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Development and Validation of Simultaneous Estimation of Enalapril and Irbesartan by Spectroscopic Method

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ABSTRACT:
This research aimed to create and verify a new spectroscopic technique for the simultaneous measurement of two commonly used antihypertensive medications, enalapril and irbesartan. The method, which offered a straight forward and affordable substitute for the routine study of pharmaceutical formulations comprising both chemicals, was based on the concepts of UV-visible spectrophotometry. The ICH requirements were followed in the validation process of the created technique, taking into account parameters including linearity, precision, accuracy, specificity, and robustness. For both enalapril and irbesartan, the method demonstrated excellent linearity over a broad concentration range, with correlation values above 0.99. Recovery experiments were used to evaluate accuracy, and the findings were adequate within reasonable bounds. The developed spectroscopic method offers several advantages, including simplicity, rapidity, and cost-effectiveness, making it suitable for routine analysis in pharmaceutical quality control laboratories. This technique aids in effective drug analysis and formulation monitoring by permitting the simultaneous estimation of enalapril and irbesartan, possibly improving patient safety and therapeutic results.

INTRODUCTION

Enalapril is indeed a medication used primarily for treating hypertension and heart failure. Its chemical structure and mechanism of action are important in understanding its pharmacological effects Enalapril is indeed a medication used primarily for treating hypertension and heart failure. Its chemical structure and mechanism of action are important in understanding its pharmacological effects[1]. By influencing the renin-angiotensin-aldosterone pathway, the prodrug enalapril influences blood pressure as well as fluid and electrolyte balance[3]. One way to describe it is as an inhibitor of angiotensin-converting enzyme. The pharmaceutical drug enalapril is employed for the treatment of hypertension. It works by preventing the renin-angiotensin-aldosterone cascade from being activated. There are no sulphydryl components in this oral drug, and its effects last for a long time[4].

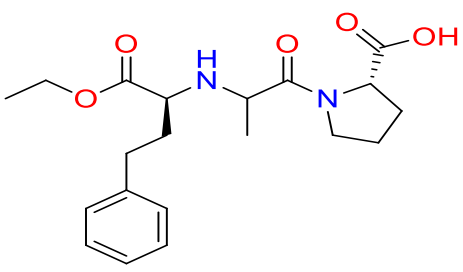

Fig 1. Structure of enalapril

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Irbesartan is a member of the ARB drug class. By specifically blocking angiotensin II's binding to its receptor subtype AT1, it inhibits both the vasoconstrictive and aldosterone-secreting effects of the drug[5]. This action results in vasodilation and a decrease in blood pressure. Blood pressure (BP) was controlled for 24 hours with irbesartan administered once daily[6]. patients with mild-to-moderate hypertension, irbesartan was superior than valsartan in terms of absolute blood pressure decrease and response rates. It was also just as effective as amlodipine, enalapril, and atenolol [7].

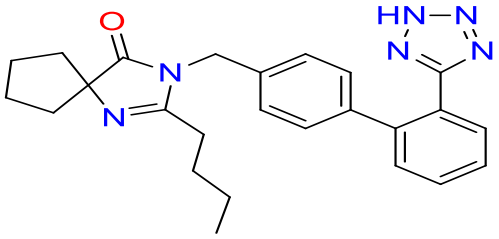

Fig 2. Structure of Irbesartan

METHOD AND MATERIAL

Procurement of Chemicals and Reagents: The analytical grade chemicals and reagents utilized in this investigation were purchased from reliable vendors. Enalapril and Irbesartan were received as gift samples from Lupin Pharmaceuticals Ltd., Bhopal, M.P., India. We purchased the fixed-dose combination of irbesartan and enalapril from a nearby medical supply store. For spectroscopic examination, all solvents and reagents were utilized without additional purification.

