



NARRATIVE REVIEW: METHODS FOR MONITORING AND ANALYZING WARFARIN IN BLOOD

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ABSTRACT

Warfarin is an oral anticoagulant with a narrow therapeutic index and high interindividual variability in pharmacokinetic and pharmacodynamic responses, requiring careful therapeutic monitoring to maintain efficacy and minimize adverse events. In clinical practice, anticoagulation monitoring is generally performed using the International Normalized Ratio (INR); however, INR values may be influenced by various factors such as drug interactions, dietary vitamin K intake, patient adherence, and genetic polymorphisms, potentially limiting its accuracy as a sole monitoring parameter. Therefore, direct measurement of plasma warfarin concentrations has gained attention as an additional approach to optimize individualized therapy. This narrative review aimed to evaluate and compare analytical methods used for monitoring and analyzing warfarin levels in blood, particularly high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Literature sources were obtained from PubMed and selected based on relevance to analytical methods for warfarin quantification published within the last ten years. The reviewed studies demonstrated that LC–MS/MS provides superior sensitivity, specificity, and low detection limits for accurate quantification of warfarin and its metabolites, while HPLC methods remain reliable, accurate, and applicable in routine clinical laboratories with more limited instrumentation. Several studies also reported that plasma warfarin concentration, especially the unbound fraction, may better reflect anticoagulant activity and bleeding risk in certain patient populations compared with INR alone. In conclusion, chromatographic methods are valuable tools for therapeutic drug monitoring of warfarin and may improve individualized anticoagulant therapy when combined with conventional INR monitoring.

Keywords: Warfarin; Therapeutic Drug Monitoring; HPLC; LC–MS/MS; Anticoagulant Therapy

1. INTRODUCTION

Warfarin is an orally administered anticoagulant that acts by inhibiting vitamin K activity and is widely used in the management of venous thromboembolism and systemic embolic conditions, particularly among individuals with atrial fibrillation or prosthetic cardiac valves. Its mechanism of action involves disruption of the vitamin K cycle, which prevents the activation of the vitamin K dependent coagulation factors, namely factors II, VII, IX, and X (Sun et al., 2006). Because the drug has a very narrow margin between effective and harmful doses, continuous evaluation of its therapeutic effect is

essential. In clinical practice, monitoring of warfarin therapy is primarily performed using the International Normalized Ratio (INR), which serves as the standard indicator for assessing anticoagulant response and dose adjustment (Barnes et al., 2018). The clinical management of warfarin remains complex due to its narrow safety range and its extensive susceptibility to interactions with other medications. Such interactions may significantly alter its metabolism, particularly through cytochrome P450-mediated oxidative pathways, and can result in severe adverse reactions, including life-threatening complications (Malátková et al., 2016). However, despite its widespread use, INR monitoring presents several important limitations. INR values may be influenced by dietary vitamin K intake, drug–drug interactions, genetic polymorphisms, hepatic function, patient adherence, and variability in anticoagulant sensitivity, which can reduce its reliability as a sole monitoring parameter. In addition, INR reflects the pharmacodynamic response to warfarin rather than directly measuring plasma drug concentrations, making it less effective in identifying certain conditions such as anticoagulant resistance, altered metabolism, or suspected toxicity.

Therapeutic drug monitoring (TDM) is an essential component of precision medicine that aims to optimize drug therapy by measuring drug concentrations in biological fluids and adjusting dosages according to individual patient needs. In current clinical practice, TDM is routinely applied to medications with a narrow therapeutic index, marked pharmacokinetic variability, or a well-established relationship between drug concentration and therapeutic response (Mernissi et al., 2023). It is widely implemented in various clinical settings, including the management of immunosuppressants in transplant recipients, antiepileptic drugs, antimicrobial agents, antineoplastic therapies, and selected cardiovascular medications. By integrating laboratory measurements with clinical assessment, TDM helps maximize therapeutic efficacy while minimizing the risk of toxicity and treatment failure. The increasing adoption of TDM has also driven the development of highly sensitive and selective analytical methods capable of accurately quantifying drug concentrations in biological matrices, particularly chromatographic techniques coupled with advanced detection systems.

