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Research article

A stability indicating method development and validation of apixaban in pharmaceutical dosage form by using RP-HPLC and *In-Vitro* evaluation of apixaban suspension delivery through enteral feeding tubes

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ABSTRACT

A simple, consistent and sensitive stability indicating reversed phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of Apixaban in pharmaceutical dosage form by using Zorbax C18 (150 mm x 4.6 mm, 5µm particle size) with a mobile phase consisting of Ammonium Formate Buffer: Acetonitrile in a ratio of 65:35% v/v and successfully validated as per the international council for harmonization (ICH) guideline. The method was found to be simple, robust, precise, sensitive, accurate; specific and stress degradation studies were performed with acidic, alkaline, oxidative, thermal, humidity and photolytic stress conditions as per ICH guidelines.

In separate *in-vitro* experiments, Apixaban suspension passed through feeding tubes to develop a clog-free suspension delivery method. Nasogastric tube (8-French [Fr]) and diluents (water, 5% dextrose in water) were tested. Recovery of Apixaban Suspension in water and 5% dextrose in water at "0 and 15" minutes incubation time were nearly 100% in 8-Fr nasogastric (NG) tubes.

Keywords: Apixaban, Stress Degradation, Stability Indicating Assay Method, Nasogastric Tube.

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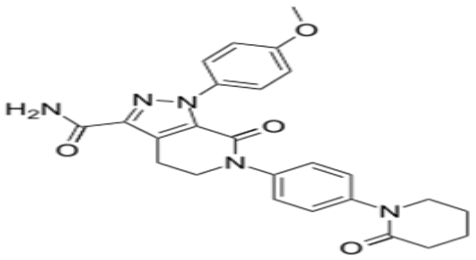
INTRODUCTION

Apixaban is chemically 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4, 5, 6, 7-tetrahydro-1H-pyrazolo [3, 4-c] pyridine-3-carboxamide, physical appearance of it is a white to pale-yellow powder with melting of 326.53 °C. It has good solubility nature in water and dimethyl sulfoxide. It is a new generation of oral anticoagulant drug that selectively inhibits coagulation factor Xa [1]. It is used in thromboprophylaxis in patients following total knee replacement surgery with a desired efficacy and safety profile [2]. Food and Drug Administration (FDA) approved Apixaban (Eliquis, Bristol-Myers Squibb/Pfizer) on December 28, 2012, for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (AF) [3]. Apixaban is not an official drug in any Pharmacopoeia. Literature survey reveals that only one marketed formulation of Apixaban is available. Some methods have been reported for their determination of Apixaban by HPLC [4-6] and hyphenated techniques such as Ultra Performance Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy [7-8], Liquid Chromatography Mass Spectroscopy [9-10], Gas Chromatography Mass Spectroscopy [11], either alone or in combination.

Limited number of research work available for stability indicating RP-HPLC and its application in *in- vitro* evaluation of Apixaban suspension delivery through enteral feeding tubes. Crushed tablet and solution formulations of Apixaban administered via a NG tube may be useful in patients unable to swallow solid dose formulations.

This paper presents analytical method development of Apixaban and validation of stability indicating assay method by using the RP-HPLC technique as per ICH guidelines as well as *in- vitro* evaluation of Apixaban suspension delivery through enteral feeding tubes. These studies evaluated the recovery of Apixaban suspension administered via enteral NG feeding tubes flushed with either 5% dextrose in water (D5W) or water and Apixaban was measured by validated stability indicating assay RP-HPLC method.

Figure 1: Chemical structure of Apixaban



MATERIALS AND METHODS

Chemicals, Reagent and Materials

The HPLC grade solvents used in this study were obtained from different suppliers (Ammonium Formate, Acetonitrile of Rankem Limited, India and HPLC grade water of SD fine-Chem ltd, Mumbai) and medical grade 5% w/v Dextrose solution, enteral nasogastric (NG) feeding tubes from local market. Commercially available tablets of Apixaban (Eliquis®contain 5 mg) were procured from local market and Apixaban active pharmaceutical ingredient (API) was obtained from Metrochem API Private Limited supplier. All excipients were obtained from Loba Chemie Pvt. Ltd., Mumbai, India.

