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UHPLC Method Development of Validation for the Estimation of Ramipril and Metoprolol Succinate in Tablet Dosage Form

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KEYWORDS

UHPLC,

Ramipril,

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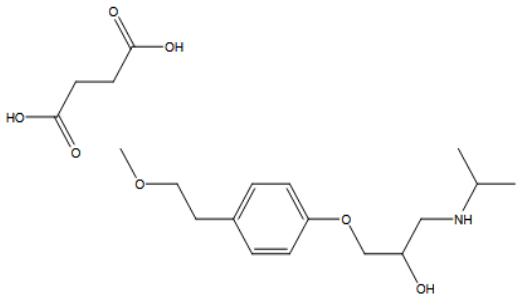
Chromatographic Method

ABSTRACT: This study aimed to develop and validate a novel Ultra-High-Performance Liquid Chromatography (UHPLC) method for the simultaneous quantification of Ramipril and Metoprolol Succinate in tablet formulations. The method, developed in compliance with International Council for Harmonization (ICH) guidelines, demonstrated high accuracy, repeatability, and low relative standard deviation (RSD) values of 0.49 for Ramipril and 0.59 for Metoprolol Succinate, confirming precision. An Agilent Technologies 1220 Infinity Series UHPLC system with a SUPELCO C18 column was used, with optimal mobile phase conditions and a flow rate of 0.7 mL/min. The method showed excellent resolution, symmetric peaks, and retention times of 0.677 minutes for Ramipril and 1.926 minutes for Metoprolol Succinate. Linearity was confirmed for concentrations ranging from 18.56 to 58.79 µg/mL for Ramipril and 25.10 to 75.15 µg/mL for Metoprolol Succinate. High recovery rates (98.95–100.19% for Ramipril and 100.05–100.18% for Metoprolol Succinate) confirmed accuracy, and the method's precision was validated by low %RSD values (0.84% for Ramipril and 0.23% for Metoprolol Succinate). This new UHPLC method is efficient, robust, and precise, offering valuable improvements for drug content assessment in pharmaceutical quality control.

1. Introduction

Metoprolol Succinate, represented by the chemical structure {butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol}, has a molecular formula of C35H56N2O10 and a molecular weight of 652.8 g/mol. This compound functions as an antihypertensive agent, specifically a β1-adrenergic blocker. Metoprolol and other adrenergic beta-antagonists are frequently used for a number of illnesses, such as anxiety, migraine headaches, glaucoma, angina pectoris, hypertension, and cardiac arrhythmias. By competing with adrenergic neurotransmitters such as catecholamines for binding at β1-adrenergic receptors in the heart, metoprolol effectively reduces heart rate, cardiac output, and blood pressure. [1]



Structure of Metoprolol succinate

Ramipril, an ACE inhibitor known for its extended half-life, is chemically identified as 4-[2-(1-ethoxycarbonyl-3-phenyl-propyl) aminopropanoyl]-4-azabicycloctane-3-carboxylic acid. Once ingested, it is converted into its active form, Ramiprilate, through hydrolysis, allowing for once-daily dosing due to its prolonged elimination half-life. Clinical studies have

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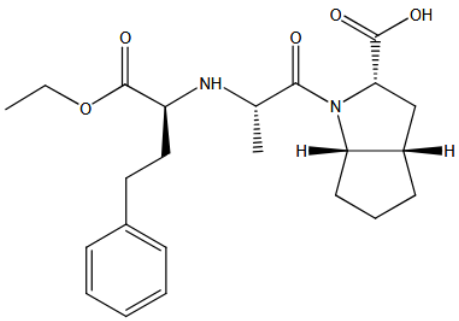
consistently demonstrated the efficacy of Ramipril, particularly benefiting patients suffering from moderate to severe congestive heart failure. [2]



Structure of Ramipril

2. Materials and Methods:

Pharmaceutical-grade samples of metoprolol succinate and Ramipril were obtained from Sigma Aldrich, a well-regarded supplier recognized for its dedication to high-quality chemical products. Additionally, analytical-grade reagents, including acetonitrile, orthophosphoric acid, and HPLC-grade water, were sourced from National Scientific Products, ensuring that the experimental procedures met rigorous standards of accuracy and reliability. To enhance the research, EMBETA R 5mg commercially available tablet formulation containing 50 mg of metoprolol succinate and 5 mg of Ramipril was purchased from a local market, allowing for the effective integration of these active ingredients into a pharmaceutical context. This meticulous selection of materials reflects a strong commitment to upholding stringent standards in pharmaceutical research and development.