Maintaining warfarin therapy within its therapeutic target is crucial because fluctuations in International Normalized Ratio values may cause inadequate anticoagulation leading to thrombosis or excessive anticoagulation resulting in bleeding complications. The International Normalized Ratio measures the time required for blood to clot and serves as a primary clinical parameter for evaluating anticoagulant effect and dosage appropriateness. Patients with atrial fibrillation are generally recommended to maintain an International Normalized Ratio between 2.0 and 3.0, while individuals with mechanical heart valves require different targets. Those with mechanical aortic valves typically require a range of 2.0 to 3.0, whereas patients with mechanical mitral valves often need a target range of 2.5 to 3.5 (Kokash et al., 2025). Although widely used, the International Normalized Ratio is influenced by dietary intake, drug interactions, reduced anticoagulant sensitivity, and patient adherence, which may limit its reliability as a sole monitoring tool. To overcome the limitations of INR monitoring, measurement of plasma warfarin concentrations has increasingly been proposed as a complementary strategy to support individualized therapy and improve clinical decision making, particularly in patients with poor adherence, suspected toxicity, renal impairment, or significant pharmacokinetic variability (Denooz et al., 2009).

Several analytical techniques have been developed to quantify warfarin concentrations, including high-performance liquid chromatography (HPLC) and high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Among these, HPLC-MS/MS with electrospray ionization provides excellent analytical sensitivity, specificity, accuracy, and relatively short analysis times. Each analytical approach has distinct advantages and limitations in terms of sensitivity, analytical performance, instrument complexity, operational cost, and suitability for routine therapeutic drug monitoring (Sun et al., 2006). Despite the widespread clinical use of warfarin, variability in analytical methods and the absence of a comprehensive synthesis of their analytical performance present challenges in selecting the most appropriate technique for clinical and laboratory applications. This review addresses

this clinical and analytical gap by evaluating the currently available methods for measuring warfarin concentrations in blood and discussing their relevance to therapeutic drug monitoring and individualized anticoagulant therapy. This narrative review aims to provide an integrated evaluation of chromatographic methods for monitoring warfarin concentrations in blood, with particular emphasis on their analytical characteristics, pharmacokinetic and pharmacodynamic relevance, and potential clinical applications in optimizing anticoagulant therapy.

2. METHODS

This narrative review was conducted through a structured literature search to identify studies describing analytical methods for warfarin quantification in blood and plasma and their clinical applications in therapeutic drug monitoring. The literature search was performed using the PubMed database with the keywords “Warfarin AND Analysis AND Blood Plasma” and “Warfarin AND Determination AND Blood Plasma.” The search included articles published between January 2015 and May 2025 and was limited to English-language, full-text original research articles. The inclusion criteria comprised original studies evaluating analytical methods for warfarin quantification in blood or plasma samples, as well as studies discussing the pharmacokinetic, pharmacodynamic, or clinical aspects of warfarin monitoring. The exclusion criteria included review articles, conference abstracts, duplicate publications, studies unrelated to warfarin analysis in blood or plasma, and articles with incomplete methodological or analytical data.

The initial search identified 52 records from the PubMed database. After removing 4 duplicate records, 48 articles underwent title and abstract screening. Of these, 28 articles were excluded because they did not meet the inclusion criteria. The remaining 20 full-text articles were assessed for eligibility, and 10 articles were further excluded due to incomplete analytical data, lack of relevance to warfarin quantification in blood or plasma, or failure to meet the predefined eligibility criteria. Finally, 10 studies were included in the narrative synthesis. The literature search and selection process are summarized in the PRISMA flow diagram ([Figure 1](#)).