Instrumentation

The method was performed on Shimadzu LC 2010 HPLC system supplied with a gradient pump connected to Ultra-violet detector and automatic injection facility. The column Zorbax C-18, (150 x 4.6) mm, 5 µm, Lab solution software, Shimadzu AY-120 balance, sonicator (Leelasonic), centrifuge machine (Remi) were used for this work. Thermal stability studies were carried out in an I-Therm dry air oven.

Method Development and Optimization of Chromatographic Conditions

To begin initially, literature methods were evaluated to check the suitability for assay and eternal feeding tubes studies of Apixaban tablets. However, we encountered with different concerns with respect to improper peak shape, shifting of retention time, placebo interference at the retention time of active peak, baseline disturbance etc. As Apixaban is having molecular weight of 459.497 which is less than 2000 g/mol; columns having C8 or C18 packing can be used. Chromatographic separation was attained on Zorbax C-18, (150 x 4.6) mm, 5 µm column using mobile phase composition of ammonium formate buffer and acetonitrile in the ratio of 65:35% (v/v).

Preparation of Standard Solution

Accurately weighed quantity of Apixaban (50 mg) standard into a 50 ml volumetric flask, dissolved and diluted up to the mark with diluent and was sonicated to get 1 mg/ml solution of Apixaban (1000 µg/ml). Further dilutions were made as per the requirement by dissolving it in diluent and mix well.

Preparation of Sample Solution

Take the Apixaban tablets equivalent to 200 mg into a 250 ml volumetric flask and add 170 ml of diluent and sonicate to disperse tablets completely, further sonicate for about 20 minutes with intermittent shaking and then make up the mark with diluent.

Assay of Marketed Formulation

Transfer 10 tablets in to 250 mL dry volumetric flask. Add about 170 mL of diluent and sonicate to disperse tablets completely, further sonicate for about 20 minutes with intermittent shaking and equilibrate to room temperature and make up volume up to the mark with diluent and mix well (Concentration: Apixaban 200µg/ml).

Stress Degradation Studies

The stability-indicating property of the developed HPLC method carried out by stress studies as per ICH recommended conditions. Stress degradation of Apixaban was carried out by forcefully subjecting the sample into acidic, alkaline, oxidative, photolytic, humidity and thermal conditions [12-14].

Method Validation

The developed method was validated for different prescribed parameters like system suitability, specificity, precision, linearity, precision at different levels, accuracy, stability of analytical solutions, robustness, filter interference as per guidelines of ICH Q2A and Q2B [15].

In Vitro Delivery of Apixaban Suspension via Enteral Feeding Tubes [16-19]

The approved labeling for the reference product and FDA draft guidance on Apixaban states that the product may be crushed and suspended in 60 ml of water or 5% dextrose in water (D5W) and promptly delivered through a NG tube. This study investigated the *in vitro* recovery of Apixaban suspension in 60 mL catheter tipped syringe with 60 mL water or 5% Dextrose (D5W) after passage through PVC 8 French Nasogastric (NG) tube with " J " holding position.

Preparation of Apixaban Suspension for Delivery through Syringe and Nasogastric Tube

The Apixaban suspensions from Eliquis tablets were prepared by crushing the tablets and suspending the obtained powder in a predetermined volume of the suspending vehicle (i.e. 5% w/v dextrose). The tablet powder suspension was transferred into the 60 mL syringe and rinsed the beaker with several washings of 20 mL 5% w/v dextrose solution ensuring complete transfer of suspension from beaker into the syringe.

'0" minute incubation

The plunger was fixed to the syringe and was gently mixed for about 30 seconds to homogenize the suspension.

"15" minutes incubation

The plunger was fixed to the syringe and was gently mixed and incubated for 15 minutes at room temperature. After incubation, the syringe was shaken gently for about 30 seconds to homogenize the suspension.

Apixaban Suspension Administration through NG tubes

NG Feeding tubes were mounted to a board to mimic the position the tube would be in a patient. An oral syringe was attached to the top of the feeding tube, the suspension was allowed to flow through the tube via gravity, and the samples were collected into 200 mL volumetric flask after passing through the tube for evaluation.