2.1. Instrumentation and Equipment:

The study used various analytical instruments and materials to ensure precise measurements and data collection. A Digital Electronic Balance, pH meter, Sonicator, Nylon membrane filter (0.45µ), Ultra-High Performance Liquid Chromatography system (Agilent technologies 1220 infinity series), CHEMSTATION OPEN LAB software, UV-Visible detector, and SUPELCO C18 analytical column (SUPELCO C18; 4.6mm X 50 mm; 3microns) were used for weighing, monitoring pH levels, and chromatographic separation and quantification.

2.2. Chromatographic method:

The selection of analytical method is influenced by factors such as the characteristics of the sample, its molecular weight, pKa value, and stability. In this research, polar drugs were the focus, leading to the use of reversed phase or ion exchange chromatography. Ultra-high-performance liquid chromatography (UHPLC) was selected for the initial separation due to its efficiency. A C18 column served as the stationary phase, while the mobile phase comprised various solvents, including acetonitrile, methanol, water, potassium dihydrogen orthophosphate, and orthophosphoric acid.

2.3. Optimized method parameters:

The chromatographic analysis was performed using an Agilent Technologies 1220 Infinity Series UHPLC system. Separation was achieved on a SUPELCO C18 analytical column with dimensions of 4.6 mm × 50 mm and a particle size of 3 microns. The column oven temperature was maintained at 40°C to ensure consistent performance and retention. Detection was carried out at a wavelength of 225 nm using a UV-Visible detector. The mobile phase consisted of a mixture of acetonitrile, methanol, and phosphate buffer (pH 5) in the ratio of 35:30:35, respectively. The system operated in isocratic mode, with a flow rate of 0.7 ml/min. An injection volume of 2 µl was used for each run, and the total runtime for the analysis was 5 minutes.

2.4. Procedure for preparation of solution:

2.4.1. Preparation of buffer:

To prepare a 0.05M KH2PO4 buffer, accurately weigh 6.8 grams of potassium dihydrogen orthophosphate and transfer it to a 1000 ml beaker. Add 900 ml of HPLC-grade water and sonicate the mixture for 20 minutes. After sonication, adjust the volume to the 1000 ml mark with additional HPLC-grade water and filter the solution using a 0.45 µm filter. Finally, adjust the pH to 5 by incorporating 1 ml of 0.1% OPA into the filtered solution.

2.4.2. Preparation of mobile Phase:

The solution is composed of acetonitrile, methanol, and a phosphate buffer at pH 5, mixed in the ratios of 350:300:350. It is essential to filter this mixture using a 0.45 µm filter under vacuum to ensure clarity and remove any particulate matter.

2.4.3. Diluent preparation:

The selection of the diluent was based on the solubility characteristics of the drugs, with methanol being utilized as the diluent in the mobile phase.

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2.5. ASSAY

Preparation of the metoprolol succinate and Ramipril standard & sample solution:

2.5.1. Preparation of standard Stock solution:

To prepare a standard solution, weigh 50 mg of Metoprolol succinate and 5 mg of Ramipril, transfer them into a 100 ml volumetric flask, dissolve them in methanol, and continue filling the flask until the total volume reaches 100 ml. This method ensures a precise standard solution, crucial for subsequent analytical procedures.

2.5.2. Sample solution preparation:

An accurately weighed amount of tablet powder equivalent to 50 mg of Metoprolol succinate and 5 mg of Ramipril was transferred into a clean, dry 100 ml volumetric flask. To this, 70 ml of the diluent was added, and the mixture was sonicated to ensure complete dissolution of the drug components. After sonication, the solution was diluted to volume with the same diluent to obtain the stock solution.

From the prepared stock solution, 3 ml was accurately pipetted into a 10 ml volumetric flask. The volume was then made up to the mark with the same diluent to obtain the final working solution for analysis.