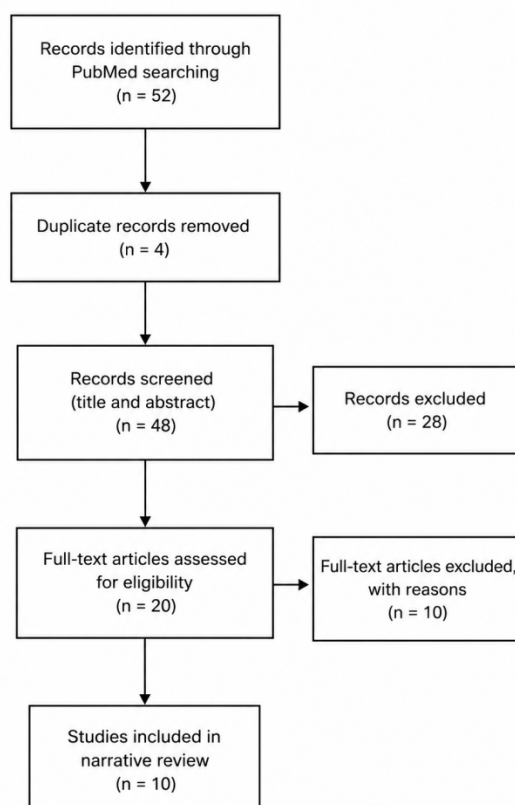


Figure 1. Flow Diagram

Data extracted from the included studies comprised publication characteristics, analytical methods, biological matrices, sample preparation procedures, chromatographic conditions, analytical performance, and clinical relevance. The findings were synthesized narratively to compare the strengths, limitations and potential clinical applications of each analytical method for monitoring warfarin concentrations in blood (Table 1 and Table 2).

Table 1. Monitoring Methods for Warfarin Drug Levels from Several Literature Studies

Analysis Methods	Results	References
Reversed-Phase LC-MS/MS with Electrospray Ionization (ESI) Mode - Human plasma - Standard: Warfarin-d6 (Isotopic Purity 98%) - Column: - Raptor Biphenyl column, size 100 × 2.1 mm, particle size 2.7 µm - Raptor Biphenyl column, size 5 × 2.1 mm, particle size 2.7 µm - Mobile Phase: - Methanol - Cold Acetonitrile 0.002% Formic Acid 2 mM Ammonium Formate - Elution: Gradient	The method has been fully validated and demonstrates rapid, sensitive, accurate, and specific analytical performance, enabling reliable characterization of variations in both free and total plasma warfarin concentrations, which are theoretically associated with its pharmacological activity and potential toxicity.	(Mernissi et al., 2023)
LC-MS/MS - Human plasma - Standard: Warfarin in sample Volumetric absorptive microsampling (VAM) using quercetin as internal standard - Column: UPLC BEH C18 - Mobile phase: A mixture consisting of 0.1 percent formic acid in water, acetonitrile, and methanol in a volumetric ratio of 30 to 69 to 1. (v/v) - Elution: Isocratic	The method demonstrated strong linearity, accuracy, and precision across the concentration range of 5 to 500 ng/mL. Warfarin concentrations measured using VAMS samples varied from 6.05 to 431.39 ng/mL. No meaningful association or strong correlation was identified between warfarin levels obtained from VAMS and International Normalized Ratio values, nor between the administered dose and International Normalized Ratio outcomes. However, a moderate yet statistically significant correlation was found between VAMS derived warfarin concentrations and the prescribed warfarin dose.	(Harahap et al., 2024)
UPHPLC - Human Plasma - Standard: - - Column: Zorbax C18 (2.1, 50mm, 2.7 µm) - Mobile phase: 65% Formic acid: Acetonitrile (65 35) - Elution: Gradient - Wavelength: warfarin: 310 nm alcohol warfarin: 390 nm	This study clarified the enzymatic pathways responsible for the reductive metabolism of warfarin in human hepatic tissue, resulting in the formation of distinct warfarin alcohol stereoisomers. The cytosolic fraction, together with the aldo ketoreductase isoenzyme AKR1C3, mediated the stereoselective reduction of the R enantiomer of warfarin, yielding SR warfarin alcohol as the predominant product. In contrast, the microsomal fraction and the carbonyl reductase enzyme CBR1 catalyzed the reduction of the S enantiomer, generating both RS warfarin alcohol and SS warfarin alcohol.	(Malátková et al., 2016)
HPLC - Human plasma - Detector: fluorescence - Standard: Warfarin - Internal Standard: Griseofulvin - Column: - Chiralcel OD RH analytical	Griseofulvin was selected as the internal standard because it shares key physicochemical properties with warfarin, including water insolubility and a comparable log P value. The retention times were 6–7.5 minutes for griseofulvin, 10–11.5 minutes for R warfarin, and 14–16 minutes for S warfarin. The method demonstrated linearity within the	(Poojar et al., 2017)

