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ANALYTICAL METHODS FOR SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND RAMIPRIL IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL MATRICES: A COMPREHENSIVE REVIEW

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ABSTRACT

Atorvastatin and ramipril are commonly used together to manage cardiovascular conditions, including high blood pressure, abnormal lipid levels, and coronary artery diseases. Accurate and reliable analytical methods are necessary for the simultaneous detection of these compounds in pharmaceutical formulations and biological samples because of their combined therapeutic effects. This review examines different analytical methods created for the measurement of atorvastatin and ramipril, both separately and together. The techniques mentioned are UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). UV spectrophotometric methods are simple and affordable, while HPLC and RP-HPLC methods offer greater sensitivity, specificity, and consistency. HPTLC is an effective option because it offers quick analysis and uses less solvent. The review also covers bioanalytical methods that use reverse phase high-performance liquid chromatography (RP-HPLC) for plasma samples, emphasizing their role in pharmacokinetic and bioavailability research. The findings from different studies suggest that these methods are dependable and appropriate for regular quality control and research purposes. This review offers a detailed summary of the analytical techniques that are currently available and highlights the significance of method validation in achieving accurate and consistent results for the simultaneous measurement of atorvastatin and ramipril.

KEYWORDS: Atorvastatin, Ramipril, HPLC, HPTLC, RP- HPLC, UV Spectrophotometry.

INTRODUCTION

Analytical Method Development

The goal of developing analytical methods is to determine the identity, purity, physical properties, and strength of drugs, as well as their bioavailability and stability.[1,2] Analytical method development and validation refer to the process of demonstrating that analytical procedures are suitable for evaluating drugs, especially the Active Pharmaceutical Ingredient (API).[3]

Method Validation

Method validation is the process of proving that an analytical method is appropriate for its intended purpose and can generate reliable and consistent results over time. The validation process includes a series of procedures and tests aimed at assessing the performance features of the method.[4]

The elements involved in method validation are

- Accuracy
- Precision
- Specificity
- Linearity
- Range
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Ruggedness
- Robustness.[5,6]

DRUG PROFILE

Ramipril

A member of the class of medications known as angiotensin-converting enzyme (ACE) inhibitors, ramipril is a derivative of 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid. Ramipril's chemical name is (2S,3aS,6aS)-1[(S)-N-[(S)-1-carboxy-3 phenylpropyl] alanyl] octahydrocyclopenta [b]pyrrole-2-carboxylic acid, 1-ethyl ester. The structure of the chemicals is seen in [Figure 1]. The liver converts the prodrug, ramipril, into ramiprilat. One powerful ACE competitive inhibitor is ramiprilat.[7] Angiotensin II is involved in blood pressure regulation and is produced from angiotensin I by the angiotensin-converting enzyme. Ramipril can be used to treat congestive heart failure and high blood pressure (hypertension) and improve survival following a heart attack by reducing the synthesis of angiotensin II.[8]

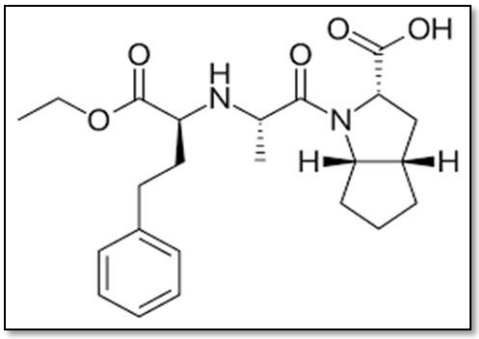

Figure: 1 Structure of Ramipril.

The quantification of ramipril alone in biological samples has been documented in the literature using kinetic spectrophotometry, visible spectrophotometry, spectrofluorimetric, atomic absorption spectrophotometry, liquid chromatography-mass spectrometry, and liquid chromatography with UV detection and medicinal dose types.[9]

