Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 340-350 | ISSN:2251-6727

A Comprehensive Review of Analytical Methods for the Quantification of Selected ACE Inhibitors (Enalapril, Lisinopril, Ramipril and Perindopril) in Pharmaceutical and Biological Matrices

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(Received: 05 January 2026 Revised: 15 February 2026 Accepted: 05 March 2026)

KEYWORDS

Analytical Methods

ABSTRACT:

Introduction: Hypertension is a major global health challenge, and the Renin–Angiotensin–Aldosterone System (RAAS) plays a crucial role in blood pressure regulation. ACE inhibitors act by blocking the conversion of angiotensin I to angiotensin II, thereby reducing vasoconstriction and peripheral resistance. Among them, Enalapril maleate, Lisinopril, Ramipril, and Perindopril are widely used due to their proven clinical efficacy and safety.

Physicochemical properties: The present section summarizes the key physicochemical properties of selected ACE inhibitors and highlights their analytical relevance in pharmaceutical evaluation. Physicochemical parameters such as molecular weight, solubility, lipophilicity, and structural features significantly influence drug stability, absorption, and chromatographic behaviour.

Methods: A comprehensive literature review was conducted to collect relevant data on the physicochemical properties of selected ACE inhibitors, namely Enalapril maleate, Lisinopril, Ramipril, and Perindopril. Information was gathered from official pharmacopoeias, peer-reviewed journal articles, standard reference textbooks, and published analytical reports. The collected data were critically evaluated and systematically compiled to compare molecular weight, solubility profile, lipophilicity, and structural characteristics, with emphasis on their analytical significance in pharmaceutical applications.

Results: The reviewed data indicate notable differences among the selected ACE inhibitors in terms of molecular weight, aqueous solubility, lipophilicity, and structural features. Enalapril maleate and Lisinopril exhibit higher aqueous solubility, whereas Ramipril and Perindopril demonstrate comparatively greater lipophilicity due to their ester functionalities. These variations significantly influence their stability profiles, chromatographic retention behaviour, and analytical method optimization. The findings highlight the importance of physicochemical characterization in selecting appropriate analytical conditions for reliable quantification.

Conclusions: The present review highlights the significance of physicochemical properties in understanding the analytical behaviour of selected ACE inhibitors. Variations in solubility, lipophilicity, molecular weight, and structural characteristics directly influence stability, chromatographic performance, and method development strategies. A systematic evaluation of these parameters supports the rational design and optimization of reliable analytical methodologies for pharmaceutical applications.

1. Introduction

Hypertension and cardiovascular diseases are major global health problems with considerable contribution to the morbidity and mortality in the whole world. Angiotensin-Converting Enzyme (ACE) inhibitors are placed in the centre stage among the list of drugs used in treating hypertension, because of their established efficacy, safety profile, and cardioprotective effects. The action of these agents is affected by inhibition of angiotensin I to angiotensin II, which is a strong vasoconstrictor, thus favouring vasodilation and reducing arterial pressure [1,2].

The renin-angiotensin-aldosterone system (RAAS) is an important part of the homeostatic regulation of blood pressure and extravascular fluids. ACE inhibitions lower the level of angiotensin II and decrease aldosterone

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JCHR (2026) 16(2), 340-350 | ISSN:2251-6727

release, which further lowers the peripheral vascular resistance and enhances cardiovascular performance [3].

An example of a prodrug ACE inhibitor which is converted to an active metabolite enalaprilat in the body is enalapril maleate. It is commonly used in the treatment of hypertension and heart failure (Figure 1) [4]. According to Figure 2, Lisinopril is a lysine analog of enalaprilat and an active ACE inhibitor without requiring a metabolic activation process, the duration of action being long (Figure 2) [5]. Ramipril is a lipophilic prodrug that has a high tissue affinity, having wide clinical applications due to cardiovascular and post myocardial infarction treatment (Figure 3) [6]. Perindopril is an ACE2 inhibitor with good long-acting nature that has good pharmacokinetic characteristics and has proved efficacy in lowering cardiovascular morbidity and mortality (Figure 4) [7].