Instruments: The examination was conducted using a Shimadzu 1900i double-beam UV-visible spectrophotometer (Shimadzu Corporation, Japan). The photometric mode was utilized to assess the transmittance or absorbance at several wavelengths. pH meter (Cole-Parmer, India) [9,10].

Simultaneous equation method:

The two wavelengths used for the method, λ1, and λ2, are enalapril and irbesartan absorption maxima in methanol 0.1N HCl (1:1) or methanol 1N HCl (1:1). A range of reference solutions, including concentrations varying from 0 to 30 µg/ml, were acquired using separate utilizing either methanol 1N HCl (1:1) or methanol to dilute the stock solutions of both medications 0.1N HCl (1:1). For both drugs, the absorbabilities (A 1%, 1cm) at both wavelengths were computed as the average of three separate estimates of the measured absorbances at the selected frequencies. The concentrations were computed using the following formulas.

$$Cx = \frac{A_1\ ay_2 - A_2\ a\ y_1}{ax_1ay_2\text{-}ax_2ay_1}$$

…… Eq. (i)

$$Cy = \frac{A_1\ ax_2 - A_2\ a\ x_1}{ay_1ax_2\text{-}ay_2ax_1}$$

…… Eq. (ii)

The absorptivities of enalapril at λ1 and λ2 are represented by Ax1 and Ax2, respectively. The absorptivities of irbesartan at M1 and λ2 are denoted by ay1 and ay2, respectively. A1 and A2 absorbances at wavelengths λ1 and λ2, respectively, are displayed by the combination. The enalapril and irbesartan concentrations that were measured are denoted by Cx and Cy, respectively.

A1 = ax1Cx + ay1Cy at λ1 It's .....(1)

A2 = ax2Cx + ay2Cy at λ2 …….. (2)

Preparation of mobile phase:

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Methanol: Mobile phase solvent is a combination of methanol and either 1N HCl or 0.1N HCl, or a 1:1 mixture of the two. A 0.22 µm membrane filter was used to filter the mobile phase after it was degassed in an ultrasonic bath before it was used.

Preparation of buffer solution:

One milliliter of triethylamine and 4.7 grams of sodium dihydrogen orthophosphate were combined with one thousand milliliters of water. The combination was subsequently treated with orthophosphoric acid to bring its pH down to 4.0±0.05.

Method development and validation by UV Spectrophotometry:

Simultaneous estimation of Enalapril and Irbesartan in tablet dosage form Using 1N HCl (1:1) as a solvent with methanol:

Stock solution preparation:

Separate 100 ml calibrated volumetric flasks were used to generate standard stock solutions for enalapril and isoproterene. 100 mg of each medication was carefully weighed and placed in the flasks. The concentration of each solution was brought to 1 mg ml-1 by gradually increasing the amount of solvent in the flasks until the desired level was reached. Dilutions ranging from 2 µg ml-1 to 30 µg ml-1 were created using the stock solution.

Absorption maxima determination:

Using a pipette, 1 mL of enalapril and 1 mL of irbesartan stock solutions (1000µg/ml) were each placed into a 100 mL calibrated volumetric flask. Later on, a 1:1 ratio of methanol to 1N HCl was used to change the volume. A final concentration of 10µg/ml was achieved by the medicines. After that, the solution was examined in the ultraviolet (UV) spectrum, which includes wavelengths between 200 and 400nm, to find out when the absorption is at its peak. The reference was a 1:1 ratio of methanol to 1N hydrochloric acid.

Preparation of standard calibration curve:

The absorbance of various drug doses was compared to a blank solvent to generate the standard calibration curve. Plotting the graph allowed researchers to examine the linear relationship between concentration and absorbance.

Procedure for preparation of standard calibration curve: Enalapril and irbesartan solutions with concentrations ranging from 2, 4, 6,... 30µg/ml (with a reference concentration of 1000µg/ml) were prepared by adding the appropriate amounts of the drugs to numerous 10 ml volumetric flasks. Then, the solvent was added to each flask until the desired concentration was reached, adjusting the capacity of the process. Two different wavelengths, 230 and 224 nm, were used to test the absorbance of the solution.

