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Analytical Method Development and Validation for the Concomitant Determination of Losartan Potassium and Hydrochlorothiazide Using Derivative Spectroscopic Techniques

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Abstract: Researchers have developed a streamlined and highly effective way to measure the combination of Losartan and Hydrochlorothiazide in synthetic mixtures using derivative UV-visible spectroscopy. By focusing on the "first derivative" of the light absorption at 296 nm and 331 nm, as well as the "second derivative" at 262 nm and 276 nm, the team was able to clear up the visual clutter where the two drugs' signals usually overlap. Using methanol as a solvent and standard quartz cells, the process proved to be remarkably accurate and followed established scientific laws for concentration. Beyond just being precise, the method is designed to be accessible—offering a fast, budget-friendly, and reliable alternative to more complex testing methods. After rigorous validation for consistency and sensitivity, it is clear that this approach is an ideal fit for routine laboratory evaluations and quality control.

Keywords: *Losartan; Hydrochlorothiazide; UV Spectroscopy; First Derivative Method, Second Derivative Method.*

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I. INTRODUCTION

➢ *Losartan Potassium*

Losartan potassium serves as a vital tool in managing cardiovascular health, primarily by tackling high blood pressure (hypertension). Think of the heart and arteries as a plumbing system; when the pressure stays too high for too long, it puts an immense strain on the "pipes" and the "pump." Over time, this wear and tear can lead to catastrophic failures like strokes, heart attacks, or kidney disease. As an angiotensin II receptor blocker (ARB), Losartan acts as a chemical signal-blocker. It stops a specific substance in your body from telling your blood vessels to tighten up. By allowing these vessels to relax and widen, the blood flows more easily, which significantly lowers blood pressure and ensures a steady supply of oxygen-rich blood reaches the heart. Beyond general hypertension, it is also a key treatment for protecting the kidneys in patients with type 2 diabetes and reducing stroke risk in those with enlarged hearts. In short, it's about more than just numbers on a monitor—it's about safeguarding the body's most essential organs.

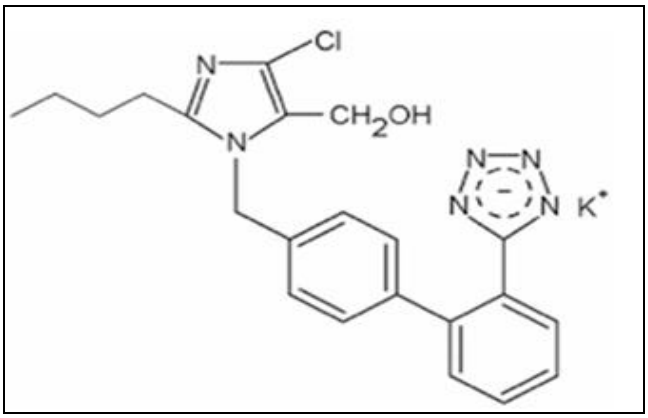

Fig 1 Structure of Losartan Potassium

➢ *Hydrochlorothaizide*

Hydrochlorothiazide is a widely used medication designed to lower high blood pressure and reduce the dangerous strain placed on the cardiovascular system. When blood pressure remains unchecked, it forces the heart to work overtime and can permanently damage the arteries. Left

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untreated, this "silent" pressure can lead to life-altering complications such as strokes, heart failure, and kidney damage. By keeping these levels under control, the medication acts as a protective shield for the body's vital organs. Beyond managing hypertension, Hydrochlorothiazide is an effective treatment for edema, or fluid retention. It helps the body shed excess salt and water that often build up due to underlying conditions like congestive heart failure, kidney disease, or liver cirrhosis. It is also frequently used to counteract the swelling that can occur as a side effect of certain steroid or hormone therapies. By helping the kidneys flush out extra fluid, it reduces physical discomfort and eases the overall burden on the heart.