Since there has been widespread clinical use of ACE inhibitors, the development of correct, precise and validated analytical methodologies is very essential towards its determination in bulk drugs, pharmaceutical dosage forms and in biological matrices. Other methods such as UV -vis spectrophotometry, HPLC, HPTLC, UPLC as well as LC-MS/MS have been reported in quality control and bioanalytical methods [8-10].

This review is systemized in a way that it provides a general summary of ACE inhibitors and the way it works, then proceeds to discuss analytical methods that have been reported to quantify Enalapril maleate, Lisinopril, Ramipril and Perindopril (Figures 1-4) in pharmaceutical and biological systems. At the end of the review, a comparative analysis is performed as well as future projections in the development of analytical methods.

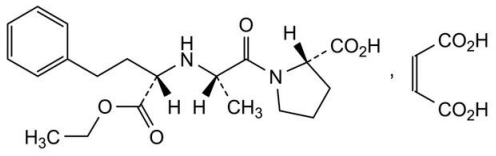

Figure 1: Chemical structure of Enalapril maleate

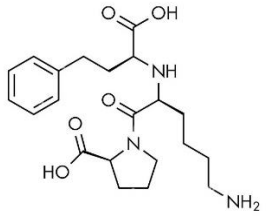

Figure 2: Chemical structure of Lisinopril

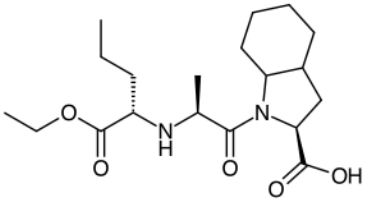

Figure 3: Chemical structure of Ramipril

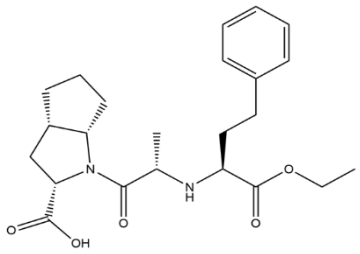

Figure 4: Chemical structure of Perindopril

2. Physicochemical Properties of Selected ACE Inhibitors

The physicochemical properties of drug substances such as molecular weight, solubility, lipophilicity, pK a and chemical structure have central roles in influencing drug absorption, bioavailability, stability, and the design of drug analysis procedures. The differences in these characteristics between angiotensin-converting enzyme (ACE) inhibitors affect their chromatographical characteristics, extraction capability, and sensitivity in the detection of angiotensin-converting enzyme in pharmaceutical and biological samples [11].

2.1 Enalapril Maleate

This is the enalapril maleate which is enalapril white to off-white crystalline powder; it is merely enalapril in

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maleate form. It has a molecular weight of about **492.5g/mol.** It dissolves freely in water and methanol, but has a low degree of solubility in ethanol. Enalapril is a prodrug, which possesses an ester functional group that is vulnerable to the hydrolysis process therefore determining its stability profile. Its moderate lipophilicity provides good absorption and good chromatographic retention in reversed-phase systems, which is demonstrated in Figure 2 [12,13].

2.2 Lisinopril

Lisinopril appears as the white to pale yellow crystalline drug, having an approximate molecular weight of **405.5 g/mol.** It is a very unusual solubility profile: its aqueous solubility is high, and at the same time, it is essentially insoluble in most organic solvents. Unlike the rest of its peers in the category of angiotensin-converting enzyme inhibitors, lisinopril is neither a prodrug nor a hydrophilic one. The strong polarity and reduced lipophilicity of this ion also cause chromatographic analyses to often require the use of an aqueous mobile phase or the addition of ion-pairing reagents, which significantly affects its analytical behavior (Figure 3) [12,14].

2.3 Ramipril

Ramipril exists as a white crystalline powder, with a molecular weight of 416.5gmol -1. It has a characteristic low aqueous miscibility and high solubility in organic solvents, including methanol and acetonitrile. Ramipril is an enzymatic hydrolysate of the lipophilic prodrug ramipril, and the active metabolite of Ramipril is the active compound ramiprollat. The strong lipophilicity of the compound enables facilitation of penetration of the tissue, but also makes it difficult to solve the compound and forces methodological optimization in the development of the analysis (Figure 4) [12,15].

