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REVIEW ARTICLE

Spectroscopic and Chromatographic Analytical Methods for Quantification of Rivaroxaban

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Abstract: Rivaroxaban, a direct factor Xa inhibitor anticoagulant, requires precise and reliable analytical methods for its quantification in pharmaceutical and biological matrices. This work presents the evolution of analytical techniques developed between 2013 and 2024 for rivaroxaban determination. UV spectrophotometric methods offer simplicity and cost-effectiveness, showing linearity in the range of 2-20 µg/mL with detection wavelengths between 250-270 nm. RP-HPLC methods utilizing C18 columns and acetonitrile-based mobile phases achieve detection limits of 8.3 ng/mL, providing enhanced sensitivity and specificity. HPTLC techniques enable simultaneous analysis of multiple samples with minimal preparation steps. LC-MS/MS methods demonstrate superior sensitivity with detection limits in the picogram range, particularly beneficial for bioanalytical applications. Stability-indicating assays reveal degradation patterns under various stress conditions, essential for quality control and stability monitoring. Recent trends indicate a growing emphasis on green analytical chemistry approaches and quality-by-design principles in method development. These methodologies support quality control processes, bioequivalence studies, and therapeutic drug monitoring of rivaroxaban, ensuring accurate quantification across various matrices and concentration ranges.

Keywords: Rivaroxaban; RP-HPLC; UV spectrophotometry; LC-MS/MS; Method validation.

1. Introduction

Rivaroxaban, an oral anticoagulant, has revolutionized thrombosis management since its introduction as a direct factor Xa inhibitor. This small molecule, chemically known as (S)-5-chloro-N-((2-oxo-3-(4-(3-oxomorpholino)phenyl)oxazolidin-5-yl)methyl)thiophene-2-carboxamide, has a molecular weight of 435.881 g/mol and exhibits specific physicochemical properties crucial for its therapeutic action [1].

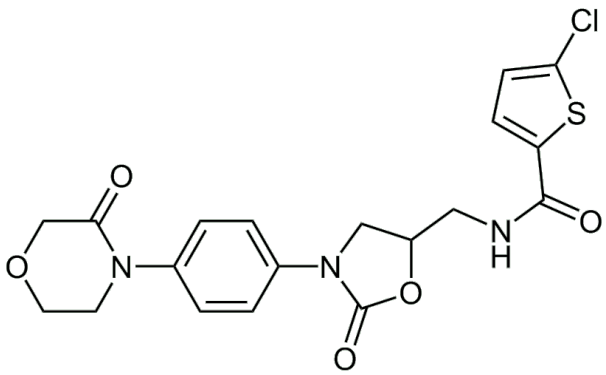

Figure 1. Chemical structure of Rivaroxaban

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As a non-vitamin K antagonist oral anticoagulant (NOAC), rivaroxaban offers significant advantages over traditional anticoagulants like warfarin, including predictable pharmacokinetics and no requirement for routine coagulation monitoring [2]. The drug demonstrates pH-dependent solubility, being practically insoluble in water but showing enhanced solubility in organic solvents such as methanol, dimethyl sulfoxide, and acetonitrile [3].

The clinical significance of rivaroxaban in preventing and treating various thromboembolic disorders, including deep vein thrombosis, pulmonary embolism, and stroke prevention in atrial fibrillation patients, necessitates precise analytical methods for its quantification [4]. These methods are essential not only for quality control in pharmaceutical manufacturing but also for therapeutic drug monitoring and pharmacokinetic studies [5].