Quantification of Apixaban by Liquid Chromatography after Administration through NG tubes:

About 100 mL acetonitrile was added in each flask, mixed well. The samples were sonicated for 30 minutes and the volume was

made up to the mark with 5% w/v dextrose solution and mixed well. The solution was centrifuged at 5000 RPM for 5 minutes and 3.6 mL of supernatant was transferred to a 10 mL volumetric flask and the volume was made up with diluent and mixed well. The solution was transferred to glass vial and sample vials were transferred directly to the autosampler for analysis of Apixaban using validated assay method.

Evaluation of Tube Delivery

1. Single Dose Administration: The recovery study was repeated for 12 times each for Eliquis samples.

2. Repeated Dose Administration: The recovery study was repeated for 12 times each for Eliquis samples in each nasogastric tube been used for 03 administrations.

3. Repeated the eternal feeding tube studies using 60 mL of water in place of 5% w/v dextrose solution.

RESULTS AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by changing mobile phase composition. The chromatogram of Apixaban is shown in figure 3 and optimized chromatographic conditions are mentioned in table 1.

Table 1: Optimized Chromatographic Conditions

Parameters	Details
Mobile phase	Ammonium formate Buffer: Acetonitrile (650:350)
Column	Zorbax C-18, (150 x 4.6) mm, 5 µm
Flow rate	1.1-ml / minute
Detection	280 nm
Injection volume	5-µL (For Assay), 100-µL (For Recovery Study)
Run time	7 min
Retention time	4.0 min
Diluent	Ammonium formate Buffer: Acetonitrile (50:50)

Assay of Tablet Formulation

The drug content was calculated as an average of six determinations and assay results were shown in table 2.

Table 2: Results of Assay of Apixaban

Actual Concentration found	Amount of Drug Estimated mean±SD*
98.5	98.1±0.46
98.1	
97.4	
97.9	
98.7	
98.1	

*The value is represented as a mean±SD (standard deviation) of 6 observations.

Stress Degradation Studies

As per the guidelines of ICH, stressed degradation studies were conducted to establish the stability indicating characteristic of the developed method and identify the ability of the drug to withstand the different physical and chemical conditions. The assay of Apixaban was calculated and reported under acid and alkaline hydrolysis, oxidation, thermal, humidity and photolytic stress conditions respectively in table 3.

Table 3: Forced Degradation results of Apixaban

Degradation Conditions	% Degradation of Apixaban
Acid Degradation (5N HCl, heated at 50°C)	7.2
Alkali Degradation (5N NaOH, heated at 50°C)	9.5
Oxidative Degradation (30 % Peroxide, heated at 40°C)	No Degradation
Thermal Degradation (60°C, 7 days)	1.1
Humidity Degradation (40°C/75% RH, 7 days)	0.8
Photolytic Degradation (1.2 million lux hours and the light intensity not less than 200 watt-hours per sq. meter)	No Degradation

The degradation products were well separated from the parent drug, and no interference of the parent drug peak with those of the degradation products was observed. Therefore, the developed HPLC method was considered stability indicating and suitable for the proposed stability and enteric tubes delivery study of Apixaban suspension.

Validation of the Method

The developed chromatographic method was validated as per ICH guidelines.

System Suitability

The values of system suitability study are represented in table 4.

Table 4: Results of System Suitability

System Suitability Parameter	Acceptance Criteria	Apixaban
Theoretical Plate Count	NLT 5000	9116
Tailing Factor	NMT 2.0	1.12
% RSD	NMT 2.0	0.69

RSD: Relative Standard Deviation, NLT: Not Less Than, NMT: Not More Than

Specificity

The below figure 3 shows that the active ingredient was well separated from blank and placebo and there was no interference of placebo with the principal peak. Hence the method is specific.

Precision

The method and intermediate precision results were shown in table 5 and 6.