2.3 Perindopril

Being a white to a bit yellow crystalline powder with a molecular weight of about 368.5g/mol -1 (free base) perindopril is only slightly soluble in water but is well soluble in organic solvents. This compound is an ester prodrug, which is metabolised in vivo to its active component, perindoprilat. Its physicochemical characteristics such as a moderate lipophilicity and an ester bond significantly influence its pharmacokinetic behaviour as well as its chromatographic characteristics (see Figure 5) [12,16].

2.5 Analytical Relevance

The differences in solubility, lipophilicity, and chemical architecture of these ACE inhibitors are crucial factors in informing the carrying out and further development of analysis procedures. Such good understanding of these physicochemical properties is essential to optimise extraction procedures, to customise the composition of the mobile phase and to provide reliable quantification in pharmaceutical dosage forms and in complex biological systems [11,16].

3. Review of Analytical Methods

3.1 Enalapril Maleate

Enalapril maleate is a common ACE inhibitor and a mass of academic literature has explored its determination in bulk drug and pharmaceutical dosage delivery systems and in biological systems using varied analytical systems. The existing body of literature mainly outlines the ideas of approaches developed to provide the typical quality control, stability evaluation, impurity characterisation, and pharmacokinetic retrospectives researches.

3.1.1 UV–Visible Spectrophotometric Methods

Several UV-visible spectrophotometric methods have been developed to determine the amount of enalapril maleate in bulk and tablet preparations. These methods are usually based on the direct measurement of absorbance in the 210 to 220Nm wavelength range which is the range of maximum absorption of the compound. The types of methodologies described in the literature are not only simplified, fast, and cost-effective but also well linearized and adequately accurate and precise. However, due to their lack of selectivity and interference by excipient substances, their utility in practice has been restricted to conventional dosage-form pharmacological analysis and is not suitable with biological samples in general [17,18].

3.1.2 High Performance Liquid Chromatography (HPLC) Methods

HPLC continues to be the most common mechanism of analysis used in the determination of enalapril maleate using precision analysis. Most modern-day approaches

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JCHR (2026) 16(2), 340-350 | ISSN:2251-6727

rely on C18 stationary phases in a reversed format, and make use of mobile phases consisting of methanol or acetonitrile along with phosphate or acetate buffers that are kept at an acidic pH. Detecting UV is traditionally done at wavelengths known to be 215-220nm. These methods have excellent sensitivity, specificity and reproducibility making them essential in determining an assay, stability indicating investigations and impurity characterizing enalapril maleate over a range of pharmaceutical preparations [19-21].

3.1.3 HPTLC Methods

High-performance liquid chromatography (H PL / HPTLC) has been used in determination of enalapril maleate in tablet dosage forms. These processes consist of the use of the silica gel plates and the correspondingly selected mobile-phase systems, then the densitometric identification. The method has a number of benefits such as the ability to independently analyze many samples, less use of solvents and less total time of analysis. The approach is confirmed to work as per the ICH guidelines and displays reasonable precisions and accuracy.

3.1.4 Bioanalytical Methods

Researchers have expressed that in the studies involving a biological matrix like the plasma, serum they apply more sensitive and selective techniques of analysis, such as the high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry (HPLC and LC-MS/MS). Preparative procedures normally involve solids-phase extraction or protein precipitation. LC-MS/MS methods easily allow the accurate determination of enalapril and enalaprilat even in the most dilute form and therefore will be best applicable to pharmacokinetic, bio -availability and bioequivalence studies [23, 24].