Atorvastatin

Atorvastatin is a member of the class of medications known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or "statins," which lower levels of "bad" cholesterol (low-density lipoproteins, or LDL) and triglycerides in the blood while raising the amount of HDL, or high-density lipoproteins, or "good cholesterol".[10] In individuals with type 2 diabetes, coronary heart disease, and other risk factors, this medication is used to treat excessive cholesterol and lower the risk of stroke, heart attack, or other cardiac problems. (3R,5R)-7- [2-(4 Fluorophenyl-5-isopropyl-3-phenyl-4-(phenylcarbinol)-1H-pyrrol 1-yl]-3,5-dihydroxyheptanoic acid calcium salt (2:1) is the IUPAC nomenclature for atorvastatin calcium (Figure 2).[11]

A review of the literature indicates that there are various methods available for measuring atorvastatin in biological fluids and formulations. These methods include spectrophotometry, HPLC with UV detection, HPLC with fluorescence detection, TLC-densitometry, LC-MS/MS, capillary electrophoresis, and microchip electrophoresis.[12]

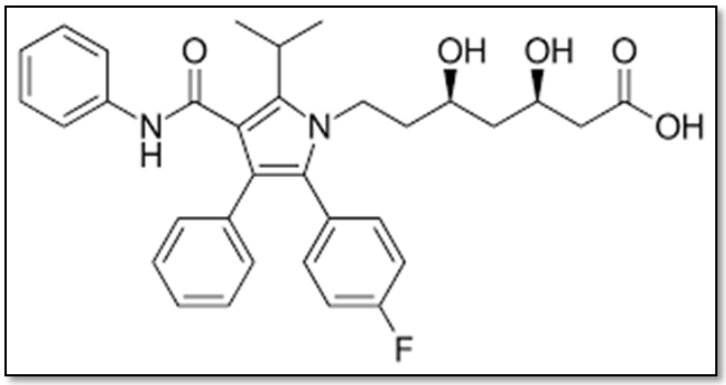

Figure: 2 Structure of Atorvastatin.

Combination Therapy

The combination of ATO and RAM has beneficial effects that enhance cardiac syndrome X, which includes patients experiencing chest pain and ischemia but with normal angiograms. This combination improves endothelial function and overall quality of life, including exercise ability and symptoms related to angina. It also reduces oxidative stress. It has positive effects on heart-related diseases. The combination of ATO and RAM is available for purchase in capsule form.[13]

Currently, there is one reported method using HPLC and spectrophotometry for the simultaneous determination of ATO and RAM in formulations, but no HPTLC method has been documented. The suggested HPLC method has been created and confirmed using various stationary and mobile phases. The reported method did not include an evaluation of intra- and intraday precision or robustness. However, it is noted that the method is precise and robust. Additionally, details about retention time, limit of detection (LOD), limit of quantification (LOQ), and assay percentage were not provided. The goal was to create validated methods that can accurately, precisely,

and sensitively measure both components in commercial pharmaceutical dosage forms at the same time. This paper outlines straightforward, accurate, sensitive, and precise methods using HPLC and HPTLC for the simultaneous measurement of ATO and RAM in capsule formulations.[14]

MATERIALS AND METHODS

1. Analytical Method for Pharmaceutical Formulation

a] UV Spectrophotometric Methods

The contents of twenty capsules were measured, and a powder equivalent to 10 mg of ATR was added to 60 ml of a solvent system. This mixture was sonicated for 10 minutes, and after sonication, the total volume was adjusted to 100 ml. One millilitre of this stock solution was diluted to a total volume of 10 millilitres to achieve a concentration of 10 µg/ml of ATR and 5 µg/ml of RAM. This solution is scanned within the wavelength range of 200 to 350 nm, using the solvent system as a blank reference. The first-order derivative spectra were obtained, and the absorbance values were recorded. Concentrations were calculated using regression equations derived from the calibration graph. The sampling wavelengths were 294 nm for ATR, at which point RAM indicated a zero crossing, and 229 nm for RAM, where ATR also showed a zero crossing. Calibration graphs were created using the absorbance values at the corresponding wavelengths.[15]

b] High Performance Liquid Chromatography [HPLC] and High-Performance Thin Layer Chromatography [HPTLC]