Sample preparation:

Twenty Lupinace tablets, each with 5 milligrams of enalapril and 5 milligrams of irbesartan, were ground into a fine powder after being measured. A conventional approach involving addition was used for the substance's analysis. Forty milliliters of a mixture of methanol and 1N HCl was used to dissolve 5 milligrams of enalapril and irbesartan powders, and then the mixture was sonicated for 10 minutes. Once the solution had been filtered using Whatman filter paper, it was diluted to a final volume of 100 ml by mixing equal parts methanol and hydrochloric acid. A solution was made up of enalapril and irbesartan, with a concentration of 1000 µg/ml of each. When the solvent level reached the appropriate level, the sample solution was carefully transferred to a 100-milliliter volumetric flask. After that, 230 and 224 nm were used for the absorbance measurement. Utilizing the standard curves for each medication, the absorbance was measured to quantify the amounts of enalapril and irbesartan in the sample solution.

Simultaneous estimation of Enalapril and Irbesartan in tablet dosage form Using 0.1N HCl (1:1) as a solvent with methanol:

Stock solution preparation:

To make the standard stock solutions (1 mg ml-1) of Enalapril and Irbesartan, 100 mg of each component was added to a 100 ml calibrated volumetric flask. After that, solvent was added until the volume was reached that was wanted. The stock solution was used to create dilutions with concentrations ranging from 2 µg ml-1 to 30 µg ml-1.

Absorption maxima determination:

For the preparation of each stock solution of Enalapril and Irbesartan, a volume of 1 milliliter of a 1000µg/ml

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concentration of each medication was placed into separate 100 ml calibrated volumetric flasks. The volume was subsequently modified by employing a 1:1 blend of methanol and 0.1N hydrochloric acid. The concentration of the medication ultimately reached 10 micrograms per milliliter. To determine the absorption maxima, the solution was analyzed by scanning it in the ultraviolet (UV) region ranging from 200 to 400 nm, using a blank solution consisting of a mixture of methanol and 0.1N HCl in a 1:1 ratio. The results indicated that the wavelengths of 230 nm and 224 nm exhibited the highest level of absorption.

Preparation of standard calibration curve:

The preparation of the standard calibration curve involved comparing the absorbance of different drug concentrations to a blank solvent. In order to examine the linear relationship between absorbance and concentration, a graph was created.

Procedure:

Separate 10 ml volumetric flasks were filled with 2, 4, 6,..., 20 µg/ml (1000µg/ml) solutions of irbesartan and enalapril, and the volume was adjusted with solvent to reach the appropriate level. Two different wavelengths, 230 and 224 nm, were used to test the absorbance of the solution.

Sample preparation:

Weighed and ground into a fine powder were twenty Lupinace pills, each of which contained 5 milligrams of enalapril and 5 milligrams of irbesartan. A conventional addition technique was employed for the substance's analysis. In a solution of 40 milliliters of methanol and 0.1N HCl in a 1:1 ratio, a dose of powder containing enalapril of 5mg and irbesartan of 5mg of irbesartan was dissolved. After that, the mixture was sonicated for 10 minutes. Following filtering via Whatman filter paper, the solution was subsequently supplemented with 100 ml of a 1:1 ratio of methanol to hydrochloric acid. A solution was made up of enalapril and irbesartan, with a concentration of 1000 µg/ml of each. Two milliliters of the sample solution were added to a one-hundred-milliliter volumetric flask after the solvent had been added to the correct volume. A further absorbance measurement was performed at 232 and 228 nm. The absorbance measurements were recorded, and the amounts of enalapril and irbesartan in the sample solution were calculated using the standard curves for the two medications.