Table 5: Precision results of Apixaban

Conc. (µg/ml)	Method Precision	Intermediate Precision
200	98.5	99.2
	98.1	98.8
	97.4	99.0
	97.9	98.9
	98.7	98.7
	98.1	98.9
Mean	98.1	98.9
% RSD	0.47	0.17
SD	0.46	0.17

Table 6: Precision at Different Levels Results of Apixaban

	Apixaban Concentration (µg/ml)		
	50 µg/ml	200 µg/ml	300 µg/ml
	544248	2167432	3243726
	542463	2161676	3235170
	542591	2152925	3238829
	541433	2153216	3244270
	539615	2145191	3242755
	538454	2148947	3238185
Mean	541467	2154898	3240489
% RSD	0.39	0.38	0.11

Figure 2: Chromatogram of placebo

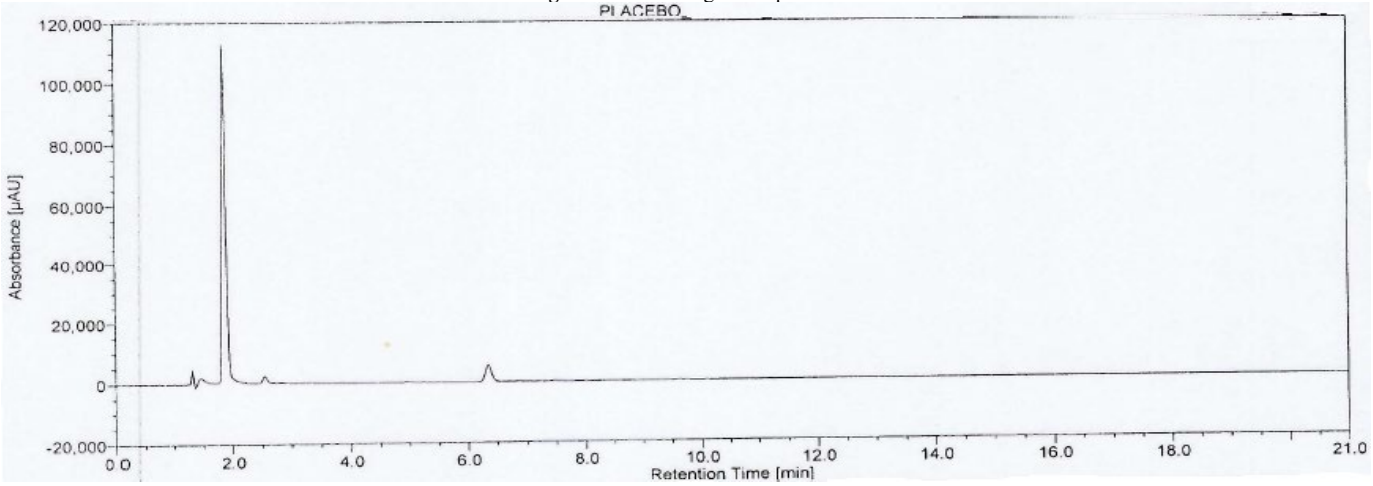
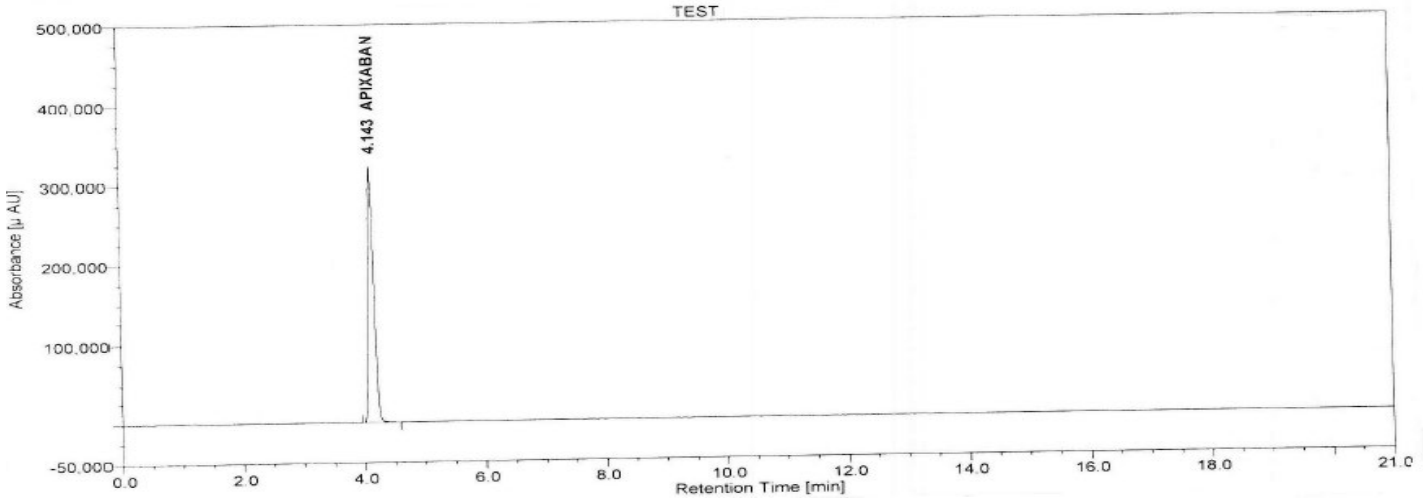