To find the amounts of ATO and RAM in a capsule, 20 capsules were weighed, and the average weight was calculated. A precise amount of powder, corresponding to 10 mg of ATO and 5 mg of RAM, was placed into a 50 ml volumetric flask, and 30 ml of methanol was added. The solution was subjected to sonication for 15 minutes. The flask was permitted to cool to room temperature, and the volume was adjusted to the mark with methanol to create the sample stock solution for ATO at a concentration of 200 g/ml and RAM at a concentration of 100 g/ml. The solution was passed through a 0.45 micrometre, 47-millimeter membrane filter.[16] A 2.5 ml aliquot was moved to a 50 ml volumetric flask and diluted to the mark with the mobile phase with methanol for HPLC and methanol: benzene: glacial acetic acid (19.6:80.0:0.4 v/v/v) for HPTLC, creating working sample solutions for ATO at 10 g/ml and RAM at 5 g/ml. A 1 ml portion of the working test solution was placed in a 10 ml volumetric flask and diluted with the mobile phase up to the mark to prepare the sample solution for ATO at a concentration of 1 g/ml and RAM at a concentration of 0.5 g/ml. For HPTLC, a 10 ml portion of the working test solution was applied to the plates to achieve sample concentrations of 100 ng per spot for ATO and 50 ng per spot for RAM.[17]

C] Reversed phase High performance liquid chromatography [RP-HPLC]

The pharmaceutical formulation of a specific brand was evaluated, with each capsule having an average weight of 176.9 mg. The weight of twenty capsules was measured separately, and the average weight was calculated. A measured amount of the capsule powder equivalent to the weight of one capsule was transferred into a 100.0 ml volumetric flask. The volume was brought to 100.0 ml using methanol. The flask was placed in a water bath and underwent sonication for 10 minutes at a temperature of 37 degrees Celsius. A 10.0 ml sample of the solution was diluted to a final volume of 100.0 ml with methanol, producing concentrations of 10.0 µg/ml of AB and 5.0 µg/ml of RM. The process was repeated using five portions of the capsule powder. The solutions were filtered using a 0.45 µm nylon membrane filter before analysis.[18]

2. ANALYTICAL METHODS FOR BIOLOGICAL MATRICES

RP-HPLC in plasma

Preparation of Sample Solution

A 5ml blood sample had 10 mg of ATR, 20 mg of RAM, and 0.2 ml of trichloroacetic acid at a concentration of 10% added to it. The sample was centrifuged at 3000 revolutions per minute for 35 minutes. to allow red blood cells to settle at the bottom. The supernatant was isolated, and the volume was adjusted to 10 ml to create a solution with a concentration of 1000 µg/ml for each drug. This solution was passed through a 0.45 µm syringe filter. The solutions were mixed with a mobile phase to achieve stock concentrations of 100 µg/ml for ATR and 200 µg/ml for RAM. A standard stock solution of VAL was created by dissolving 10 mg of the drug in 80 ml of the mobile phase. The mixture was subjected to sonication for 10 minutes, after which the total volume was adjusted to 100 ml with the mobile phase to achieve a concentration of 100 µg/ml in a 100 ml volumetric flask.[19]

Quality Control Sample Analysis

Quality control sample analysis involved taking appropriate amounts from each standard stock solution of the medication and placing them into three 10 ml volumetric flasks. The volume was modified using the mobile phase to prepare solutions with concentrations of 0.5, 2.5, and 25 µg/ml for ATR; 1, 7, and 50 µg/ml for RAM; and 25 µg/ml for VAL in each flask. About 20 µl of each concentration of each drug was injected separately into the RP HPLC system and analysed according to the specified conditions. Both drugs were evaluated using a UV detector calibrated to 217 nm. Each peak was identified with its corresponding peak area. Retention factors were identified. The quantities of both drugs were measured using information obtained from calibration curves.[20]

RESULT AND DISCUSSION

Results of UV Spectrophotometric Methods

Table 1: SD: Standard Deviation, RSD: Relative Standard Deviation.

Analyte	Label claim (mg/tab)	% Label claim estimated (Mean ± SD*)	RSD*
Atorvastatin	10	100.22±0.8722	0.8702
Ramipril	5	100.10±0.8064	0.8055

*Denotes average of six determinations.

