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Research Article

Development and Validation of a Stability Indicating RP-HPLC Method for Quantitative Estimation of Apixaban in Tablet Dosage Form

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ABSTRACT
Objective: The present study aimed to develop and validate a simple, accurate, precise, and stability-indicating RP-HPLC method for the quantitative estimation of Apixaban in tablet dosage form.
Methods: Chromatographic separation was achieved using a Phenomenex ODS-3 C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of Methanol (70:30 v/v) at a flow rate of
1.0 mL/min. Detection was carried out at 249 nm using a UV detector. The developed method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, LOD, LOQ, and system suitability.
Results: The developed method showed satisfactory chromatographic performance with good peak symmetry and acceptable retention time. Validation studies demonstrated excellent linearity, accuracy, and precision within the selected concentration range. The method was found to be robust and stability-indicating, capable of separating Apixaban from its degradation products.
Conclusion: The developed RP-HPLC method is simple, rapid, accurate, precise, and suitable for routine quality control analysis of Apixaban in pharmaceutical dosage forms.

Keywords: Apixaban, Rp-Hplc, Method Validation, Stability-Indicating Method, Ich Guidelines, Pharmaceutical Analysis.

INTRODUCTION

Apixaban is a direct oral anticoagulant that selectively inhibits Factor Xa and is widely used for the prevention and treatment of thromboembolic disorders. Reliable analytical methods are required for routine quality control and stability assessment of pharmaceutical formulations. RP-HPLC is one of the most preferred analytical techniques due to its accuracy, precision, sensitivity, and reproducibility. Therefore, the present study focuses on the development and validation of a
Stability-indicating RP-HPLC method for quantitative estimation of Apixaban in tablet dosage forms.

MATERIALS AND METHODS

Sr. No.	Chemicals/ Reagents/ Solvents	Supplier	Grade
1	Methanol	Merck	HPLC grade
2	Acetonitrile	Merck	HPLC grade
3	Water	Siddhi Lab	HPLC grade

1.1 Chemicals and Reagents Apixaban reference standard was used. Methanol, acetonitrile, and HPLC-grade water were employed throughout the study.

1.2 Instrumentation Analysis was performed using an Agilent 1260 Infinity II HPLC system equipped with UV detection and OpenLab EZChrome software.

1.3 Chromatographic Conditions

Column: Phenomenex ODS-3 C18 (250 × 4.6 mm, 5 µm)
Mobile Phase: Methanol (70:30 v/v)
Flow Rate: 1.0 mL/minDetection Wavelength: 249 nm
Injection Volume: 20 µL Column Temperature: 35°C

1.4 Preparation of Standard and Sample Solutions Standard and tablet sample solutions were prepared using methanol and DMSO as diluents according to the developed analytical procedure.

1.5 Method Validation

Validation was carried out according to ICH Q2(R1) guidelines including:

- Specificity
- Linearity
- Accuracy
- Precision
- Robustness
- LOD and LOQ
- System Suitability

RESULTS AND DISCUSSION

Solubility Study Apixaban

Table

Sr. No.	Name OF Solvent	Observation	Conclusion	Summary
1	Water	Drug Particles SEEN AFTER SONICATION	Drug WAS NOT FOUND SOLUBLE IN WATER.	Dmso USED AS A DILUENT FOR PREPARING STOCK SOLUTION.
2	Methanol	Drug Particles SEEN AFTER SONICATION	Drug WAS NOT FOUND SOLUBLE IN METHANOL.	
3	Ethanol	Drug Particles SEEN AFTER SONICATION	Drug WAS NOT FOUND SOLUBLE IN Ethanol.	
4	Acetonitrile	Drug Particles SEEN AFTER SONICATION	Drug WAS NOT FOUND SOLUBLE IN Acetonitrile	
5	0.1 N Hcl	Drug Particles SEEN AFTER SONICATION	Drug WAS NOT FOUND SOLUBLE IN 0.1 N Hcl.	
6	0.1 N NaOH	Drug Particle seen after sonication	Drug was not found soluble in 0.1 N NaOH.	
7	DMSO	No Drug Particles seen after sonication	Drug was found soluble in DMSO.	

Conclusion

DMSO was selected as the solvent for dissolving Apixaban.