column, 4.6×150 mm, $5 \mu\text{m}$ 2. Chiralcel OD RH guard column, 4.0×10 mm, $5 \mu\text{m}$ • Mobile phase: Acetonitrile and phosphate buffer at pH 2 in a 40 to 60 ratio, delivered isocratically at 1 mL per minute Injection volume: 20 μL • Elution: Gradient • Wavelength: Warfarin: excitation at 310 nm and emission at 350 nm Griseofulvin: excitation at 300 nm and emission at 400 nm	concentration range of 2.2 to 3 ppm. The calibration curve for R warfarin yielded the equation $y = 0.0705x + 0.0704$ with a coefficient of determination of 0.978, whereas S warfarin produced $y = 0.0513x + 0.0297$ with a coefficient of determination of 0.9924. Validation results confirmed that the analytical method met the required performance criteria, with limits of detection of 0.0674 ppm for R warfarin and 0.0897 ppm for S warfarin, and limits of quantification of 0.225 ppm and 0.298 ppm, respectively. The mean plasma concentrations were 0.76 ± 1.87 micrograms per millilitre for R warfarin and 0.59 ± 0.81 micrograms per millilitre for S warfarin. In participants receiving the standard dose, the average concentrations were 0.68 ± 0.61 micrograms per millilitre for R warfarin and 0.52 ± 0.42 micrograms per millilitre for S warfarin. Overall, these findings indicate that the method is reliable and appropriate for determining warfarin concentrations in pharmacokinetic studies.	
LC - MS/MS • Rat plasma • Standard: Warfarin • Internal Standard: Diclofenac sodium • Column: Hypersil Chiral - OT (4.6 mm x 150 mm, $3 \mu\text{m}$) • Mobile phase: Formic acid 0.1%: Acetonitrile V/V (60%:40%) • Elution: Isocratic	This method successfully resolves the major warfarin metabolites, including 7 hydroxy warfarin and 10R hydroxy warfarin, thereby demonstrating the prominent contributions of the CYP2C9 and CYP3A enzyme systems in warfarin biotransformation. The results further indicate that the plasma concentration of S warfarin is consistently higher than that of R warfarin.	(Jin et al., 2022)
HPLC with fluorescence detector (HPLC - FLD) • Human plasma • Standard: Pure Warfarin from WAKO brand • Internal Standard: Griseofulvin • Column: Chiralcel OD-RH analytical column (4.6×150 mm, $5 \mu\text{m}$) equipped with a Chiralcel OD-RH guard column., • Mobile phase: Acetonitrile and phosphate buffer pH 2.0 in a 40:60 ratio. • Elution: Isocratic	A robust analytical method was successfully established to quantify the enantiomers of warfarin, namely R-warfarin and S-warfarin, in human plasma in order to characterize differences in their metabolic pathways mediated by cytochrome P450 enzymes. The method demonstrated high sensitivity, selectivity, precision, and overall validity, employing a protein-precipitation sample preparation procedure with acetonitrile that met the validation requirements set by the European Medicines Agency. Excellent linearity was obtained across the concentration range of 100 to 2500 ng/mL, supported by acceptable intra-day and inter-day precision and accuracy values, with percent relative standard deviation and percent difference remaining below 15%.	(Putriana et al., 2022)
HPLC-MS/MS • Human plasma • Standard: Warfarin • Column: Chiral column • Mobile phase: Formic acid 0.1% in water and acetonitrile (60:40, v/v) • Elution: Isocratic	Through a genome-wide association study (GWAS) approach involving research subjects from Sub-Saharan African patients, it was successfully identified that genetic variants in the CYP2C9 gene, namely the CYP2C98, CYP2C99, CYP2C911 genes, could affect the metabolite levels of warfarin drugs and their therapeutic effects.	(Asiimwe et al., 2022).
HPLC • Human plasma • Standard: Warfarin and Phenytoin	The comparison of the CYP2C911 allele in relation to phenytoin and S-warfarin shows an effect on the metabolism of S-warfarin, influencing enzyme	(Wanounou et al.,

