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METHOD DEVELOPMENT AND VALIDATION OF APIXABAN USING RP HPLC IN BULK DOSAGE FORMS

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ABSTRACT

A simple and selective LC method is described for the determination of Apixaban dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of HPLC water (pH 5.8): ACN (55:45v/v), with detection of 281 nm. Linearity was observed in the range 75-150 µg /ml for Apixaban (r^2 =0.994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Apixaban, LC method, pharmaceutical dosage form

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INTRODUCTION

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer Analytical methods for such drugs. Basic criteria for new method development of drug analysis- The drug or drug combination may not be official in any pharmacopoeias. A proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients. Analytical methods for a drug in combination with other drugs may not be available. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. Analytical method development provides the support to track the quality of the product from batch to batch. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying

with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task (1-3).

Apixaban is 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3-carboxamide. Apixaban acts by directly inhibiting, in a reversible manner, free and clot-bound factor Xa to inhibit coagulation. Apixaban is to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. It has also been used to lower the risk of developing venous thrombosis post-orthopedic surgical procedures

MATERIALS AND METHODS

Preparation of standard stock solution of Apixaban (4-8)

5mg of Apixaban was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 2ml to 10ml with methanol.

Preparation of samples for Assay

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.

Preparation of sample solution

5tablets (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.

Fig-1 and 2 shows the chromatogram of standard and sample.

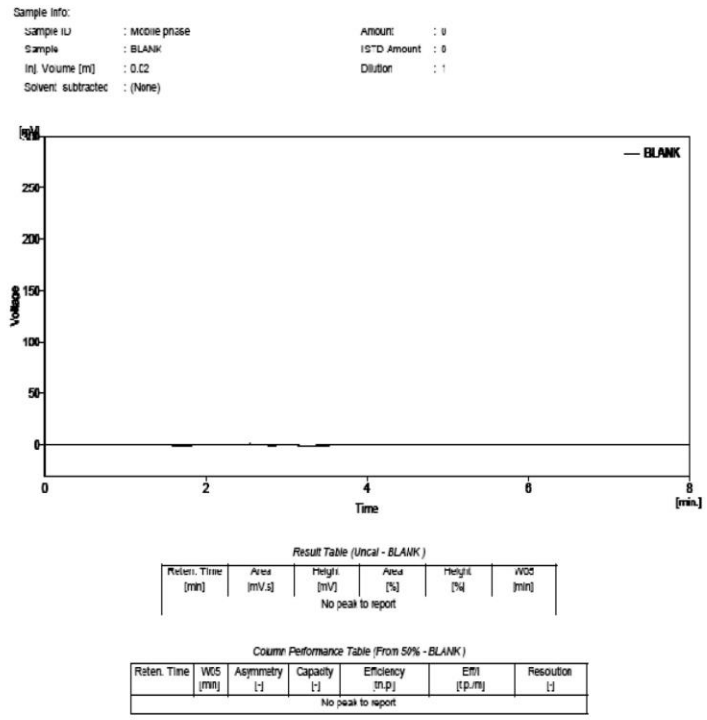


Fig-1 Blank chromatogram for specificity

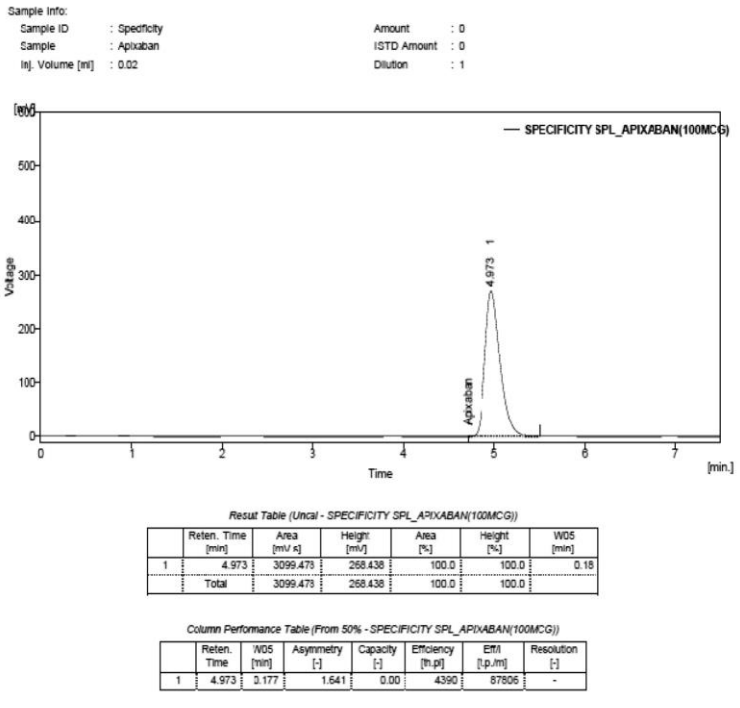


Fig-2 Chromatogram for specificity of Apixaban sample

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 281nm for Apixaban.

The amount of Apixaban present in the taken dosage form was found to be 99.08% (Table-1).