Validation of method:

Analytical chemistry relies heavily on method validation. By checking for correctness, consistency, and reliability, it ensures the analytical method is suitable for its intended use. The term "technique validation" describes the steps taken to ensure that an analytical method is well-suited to its job and can reliably produce accurate results. The technique involves a series of tests and assessments to identify the approach's performance traits. To validate a process, one must first provide detailed written documentation that assures the approach will reliably produce a product that matches its specified quality characteristics and standards. Following ICH guidelines, the method for simultaneous estimation was approved for use.

Parameters for Validation of method:

Linearity:

In analytical chemistry, linearity is a crucial statistic. If an analytical procedure can produce results that are directly proportional to the concentration of the material under study, within a certain range, we say that it is linear. Determination: A distinct set of solutions was created from the stock solutions and examined in order to demonstrate the linearity of the suggested approach. Five to six estimates were made in order to determine linearity. It was discovered that the responses matched the analyte concentrations exactly.

Range:

The interval that includes the maximum and minimum concentrations of the analyte in a given sample is called the range of the analytical method, according to ICH. Showing that the analytical procedure is sufficiently precise, accurate, and linear determines the range.

Accuracy:

According to ICH, an analytical procedure's correctness is determined by how closely the value discovered and the conventional true value or recognized reference value agree. Accuracy can be ascertained by recovering a known standard solution that has been "spiked" or added to the sample. In other words, an aliquot of the sample is added

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to a known amount of the chemical to be analyzed—typically in the form of a solution—before the analysis. the concentration of the analyte in the sample's spiking solution is determined. The next step is to calculate the spike recovery percentage.

$$\text{Percentage recovery} = \frac{100\ (Xs - Xu)}{K}$$

Where,

Xs = The spiked sample's measured value

Xu = Measured value for the unspiked sample modified to account for spike dilution

K= The sample's known spike value.

Recovery trials were carried out in triplicate using the standard addition method at 80%, 100%, and 120% in order to assess the method's degree of accuracy. Pre-analyzed samples were treated following the suggested procedure after known quantities of standard were added.

Precision:

Duplicate or repeat testing on one sample from a batch of samples is typically used to assess the precision of analysis. The terms standard deviation, CV, standard error of the mean (M), RSD, and relative percent its statistical expressions are difference (RPD), However, the analyte concentrations can alter the readings' standard deviation. Conversely, RSD, which can be defined as the ratio of the arithmetic mean of replicate analyses to the standard deviation, and is expressed as a percentage, has no such issue, and is a more logical method of stating accuracy.

$$\text{RSD} = \frac{\text{Standard deviation}}{\text{Arithmetic mean of replicate analysis}} \times 100\%$$

Six separate tests of the test sample preparation were run, and the percentage RSD was calculated, in order to assess the method's precision as well as its intra-day repeatability. By having a separate person carry out the same operation under the same experimental settings on different days, the intermediate (interday) precision of the method was verified.

Robustness:

Three injections of the standard solution were made under the following conditions:

- The mobile phase's aqueous phase ratio has altered by ± 1%.
- The flow rate is now off by ± 10%.
- Wavelength shift

RESULTS

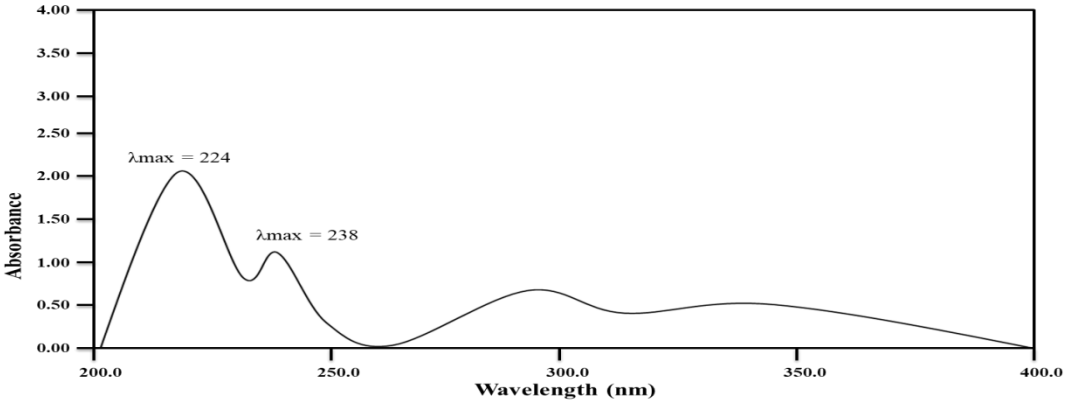
Method development and validation by UV spectrophotometry:

Simultaneous estimation of Enalapril and Irbesartan in tablet dosage form using methanol: 1N HCl (1:1) as solvent.

Absorption maxima determination:

The absorbance max of Enalapril was found to be 238 nm.

The absorbance max of Irbesartan was found to be 224 nm.



Graph 1. Curve of Enalapril and Irbesartan in commercialproduct

Sr. No.	Concentration (mcg/ml)	Absorbance

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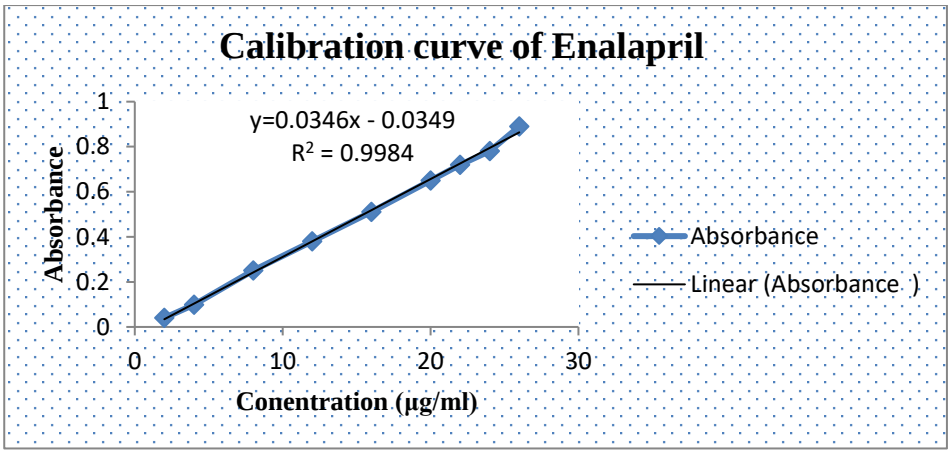
I.	2	0.04
II.	4	0.098
III.	8	0.25
IV.	12	0.38
V.	16	0.51
VI.	20	0.65
VII.	22	0.72
VIII.	24	0.78
IX.	26	0.89

Table 1. Dilutions for the calibration curve of Enalapril



Graph 2. Calibration curve of Enalapril

The calibration curve, which has a regression coefficient value (R) of 0.9984 and a straight line equation y = 0.0346x - 0.0349, demonstrates that Beer's Law is followed in the concentration range of enalapril 2-26 mcg/ml.

Table 2. Dilutions for the calibration curve of Irbesartan

Sr. No.	Concentration (mcg/ml)	Absorbance
I.	2	0.015
II.	4	0.22
III.	6	0.41
IV.	8	0.63
V.	10	0.8
VI.	12	1.01

