Analytical Method Development and Validation of Ramipril Using Uv-Visible Spectroscopy: Single Point Standardization, Standard Absorptivity, And Calibration Curve Approach

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***Abstract*— UV spectrophotometric techniques play a crucial role in pharmaceutical analysis for the estimation of active compounds. This study presents the development and validation of a UV-Visible Spectroscopy method for Ramipril using methanol as a solvent. The method adheres to Beer-Lambert's law and ICH guidelines, exhibiting a maximum absorbance at 207 nm. Excellent linearity (R^2 = 0.9992) was observed over the tested concentration range, with precise LOD and LOQ values confirming the method's sensitivity. Additionally, the method demonstrated high precision, accuracy, and robustness, making it a reliable choice for routine pharmaceutical quality control applications.**

***Index Terms*— UV spectrophotometry, Ramipril, Methanol, Validation, ICH, Pharmaceutical Analysis**

I. INTRODUCTION [1,2,3]

Ramipril is an inhibitor of the angiotensin-converting enzyme (ACE). The renin-angiotensin-aldosterone system is affected. It decreases the breakdown of bradykinin and prevents the conversion of the inactive angiotensin I to the extremely powerful vasoconstrictor angiotensin II. chemically2-[N-[(S)-1-(ethoxy carbonyl) -3-phenylpropyl)] -L-alanyl] -(1S,3S,5S) -2azabicyclo [33-0] octane carboxylic acid. The British Pharmacopoeia, which specifies a potentiometric titration method for its assay in bulk and dose form, officially recognizes the medication. Publications pertaining to the determination of Ramipril include: Derivative compensation approach and zero crossing derivative technique were used to estimate the amount of Ramipril and hydrochlorothiazide in a binary mixture.

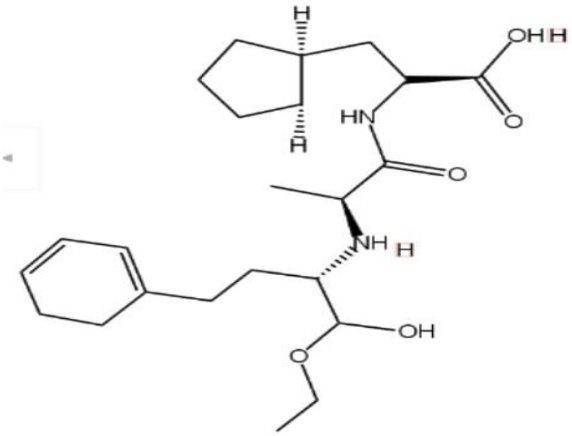

Fig. No. 1 Ramipril

UV-Visible Spectroscopy is a simple, cost-effective, and efficient technique extensively used for pharmaceutical analysis. This technique operates based on Beer-Lambert's law, which relates absorbance to concentration, making it highly suitable for quantitative drug estimation. It allows rapid detection of drug purity and concentration with minimal sample preparation, making it a widely accepted tool in quality control laboratories.

Several analytical methods, including spectrophotometric and chromatographic techniques, have been reported for the quantification of RAM. However, there remains a need for a validated, simple, and economical method with high precision and accuracy. This study aims to develop and validate a UV spectrophotometric method for the estimation of RAM using methanol as a solvent, following ICH guidelines.

II. MATERIALS AND METHODS

2.1 Instrumentation

The study was performed using a Systronics (India) Limited UV-visible spectrophotometer, Model AU-270, featuring a double beam and double detector arrangement with a 1 cm matched cell. The software used was UV Professional Double Beam. The mobile phase was degassed using an ultrasonicator cleaner, and an electronic balance was used for weighing.

2.2 Chemicals and Reagents

RAM standard was obtained from CTX Lifesciences Pvt. Limited, batch no. 24MS000014, with a manufacturing date of February 2024 and an expiration date of 2029. Analytical-grade reagents were gifted by Ajanta Pharmaceutical PPIC department, Mumbai. Calibrated glassware from Borosil Company was used throughout the analysis.

III. METHODOLOGY

3.1 Selection of Solvent

Methanol was chosen as the solvent due to its superior solubility properties for RAM

3.2 Preparation of Standard Stock Solution

A 25 mg quantity of pure RAM was accurately weighed and dissolved in methanol in a 25 ml volumetric flask to obtain a final concentration of 1000 ppm. A working standard of 20 ppm was prepared from this stock solution using distilled water.

3.3 Determination of Lambda Max

The UV spectrum of RAM was scanned between 200 and 400 nm at a concentration of 20 ppm. The maximum absorbance wavelength was observed at 207 nm.