Procedure:

In the procedure, 2 µL of both the standard and sample solutions were injected into the chromatographic system, where the areas corresponding to the peaks of Metoprolol and Ramipril were measured. The percentage assay was then calculated using the appropriate formula.

Calculation:

For Ramipril: Calculate the amount of Ramipril by using the following formula:

$$\text{Content of Ramipril} = \frac{AT}{AS} \times \frac{Ws}{100} \times \frac{5}{50} \times \frac{P}{WT} \times \frac{\text{Average Weight}}{5} \times 100.00$$

AT = Average area counts of peaks due to Ramipril in the chromatogram of Sample solution.

AS = Average area counts of peaks due to Ramipril in the chromatogram of Standard solution.

WS = Weight of Standard Preparation

WT = Weight of sample preparation

P = Potency of Ramipril working standard

For Metoprolol Succinate:

Calculate the amount of Metoprolol Succinate by using the following formula:

$$\text{Content of Metoprolol} = \frac{AT}{AS} \times \frac{Ws}{100} \times \frac{5}{50} \times \frac{P}{WT} \times \frac{\text{Average Weight}}{5} \times 100.00$$

AT = Average area counts of peaks due to Metoprolol succinate in the Chromatogram of Sample solution.

AS = Average area counts of peaks due to Metoprolol succinate in the chromatogram of Standard solution

WS = Weight of Standard Preparation

WT = Weight of Sample preparation

P = Potency of Metoprolol Succinate working standard

Validation of Analytical method:

Accuracy:

Accuracy was assessed through percentage recovery studies conducted at three distinct concentration levels. In this process, a known quantity of standard drug powders of METO and RAMI was added to the reanalyzed sample solution at levels of 80%, 100%, and 120%.

Precision:

The precision of the method was assessed through five replicate injections of a standard solution containing METO and RAMI. To evaluate method precision, the analyst was analyzed five times using the proposed approach. Additionally, repeatability was determined by conducting multiple injections of a homogeneous sample of METO and RAMI.

Linearity:

The linearity of METO and RAMI was evaluated by analysing five distinct concentrations of each drug in methanol. Calibration curves were constructed by plotting the peak area against the corresponding drug concentrations.

Robustness:

Robustness was evaluated by intentionally altering key technique parameters, including wavelength, flow rate, and mobile phase composition. The investigation focused on the robustness of the method for both RAMI and METO.

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Result and Discussion:

Selection of wavelength:

The optimal wavelength for the simultaneous estimation of Metoprolol Succinate and Ramipril was determined based on their absorbance values. Although the highest absorbance was at 230.0 nm, 219.0 nm was selected for analysis as it provided strong absorbance and was suitable for detecting both drugs with good sensitivity, making it ideal for spectrophotometric measurement. **(Fig. no. 1)**

Optimized method parameters:

Chromatographic conditions:

Mobile phase: Acetonitrile: Methanol: Phosphate buffer PH 5 (350:300:350)

Flow rate: 0.7ml/min

Column: SUPELCO C18; 4.6mm X 50 mm; 3µ

Detector wave length: 225nm Lµ

Injection volume: 2

Run time: 5 min

Diluent: Methanol & mobile phase

Results: Retention time and peak shape is good.

Temperature: 40ºC

Ramipril was eluted at 0.677 min and Metoprolol succinate was eluted at 1.926 min with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. Out of 4 trails was selected for further studies because when compared to other trails 4th trails was found less in retention time due to the ratio or organic solvent in mobile phase. The method was found, based on the system suitability parameters like resolution, tailing factor and theoretical plates. **(Fig. no: 2)**

Discussion:

The pharmaceutical formulation analysis showed high purity for both drugs, with Ramipril at 101.64% (%RSD 0.49) and Metoprolol Succinate at 100.18% (%RSD 0.59), indicating minimal variability. Ramipril (label claim 5 mg) yielded 5.03 mg, and Metoprolol Succinate (label claim 50 mg) yielded 50.095 mg, confirming the accuracy and consistency of the assay results.**(Table no: 1)**

