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# AN RP- UPLC METHOD DEVELOPMENT AND VALIDATION OF APIXABAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Pharmacy

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## ABSTRACT

A simple and selective UPLC method is described for the determination of Apixaban. Chromatographic separation was achieved on Inertsil ODS 3V C18(150x4.6 ID) 5µm column using mobile phase consisting of a mixture of Phosphate buffer : Acetonitrile (40 :60) with detection of 269 nm. Linearity was observed in the range 50-150 µg /ml for Apixaban (r2 =0.999) for the amount of drug estimated by the proposed method was in good agreement with the label claim.

The proposed method was 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.

## KEYWORDS

Apixaban, UPLC.

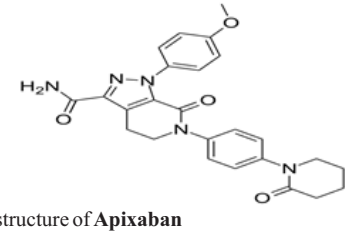
## INTRODUCTION:

UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time. According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linear velocities.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, 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. Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B).

## DRUG PROFILE:

**Apixaban**, sold under the tradename **Eliquis**, is an anticoagulant for the treatment of venous thrombo embolic events. It is taken by mouth. It is a direct factor Xa inhibitor. It was approved in the U.S. in 2014 for treatment and secondary prophylaxis of deep vein thrombosis (DVT) and pulmonary embolism (PE).



Chemical structure of **Apixaban**

**IUPAC Name:** 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3-carboxamide

**Molecular Formula:** $C_{25}H_{25}N_5O_4$

**Molecular Weight:** 459.4971 g/mol

**Mechanism of Action:**

Apixaban is a highly selective, orally bio-available and reversible direct inhibitor of free and clot-bound factor Xa. Factor Xa catalyzes the conversion of prothrombin to thrombin, the final enzyme in the coagulation cascade that is responsible for fibrin clot formation. Apixaban has no direct effect on platelet aggregation, but by inhibiting factor Xa, it indirectly decreases clot formation induced by thrombin.

**Literature Review:**

- **D. Shravan Kumar *, Dr. M. Ajitha, Mahesh Rajendra Awate, K.S.L. Harika** A simple, accurate and precise isocratic RP-HPLC method was developed using Inertsil ODS 3V 250 mm x 4.6 mm 5µm column for estimation of Apixaban in tablet dosage form.
- **M. Kashid, M.B. Vidhate** A simple, fast and precise high performance liquid chromatographic method is developed and validated for apixaban as per ICH guideline. Chromatographic separation of drugs was performed on a Kromasil C18 column (250 mm x 4.6 mm, 5 µ) with a mobile phase comprising of sodium acetate: acetonitrile in the ratio 50:50 (V/V) at a flow rate of 1 mL/min.
- **Md. Abdul Majeed, Dr.K.Vijaya, B. Anitha** A simple and precise RP-HPLC method was developed for the determination of Apixaban dosage form the chromatographic separation was achieved on an Zorbax Bonus RP (250 X 4.6mm) column with a mobile phase contain the gradient mixture of the solvents (Acetonitrile: water) in the ratio of 90:10(v/v).

## MATERIALS AND METHODS:

**Preparation of standard solution:**

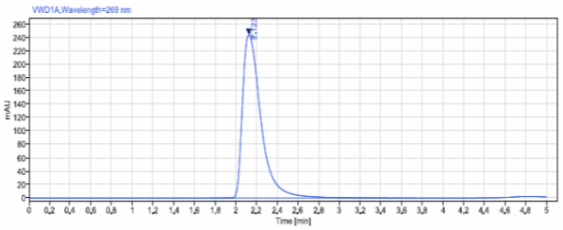
Weigh accurately 10 mg of Apixaban in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Apixaban is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

**Preparation of Sample solution:** Weigh accurately and powder 10 Tablets of Apixaban **(Eliqis - 5 mg).** Weigh accurately 10 mg of Apixaban in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Apixaban is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

**Optimized Chromatographic conditions:**

| | |
|---|---|
| Mobile phase | Phosphate buffer : Acetonitrile |
| pH | 5 |
| Column | Inertsil ODS 3V column,C18(150x4.6 ID) 5µm |

| | |
|---|---|
| Flow rate | 1.0 ml/min |
| Column temperature | Room temperature(20-25oC) |
| Sample temperature | Room temperature(20-25oC) |
| Wavelength | 269nm |
| Injection volume | 20 µl |
| Run time | 5 min |




**Chromatogram of Apixaban (Optimized)**
**s**

**RESULT AND DISCUSSION:**

**Determination of Absorption maxima:**

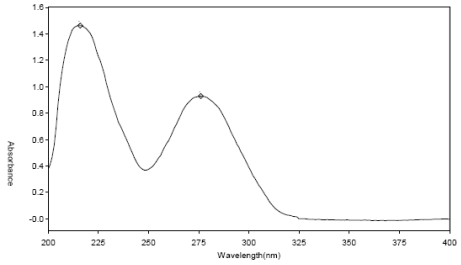



***Fig.* 1: UV-VIS spectrum of APIXABAN (269 nm)**

**ASSAY: RESULTS**

| | Apixaban | |
|---|---|---|
| | **Standard Area** | **Sample Area** |
| **Injection-1** | 3057.38 | 3055.23 |
| **Injection-2** | 3056.29 | 3054.42 |
| **Injection-3** | 3055.46 | 3054.73 |
| **Injection-4** | 3055.13 | 3055.26 |
| **Injection-5** | 3056.22 | 3053.97 |
| **Average Area** | 3056.10 | 3054.72 |
| **Standard deviation** | 0.87 | |
| **%RSD** | 0.03 | |
| **Assay(%purity)** | 99.96 | |