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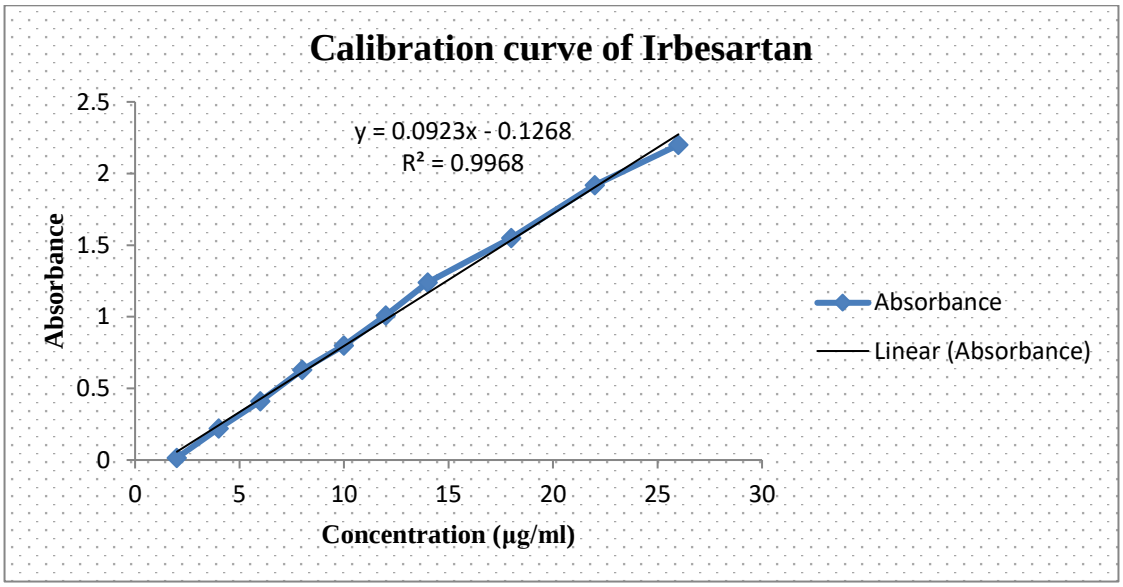
VII.	14	1.24
VIII.	18	1.55
IX.	22	1.92
X.	26	2.2

Graph 3. Calibration curve of Irbesartan



The calibration curve, which has a regression coefficient value (R) of 0.9968 and a straight line equation y = 0.0923x - 0.01268, demonstrates that Beer's Law is followed in the Irbesartan concentration range of 2-26 mcg/ml.

Molar absorptivity and Percent molar absorptivity:

Table 3. Molar absorptivity and Percent molar absorptivity

Wavelength (nm)	**Enalapril**			**Irbesartan**		
	Absorbance	€1%,1cm	€max	Absorbance	€1%,1cm	€max
238	0.38	445	28101.75	0.63	507.5	27009.375
224	0.34	400	22684	0.48	312.5	15190.625

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Simultaneous equation method:

At $\lambda_{238\ nm}$: $5.79 = 0.37C_x + 0.34C_y$ ..... (3)

At$\lambda_{224\ nm}$: $4.78 = 0.16C_x + 0.41C_y$ ..... (4)

Preparation of sample solution:

Table 4. Weight of tablet taken for analysis

Particulars Groups	**Weight of Tablet**
Total wt. of 20 tablets	5.4557 g
Average weight	0.272785 g

Table 5. Results of tablet analysis

Particulars Groups		**Results**	
Absorbance of 20µg/ml (Enalapril) solution of tablet at	238nm	1.1954	
	224nm	0.9959	
Drug found/claim label(mg)		5.22/5	5.14/5
%found/%limit		102.4/90-110	102.8/90-110

The result shows that the con. of Enalapril and Irbesartan in tablets were found in the prescribed official range.

Validation of developed method:

By ICH recommendations, the established approach for the simultaneous estimate of enalapril and irbesartan was verified.