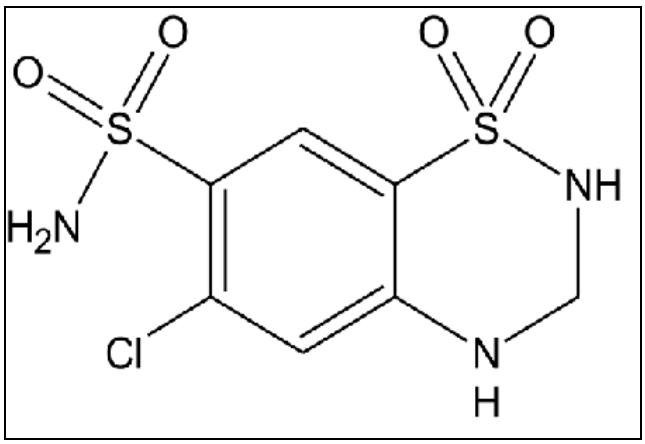

Fig 2 Structure of Hydrochlorothiazide

➢ *UV/Vis Spectrophotometry*

UV/Vis spectrophotometry is a foundational analytical technique that uses light to "see" and measure chemical substances. It works by passing electromagnetic radiation—specifically in the ultraviolet (200–380 nm) and visible (380–800 nm) ranges—through a sample. When a molecule contains specific features, like π electrons or unshared electron pairs (often found in complex organic structures), it absorbs certain wavelengths of that light.

II. PREPARATION OF STANDARD CALIBRATION CURVES BY FIRST DERIVATIVE & SECOND DERIVATIVE SPECTROSCOPY

➢ *Solvent Used*

Methanol was used as the solvent

➢ *Preparation of Standard Stock Solution*

To prepare the standard stock solutions, 10 mg of Losartan potassium and 10 mg of Hydrochlorothiazide were accurately weighed and placed into two separate 10 ml volumetric flasks. About 5 ml of methanol was added to each flask, and the mixtures were shaken vigorously to ensure the drugs were completely dissolved. Once fully in solution, additional methanol was added to reach the 10 ml graduation mark on each flask. This precise dilution resulted in two individual stock solutions, each with a final concentration of 1000 µg/ml.

➢ *Preparation of Mixture Solution*

The preparation of the analytical mixture involves a systematic serial dilution process to ensure the final solution maintains a precise 4:1 ratio of the two active ingredients. To achieve this, a portion of the 1000 ppm Losartan stock was first reduced to 100 ppm. For the Hydrochlorothiazide, a more rigorous two-step dilution was required, bringing it down from 1000 ppm to 10 ppm to account for its lower concentration in the final blend. By combining 0.4 ml of the Losartan intermediate with 1 ml of the Hydrochlorothiazide working solution and adjusting the total volume to 10 ml with methanol, a standardized synthetic mixture was created. This meticulous approach is essential in pharmaceutical analysis to ensure the developed spectroscopic method can reliably detect and quantify each drug within its specific therapeutic range.

➢ *Selection of Analytical Wavelength*

To identify the most effective points for measurement, standard solutions of Losartan (LOSA) and Hydrochlorothiazide (HCT) were scanned across the ultraviolet range from 400 nm down to 200 nm. Because their natural absorbance patterns often overlap, the raw data was transformed into derivative spectra to "clean up" the signals and eliminate interference. The goal was to find "zero-crossing points"—specific wavelengths where one drug shows no absorbance while the other shows a significant signal. For the first derivative method, 331 nm and 296 nm were chosen; at 331 nm, HCT has zero absorbance, allowing for the isolated measurement of LOSA, while at 296 nm, LOSA reaches zero, leaving a clear path to measure HCT. A similar strategy was applied to the second derivative spectra, where 262 nm and 276 nm were selected as the optimal working wavelengths to ensure that each component could be quantified without being masked by the other. This careful selection ensures that even in a combined mixture, the results for each drug remain distinct and highly accurate.

➢ *Preparation of Calibration Curve*

- *Calibration Curve for Losartan*

To establish the calibration curve for Losartan (LOSA), a series of standard solutions were prepared with concentrations ranging from 2 to 10 µg/ml. This was achieved by accurately pipetting 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the 100 µg/ml intermediate stock into separate 10 ml volumetric flasks, then diluting each to the mark with methanol. By measuring the absorbance of these specific concentrations at the previously identified wavelengths of 331 nm and 262 nm, a mathematical relationship—the calibration curve—can be plotted. This curve serves as the essential reference point for the study, allowing the concentration of unknown samples to be calculated based on their light absorption intensity.