Table-1 Assay Results of apixaban

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	

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 (Table-2 and fig-3).

Table-2 Linearity of Apixaban

S.No.	Conc.(µg/ml)	Area
1	50	1904.438
2	75	3061.665
3	100	3680.717
4	125	4770.500
5	150	5220.440

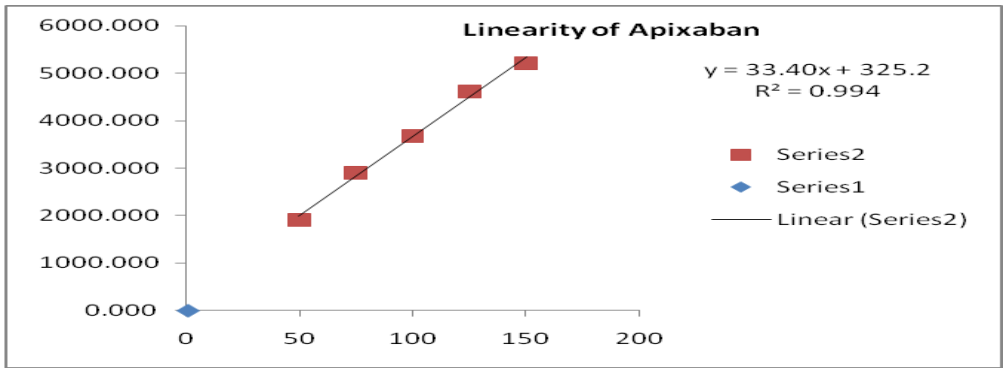
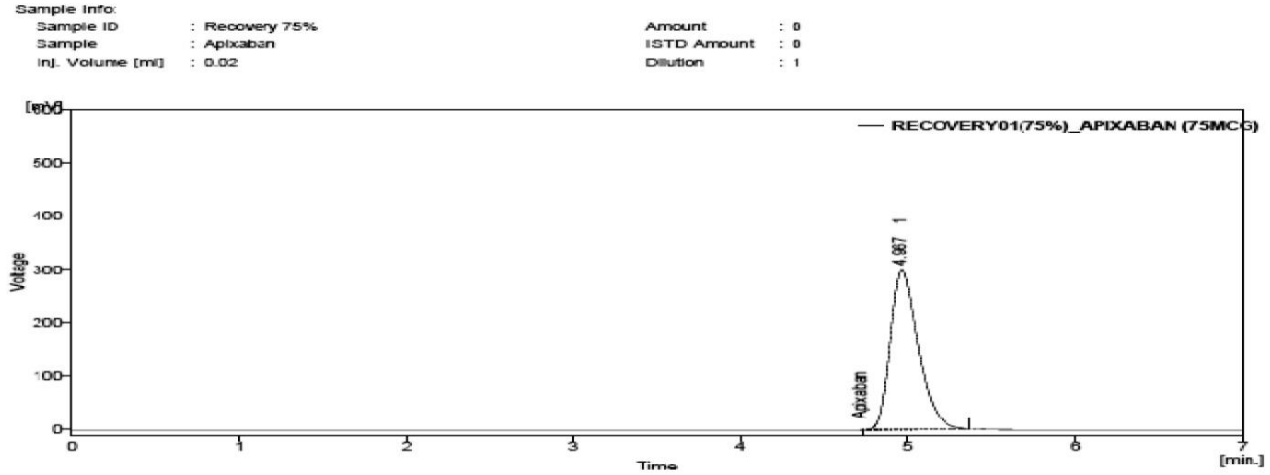


Fig-3 Linearity graph of Apixaban

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-4-6).



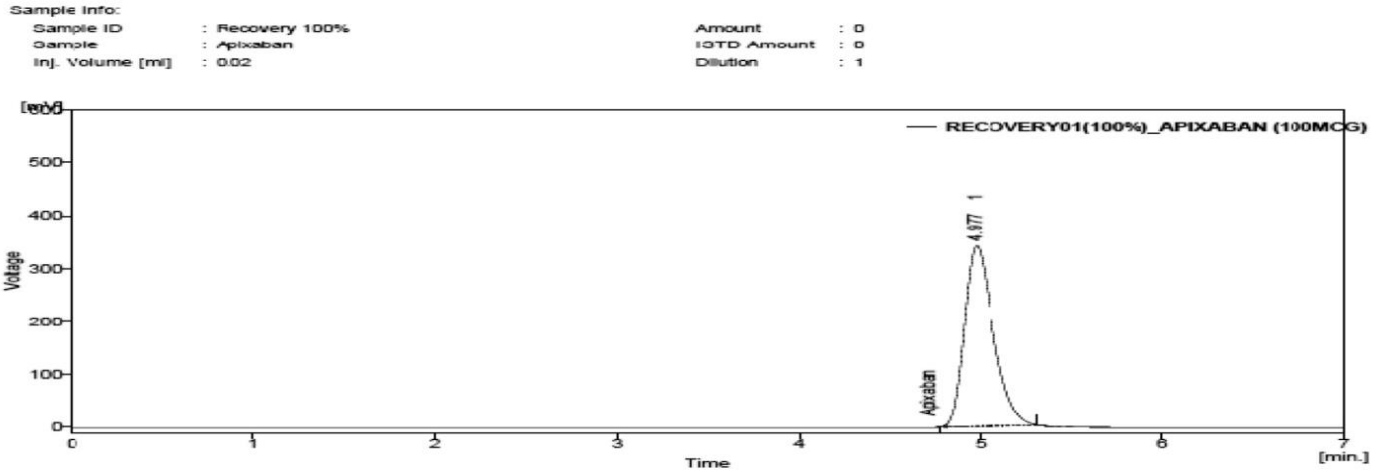
Result Table (Uncal - RECOVERY01(75%)_APIXABAN (75MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.967	3404.393	298.664	100.0	100.0	0.18
	Total	3404.393	298.664	100.0	100.0	