- **Linearity:**

(r^2) for Enalapril = 0.9984

(r^2) for Irbesartan = 0.9968

Table 6. Accuracy: Recovery study of enalapril

Sr. No	**Con.of stock µg/ml**	**Con. spiked µg/ml**	**Total Con./ml**	**Conc. Found**	**Abs. at 224 nm**	**Abs. at 238 nm**	**Percentage Recovery**	**Average**
1.	50	0	50	50	0.5072	0.6070	91%	
2.	50	2	52	51.89	0.5199	0.6735	94.5%	
3.	50	2	52	51.85	0.5199	0.6738	92.5%	
4.	50	2	52	51.95	0.5199	0.6735	94.5%	

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5.	50	2.5	52.5	52.31	0.5285	0.6974	92.4%	93.24%
6.	50	2.5	52.5	52.29	0.5285	0.6975	93.6%	
7.	50	2.5	52.5	52.37	0.5289	0.6979	94.8%	
8.	50	3	53	52.81	0.5372	0.7186	93.66%	
9.	50	3	53	52.9	0.5370	0.7183	96.66%	
10.	50	3	53	52.79	0.5372	0.7185	93%	

The recovery was found between 93.6% to 94.5%, with an average of 93.24%. Standard deviation was found to be 1.339. Percent relative standard deviation was found to be 1.25. This obeys the acceptance criterion.

Table 7. Recovery study of Irbesartan

Sr. No	Con. Of stock µg/ml	Con. Spiked µg/ml	Total conc./ml	Conc. Found	Abs. at 238 nm	Abs. at 224 nm	Percentage Recovery	Average
1.	50	0	50	50	0.5036	0.6083	94%	
2.	50	2	52	51.97	0.8508	0.9707	98.5%	
3.	50	2	52	51.89	0.8504	0.9708	94.5%	
4.	50	2	52	51.78	0.8508	0.9706	99%	
5.	50	2.5	52.5	52.38	0.9384	1.0689	95.2%	97.64%
6.	50	2.5	52.5	52.49	0.9385	1.0686	99.6%	
7.	50	2.5	52.5	52.46	0.9387	1.0686	98.4%	
8.	50	3	53	52.86	1.0232	1.1581	95.3%	
9.	50	3	53	52.89	1.0235	1.1584	96.3%	
10.	50	3	53	52.95	1.0233	1.1586	98.3%	

The recovery was found between 94.5% to 99.6%, with an average of 97.64%. The standard deviation was found to be 1.439. Percent relative standard deviation was found to be 1.16. This obeys the acceptance criterion.

Table 8. Precision of Intraday

Sr. No.	Sample conc. (µg/ml)	Absorbance		Label claim mg/tab		Label claim estimated		% Label claim estimated	
		238 nm	224 nm	Enalapril	Irbesartan	Enalapril	Irbesartan	Enalapril	Irbesartan
1.	10	0.5092	0.6121	5	5	5.22	5.16	104.4	103.2
2.	10	0.5089	0.6124	5	5	5.17	5.11	103.4	102.2
3.	10	0.5095	0.6122	5	5	5.16	5.20	103.2	104
4.	15	0.7638	0.9181	5	5	5.08	5.13	101.6	102.6

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5.	15	0.7636	0.9178	5	5	5.14	5.16	102.8	103.2
6.	15	0.7637	0.9180	5	5	5.18	5.19	103.6	103.8
7.	20	1.0166	1.2231	5	5	5.21	5.14	104.2	102.8
8.	20	1.0168	1.2228	5	5	5.12	5.21	102.4	104.2
9.	20	1.0174	1.2231	5	5	5.14	5.15	102.8	103

Table 9. Relative standard deviation of Precision of Intraday

Sr. No	Drugs	Average ± Standard deviation	Percent relative standard deviation	Error
1.	Enalapril	104.35 ± 0.676	0.65	0.282
2.	Irbesartan	104.32 ± 0.467	0.45	0.242