Selection of Analytical Wavelength

1) Blank (Solution 1):

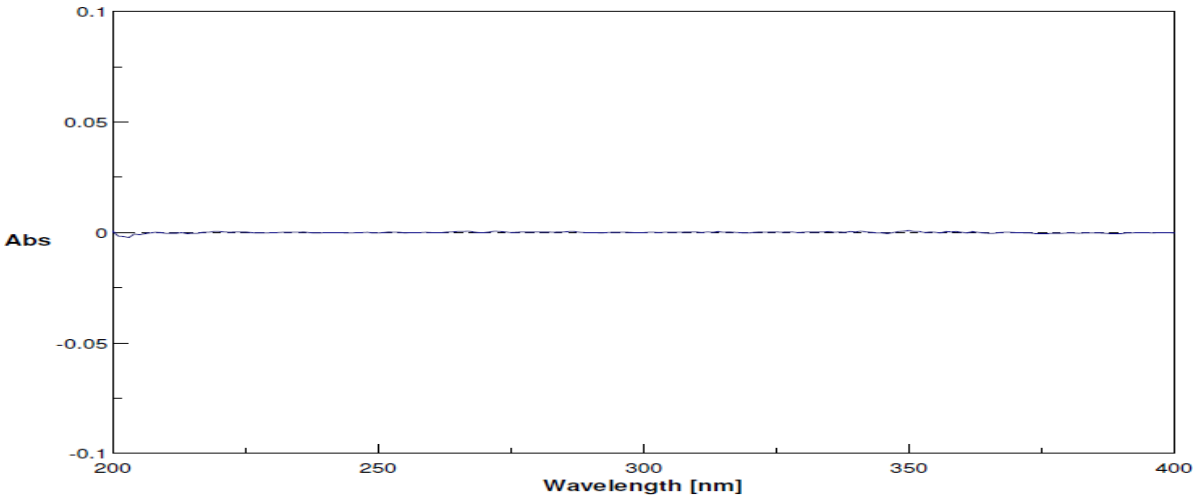

Fig. UV Spectrum of Solution 1 as a Blank

The image displays a UV-Visible absorption spectrum plotted on a graph where the x-axis represents the Wavelength [nm] ranging from 200 to 400 nm, and the y-axis represents Absorbance (Abs**)** ranging from -0.1 to 0.1. The most striking observation is that the spectral line is essentially flat and positioned directly on the zero baseline across the entire measured range. There are no discernible peaks, valleys, or significant absorption features, which indicates that the sample being measured (or perhaps a blank solvent) does not absorb light in the ultraviolet or visible spectrum between 200 nm and 400 nm

Method Development by RP – HPLC

Optimization of HPLC method Trial 1:

Chromatogram:

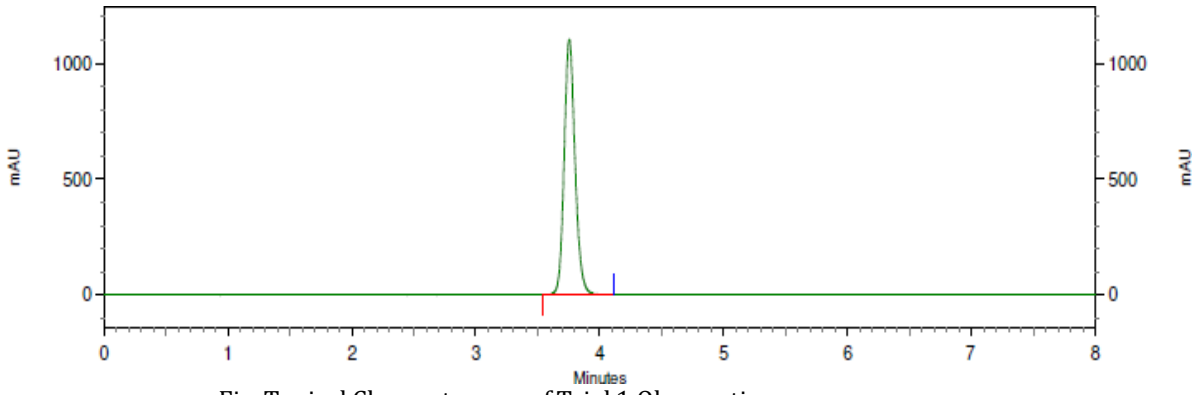

Fig. Typical Chromatogram of Trial 1 Observation:

Apixaban eluted with Good Chromatography.

Conclusion: Method Accepted

The optimized chromatographic conditions produced a sharp and symmetrical peak for Apixaban with satisfactory retention characteristics. The developed method exhibited excellent linearity over the selected concentration range. Accuracy studies demonstrated acceptable percentage recovery values, while precision studies showed low %RSD values. Robustness testing confirmed that minor changes in chromatographic conditions did not significantly affect method performance. The developed method effectively distinguished Apixaban from degradation products, confirming its stability-indicating nature.