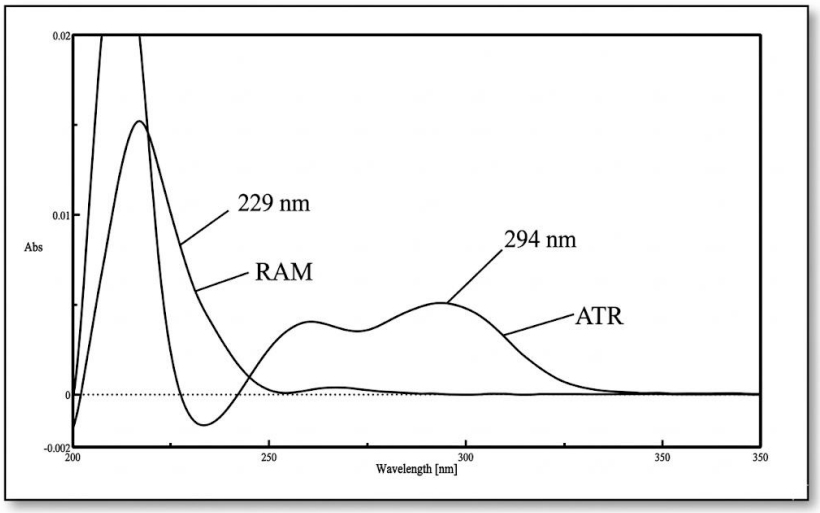

Figure 3: Overlain spectra of Atorvastatin and Ramipril in first order derivative mode.

The UV Spectroscopy method showed in [Table: 1] and the first order derivative model overlain spectra of Atorvastatin and Ramipril is shown in [Figure: 3]

Result of High-Performance Liquid Chromatography [HPLC] and High-Performance Thin Layer Chromatography [HPTLC]

Table 2: Parameter of Atorvastatin & Ramipril in Proposed HPLC and Reported HPTLC.

PARAMETER	HPLC		HPTLC	
	ATORVASTATIN	RAMIPRIL	ATORVASTATIN	RAMIPRIL
Linearity range	0.5- 5µg/ml	0.5- 5µg/ml	50- 500ng/spot	50- 500 ng/spot
Slope	59785.4904	33790.2356	9.3332	4.0682
Standard slope	25.2963	0.1549	0.0158	0.0221
Intercept	-535.1836	-1471.7753	71.6687	-27.7690
Standard Intercept	127.6127	7.2648	6.5140	5.4183
r	0.9992	0.9991	0.9992	0.9989

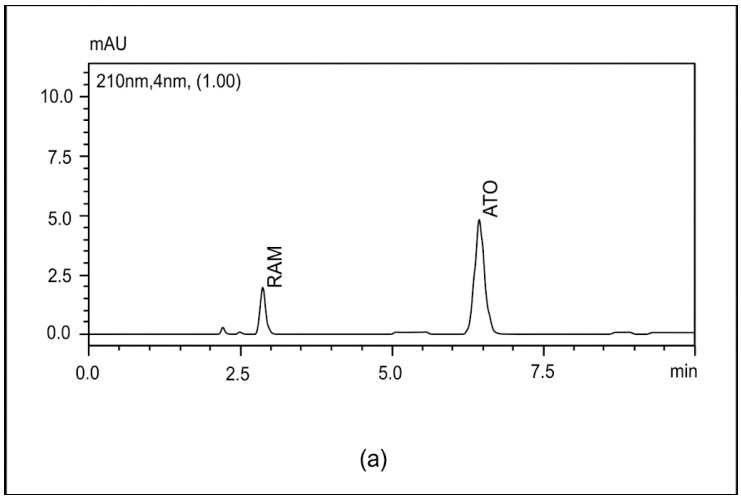

Figure 4. [a] High Performance Liquid Chromatography [HPLC].