- *Calibration Curve for Hydrochlorothiazide*

To build the calibration curve for Hydrochlorothiazide (HCT), a set of standard solutions was created with concentrations spanning from 0.5 to 2.5 µg/ml. These were prepared by taking 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the 10

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µg/ml working stock and diluting them to a final volume of 10 ml using methanol. By measuring the absorbance at 296 nm and 276 nm, the researchers can ensure the method is accurate even at these much lower concentration levels. This step confirms that the equipment can reliably detect HCT with high sensitivity, which is critical since it is present in smaller amounts compared to Losartan.

➢ *Limit of Detection (LOD) and Limit of Quantitation (LOQ)*

The LOD and LOQ were separately determined which is based on calibration curve. The standard deviation of y-intercepts of regression lines may be used as standard deviation.

LOD= (3.3 ×D)/S
LOQ = (10 ×D)/S

Where, D = Standard deviation of the y- intercepts of regression line

S = Slope of the calibration curve

➢ *Accuracy and Precision*

- *Accuracy*

The accuracy of the method was tested by "spiking" known amounts of Losartan and Hydrochlorothiazide into samples of a known concentration. By comparing the amount actually measured by the spectrophotometer against the amount that was originally added, the team could calculate the percent deviation. This confirms whether the method truly measures what it claims to measure without being affected by other ingredients in the mixture.

- *Precision*

The ability of the method to produce the same results multiple times—was evaluated using two different approaches:

✓ *Intra-Day Precision:*

The analysis was repeated three times for three different concentration levels throughout a single day.

✓ *Inter-Day Precision:*

The same process was repeated over three consecutive days to ensure that the results remained stable regardless of small daily variations in lab conditions.

The consistency of these results was measured using %RSD (Relative Standard Deviation). A low %RSD indicates high repeatability, proving that the method is robust enough for routine pharmaceutical testing.

III. RESULT AND DISCUSSION

➢ *Simultaneous Spectrophotometric Determination of Losartan potassium and Hydrochlorothiazide by First Derivative Spectroscopy*