CONCLUSION

A simple, rapid, accurate, precise, and stability-indicating RP-HPLC method was successfully developed and validated for quantitative estimation of Apixaban in tablet dosage forms. The method complies with ICH validation requirements and is suitable for routine quality control and stability studies in pharmaceutical industries.

From the observations of trials first, it was concluded that chromatographic conditions in trial first gives better peak, good retention time and tailing factor therefore chromatographic conditions in trial first was used for method validation.

Optimized Chromatographic Conditions

Table

Parameter	Description
Mode	Isocratic
Column Name	Phenomenex ODS-3, 250 Mm X 4.6mm ID, 5 Mm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	249 Nm
Column Oven Temp	35ºc
Mobile Phase	Methanol : Water (70:30% V/V)
Flow Rate	1.0 Ml/Min
Run Time	08 Minutes

REFERENCES

1. Kasture A.V., Wadodkar S. G., K. R. Mahadik., H. N. More., "PharmaceuticalAnalysis", Nirali Prakashan ,2016; 2 (23):2.1- 2.2.
2. Chatten LG, "Pharmaceutical chemistry" Marcel Dekker Inc,1996; 2:23-25.
3. Beckett AH, Stenlake JB, "Practical pharmaceutical chemistry", CBS publisher distributors,1986;2:13-17.
4. David CL, "Pharmaceutical analysis",Black well publishing, 1994;6:2-4.
5. Ahuja S, "Handbook of modern pharmaceutical analysis", Eds.Academic Press,2001; 3 : 383.
6. Basic Education in Analytical Chemistry, Analytical Science, 2001:17
7. Ohannesian L, Streeter J.A., "Drugs and pharmaceutical science", Handbook of pharmaceutical Analysis, 130-149
8. Douglas A; Skoog F;james , H.stanley, "Liquid chromatography in instrumental analysis",Cengage learning India pvt.ltd;2007;9:893-934
9. Malviya R, Bansal V, Pal O.P. and Sharma P.K, "High Performance Liquid Chromatography: A SHORT REVIEW", Journal of Global Pharma Technology. 2010; 2(5): 22-23.
10. Verma Vikrant, "A Review on HPLC and RP-HPLC Analysis Method" International Journal of institutional pharmacy and life sciences,2014;4(4):50-64.
11. Mr. Gorhe S G, Miss. Pawar G R, "A Review on High Performance Liquid Chromatography (HPLC)", International Journal of Advance Scientific Research And Engineering Trends,2018; 3(1):6-7.
12. Santosh Kumar Bhardwaj, K. Dwivedia and D. D. Agarwala, "A Review: HPLC Method Development and Validation", International Journal of Analytical and Bioanalytical Chemistry,2015; 5(4),76-79.
13. Nischal K, Somshekar B, Abhilekha P M, Sharadamma K C, Radhakrishna P M, "A Simple RP-HPLC Method for Estimation of Apixaban and Ivermectin in a
14. Meher saurabhi, MohmmadAbu, Meher Salim, "Development and Validation of a New HPLC-UV Method for the Simultaneous Determination of Apixaban and Ivermectin B1a in a Pharmaceutical Formulation", Journal oF AOAC ,2010; 6(93):1868-1873
15. Lourdes Mottiera,, Laura Morenoa,, Luis Alvareza,, Guillermo Virkela,, Carlos Lanusse, " Measurement of triclabendazole and its metabolites in liver flukes: method development and full validation", Journal of Pharmaceutical and Biomedical Analysis,34(2004);5:991-999
16. Boyka Tsvetkova, Ivanka Pencheva,

Alexander Zlatkov and Plamen Peikov, “Simultaneous high-performance liquid chromatography determination of paracetamol and ascorbic acid in tablet dosage forms”, African Journal of Pharmacy and Pharmacology,2012;6(17):1332-1336.

17. M. Sharaf El-Din, M.Eid, A.M. Zeid, “Development and Validation of RP-HPLC Method for Simultaneous Determination of Ascorbic Acid and Salicylamide in their Binary Mixtures: Application to Combined Tablets”, J Chromat Separation Technique,2012; 3(5):2-7.
18. Shantaram Bonde, Ranjana Bhadane, Sandhya Walke, Nikitra Gupta, “Determination of Vitamin C from formulated drug by HPLC and UV/VIS-Spectrophotometry Techniques”, International Journal f Analytical Pharmaceutical and Biomedical Sciences,2015;4(5):9-13
19. Raymond C Rowe et al, Handbook of Pharmaceutical Excipients,Published by the Pharmaceutical Press;6:71-75.