Table 1. Summary of Reported Analytical Methods for Enalapril Maleate

Analytical Techniques	Sample Matrix	Column / Conditions	Detection (λ / Mode)	Application
UV–Visible Spectrophotometry	Bulk drug, Tablets	Direct UV method	210–220 nm	Routine quality control
UV–Visible Spectrophotometry	Tablets	Zero-order UV	215 nm	Assay of formulations
RP-HPLC	Bulk drug, Tablets	C18 column; ACN–buffer	UV, 215–220 nm	Assay, stability studies
RP-HPLC	Tablets (combination)	C18 column; ACN–phosphate buffer	UV, 215 nm	Simultaneous estimation
Stability-indicating HPLC	Bulk & Formulation	C18 column; acidic buffer–ACN	UV detection	Degradation studies
HPTLC	Tablets	Silica gel 60 F254	Densitometric scanning	Rapid routine analysis
HPLC (Bioanalytical)	Human plasma	C18 column; buffer–ACN	UV detection	Pharmacokinetic studies
LC–MS/MS	Plasma / Serum	RP column; gradient elution	MS/MS (ESI mode)	Bioavailability studies

3.2 Lisinopril

Lisinopril is an Angiotensin-Converting Enzyme (ACE) inhibitor of the second generation which is widely used in the clinical treatment of hypertension and heart failure. Uniquely, in contrast to many other representatives of this group, lisinopril is not a prodrug but it is a substance having a significantly high polarity and hydrophilicity, which naturally predetermines good pharmacokinetic characteristics. Considering its widespread use in therapy, a variety of analytical methodological procedures have been reported systematically to accurately determine lisinopril. These include bulk drug observation, testing in a pharmaceutical dosage formulation, and testing in various biological matrices hence stipulating the strong applicability of the compound in pharmaceutical sciences as well as in clinical practice.

3.2.1 UV–Visible Spectrophotometric Methods

Various UV visible spectrophotometric procedures have been formulated in the analysis of the lisinopril in its bulk state and tablets. These methods mainly depend on direct measurements of absorbance in the ultraviolet band of 210220nm or make use of derivatization methods to provide increased sensitivity of detection. The reported strategies are simplicity, fastness and cost-effectiveness and portray acceptable linearity and accuracy. However, due to a lack of selectivity and confusion with formulation excipients, they could only be used in the theoretical scope to the standard pharmaceutical analysis

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and are inapplicable in the analysis of biological matrices [25,26].

3.2.2 High Performance Liquid Chromatography (HPLC) Methods

High-performance liquid chromatography is the most extensive procedure that is being used to quantitatively analyze lisinopril. Most of the reported procedures utilize reversed -phase C18 columns, and the mobile phases are made up of aqueous buffers mixed with either methanol or acetonitrile. Due to the ionic nature and high polarities of lisinopril, numerous protocols also add an ion-pairing reagent or add an increased fraction of aqueous phase in the mobile phase to establish reasonable analyte retention. The detection is traditionally carried out in UV absorbance usually in the 215 -225nm spectrum. These methods of analysis are habitually utilized in the assessment of determination, stability assay, and simultaneous analysis of lisinopril and other antihypertensive medications [27-29].

3.2.3 HPTLC Methods

It has also been reported that estranged use of high-performance thin layer chromatography (HPTLC) methods in the estimation of lisinopril in pharmaceutical dosage preparations. These techniques utilize silica gel plates asserted with the right mobile phase ratios, and is then densitometrically detected to identify the analyte in number. HPTLC has such benefits as it consumes less time to analyze, less solvent is used and multiple samples could be analyzed at the same time. The reported techniques are justified based on the directives of the International Council for Harmonisation (ICH) and show adequate accuracy and precision [30].

3.2.4 Bioanalytical Methods

Within the framework of determining lisinopril in the context of biological fluids, in particular plasma and serum, there is a long-established use of high-sensitivity analysis methods, where the most evident is high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The conventional methods used in sample preparation include protein precipitation or extraction in solid phase. Though LC-MS/MS specifically, offers better sensitivity and selectivity, which allows the precise identifying of lisinopril at infamous levels; this property makes it particularly suitable in the testing of pharmacokinetic, bioavailability and bioequivalence [31,32].