3.4 Estimation of Ramipril [4]

Table No. 1 Absorbance

Concentration	Absorbance
10 ppm	0.288
20 ppm	0.584
30 ppm	0.902
40 ppm	1.198
50 ppm	1.451
Tablet 20 ppm	0.527

3.4.1 Standard Absorptivity Method

- Prepare standard solutions of RAM in methanol at concentrations ranging from 10 ppm to 50 ppm.
- Measure the absorbance of each solution at 207 nm using a UV-visible spectrophotometer.
- Calculate the absorptivity (a) using Beer-Lambert's law:

A = abc

Where:

- A = Absorbance
- a = Molar Absorptivity
- b = Path length (1 cm)
- c = Concentration in ppm

The standard absorptivity method is based on the principle of Beer-Lambert's law, which states that absorbance is directly proportional to concentration by determining the absorptivity coefficient (a), unknown sample concentrations can be estimated accurately. This method is widely used due to its simplicity and ability to provide reliable quantitative results. By using formula, it was noted that the percentage purity of drug by using this method was found 90.25 %. (By using Table No. 1)

3.4.2 Single Point Standardization Method

- Prepare a standard solution of RAM in methanol at a known concentration (e.g., 20 ppm).
- Prepare the test sample solution in methanol.
- Measure the absorbance of both the standard and test sample at 207 nm using a UV-visible spectrophotometer.
- Calculate the concentration of the test sample using the equation:

Ctest = (Atest X Cstd) / Astd

Where, C_{test} = concentrations of sample

C_{std} = concentrations of standard

A_{test} = absorbance of the sample

A_{std} = absorbance of the standard

The Single Point Standardization Method is a direct and simple approach for estimating the concentration of an unknown sample by comparing its absorbance with that of a standard solution of known concentration. It is particularly useful for quick and routine analysis where high precision is not required. By using formula, it was noted that the percentage purity of drug by using this method was found 90.25 %. (By using Table No. 1)

3.4.3 Calibration Curve Method

- Prepare standard solutions of RAM in methanol at concentrations ranging from 10 ppm to 50 ppm.
- Measure the absorbance of each solution at 207 nm using a UV-visible spectrophotometer.
- Plot a calibration curve by graphing absorbance against concentration.
- Determine the equation of the calibration curve using linear regression analysis.
- Use the calibration curve equation to estimate the concentration of unknown samples by interpolating their absorbance values.

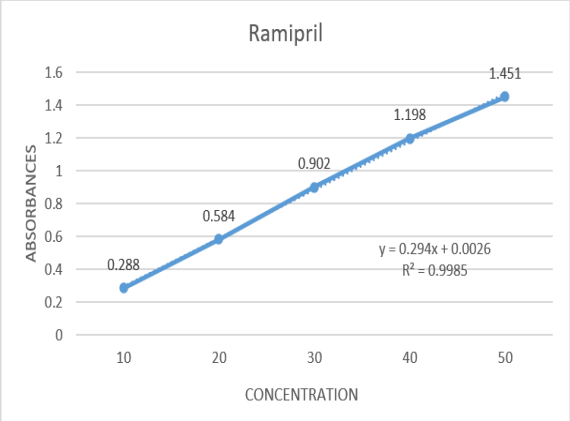

Fig. No. 2 Calibration Curve

The Calibration Curve Method is based on Beer-Lambert's law, where absorbance is directly proportional to concentration. By constructing a standard calibration curve, the concentration of unknown samples can be accurately determined. This method ensures high precision and reliability in quantitative analysis. By using equation of line after calculating by using formula, it was noted that the percentage purity of drug by using this method was found 90.25 %.

IV. METHOD VALIDATION[5]

4.1 Linearity[6-9]

The closeness of the test results obtained by the method to the true value or an accepted reference value. It is usually expressed as a percentage recovery. The regression coefficient (R^2) from the linearity curve was found to be 0.9992, indicating excellent linearity

Table No. 2 Linearity

Concentration	Absorbance	Calculations	
10	0.288	MEAN	0.8846
20	0.584	SD	0.465194
30	0.902	RSD	0.5258
40	1.198	%RSD	52.58%
50	1.451	Slope	0.0294
TABLET 20 ppm	0.527	Intercept	0.0012

4.2 Precision[10-13]

The degree of repeatability of an analytical method under normal operating conditions. It is divided into: Intraday and Interday Precision

Results showed minimal variation in absorbance values over different time intervals and on different days, demonstrating the method's precision

Table No. 3 Intraday Precision

Time	Concentration	Absorbance	Calculations	
0 Hrs	20 PPM	0.584	Mean	0.5866
2 Hrs	20 PPM	0.591	SD	0.00422
4 Hrs	20 PPM	0.581	RSD	7
8 Hrs	20 PPM	0.584	%RSD	0.00720
12 Hrs	20 PPM	0.591		5
24 Hrs	20 PPM	0.589		0.7205