Table 10. Precision interday

Sr. No.	Sample conc. (µg/ml)	Absorbance		Label claim mg/tab		Label claim estimated		% Label claim estimated	
		238 nm	224 nm	Enalapril	Irbesartan	Enalapril	Irbesartan	Enalapril	Irbesartan
1.	10	0.5092	0.6121	5	5	5.22	5.16	104.4	103.2
2.	10	0.5089	0.6124	5	5	5.17	5.11	103.4	102.2
3.	10	0.5095	0.6122	5	5	5.16	5.20	103.2	104
4.	15	0.7638	0.9181	5	5	5.28	5.13	101.6	102.6
5.	15	0.7636	0.9178	5	5	5.14	5.16	102.8	103.2
6.	15	0.7637	0.9180	5	5	5.18	5.19	103.6	103.8
7.	20	1.0166	1.2231	5	5	5.21	5.14	104.2	103.8
8.	20	1.0168	1.2228	5	5	5.12	5.21	102.4	104.6
9.	20	1.0174	1.2231	5	5	5.34	5.15	102.8	104.2

Table 11. Relative standard deviation of Precision of Interday

Sr. No	Drugs	Average ± Standard deviation	Percent relative standard deviation	Error
1.	Enalapril	103.35 ± 0.676	0.85	0.382
2.	Irbesartan	103.32 ± 0.467	0.65	0.262

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Table 12. Precision interanalyst

Sr. No.	Sample conc. (µg/ml)	Absorbance		Label claim mg/tab		Label claim estimated		% Label claim estimated	
		238 nm	224 nm	Enalapril	Irbesartan	Enalapril	Irbesartan	Enalapril	Irbesartan
1.	10	0.5092	0.6121	5	5	5.22	5.16	104.4	103.4
2.	10	0.5089	0.6124	5	5	5.17	5.11	103.4	104.2
3.	10	0.5095	0.6122	5	5	5.16	5.20	103.2	103.2
4.	15	0.7638	0.9181	5	5	5.28	5.13	102.6	101.6
5.	15	0.7636	0.9178	5	5	5.14	5.16	102.8	103.2
6.	15	0.7637	0.9180	5	5	5.18	5.19	102.3	102.8
7.	20	1.0166	1.2231	5	5	5.21	5.14	104.2	103.8
8.	20	1.0168	1.2228	5	5	5.12	5.21	102.4	102.5
9.	20	1.0174	1.2231	5	5	5.34	5.15	103.1	102.2

Table 13. Relative standard deviation of Precision of Interanalyst

Sr. No	Drugs	Average ± Standard deviation	Percent relative standard deviation	Error
1.	Enalapril	102.55 ± 0.376	0.65	0.482
2.	Irbesartan	103.42 ± 0.267	0.55	0.362

Table 14. Common Characteristic parameters of method

Parameters	Intraday		Interday		Interanalyst	
	Enalapril	Irbesartan	Enalapril	Irbesartan	Enalapril	Irbesartan
SD	0.826	0.567	0.938	0.5607	0.615	0.764
RSD	0.65	0.66	0.80	0.54	0.78	0.75
Error	0.362	0.222	0.327	0.169	0.216	0.258

The percentage RSD of intraday, interday, and interanalyst precision was 1>, which shows that the method is precise, reproducible, and repeatable.

CONCLUSION

An effective spectroscopic technique for the simultaneous determination of enalapril and irbesartan has been devised and verified by the research. The technique meets the

strict standards specified by regulatory bodies and demonstrates outstanding analytical performance characteristics. Its accuracy, precision, linearity, specificity, and robustness have been thoroughly evaluated and found to be satisfactory, thus confirming its suitability for routine analysis in pharmaceutical laboratories. By enabling the simultaneous quantification of both drugs in a single analysis, this method offers significant advantages in terms of time and resource efficiency compared to traditional methods that require separate analyses. Moreover, the method has been successfully applied to the analysis of commercially available formulations, demonstrating its practical utility in pharmaceutical quality control. All things considered, the established spectroscopic method constitutes a significant contribution to the field of pharmaceutical analysis, offering a dependable and effective way to guarantee the integrity and quality of products containing enalapril and irbesartan.

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