Table 2. Summary of Reported Analytical Methods for Lisinopril

Analytical Technique	Sample Matrix	Column / Key Conditions	Detection (λ/Mode)	Application
UV–Visible Spectrophotometry	Bulk drug, Tablets	Direct UV / derivatization	210–230 nm	Routine assay
UV–Visible Spectrophotometry	Tablets	Zero / derivative UV	~215 nm	Quality control
RP-HPLC	Bulk drug, Tablets	C18 column; ACN–buffer	UV, 210–220 nm	Assay determination
RP-HPLC	Bulk & Formulation	C18 column; acidic buffer–ACN	UV detection	Forced degradation studies
RP-HPLC (Simultaneous)	Tablets (with other drugs)	C18 column; gradient elution	UV detection	Simultaneous estimation
HPTLC	Tablets	Silica gel 60 F254	Densitometric scanning	Rapid routine analysis
HPLC (Bioanalytical)	Human plasma	RP column; buffer–organic phase	UV detection	Pharmacokinetic studies
LC–MS/MS	Plasma / Serum	RP column; gradient elution	MS/MS (ESI mode)	Bioavailability & bioequivalence

3.3 Ramipril

Ramipril is an ester prodrug derivative of ACE-inhibitors that is lipophilic and widely used in clinical practice of hypertension, heart failure, and post-myocardial infarction syndromes. Its widespread use as a therapeutic agent, combined with its metabolic transformation to the active form ramiprilat has led to a plethora of methods that can be used to determine the concentration of the parent compound in bulk samples, pharmaceutical preparations and biological samples.

3.3.1 UV–Visible Spectrophotometric Methods

The determination of ramipril in bulk drug and tablet dosage form has been reported using UV-visible spectrophotometric methods. The principles of these methods are either direct measurement of absorbance in the UV, usually 210230nm, or derivatization to increase sensitivity. The procedures are easy, quick, and cost effective and demonstrate good linearity and accuracy. Nevertheless, their use is limited by poor selectivity and the excipient interference, which limits their use

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primarily to quality control of a formulation on a routine basis [33,34].

3.3.2 High Performance Liquid Chromatography (HPLC) Methods

High-performance liquid chromatography (HPLC) is the most used mode of analysis that is used to quantify ramipril. Majority of the assay procedures involve the use of reversed-phase C18 stations whereby the mobile phases consist of acetones/methane significantly mixed with acidic buffers. The spectral window of 210220nm is performed on a regular basis to carry out ultraviolet detection. Several stability-indicating HPLC procedures have also been established to determine degradation schemes in forced-degradation environments. The methods are strong in accuracy, precision and reproducibility and are widely used in determination of assays and also stability studies [35-37].

3.3.3 HPTLC Methods

The procedure of the high-performance thin-layer chromatography (HPTLC) assay has been optimized to quantitatively determine the amount of ramipril that is present in commercial dosage forms. These analytical plans use silicagel plates along with rationally selected compositions of mobile phase after which these are densitometrically detected on calibrated equipment. The natural virtues of HPTLC such as the extreme decrease in solvent consumption, the ability to run multiple specimens simultaneously, and the ability to reduce the total analytical run time make it a desirable alternative to traditional methods of chromatography. The methods are strictly tested by meeting the guidelines of international council of harmonisation (ICH), demonstrating satisfactory accurateness and precision [38].

3.3.4 Bioanalytical Methods

In the case of the biological matrices, e.g., plasma and serum, sensitive and selective methods of analysis, e.g. using high-performance liquid chromatography (HPLC) and tandem liquid chromatography-mass spectrometry (LC-MS/MSs), have been employed to determine both ramipril and ramiprilat active metabolite. The preparative step typically involves protein precipitation/solid phase extraction and thus reducing endogenous interferences. Due to its high sensitivity and specificity, LC-MS/MS is highly suitable especially in pharmacokinetic experiments, bioavailability determination and in bioequivalence studies [39,40].