• Column: Chiral ART Cellulose-SC column, 250 × 4.6 mm, 5 µm particle size • Mobile Phase: n-hexane, ethanol, and acetic acid (90:10:1, v/v/v) • Elution: Isocratic • Wavelength: 308 nm for warfarin	polymorphism and warfarin concentration in the blood, but not affecting phenytoin. (2022)
LC/MS • Rat plasma • Standard: Warfarin • Internal Standard: Caffeic acid • Column: Chiralpak AD-RH column, 4.6 × 150 mm, 5 µm particle size • Mobile Phase: - Phase A: 0.01% formic acid in water - Phase B: Formic acid in acetonitrile • Elution: Gradient 0.0 – 1.0 min: A/B 80:20 1.1 – 4.0 min: A/B 20:80 4.1 – 8.0 min: A/B 80:20	The concurrent consumption of cranberry juice with warfarin may lead to pharmacokinetic and pharmacodynamic interactions. Cranberry can reduce warfarin levels, decrease AUC and C _{max} , and diminish its anticoagulant effect as indicated by a lower INR value. (Yu et al., 2021)
HPLC–FLD • Rat plasma • Standard: Warfarin • Internal Standard: Naproxen • Diphenyl column, 150 × 4.6 mm, 3 µm particle size • Mobile Phase: 25 mmol/L phosphate buffer, methanol, and acetonitrile (70:20:10, v/v/v), pH 7.4 • Elution: Isocratic	The method developed in this study was capable of accurately measuring and separating warfarin and 7-hydroxywarfarin levels, with method validation showing acceptable accuracy (% error) and precision (% RSD) within the permissible limit of <15%. (Zayed et al., 2020)

Table 2. Summary of Warfarin Level Monitoring Studies

Sample (N)	Pharmacokinetic Parameters	Pharmacodynamic Parameters	Clinical Values and Implications	Reference
50 end-stage CKD patients undergoing regular hemodialysis	Free and total warfarin concentrations in hemodialysis patients' plasma Interindividual variability in warfarin concentrations Influence of plasma albumin on total warfarin levels	International Normalized Ratio (INR) values Correlation between free drug levels and INR INR stability in chronic kidney disease (CKD) patients	Warfarin-treated CKD patients require drug level monitoring to reduce the risk of bleeding not reflected by INR values and to optimize therapy outcomes	(Mernissi et al., 2023)
25 patients receiving maintenance warfarin anticoagulant therapy	Steady-state concentration (C _{ss}) of warfarin in blood	25 patients receiving maintenance warfarin anticoagulant therapy	Steady-state concentration (C _{ss}) of warfarin in blood	(Harahap et al., 2024)
77 outpatients from the cardiology clinic of a hospital in Bandung	C _{max} , T _{max} , t _{1/2} , AUC, Cl, V _d	INR values Relationship between R- and S-warfarin enantiomer concentrations and INR values	Monitoring warfarin levels based on R- and S-enantiomer analysis can optimize individualized dosing and reduce the risk of therapeutic failure	(Poojar et al., 2017)

8 male rats (200 g)	C _{max} , T _{max} , t _{1/2} , AUC, Cl, F, V _d	Not reported	Pharmacokinetics preclinical trials	(Jin et al., 2022)
6 healthy individuals (not specified in detail)	Not reported	Not reported	Validated the quantitative method for warfarin measurement in plasma	(Putriana et al., 2022)
548 patients	Plasma concentration of warfarin enantiomers Total concentration of warfarin levels Concentration ratio of warfarin	Not reported	Genetic variants in CYP2C9 and CYP2C92 influence warfarin metabolite levels	(Asiimwe et al., 2022)
150 patients	AUC, Cl, F, t _{1/2}	Genotype distribution of VKORC1 in CYP2C9 polymorphisms	Genetic variants in the CYP2C9 gene can affect warfarin levels and increase the risk of toxicity	(Wanounou et al., 2022)
21 rats for pharmacokinetic testing 32 rats for pharmacodynamic testing	C _{max} , AUC, t _{1/2}	INR values	Warfarin and cranberry juice if consumed simultaneously can cause interactions that can affect warfarin levels	(Yu et al., 2021)
6 male rats	C _{max} , T _{max} , AUC, AUM, MRT, t _{1/2} , CL/F, V _d /L	Not reported	Warfarin therapy can be optimized by monitoring warfarin and its metabolites to better predict anticoagulant effects	(Zayed et al., 2020)

3. RESULTS AND DISCUSSION

Warfarin is an anti-coagulant drug with a narrow therapeutic index which in each individual has different pharmacokinetic variability and the risk of drug and food interactions is high, therefore it is necessary to monitor warfarin drug levels in plasma to optimize therapy and minimize the risk of toxicity. The parameter for successful warfarin therapy is the International Normalized Ratio (INR) value. Many factors can affect the INR, namely variability in vitamin K diet, patient compliance, other drug interactions and genetic polymorphisms that affect metabolism (CYP2C9 and VKORC1). This causes the monitoring of warfarin therapy should be done directly by measuring warfarin levels in plasma so as to see the pharmacokinetic and pharmacodynamic parameters.