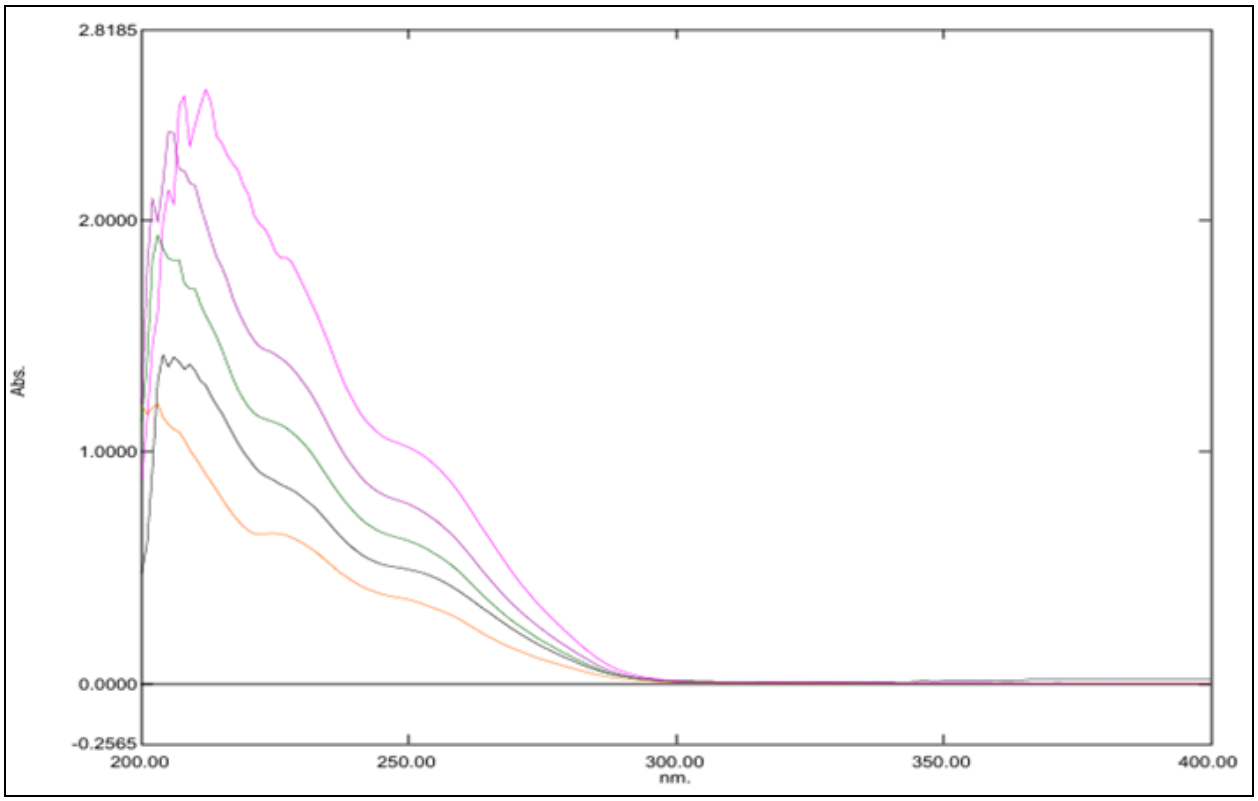

Fig 3 Normal Overlay of Losartan Potassium

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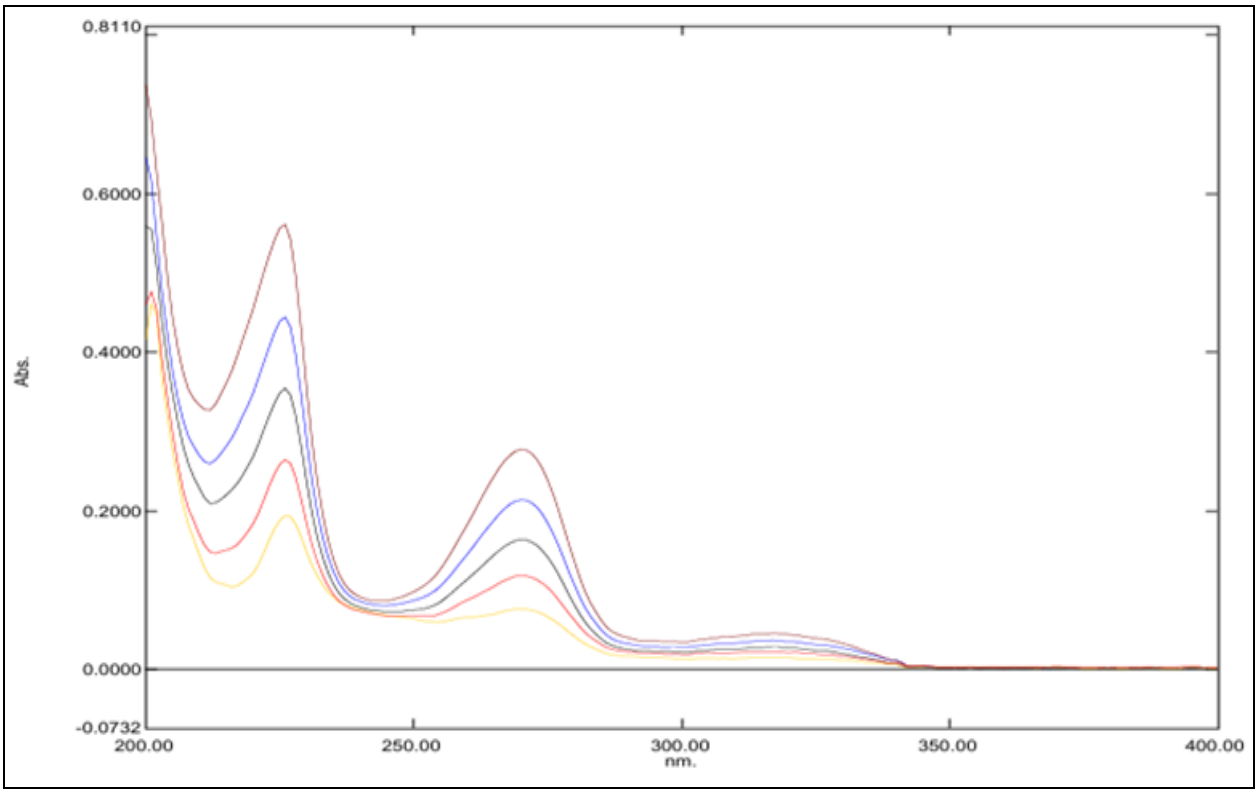