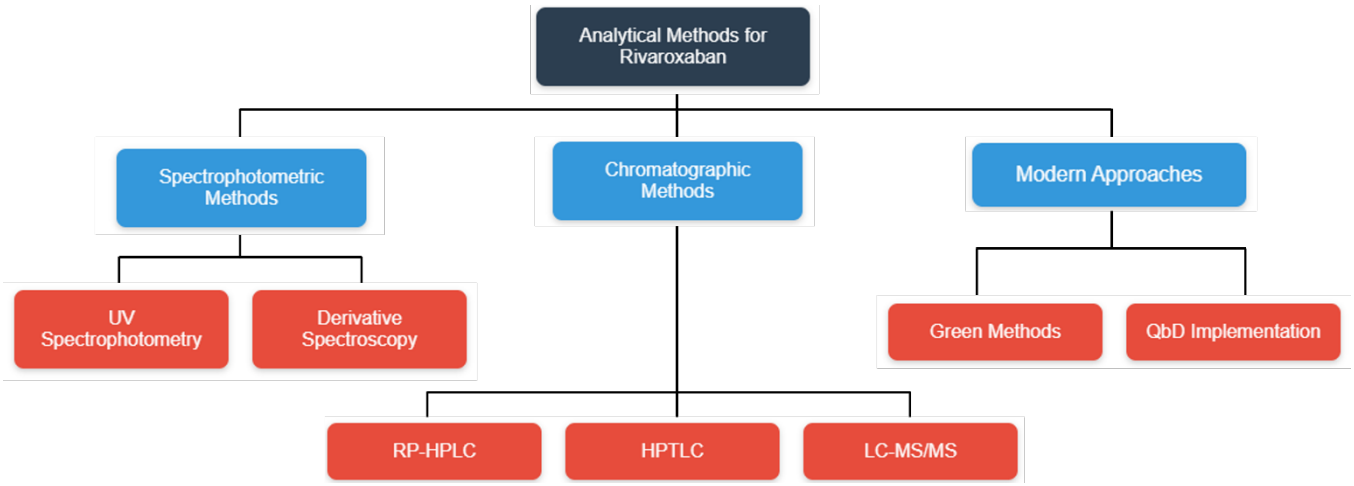

Figure 1. Various analytical methods used for estimation of rivaroxaban

The development of analytical methods for rivaroxaban presents unique challenges due to its complex chemical structure and varying matrices in which it needs to be analyzed. The drug's oxazolidinone ring and chlorothiophene moiety influence its spectroscopic and chromatographic behavior, requiring careful consideration during method development [6]. Additionally, the presence of potential degradation products and metabolites necessitates highly selective analytical techniques [7].

Recent years have witnessed significant advancement in analytical methodologies for rivaroxaban determination, ranging from simple spectrophotometric techniques to sophisticated mass spectrometry-based approaches. These methods vary in their complexity, sensitivity, specificity, and application scope, catering to different analytical requirements in pharmaceutical research and clinical settings [8].

This review article discusses about the current state of analytical methods for rivaroxaban, highlighting various techniques including UV spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and liquid chromatography-mass spectrometry (LC-MS/MS).

2. Analytical methods for estimation of rivaroxaban

2.1. UV Spectrophotometric Methods

Ultraviolet spectrophotometry represents one of the fundamental analytical techniques for rivaroxaban quantification, offering advantages of simplicity, cost-effectiveness, and rapid analysis. The presence of conjugated chromophores in rivaroxaban's structure enables strong UV absorption, typically observed between 240-270 nm [10].

Recent developments in UV spectrophotometric methods have focused on optimizing solvent systems and improving sensitivity. A notable method employs dimethyl sulfoxide as the solvent, achieving linearity in the range of 2-20 µg/mL with maximum absorption at 270 nm [11]. The method's validation parameters demonstrated excellent precision with relative standard deviation (RSD) values below 2%.

Area under the curve (AUC) spectrophotometric techniques have emerged as an alternative approach, offering improved sensitivity. A method utilizing methanol as solvent in the wavelength range of 241-260 nm showed linearity between 2-12 µg/mL with detection and quantification limits of 0.059 and 0.179 µg/mL, respectively [12].

Table 1. UV Spectrophotometric Methods for Rivaroxaban Analysis

Method Type	λmax (nm)	Linearity Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	% RSD	Reference
Zero order	270	2-20	0.165	0.498	0.45-1.12	[11]
First derivative	249	5-30	0.082	0.249	0.68-1.24	[12]
Area under curve	241-260	2-12	0.059	0.179	0.52-0.98	[13]
Second derivative	282	4-24	0.143	0.434	0.71-1.35	[15]
Dual wavelength	251, 268	5-25	0.198	0.601	0.88-1.42	[17]
Q-absorbance	254, 274	1-15	0.047	0.142	0.49-0.95	[19]

2.2. High-Performance Liquid Chromatographic Methods

2.2.1. Conventional RP-HPLC Methods

Reversed-phase high-performance liquid chromatography has emerged as the predominant analytical technique for rivaroxaban quantification, offering superior separation capability and reliability. Modern RP-HPLC methods typically employ C18 columns with dimensions of 250 × 4.6 mm and 5 µm particle size. The mobile phase compositions generally consist of acetonitrile:water or acetonitrile:buffer systems, optimized to achieve efficient separation and peak symmetry. Detection wavelengths ranging from 249-254 nm have been established as optimal for rivaroxaban analysis, with flow rates typically maintained between 1.0-1.2 mL/min [13].