Therapeutic drug monitoring (TDM) is an essential component in optimizing warfarin therapy, particularly to achieve and maintain the International Normalized Ratio (INR) within the therapeutic range. Warfarin has a narrow therapeutic index and exhibits high interindividual variability in response, meaning that the same dose may produce different anticoagulant effects among patients. Therefore, the implementation of TDM is necessary to enable individualized dose adjustment and to improve therapeutic outcomes (N. A. Putriana et al., 2025).

Research findings indicate that patients with chronic kidney disease (CKD) receiving warfarin exhibit high variability in plasma drug concentrations, with a tendency toward an association between free warfarin levels and INR values. These findings suggest that the success of anticoagulant therapy, as assessed by the achievement of therapeutic INR levels, is influenced by blood drug concentrations, particularly the free fraction that directly determines

pharmacological effects. Consequently, TDM plays a crucial role as part of therapeutic evaluation to support a more comprehensive assessment of warfarin therapy outcomes in patients with CKD (Mernissi et al., 2023).

Harahap et al. (2024) emphasize that INR monitoring alone is insufficient to fully describe the effectiveness and safety of warfarin therapy and support the use of therapeutic drug monitoring based on warfarin blood concentration measurements as an additional approach for therapy evaluation, especially in clinical conditions characterized by unstable or dose-incongruent responses. Numerous studies have demonstrated that warfarin therapy is associated with high interindividual variability in response, particularly in patients with complex clinical conditions, and that INR monitoring alone does not fully reflect the pharmacological profile or bleeding risk. In outpatient clinical practice, the use of relatively uniform warfarin doses still results in wide variability in plasma concentrations of R-warfarin and S-warfarin (N. Putriana et al., 2022). These findings underscore that the success of warfarin therapy, as evaluated by the attainment of therapeutic INR levels, is influenced by blood drug concentrations. Therefore, studies examining the relationship between plasma warfarin concentrations and therapeutic response (INR) are essential to strengthen the role of therapeutic drug monitoring as part of the evaluation and optimization of warfarin therapy.

3.1. LC-MS/MS Analytical Method

The quantification of warfarin using this method has been shown to be highly sensitive and specific for detecting both free and total warfarin levels in plasma, with high accuracy and precision and a short retention time. Several studies (Mernissi et al., 2023; Harahap et al., 2024) have shown that LC-MS/MS provides high accuracy for measuring both free and total warfarin concentrations within the ng/mL range, with a very low limit of detection (LOD). This method can be used to monitor variability in warfarin levels, which may not always correlate with INR values from both pharmacokinetic and pharmacodynamic perspectives. It is applicable in clinical practice for specific populations, particularly patients undergoing haemodialysis who are on warfarin therapy, where close monitoring is necessary to detect potential bleeding risks (Mernissi et al., 2023). In addition, this method has demonstrated that S-warfarin concentrations are higher than R-warfarin in rat plasma, using a Chiral-OT column capable of separating hydroxylated warfarin metabolites such as 7-hydroxywarfarin and 10(R)-hydroxywarfarin, as well as distinguishing the metabolic activity of CYP2C9 and CYP3A enzymes (Jin et al., 2022). Warfarin is a drug with a narrow therapeutic index and is prone to interactions when taken concurrently with certain drugs or foods. A study using LC/MS analysis in rat plasma showed that co-administration of cranberry juice with warfarin resulted in pharmacokinetic changes, such as decreased AUC and C_{max}, and a reduced pharmacodynamic response, indicated by lower INR values (Yu et al., 2021).