Fig 4 Normal Overlay of Hydrochlorothiazide

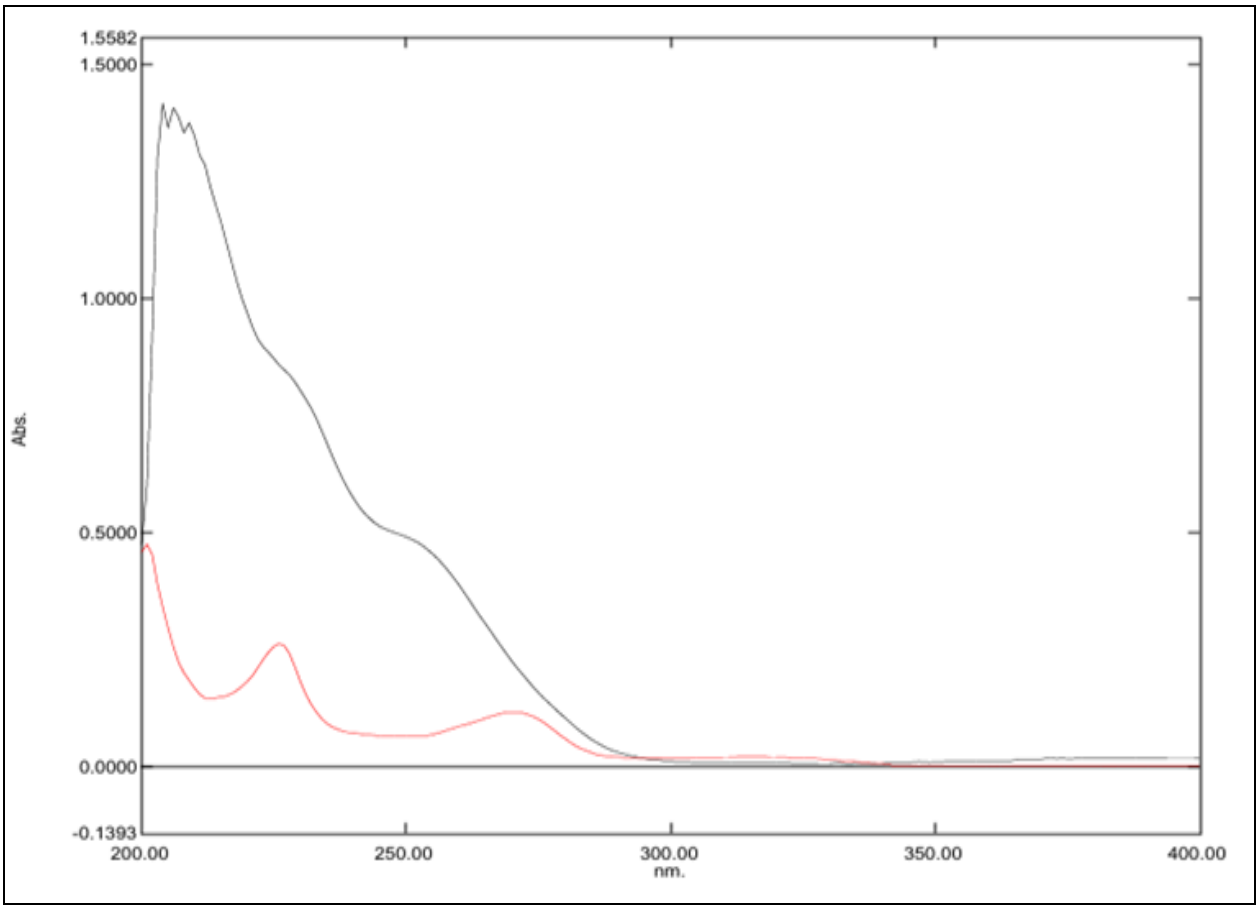

Fig 5 Normal Overlay of LOSA and HCT

