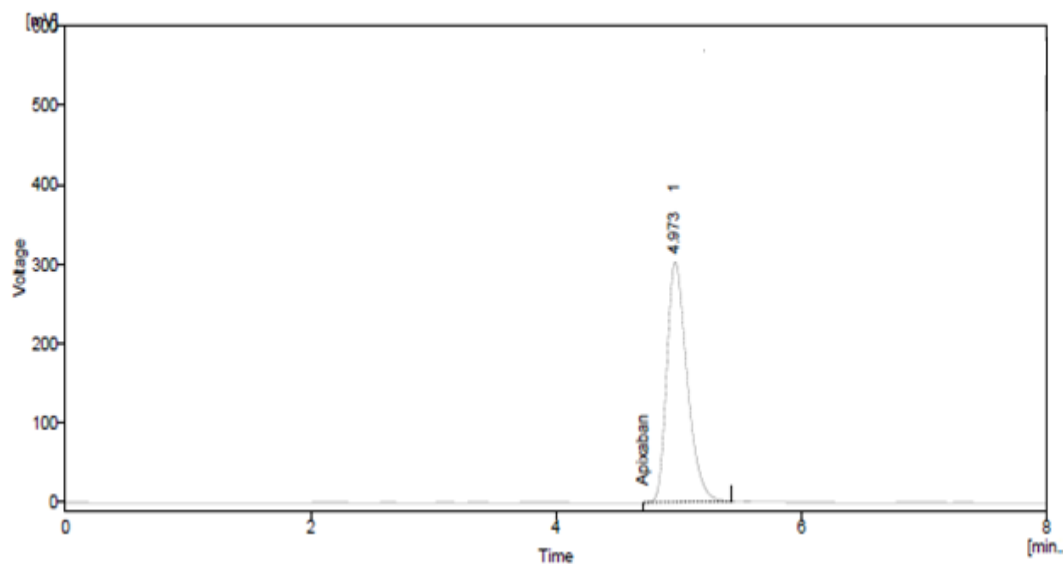


Fig: Chromatogram for decreased wavelength 279nm

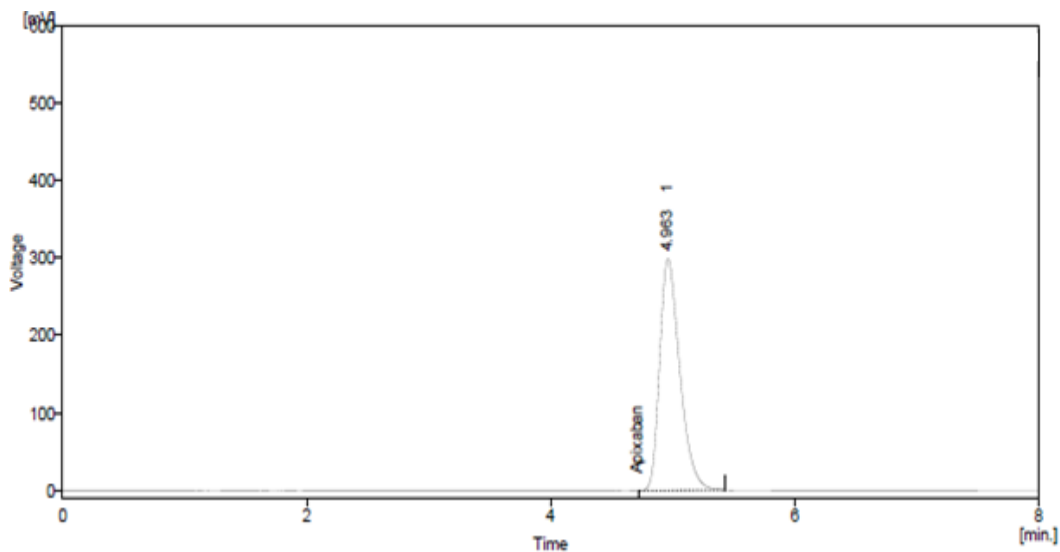


Fig: Chromatogram for change in wave length 283nm

Table: Results of Robustness study

Parameter	Apixaban	
	Retention time(min)	Tailing factor
Flow		
0.8ml/min	6.080	1.783
1.0 ml/min	4.960	1.641
1.2ml/min	4.233	1.722
Wavelength		
279nm	4.973	1.641
281nm	4.960	1.641
283nm	4.963	1.641