Table 3. Summary of Reported Analytical Methods for Ramipril

Analytical Technique	Sample Matrix	Column / Key Conditions	Detection (λ / Mode)	Application
UV–Visible Spectrophotometry	Bulk drug, Tablets	Direct UV / derivatization	210–230 nm	Routine assay
UV–Visible Spectrophotometry	Tablets	Zero / derivative UV	~215 nm	Quality control
RP-HPLC	Bulk drug, Tablets	C18 column; ACN–buffer	UV, 210–220 nm	Assay determination
Stability-indicating RP-HPLC	Bulk & Formulation 5	C18 column; acidic buffer–ACN	UV detection	Forced degradation studies
RP-HPLC (Combination)	Tablets (with other drugs)	C18 column; gradient elution	UV detection	Simultaneous estimation
HPTLC	Tablets	Silica gel 60 F254	Densitometric scanning	Rapid routine analysis
HPLC (Bioanalytical)	Human plasma	RP column; buffer–organic phase	UV detection	Pharmacokinetic studies
LC–MS/MS	Plasma / Serum	RP column; gradient elution	MS/MS (ESI mode)	Bioavailability & bioequivalence

3.4 Perindopril

Long-acting angiotensin converting enzyme inhibitor perindopril is still at the center stage in the management of hypertension, chronic heart failure, and stable coronary artery disease. It is an ester pro-drug which functionally is broken down by esterase in the body to release the active drug, perindoprilat. Due to the wide clinical use, and positive pharmacokinetics properties, numerous analytical guidelines have been established in order to determine the concentration of perindopril in raw pharmaceutical and various biological samples.

3.4.1 UV–Visible Spectrophotometric Methods

Currently, the UV–visible spectrophotometric techniques have been advocated to the quantification of perindopril in the bulk drug and tablet doses. The major dependence is made on the direct measurement of absorbance at the ultraviolet region, typically, 210-230

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nm, or use of derivatization of the assays as strategies to increase the sensitivity of the assays. The nature of these methods is that they are operational in a simple way, cost-efficient, and display reasonable linearity and precision. Nevertheless, owing to the selectivity as a limitation inherent in their application, and the possibility of interference as a result of formulation excipients, their practical application is mostly limited to regular quality-control assessments of pharmaceutical preparations, as defined in the references cited [41,42].

3.4.2 High Performance Liquid Chromatography (HPLC) Methods

HPLC is the most widely used method of analysis in determining perindopril. Most of the published protocols use reversed phase C18 columns, and mobile phase is a mixture of methanol or acetonitrile and buffered aqueous solutions at an acidic pH. Ultraviolet measurement is a regularly performed measurement at wavelengths near 215-220nm. Various stability predictive HPLC systems have been established in order to determine the behaviour of degradation of perindopril under stress cases. These procedures have a high accuracy, precision, and robustness and thus apply in the normal quality control and stability tests [43-45].

3.4.3 HPTLC Methods

The use of high-performance thin-layer chromatography (HPTLC) methods has also been developed to determine perindopril in formulated pharmaceutical compounds in quantities. These methods utilize silica gel plates using judiciously chosen mobile-phase systems, and then are densitometrically detected. The HPTLC procedures present a variety of advantages, which include reduced solvent use, simultaneous analysis of several specimens, and shorter period of the analytic time. The practice of procedures described in the literature is confirmed, as the assurance of high accuracy and precision was realised in compliance with international Council of harmonisation (ICH) principles [46].

3.4.4 Bioanalytical Methods

A number of analytical methods have been reported in sensitive mode such as HPLC and LC -MS/MS to measure perindopril and its active metabolite, perindoprilat, in biological samples like plasma and serum. Protein precipation or solid-phase extraction is the most frequent method of sample preparation. The sensitivity and selectivity of LC–MS/MS methods are very high, so the method can easily have a precise quantification at extremely low concentrations, and it is widely used in pharmacokinetic and bioequivalence studies [47,48].