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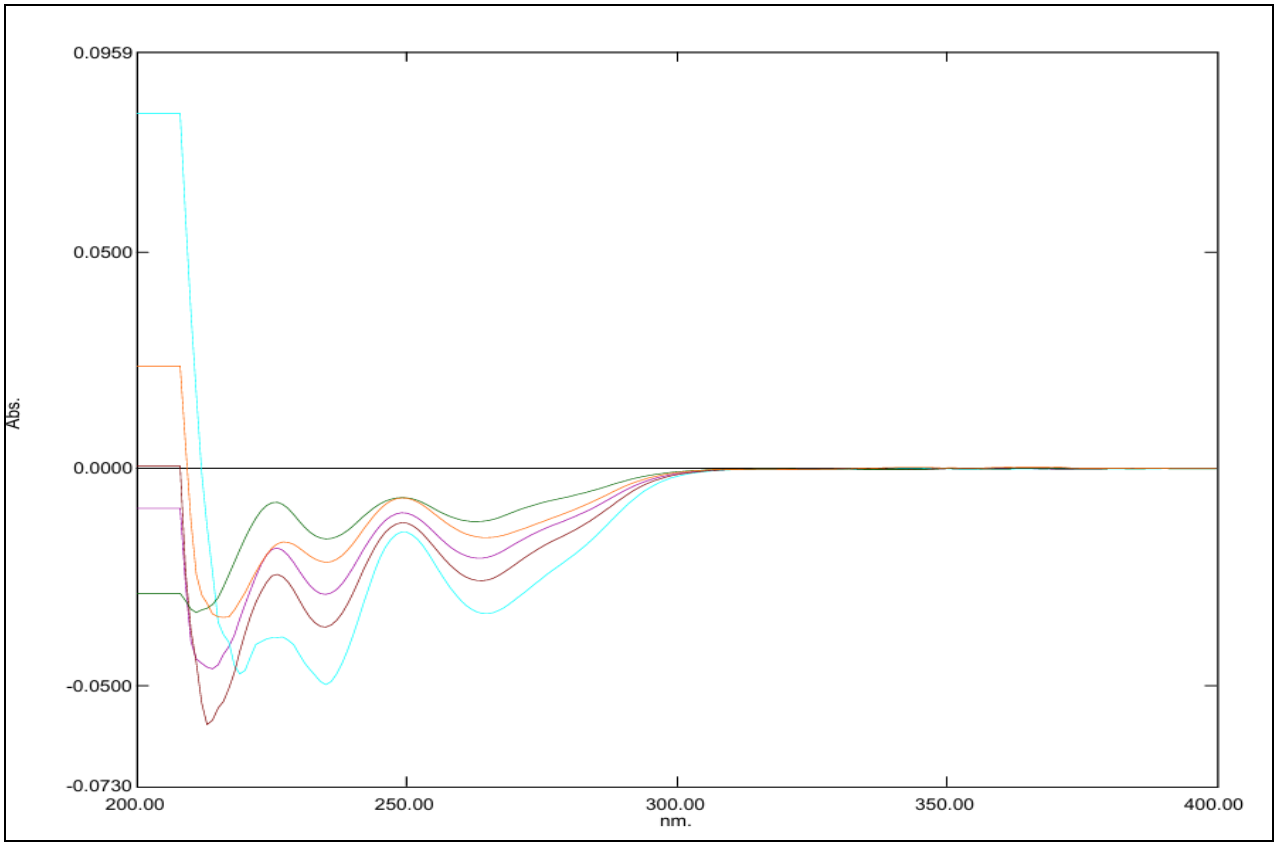