Column Performance Table (From 50% - RECOVERY01(75%)_APIXABAN (75MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.967	0.177	1.615	0.00	4379	87571	-

Fig-4 Chromatogram of 75%recovery



Result Table (Uncal - RECOVERY01(100%)_APIXABAN (100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.977	3838.430	341.404	100.0	100.0	0.18
	Total	3838.430	341.404	100.0	100.0	

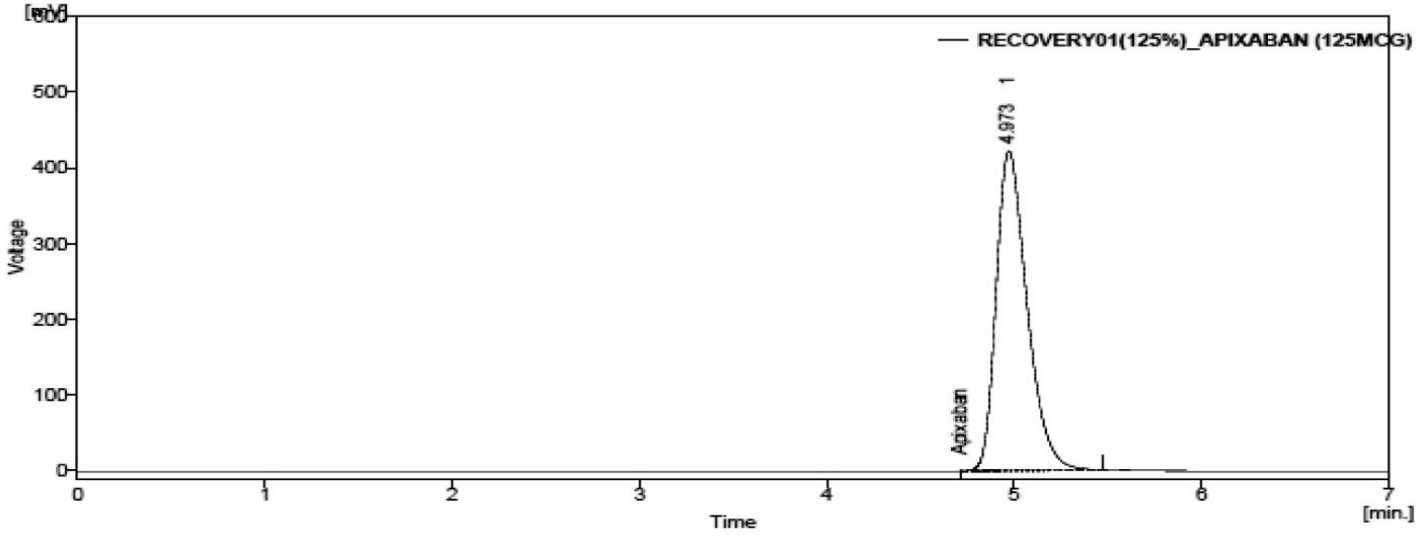
Column Performance Table (From 50% - RECOVERY01(100%)_APIXABAN (100MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.977	0.177	1.632	0.00	4396	87924	-

Fig-5 Chromatogram of 100% recovery

Sample Info:

Sample ID	: Recovery 125%	Amount	: 0
Sample	: Apixaban	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1



Result Table (Uncal - RECOVERY01(125%)_APIXABAN (125MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.973	4838.317	422.395	100.0	100.0	0.18
	Total	4838.317	422.395	100.0	100.0	

Column Performance Table (From 50% - RECOVERY01(125%)_APIXABAN (125MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.973	0.177	1.641	0.00	4390	87806	-

Fig-6 Chromatogram of 125% recovery

The percentage mean recovery of Apixaban is 98.40%. Test results for Apixaban are showing that the %RSD of Assay results are within limits. The results were shown in table-3. The LOD for this method was found to be 44.18 µg/ml of Apixaban. The LOQ for this method was found to be 133.89 µg/ml for Apixaban. From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table-3 Results for Method precision of Apixaban

Apixaban		
S.No.	Rt	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
avg	4.9767	3290.152
stdev	0.0141	23.203
%RSD	**0.28**	**0.70**

CONCLUSION

Experimental results and parameters it was concluded that, this newly developed method for the estimation of Apixaban was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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