The system suitability results for Ramipril and Metoprolol Succinate met ICH guidelines. Resolution was 2.459 (above the minimum), tailing factors were well below 2, and theoretical plate counts exceeded 2000, indicating good peak symmetry and column efficiency. Repeatability was confirmed with five consistent injections, showing stable retention times and peak areas. %RSD values for both drugs were low (0.06%), indicating excellent precision. **(Table no: 2 & 3)**

According to the specificity data indicated that no peaks were detected in the blank or placebo samples at the retention times corresponding to Ramipril and Metoprolol Succinate. In contrast, the standard samples exhibited distinct peaks, with Ramipril showing a retention time of 1.910 minutes and an area of 1330.341, while Metoprolol Succinate had a retention time of 1.926 minutes and an area of 731.623. **(Table no: 4).**

In order to evaluate linearity, the study examined five concentrations of metoprolol succinate and Ramipril. The results showed exceptional linearity for Ramipril, with a correlation coefficient of 1.000 and a standard deviation of 399.602. Metoprolol Succinate, on the other hand, showed a robust linear relationship between concentration and peak area, with a standard deviation of 257.812, an intercept of 0.02811, and a slope of 0.0686. Both compounds had a strong correlation coefficient. **Table no: 5 & 6, Fig. no: 3 & 4**

The accuracy of the UHPLC method was validated using the standard addition technique at 80%, 100%, and 120% concentration levels. Mean recoveries for Ramipril (99.73%–100.41%) and Metoprolol Succinate (99.88%–100.17%) were within acceptable limits, confirming the method's high accuracy and reliability for simultaneous drug estimation. **Table no: 7 & 8**

The precision of the method for Ramipril and Metoprolol Succinate was confirmed through system, method, and intermediate precision studies. System precision showed excellent reproducibility with %RSD values of 0.08% (Ramipril) and 0.10% (Metoprolol). Method precision yielded %RSDs of 0.845% and 0.23%, while intermediate precision under varying conditions showed %RSDs of 0.940% and 0.25%, all well within acceptable limits, confirming the method's reliability. **(Table no: 9, 10 & 11)**

The robustness of the method was confirmed by introducing slight variations in flow rate and detection wavelength. Despite these changes, system suitability parameters remained stable, and all results were within acceptable limits. %RSD values for Ramipril (0.27–0.47)

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and Metoprolol Succinate (0.20–0.63) demonstrated that the method is robust and reliable under varied conditions. **(Table no: 12)**

Result:

A novel UHPLC method was developed and validated for the simultaneous analysis of Ramipril and Metoprolol Succinate in combined formulations. The method showed excellent specificity, linearity, precision, accuracy, and robustness per ICH guidelines. Using a SUPELCO C18 column and an optimized mobile phase, it achieved sharp, well-resolved peaks with short retention times. With minimal sample preparation and a run time under 10 minutes, the method is well-suited for routine quality control, dissolution testing, raw material analysis, and bioanalytical applications. Overall, it is a reliable and efficient tool for pharmaceutical analysis.

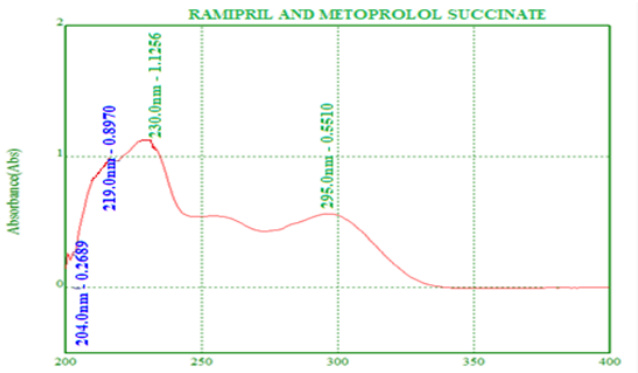

Fig. no: 1. Wavelength of Ramipril and Metoprolol succinate

Table No.01 Assay of Ramipril and Metoprolol succinate

Sample	Label claim	Peak area*	Amount obtained*	Percent label claim % w/w*	SD	%RSD
Ramipril	**5mg**	1320.935	5.03	101.64%	0.49	0.04
Metoprolol succinate	**50 mg**	730.197	50.095	100.18%	0.59	0.08