Observation

From the observation it was found that the system suitability parameters were within limit at variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

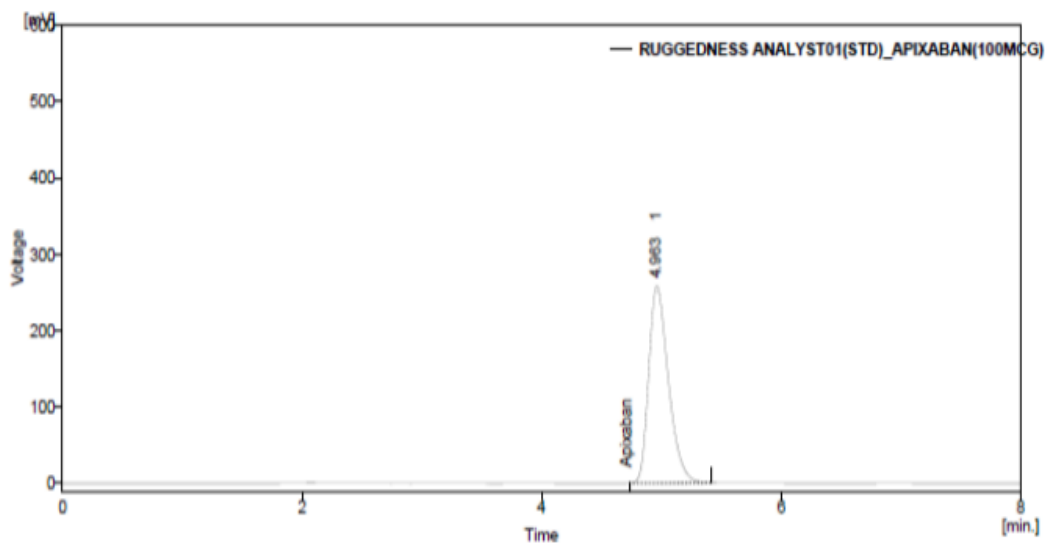


Fig: Chromatogram of Analyst 01 standard preparation

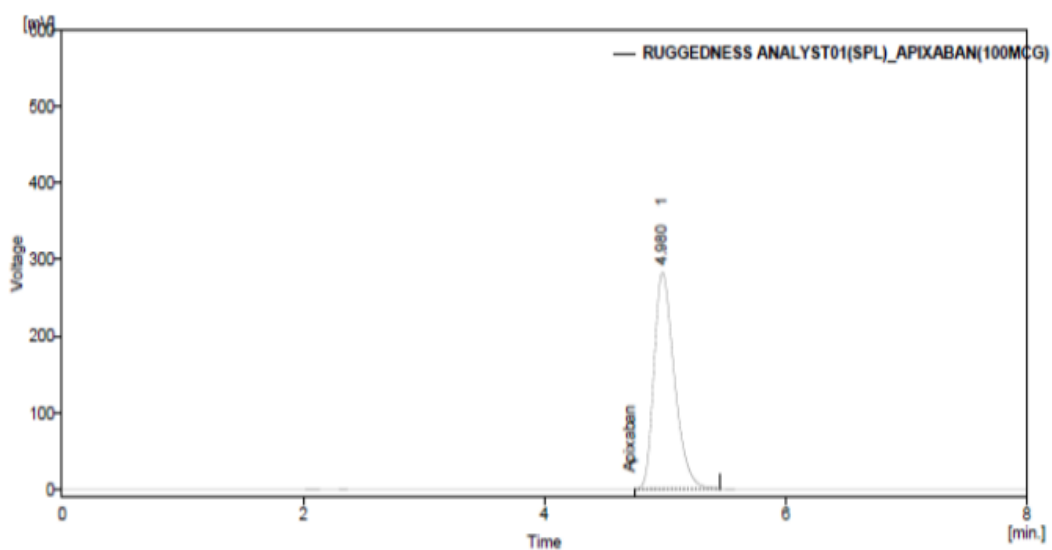


Fig: Chromatogram of Analyst 01 sample preparation