3.2. HPLC Analytical Method

HPLC has been validated with good linearity and sensitivity values using Zorbax C18 columns at different wavelengths, enabling the detection of warfarin stereoisomeric metabolites in different forms to see the variability of metabolism between patients on warfarin therapy, in patients with high risk of toxicity even though INR values appear normal can be identified (Malátková et al., 2016). Using a Fluorescence detector, this method can separate the R- and S-enantiomers of warfarin used in pharmacokinetic studies because it is able to accurately measure enantiomer levels in patient plasma samples so as to determine individual warfarin dose adjustments (Poojar et al., 2017). (Putriana et al., 2022) in their research developed a selective and sensitive HPLC - FLD method that has been validated according to EMA standards is very suitable for monitoring warfarin enantiomer concentrations and proven to be precise and accurate. It can be applied to patients with bleeding risk who need initial dose

titration so that very low warfarin concentrations can be detected. In a pharmacogenomic study on the individualization of warfarin therapy in African - Sub Saharan populations, this method links genetic variants (CYP2C98, CYP2C99, CYP2C911) can affect the metabolism of warfarin levels in blood plasma (Asiimwe et al., 2022). This method can also compare the effect of CYP2C911 allele variants on the metabolism of S-warfarin and phenytoin, the results showed that S-warfarin was more affected by the polymorphism than phenytoin and emphasized the need for monitoring levels based on patient genotype (Wanounou et al., 2022).

The available evidence consistently demonstrates that direct measurement of warfarin plasma concentrations provides complementary information to INR monitoring and may improve the evaluation of anticoagulant therapy in selected patient populations. Although INR remains the clinical gold standard for dose adjustment, it reflects the pharmacodynamic response rather than the actual drug exposure. Consequently, discrepancies may occur between plasma warfarin concentrations and INR values, particularly in patients with chronic kidney disease, liver dysfunction, polypharmacy, genetic polymorphisms affecting CYP2C9 or VKORC1, or unstable dietary vitamin K intake. These findings indicate that integrating pharmacokinetic data obtained through therapeutic drug monitoring with conventional INR assessment may provide a more comprehensive understanding of individual patient responses and support precision dosing strategies.

The reviewed studies also highlight important differences among analytical methods used for therapeutic drug monitoring. High-performance liquid chromatography with ultraviolet detection (HPLC-UV) remains a practical and relatively inexpensive technique suitable for routine laboratory analysis; however, its sensitivity and selectivity are generally lower than those of liquid chromatography-tandem mass spectrometry (LC-MS/MS). In contrast, LC-MS/MS consistently demonstrated superior analytical performance, including lower limits of detection, higher specificity, shorter analysis times, and the ability to simultaneously quantify R-warfarin, S-warfarin, and their metabolites. Nevertheless, the widespread implementation of LC-MS/MS is constrained by high instrument costs, specialized maintenance requirements, and the need for trained personnel, limiting its availability in many clinical laboratories, particularly in resource-limited healthcare settings. Therefore, the selection of an analytical method should consider not only analytical performance but also laboratory infrastructure, cost-effectiveness, and the intended clinical application.

From a clinical perspective, the implementation of therapeutic drug monitoring for warfarin should not be considered a replacement for INR monitoring but rather a complementary strategy for patients with unpredictable anticoagulant responses or those at high risk of adverse events. Current evidence suggests that TDM may be particularly valuable in complex clinical scenarios, including patients with chronic kidney disease, significant drug-drug interactions, suspected non-adherence, extreme pharmacokinetic variability, or recurrent thromboembolic or bleeding events despite therapeutic INR values. However, despite promising analytical advances, routine implementation of warfarin TDM remains limited because standardized therapeutic concentration ranges, universally accepted clinical decision thresholds, and cost-effectiveness data are still insufficient.

4. CONCLUSION

Warfarin therapy requires careful monitoring because of its narrow therapeutic index and considerable interindividual variability. Although the International Normalized Ratio (INR) remains the primary parameter for assessing anticoagulant response, direct measurement of plasma warfarin concentrations provides complementary pharmacokinetic information that may improve therapeutic evaluation in selected patients. Among the available analytical techniques, HPLC and LC-MS/MS demonstrate high analytical performance, with LC-MS/MS offering superior sensitivity and specificity for therapeutic drug monitoring. Overall, integrating plasma

warfarin concentration measurements with conventional INR monitoring has the potential to support individualized dose optimization and enhance the effectiveness and safety of anticoagulant therapy.

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6. CONFLICT OF INTEREST

All authors declare that there is no conflict of interest associated with this manuscript.

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