***Each value is a Mean of Two readings**

Table No: 02 System suitability for Ramipril

Sample ID	Ramipril	
	RT	**AREA**
Average	0.675	1319
SD	0.00	0.836
RSD%	0.12%	0.06%

Table No: 03 System suitability for Metoprolol

Sample ID	Ramipril	
	RT	**AREA**
Average	0.675	1319
SD	0.00	0.836
RSD%	0.12%	0.06%

Table No 04. Specificity table for Ramipril and Metoprolol Succinate

Sample ID	Ramipril		Metoprolol Succinate	
	RT	**Area**	**RT**	**Area**
BLANK	1.910	No peak observed	1.926	No peak observed
STANDARD	1.910	1330.341	1.926	731.623
PLACEBO	1.910	No peak observed	1.926	No peak observed

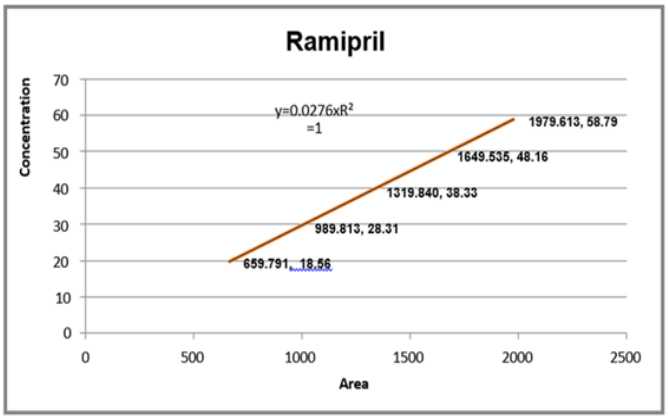

Fig. no: 2: Optimized Chromatogram of Ramipril and Metoprolol succinate

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Table No. 05. Linearity Data of Ramipril

S.no	Levels	Conc(µg/ml)	Area of Ramipril
1.	50%	18.56	659.791
2.	75%	28.31	989.813
3.	100%	38.33	1319.840
4.	125%	48.16	1649.535
5.	150%	58.79	1979.613
		SD	399.602
		Intercept	0.99203
		Slope	0.0276
		Correlation coefficient	0.9998

Table No.06 Linearity Data of Metoprolol Succinate

S.no	Levels	Conc(µg/ml)	Area of Metoprolol Succinate
1.	50%	25.10	366.142
2.	75%	38.17	546.734
3.	100%	50.10	729.916
4.	125%	62.30	913.142
5.	150%	75.15	1094.91
		SD	257.812
		Intercept	0.02811
		Slope	0.0686

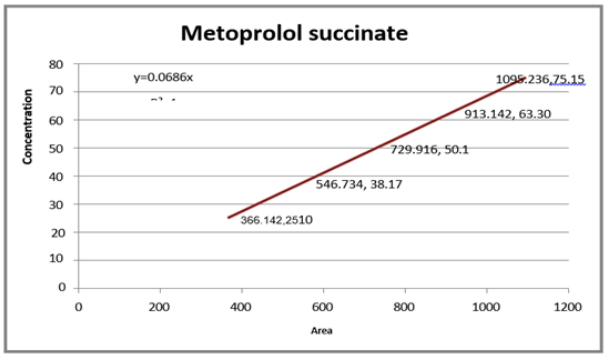

Fig. no: 3 Linearity of Ramipril

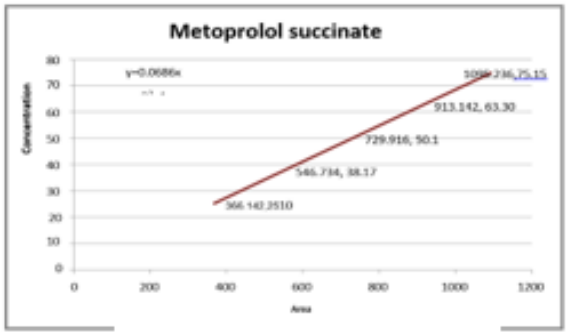

Fig. no: 4 Linearity of Metoprolol

Table No .07. Accuracy table of Ramipril

Concentration in %	Sample Weight	Sample area	Average area	Calculated content (in mg)	Recovery (%)	Average Recovery (%)
80%	200.96	1063.236	1067.571	4.9818	99.63	100.41
	200.85	1075.213		5.0496	100.99	
	200.65	1064.263		5.0313	100.62	
100%	250.12	1339.845	1336.618	5.0158	100.20	99.90
	250.24	1329.745		4.9546	99.09	
	250.34	1340.263		5.0330	100.63	
	300.23	1594.632		4.996	99.94	