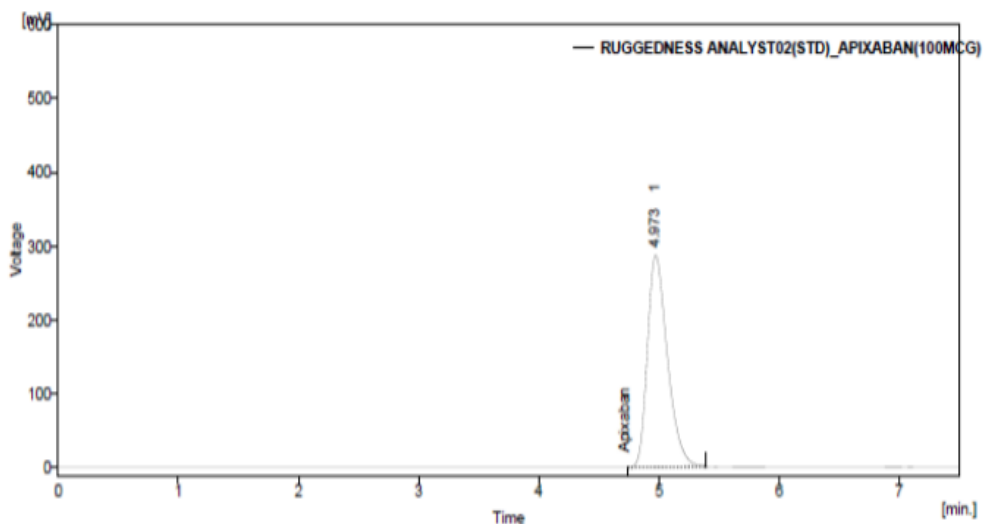


Fig: Chromatogram of Analyst 02 standard preparation

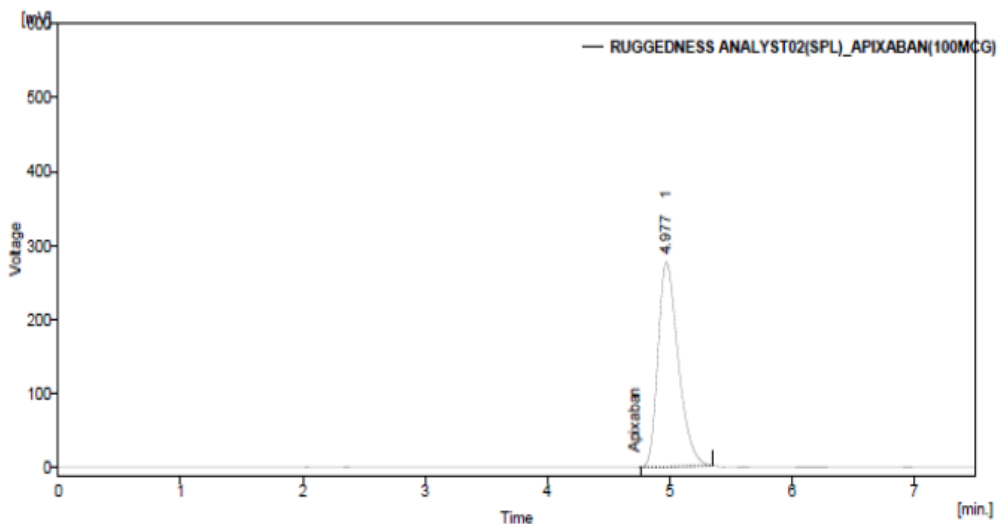


Fig: Chromatogram of Analyst 02 sample preparation

Table: Results for Ruggedness

Apixaban	%Assay
Analyst 01	98.98
Analyst 02	98.10

OBSERVATION

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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