Table 4. Summary of Reported Analytical Methods for Perindopril

Analytical Technique	Sample Matrix	Column / Key Conditions	Detection (λ / Mode)	Application
UV–Visible Spectrophotometry	Bulk drug, Tablets	Direct UV / derivatization	210–230 nm	Routine assay of formulations
UV–Visible Spectrophotometry	Tablets	Zero / derivative UV	~215 nm	Quality control
RP-HPLC	Bulk drug, Tablets	C18 column; ACN/MeOH –buffer	UV, 215–220 nm	Assay determination
Stability-indicating RP-HPLC	Bulk & Formulation	C18 column; acidic buffer–ACN	UV detection	Forced degradation studies
RP-HPLC (Combination)	Tablets (with other drugs)	C18 column; gradient elution	UV detection	Simultaneous estimation
HPTLC	Tablets	Silica gel 60 F254	Densitometric scanning	Rapid routine analysis
HPLC (Bioanalytical)	Human plasma	RP column; buffer–organic phase	UV detection	Pharmacokinetic studies
LC–MS/MS	Plasma / Serum	RP column; gradient elution	MS/MS (ESI mode)	Bioavailability & bioequivalence

3.5 Simultaneous Estimation of Enalapril, Lisinopril, Ramipril and Perindopril (with citations)

Parallel estimation of ACE inhibitors has been subjected to high levels of examination in the chromatographic field and the rationale has been to increase the level of analytical throughput and at the same time reduce the length of time it runs. Spread throughout literature a number of RP-HPLC methods have been outlined along with the determination and appearance of Enalapril maleate, Lisinopril, Ramipril and Perindopril, either single-handedly or in combination with complementary antihypertensive agents. They traditionally use C18 stationary phases and buffered mobile phases, they watch the absorption 210 225nm and have been verified in

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JCHR (2026) 16(2), 340-350 | ISSN:2251-6727

compliance with ICH guidelines, thus proving acceptable accuracy, precision, and strength [49-52].

Due to the significant differences in polarity and lipophilicity of the analytes under the study, careful optimization of the chromatography parameters is dictated as the method of ensuring an acceptable resolution. Similar technological improvements in the LC-MS/MS field have produced methodologies that can simultaneously measure both ACE inhibitors and their active metabolites in biological samples at both high sensitivity and selectivity needed in pharmacokinetic and bioequivalence studies cannot be done without [53].

4. Comparative Discussion of Analytical Methods

As has been recorded, the literature has attributed the precise quantification of the angiotensin-converting enzyme inhibitors Enalapril maleate, Lisinopril, Ramipril and Perindopril in both the pharmaceutical dosage forms as well as the relevant biological matrices. The restricted choice of a suitable analytical method is predetermined by a set of factors that include the physicochemical characteristics of active pharmaceutical ingredient, the sensitivity of analysis needed, complexity of the sample matrix, and the purpose, which a specific method will be used (nondestructive monitoring of quality of a product, thorough stability studies, or full pharmacokinetic examination).

4.1 Comparison of Spectrophotometric Methods

The ultraviolet-visible spectrophotometric can be widely reported in the round four angiotensin-converting enzyme (ACE) inhibitors, and are most commonly used in the process of routinely quantifying bulk pharmaceutical preparations and tablet formulations. This makes such methods inherently simple, fast and relatively inexpensive, therefore, benefiting this sector especially in the quality-control laboratories that might not elaborate instrumentation. However, because of their low selectivity and possibility of interferences caused by formulation excipients, UV-visible spectrophotometry fails in terms of use in a biological sample and in studies of stability indicators. Among the four of them, lisinopril and enalapril maleate, due to their higher hydrophilicity, will exhibit a larger UV absorbance, and ramipril and perindopril regularly require chemical derivatization to give good sensitivity.

4.2 Comparison of Chromatographic Methods

Reverted-phase high-performance liquid chromatography is the most ideal analytical modality of all the four angiotensin-converting-enzyme (ACE) inhibitors. Relative to spectrophotometric analysis, HPLC has significantly greater selectivity, accuracy, and reproducibility. There are various stability indicating HPLC methods reported in literature in the case of enalapril maleate, which is prone to hydrolytic degradation. An example of such a case is the polarity of the drug Lisinopril, which is often of such high polarity that aqueous composition is required, or it may be necessary to add an ion-pairing reagent. On the other hand, ramipril and perindopril, lipophilic ester prodrug, show increased retention on C18 phases and therefore best fitted on reversed-phase chromatography systems.