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120%	300.54	1599.896	1596.590	4.998	99.96	99.73
	300.68	1595.243		4.995	99.90	

Table No. 8. Accuracy table of Metoprolol Succinate

Concentration in %	Sample Weight	Sample area	Average area	Calculated content (in mg)	Recovery (%)	Average Recovery (%)
80%	200.96	600.230	600.981	49.8415	99.68	99.88
	200.85	601.254		49.9539	99.91	
	200.65	601.458		50.0207	100.04	
100%	250.12	750.312	751.067	50.0584	100.12	100.17
	250.24	751.245		50.0966	100.19	
	250.34	751.643		50.1031	100.21	
120%	300.23	900.326	900.032	50.0413	100.08	99.96
	300.54	899.645		49.9519	99.90	
	300.68	900.125		49.9553	99.91	

Table no: 9 System Precision for Ramipril & Metoprolol

Sample ID	Ramipril		Metoprolol succinate	
	RT	**AREA**	**RT**	AREA
Average Value	0.670	1321.812	1.926	**731.662**
SD:	0.00	1.102	0.02	**0.702**
%RSD:	0.12	0.08	0.93	0.10

Table No.10. Method precision of Ramipril & Metoprolol

Sample ID	Ramipril		Metoprolol succinate	
	Calculated Assay (in mg)	**Calculated Assay %**	**Calculated Assay (in mg)**	Calculated Assay %
Average Value	4.936	98.72	49.93	99.87
SD:	0.041	0.83	0.114	0.23
%RSD:	0.845	0.847	0.23	0.23

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Table No.11.Intermediate precision of Ramipril & Metoprolol Succinate

Sample ID	Ramipril		Metoprolol succinate	
	Calculated Assay (in mg)	**Calculated Assay %**	**Calculated Assay (in mg)**	Calculated Assay %
Average Value	4.9667	99.328	50.08	100.34
SD:	0.046	0.931	0.08	0.25
%RSD:	0.940	0.93	0.16	0.25

Table No.12.Robustness data for Ramipril and Metoprolol Succinate

S. No	Condition	%RSD of Ramipril Assay	%RSD of Metoprolol Succinate Assay
1	(-)Flow rate-0.6ml/min	0.30	0.44
2	(+)Flowrate-0.8ml/min	0.47	0.20
3	(-)Wavelength-224nm	0.32	0.63
4	(+)Wavelength-226nm	0.27	0.27

Conclusion:

In this research was conclude, a novel and efficient UHPLC method was developed and validated for the simultaneous quantification of Ramipril and Metoprolol Succinate in a combined pharmaceutical formulation. The method exhibited outstanding characteristics, including specificity, linearity, precision, accuracy, and robustness, in accordance with ICH guidelines. Utilizing a SUPELCO C18 column and an optimized mobile phase of Acetonitrile, Methanol, and Phosphate Buffer (pH 5) in a 35:30:35 ratio, the method achieved sharp, well-resolved peaks with retention times of 0.677 minutes for Ramipril and 1.926 minutes for Metoprolol Succinate. This approach offers significant practical benefits, such as minimal sample preparation, a total run time of under 10 minutes, and the absence of complex extraction steps, making it suitable for routine quality control, dissolution testing, content uniformity assessments, raw material analysis, and bioanalytical applications, including bioequivalence and pharmacokinetic studies. Overall, the developed UHPLC method is robust, reliable, and versatile, positioning it as a valuable tool in pharmaceutical research and industrial quality control for the effective analysis of Ramipril and Metoprolol Succinate in combined dosage forms.

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