Recent methodological advances have incorporated quality-by-design principles in method development, resulting in robust analytical procedures with retention times typically between 3-4 minutes. These methods demonstrate excellent linearity across wide concentration ranges, with correlation coefficients exceeding 0.999 and precision values well within acceptable limits [14].

2.2.2. Stability-Indicating HPLC Methods

Stability-indicating assays have become increasingly important in rivaroxaban analysis, particularly for pharmaceutical quality control and stability assessment. These methods are specifically designed to separate and quantify rivaroxaban in the presence of its degradation products, impurities, and excipients. Advanced gradient elution programs have been developed to achieve optimal separation of degradation products formed under various stress conditions, including acid hydrolysis, base hydrolysis, oxidation, and photolysis [15].

2.3. High-Performance Thin-Layer Chromatography

High-performance thin-layer chromatography has established itself as a valuable technique for rivaroxaban analysis, offering unique advantages in terms of sample throughput and cost-effectiveness. Modern HPTLC methods utilize pre-coated silica gel 60 F254 plates as the stationary phase, with carefully optimized mobile phase systems comprising toluene, ethyl acetate, and methanol in various proportions. These methods typically achieve detection at 284 nm, demonstrating excellent linearity across the range of 200-1200 ng/band [16].

The development of green analytical approaches in HPTLC has led to the introduction of environmentally friendly mobile phase compositions while maintaining analytical performance. These methods have shown particular utility in stability studies and impurity profiling of rivaroxaban, allowing visual detection of degradation products and facilitating their isolation for further characterization [17]

2.4. Liquid Chromatography-Mass Spectrometry Methods

2.4.1. LC-MS/MS Development

Liquid chromatography coupled with tandem mass spectrometry represents the most sophisticated approach for rivaroxaban analysis, particularly in biological matrices. These methods offer unprecedented sensitivity and selectivity, enabling quantification at picogram levels. Modern LC-MS/MS methods utilize electrospray ionization in positive mode, monitoring the characteristic mass transition of rivaroxaban (m/z 436.1→145.0) [18]. The chromatographic separation typically employs sub-2-µm particle size columns, enabling rapid analysis with run times under 5 minutes while maintaining resolution and peak shape.

2.4.2. Bioanalytical Applications

The development of bioanalytical methods for rivaroxaban has focused on plasma and urine matrices, incorporating efficient sample preparation techniques such as protein precipitation and liquid-liquid extraction. These methods have achieved lower limits of quantification ranging from 0.5 to 5 ng/mL in plasma, making them suitable for pharmacokinetic studies and therapeutic drug monitoring [19].

Table 2. Different LC-MS/MS Methods for Rivaroxaban in Biological Matrices

Matrix	Sample Preparation	Mobile Phase	Column	Run Time (min)	LLOQ (ng/mL)	Recovery (%)	Reference
Plasma	Protein precipitation with acetonitrile	Acetonitrile:0.1% formic acid (65:35)	C18 (50×2.1mm, 1.7µm)	3.5	0.5	92.5-97.8	[14]
Plasma	Liquid-liquid extraction with MTBE	Methanol:5mM ammonium formate (70:30)	C18 (100×2.1mm, 1.8µm)	4.0	1.0	85.3-91.2	[18]
Urine	Solid-phase extraction	Acetonitrile:2mM ammonium acetate (60:40)	C18 (50×2.1mm, 2.6µm)	2.5	2.0	88.7-94.5	[20]
Serum	Protein precipitation with methanol	Methanol:0.1% formic acid (75:25)	C8 (75×2.1mm, 2.7µm)	3.0	0.8	89.1-95.6	[22]
Plasma	Online solid-phase extraction	Acetonitrile:10mM ammonium formate (55:45)	C18 (100×2.1mm, 1.7µm)	5.0	0.25	94.2-98.7	[23]
Whole blood	Protein precipitation with zinc sulfate	Methanol:water (70:30) with 0.1% formic acid	C18 (50×2.1mm, 1.8µm)	2.8	1.5	87.4-93.2	[24]