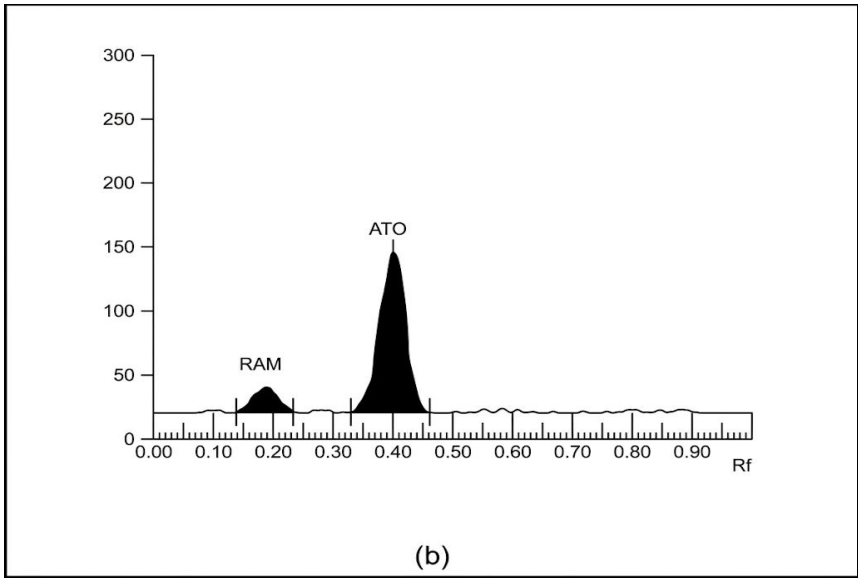

Figure 5. [b] High Performance Thin Layer Chromatography [HPTLC].

High-Performance Liquid Chromatography analysis of Atorvastatin at a concentration of 1 mg/mL and Ramipril at 0.5 mg/mL from a capsule formulation was conducted at 210 nm, showing retention times of 6.42 minutes for Atorvastatin and 2.86 minutes for Ramipril. The result and graphical represent in [Table: 2] and [Figure: 4]. High-Performance Thin-Layer Chromatography analysis of Atorvastatin at 100 ng per spot and Ramipril at 50 ng per spot from the same capsule formulation was performed at 210 nm, yielding retention factors (Rf) of 0.40 for ATO [Atorvastatin] and 0.19 for RAM [Ramipril].

Result of Reversed Phase High Performance Liquid Chromatography [RP-HPLC]

Table 3: The RP-HPLC recovery value of Atorvastatin & Ramipril capsule formulation.

FORMULATION	DRUG	INJECTED SAMPLE [in mg]	AMOUNT STANDARD ADDED [in mg]	AMOUNT RECOVERED [in mg]	% RECOVERY
CAPSULE	Atorvastatin	17.59	1.5	1.6	98.5
	Ramipril	17.59	0.75	1.2	99.0

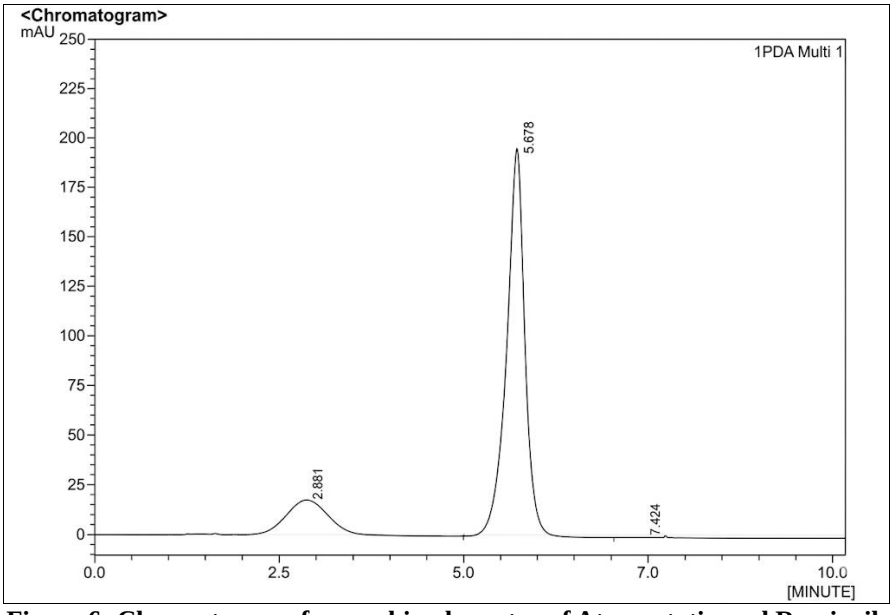

Figure 6: Chromatogram for combined spectra of Atorvastatin and Ramipril.