Table No. 4 Interday Precision

Time	Concentration	DAY 1	DAY 2	DAY 3
0 Hrs	20 PPM	0.584	0.581	0.580
2 Hrs	20 PPM	0.587	0.583	0.582
4 Hrs	20 PPM	0.580	0588	0.579
8 Hrs	20 PPM	0.585	0.579	0.584

12 Hrs	20 PPM	0.588	0.582	0.585
24 Hrs	20 PPM	0.591	0.589	0.589
Mean		0.585833	0.583667	0.583167
SD		0.003764	0.003983	0.003656
RSD		0.006425	0.006824	0.006269
%RSD		0.6425	0.6824	0.6269

4.3 Accuracy[13-15]

Accuracy is often assessed at different levels, typically 80%, 100%, and 120% of the target concentration in validation studies. This ensures that the analytical method provides reliable results across a range of concentrations. The accuracy is determined by recovery studies, where known amounts of the analyte are added to the sample, and the percentage recovery is calculated.

Table No.5 Accuracy

Spiked Level (%)	Added Concentration	Measured Concentration	% Recovery
80	8 ppm	7.9	98.8
100	10 ppm	10.1	101
120	12 ppm	12.2	101.7

Result shows mean of 3 readings

4.4 Ruggedness[16-20]

The reproducibility of the method under normal conditions by different analysts, instruments, or laboratories. Two analysts performed the study independently, and % RSD was found to be within acceptable limits

Table No. 6 Ruggedness

Analyst	X	Y
System	Systronics	Shimadzu
Day	Monday	Thursday
Time	Morning	Evening
Concentration	20 PPM	20 PPM
Absorbance	0.584	0.585
	0.581	0.579
	0.589	0.588
Mean	0.584667	0.584
SD	0.004041	0.004583
RSD	0.006911	0.007847
%RSD	0.6911	0.7847

4.5 Robustness[21-23]

The ability of the method to remain unaffected by small but deliberate variations in method parameters (e.g., pH, temperature, flow rate) and still provide accurate results. The method's robustness was assessed at three wavelengths (207, 209, and 211 nm), showing minimal variation in absorbance values

Table No. 7 Robustness

Wavelength	Concentration	Absorbance	Calculations	
207 nm	20PPM	0.584	SD	0.003512
	20PPM	0.581	RSD	0.006010
	20PPM	0.588	%RSD	0.6010
209 nm	20PPM	0.589	SD	0.005568
	20PPM	0.578	RSD	0.009550
	20PPM	0.582	%RSD	0.9550
211 nm	20PPM	0.588	SD	0.004041
	20PPM	0.583	RSD	0.006880
	20PPM	0.591	%RSD	0.6880

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)[24-27]

LOD is the lowest amount of analyte that can be detected but not necessarily quantified under stated conditions and LOQ is the lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy.

$LOD = \frac{3.3 \text{ X } \sigma}{S}$ $LOQ = \frac{10 \text{ X } \sigma}{S}$

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

LOD and LOQ were calculated as 0.098 µg/ml and 0.297 µg/ml, respectively.

V. ESTIMATION OF RAMIPRIL FROM TABLET DOSAGE FORM

Weigh and finely grind 20 tablets. Weigh a portion equivalent to 10 mg of RAM.
Dissolve in 10 mL methanol (Sonicate if needed). Filter the solution to remove excipients. Take 1 mL of the filtrate and dilute to 10 mL (final concentration ~100 ppm). Check the absorbance of this resulting solution at 280 nm. By using formula, it was noted that the percentage purity of drug was found 90.25 %.

Table No. 8 Assay of Marketed Formulation

Concentration	Absorbance at 207 nm	Specifications
20 ppm	0.584	Brand Name: Ramipres-5
	0.581	Label Claim: 5 mg
	0.588	Manufacturer: CIPLA Pvt. Ltd.
Mean	0.58433	Manufacturing date: Feb 2024
SD	0.003512	Expiry Date: Feb 2029
RSD	0.006010	Batch No: 24MS000014
%RSD	0.6010	% Purity of drug: 90.25 %.

VI. DISCUSSION

The developed UV spectrophotometric method for RAM adheres to ICH guidelines. It demonstrates high precision, accuracy, linearity, robustness, and sensitivity. The proposed method provides an efficient and cost-effective alternative for routine pharmaceutical analysis.

VII. CONCLUSION

A simple, accurate, and economical UV-spectrophotometric method for the determination of RAM was successfully developed and validated according to ICH guidelines. This method offers a reliable approach for routine pharmaceutical quality control.

VIII. CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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