4.3 Comparison of HPTLC Methods

Since analytical methods have upgraded recently, high-performance thin-layer chromatography (HPTLC) methodologies have been developed on each of the four agents, mostly to analyse pharmaceutical dosages. This type of technique allows the simultaneous analysis of specimens with a large number, which minimizes the use of solvents and also means the reduction in time of analysis. Irrespective of the fact that the sensitivity of HPTLC is relatively less as compared to that of high-performance liquid chromatography (HPLC), both techniques provide specific advantages when it comes to quality control in routine and initial screening. It is worth mentioning that the HPTLC protocols used with enalapril maleate and ramipril have better chromatographic resolution compared to lisinopril, which could be explained by the natural differences in the polarity of compounds.

4.4 Comparison of Bioanalytical Methods

When working with the biomedical analysis, especially with biological samples of plasma and serum, we usually use highly sensitive methods of analysis, first and foremost HPLC and LC-MS/MS. Such LC-ms/ms methods have not only impressive sensitivity but also superior selectivity and thus allow to quantify the analytes with traces of concentration. Therefore, they are widely used in the study of the pharmacokinetics, bioavailability and bioequivalence of each of the four angiotensin-converting enzyme inhibitors. Enalapril

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along with ramipril together with their metabolites enalaprilat and ramiprilat are regularly analyzed by means of LC ms ms based analyses precisely due to their very low plasma concentrations.

5. Conclusion and Future Perspectives

In this review, I summarize the analytical methods that were used to quantitatively determine the target angiotensin-converting enzymes inhibitors i.e. Enalapril maleate, Lisinopril, Ramipril and Perindopril in both pharmaceutical dosage form as well as in biological matrices. These represent the main therapeutic mentioned in the pharmacotherapeutic armamentization of the treatment of cardiovascular diseases and their accurate measurement is of high priority in maintaining the quality of products, patient safety, and effectiveness of the therapeutic effect. A vast range of analysis methods, such as UV-visible spectrophotometry, HPLC, HPTLC, UPLC, and LC-MS/MS are recorded in the literature. Spectrophotometric methods have been appreciated because of their simplicity, speed and cost-effectiveness, which make them appropriate in regular quality-control of pharmaceutical preparations. However, this restricts their selectivity, which also makes their use in complex matrices impossible. In comparison, chromatography-based methods, especially reversed-phase high-performance liquid chromatography, offer much better sensitivity, specificity, and reproducibility and are consequently extensively exploited in the determination of an assay, impurity profiling as well as in stability-indicating experiments. Tandem liquid chromatography-mass spectrometry (LC-MS/MS) has become the standard of bioanalysis because it is the most sensitive assay due to its ability to analyze at low concentration levels ACE inhibitors, and their active metabolite species. Such methods cannot be overlooked in pharmacokinetic, bio-availability and bio-equivalent studies. The unique physicochemical nature of the four ACE inhibitors has a significant effect on the choice and optimization of the method explaining why a delicate appreciation of the characteristic of drugs in the development of the analysis method is well-primed.

Future Perspectives

Although the field of analysis has been showered in numerous techniques, a gap in substantiveness still remains, which is why the development of environmentally friendly, economically viable and high-throughput analysis methods should be subject to further academic research. Future research activities must focus on harnessing the idea of green analytical chemistry, reducing the use of solvents, and applying other improved modalities, including ultra -performance liquid chromatography (UPLC) and hyphenated methods, therefore reaching increased sensitivity in addition to cutting down on the analysis times. At the same time, the development of stability-indicating and bioanalytical technologies consistent with current regulatory requirements will continue to form a central point of interest in the scientific community, and the methodological activities need to be further perfected and authenticated according to regulatory requirements.

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Journal of Chemical Health Risks

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