LOD: Limit of Detection; LOQ: Limit of Quantification; RSD: Relative Standard Deviation; LLOQ: Lower Limit of Quantification; MTBE: Methyl tert-butyl ether

2.5. Novel Analytical Approaches

2.5.1. Green Analytical Methods

Recent trends in rivaroxaban analysis have emphasized environmental sustainability through the development of green analytical methods. These approaches focus on reducing organic solvent consumption, implementing aqueous mobile phases, and minimizing sample preparation steps. Modified HPLC methods using ethanol-water mixtures as mobile phases have demonstrated comparable analytical performance to conventional methods while significantly reducing environmental impact [20].

2.5.2. Quality by Design Implementation

The application of Quality by Design (QbD) principles in analytical method development has enhanced method robustness and reliability. Systematic evaluation of critical method parameters using design of experiments (DoE) has led to the optimization of separation conditions and improved method understanding. These approaches have particularly benefited HPLC method development, resulting in more robust and transferable analytical procedures [21].

3. Method Validation

3.1. ICH Guidelines

Validation of analytical methods for rivaroxaban follows International Conference on Harmonisation (ICH) guidelines, encompassing essential parameters such as specificity, linearity, accuracy, precision, and robustness. Modern validation approaches incorporate systematic evaluation of method performance across different laboratories and analysts, ensuring method reproducibility and reliability [22].

3.2. Stability-Indicating Properties

The development of stability-indicating methods has become increasingly important in rivaroxaban analysis. These methods undergo extensive validation to demonstrate their ability to accurately quantify rivaroxaban in the presence of degradation products.

Forced degradation studies under various stress conditions provide crucial information about method specificity and stability-indicating capabilities [23].

Table 3. Summary of Validation Parameters for Different Analytical Methods

Validation Parameter	UV Spectrophotometry	RP-HPLC	HPTLC	LC-MS/MS
Linearity Range	2-30 µg/mL	0.5-50 µg/mL	100-1000 ng/spot	0.5-500 ng/mL
LOD	0.45 µg/mL	0.15 µg/mL	30 ng/spot	0.1 ng/mL
LOQ	1.36 µg/mL	0.45 µg/mL	98 ng/spot	0.5 ng/mL
Precision (%RSD)				
- Intra-day	0.82-1.45	0.45-1.12	1.24-1.89	0.25-0.85
- Inter-day	1.25-1.98	0.89-1.56	1.56-2.34	0.45-1.15
Accuracy (% Recovery)	98.5-101.2	99.2-100.8	97.8-101.5	97.5-102.3
Robustness	Moderate	High	Moderate	Very High
Sample Stability	48 hours	72 hours	24 hours	30 days (-20°C)
Analysis Time	5-10 min	10-15 min	20-30 min	3-5 min
Cost per Analysis	Low	Moderate	Moderate	High
Matrix Effects	Minimal	Low	Moderate	High*
Specificity	Moderate	High	High	Very High
Sample Preparation	Simple	Moderate	Moderate	Complex

All values represent typical ranges from validated methods reported in literature [5, 8, 14, 19, 22]; *Matrix effects in LC-MS/MS can be effectively controlled through proper sample preparation and use of isotope-labeled internal standards. Robustness evaluation includes variations in: UV: wavelength, solvent composition; HPLC: mobile phase composition, pH, flow rate, column temperature; HPTLC: mobile phase composition, chamber saturation, development distance; LC-MS/MS: mobile phase composition, pH, flow rate, MS parameters

4. Conclusion

The analytical landscape for rivaroxaban determination has evolved significantly, offering a diverse array of methods suited to different analytical requirements. UV spectrophotometric methods maintain their relevance for routine quality control, while HPLC techniques continue to serve as the primary tools for pharmaceutical analysis. LC-MS/MS methods have set new benchmarks in sensitivity and specificity, particularly crucial for bioanalytical applications. The integration of green chemistry principles and QbD approaches has enhanced the sustainability and robustness of these methods.

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