**System Suitability:**

Results for system suitability of APIXABAN.

| Injection | RT | Peak area | Theoretical plates (TP) | Tailing factor (TF) |
|---|---|---|---|---|
| 1 | 2.118 | 3061.86 | > 2000 | < 2 |
| 2 | 2.119 | 3060.29 | > 2000 | < 2 |
| 3 | 2.125 | 3060.51 | > 2000 | < 2 |
| 4 | 2.122 | 3061.14 | > 2000 | < 2 |
| 5 | 2.123 | 3061.09 | > 2000 | < 2 |
| 6 | 2.13 | 3061.33 | > 2000 | < 2 |
| Mean | 2.123 | 3061.037 | | |
| SD | 0.00436 | 0.57 | | |
| %RSD | 0.205 | 0.02 | | |

- The plate count and tailing factor results were found to be within the limits and The % RSD was found to be 1.2 so system is suitable and giving precise results.

**METHOD PRECISION:**

Method precision was determined by injecting sample solutions of concentration APIXABAN (20µg/mL) for six timesare prepared separately.

Method precision results for APIXABAN

| | Apixaban | |
|---|---|---|
| S.No. | RT | AREA |
| 1 | 2.12 | 3054.11 |
| 2 | 2.118 | 3054.63 |
| 3 | 2.124 | 3053.91 |
| 4 | 2.119 | 3055.27 |
| 5 | 2.121 | 3057.38 |
| 6 | 2.117 | 3054.66 |
| AVG | 2.1198 | 3054.9933 |
| SD | 0.0025 | 1.2625 |
| %RSD | 0.117 | 0.041 |

- The %RSD of Assay for 6 Samples determinations of APIXABAN found to be within the acceptance criteria (less than 2.0%). hence method is precise.

**Linearity:**

Linearity data of APIXABAN

| S.No | Concentration (µg/mL) | Area |
|---|---|---|
| 1 | 50 | 1481.5 |
| 2 | 80 | 2436.5 |
| 3 | 100 | 3050.96 |
| 4 | 120 | 3678.69 |
| 5 | 150 | 4629.19 |

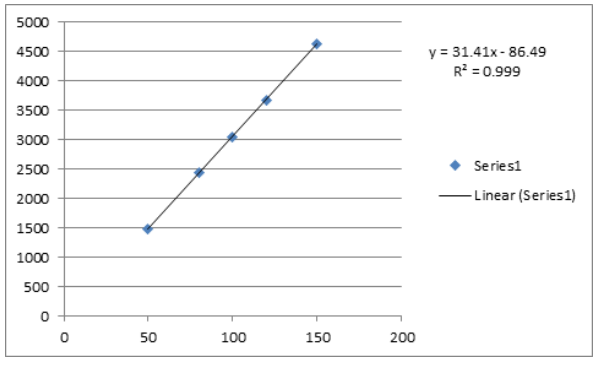



***Fig 2:* Graph for Linearity data of APIXABAN.**

**Linearity results of Apixaban:**

| S.No | Parameter | APIXABAN |
|---|---|---|
| 1 | Correlation coefficient | 0.999 |
| 2 | Slope | 31.41 |
| 3 | Intercept | 86.49 |

- The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparation 0.999.

**Accuracy:**

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for Apixaban.

| Name of the Sample | Standard Weight in mg | Area | Conc Added (µg/ml) | Conc Recovered (µg/ml) | %Recovery | Average |
|---|---|---|---|---|---|---|
| 50% Recovery_01 | 50 | 14507698 | 50 | 49.99 | 100.0 | |
| 50% Recovery_02 | 50 | 14368377 | 50 | 49.51 | 99.0 | |
| 50% Recovery_03 | 50 | 14348431 | 50 | 49.44 | 98.9 | |
| 100% Recovery_01 | 100 | 28777447 | 100 | 99.16 | 99.2 | |
| 100% Recovery_02 | 100 | 28784075 | 100 | 99.19 | 99.2 | 99.4 |
| 100% Recovery_03 | 100 | 28669699 | 100 | 98.79 | 98.8 | |
| 150% Recovery_01 | 150 | 43496072 | 150 | 149.88 | 99.9 | |
| 150% Recovery_02 | 150 | 43483295 | 150 | 149.84 | 99.9 | |
| 150% Recovery_03 | 150 | 43236035 | 150 | 148.98 | 99.3 | |

Results for Recovery of APIXABAN

- The percentage mean recovery of APIXABAN 99.4%.

**ROBUSTNESS:**

Results for Robustness of APIXABAN

| Chromatographic changes | | Rt (min) | Tailing Factor | Theoretical Plates | %RSD for Standard areas |
|---|---|---|---|---|---|
| Flow rate (mL/min) | 0.4 | 1.871 | 1.63 | 4171 | 0.03 |
| | 0.6 | 1.523 | 1.43 | 3459 | 0.49 |
| Temperature (°C) | 25 | 1.628 | 1.48 | 3786 | 0.05 |
| | 35 | 1.626 | 1.52 | 3434 | 0.42 |

- The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

**RUGGEDNESS:**

Ruggedness Results of APIXABAN

| APIXABAN | **%Assay** |
|---|---|
| **Analyst 01** | 100.17 |
| **Analyst 02** | 99.17 |
| **%RSD** | 0.48 |

- From the above results % Assay and %RSD obtained acceptance criteria so method is rugged.

**CONCLUSION:**

From the above 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 meant in industries, approved testing laboratories studies in near future.

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