The percentage recovery values and low RSD indicate reliability of the methods. The chromatogram for combined spectra and recovery values is shown above [Table: 3] and [Figure:6].

Result of RP-HPLC in Plasma

Table 4: SD: Standard Deviation; RSD: Relative Standard Deviation. *Average of fifteen determinations.

ANALYTE	MEAN RECOVERY* ± SD	RSD
Atorvastatin	92.25± 3.8973	3.9345
Ramipril	91.72 ± 4.0325	4.1925

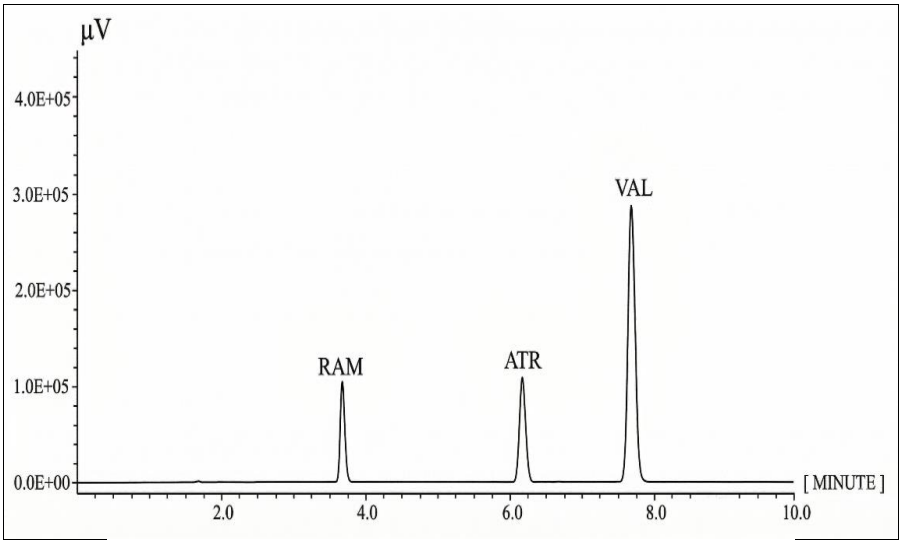

Figure: 7 Chromatogram of Quality control sample analysis.

The Plasma studies also showed acceptable recovery and reproducibility [Table 4].

The chromatogram representation of quality control analysis is shown in [Figure: 7].

CONCLUSION

This comprehensive review highlights the significance of reliable and validated analytical methods for the simultaneous determination of atorvastatin and ramipril in pharmaceutical formulations and biological matrices. These two drugs are widely used in combination therapy for the effective management of cardiovascular diseases, including hypertension, hyperlipidaemia, and coronary artery disorders. Therefore, the development of accurate, precise, and sensitive analytical techniques is essential to ensure their quality, safety, and therapeutic efficacy. The review clearly demonstrates that various analytical methods such as UV spectrophotometry, HPLC, HPTLC, and RP-HPLC have been successfully employed for the estimation of these drugs. UV spectrophotometric methods are simple, cost-effective, and suitable for routine analysis, while chromatographic techniques like HPLC and RP-HPLC provide higher sensitivity, specificity, and reproducibility. HPTLC offers advantages such as rapid analysis and reduced solvent consumption, making it an efficient alternative for simultaneous estimation.

Method validation plays a crucial role in confirming the reliability of these analytical procedures. Parameters such as accuracy, precision, linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness ensure that the developed methods meet regulatory standards and produce consistent results. The reported studies indicate low relative standard deviation (RSD) values and high percentage recovery, confirming the reliability and reproducibility of the methods. Furthermore, bioanalytical methods, particularly RP-HPLC in plasma samples, have proven effective in pharmacokinetic and bioavailability studies. These methods demonstrate acceptable recovery, sensitivity, and reproducibility, making them suitable for clinical and research applications.

Overall, the findings emphasize that the combination of advanced analytical techniques and proper validation ensures accurate simultaneous determination of atorvastatin and ramipril. These methods are highly beneficial for routine quality control, formulation development, and clinical studies. Future research may focus on developing more rapid, eco-friendly, and highly sensitive techniques to further improve analytical efficiency and support the growing demands of pharmaceutical analysis.

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