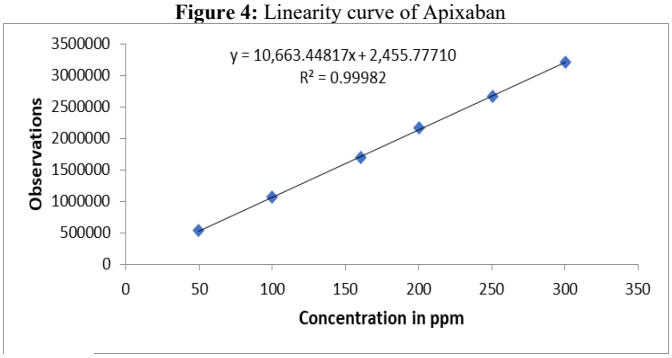
Figure 3: Chromatogram of sample



Linearity and Range

The value of correlation coefficient for Apixaban (figure 4) demonstrated the good relationship between peak areas and concentrations. Therefore, the developed method was linear in the concentration range of 50-300 µg/ml.

Figure 4: Linearity curve of Apixaban



Accuracy

The accuracy data of the proposed method is summarized in table 7.

Table 7: Results of Accuracy

	% Level	Apixaban % Recovery
	25	100.5
	100	99.7
	150	99.2
Mean		99.8
SD		0.66

Mean + SD (n=3)

Stability of Analytical Solution

The changes in response with respect to initial are calculated and data reported in table 8.

Table 8: Stability results of Apixaban

Time in Hours	Standard		Test	
	Area Response	% Deviation from Initial	Area Response	% Deviation from Initial
Initial	2169019	NA	2143564	NA
4	2183100	-0.65	2139711	0.18
8	2164851	0.19	2124564	0.89
12	2188937	-0.92	2119363	1.13
16	2162196	0.31	2123064	0.96
20	2162411	0.30	2132342	0.52
24	2163803	0.24	2150686	-0.33
28	2151921	0.79	2130954	0.59
32	2139978	1.34	2126023	0.82
36	2128956	1.85	2124606	0.88
40	2141082	1.29	2115311	1.32
44	2144550	1.13	2111513	1.50
50	2131840	1.71	2126217	0.81

Robustness

Table 9: Robustness Data of Apixaban

Parameter Name	% RSD
Flow rate: +10%	0.08
Flow rate: -10%	0.06
Wavelength: +3 nm	0.06
Wavelength: -3 nm	0.05
Column temperature: +5°C	0.09
Column temperature: -5°C	0.16
Mobile phase ratio change: +2 % absolute (Acetonitrile)	0.12
Mobile phase ratio change: -2 % absolute (Acetonitrile)	0.15

Robustness was performed by changing various method parameters and results were represented in table 9.

***In Vitro* Delivery of Apixaban Suspension via Enteral Feeding Tubes**

The prepared Apixaban suspension deliverability and compatibility with enteric feeding tubes, as indicated by Apixaban recovery in the suspensions collected after passing through the tubes, is presented in table 10. After the suspension traversed the tubes completely, the tubes were visually inspected for the presence of any residual fluid and none of the tubes exhibited any signs of blockage. After medication administration, tubes are typically flushed with 10 mL of water or 5% dextrose. The mean recovery of Apixaban in the prepared suspensions in 5% dextrose in water and water was found to be about 100%.

Figure 5: Photograph of 8F PVC NG Tube

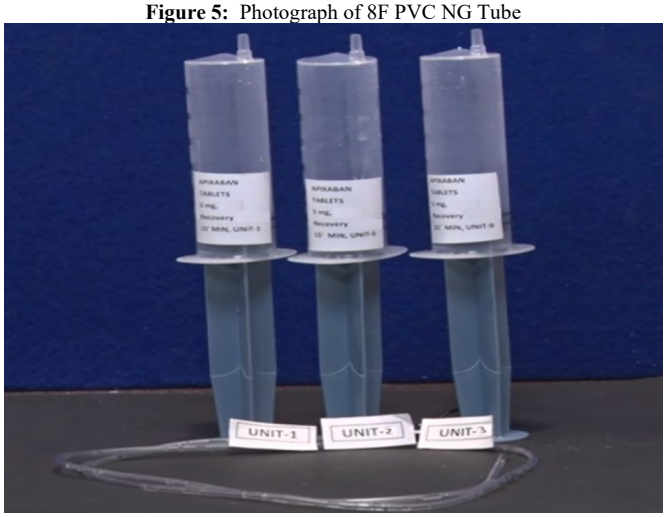


Table 10: Mean Recovery (%) of Apixaban in the Prepared Suspension after Passing through Enteric Feeding Tubes**.**

Apixaban Recovery, mean (%)					
Suspending Vehicle		5% Dextrose in water		Water	
Tube type	Administration	Incubation Time			
		"0" minute	"15" minutes	"0" minute	"15" minutes
PVC (Poly Vinyl Chloride), 8 Fr, NG Tubes	Single Dose	95.5± 0.91	96.6± 1.50	95.7± 0.81	96.1± 1.16
	Repeated Dose	Not Applicable	96.1± 0.81	Not Applicable	95.9± 1.15

The mean Apixaban content in each set expressed as a percentage of the dose administered (target dose).

CONCLUSIONS

Results obtained by validation studies suggested that the developed stability indicating assay method is simple, precise, accurate, specific, robust, rugged and possess stability indicating characteristics. Thus, this method can be used for routine analysis and to check the stability testing of Apixaban formulation. The methods developed in this study could be used to distinguish batches with suboptimal product quality for delivery using NG tubes and to confirm the substitutability of generic drug products for this alternative route of administration

The purpose of this publication was to develop a stability indicating method and provide additional information on the clinical utility of Apixaban, particularly for individuals with swallowing difficulties. A suspension of Apixaban in 5% dextrose and water were identified as suitable for administration through enteral feeding tubes. The recovery of Apixaban after passing through the selected enteric feeding tubes was found to be within acceptable range (±10%) of the label claim. The study presents the feasibility of preparing an extemporaneous suspension of Apixaban for delivery via enteric feeding tubes, using Eliquis tablets.

The possibility of mechanical tube occlusion due to administration of Apixaban suspension was further explored. Following administration of Apixaban suspension, the NG tubes were observed for occlusion; no remaining suspensions were visible in the 8Fr NG tubes (figure 5).

A limitation of these evaluations is that they were not performed in patients with feeding tubes. There are limited publications evaluating the delivery of Apixaban formulations via feeding tubes. Overall, these results support several alternative methods of Apixaban administration in patients who are unable to swallow solid oral dose formulations. The data on Apixaban suspension support the administration of crushed Apixaban tablets suspended in D5W and water via an NG tube as a flush medium. Notably, in this study, one type of NG tube was investigated. However, additional parameters such as the material and design of the NG tube could be investigated in the future. The methods developed in this study could be used to evaluate *in vitro* equivalence and to assess the potential risks of delivering oral drug products through enteral feeding tubes after suspension in 5% dextrose in water and water.

CONFLICT OF INTERESTS

There is no conflict of interest regarding the publication of this article.

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