Fig 6 First Order Derivative Spectrum of Losartan Potassium

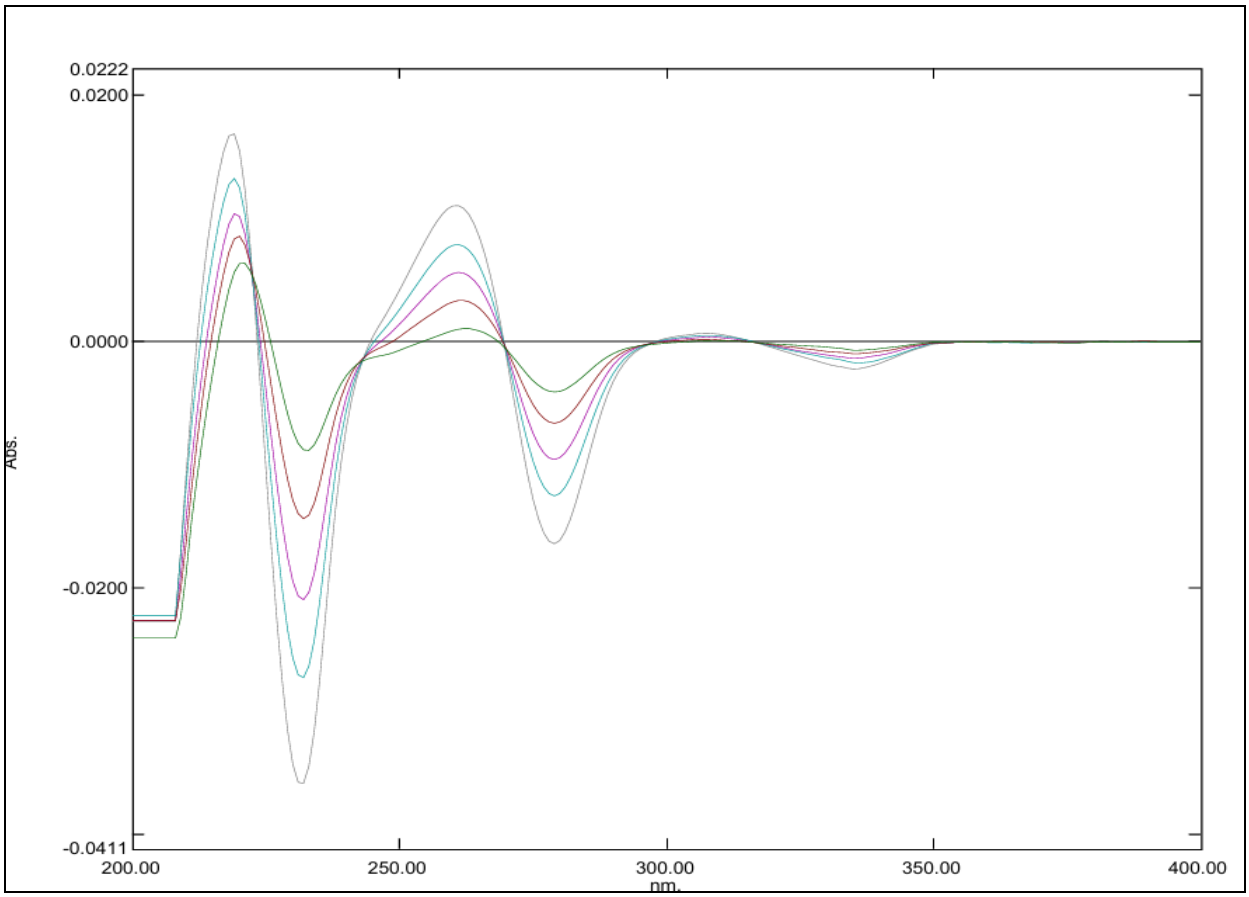

Fig 7 First Order Derivative Spectrum of Hydrochlorothiazid

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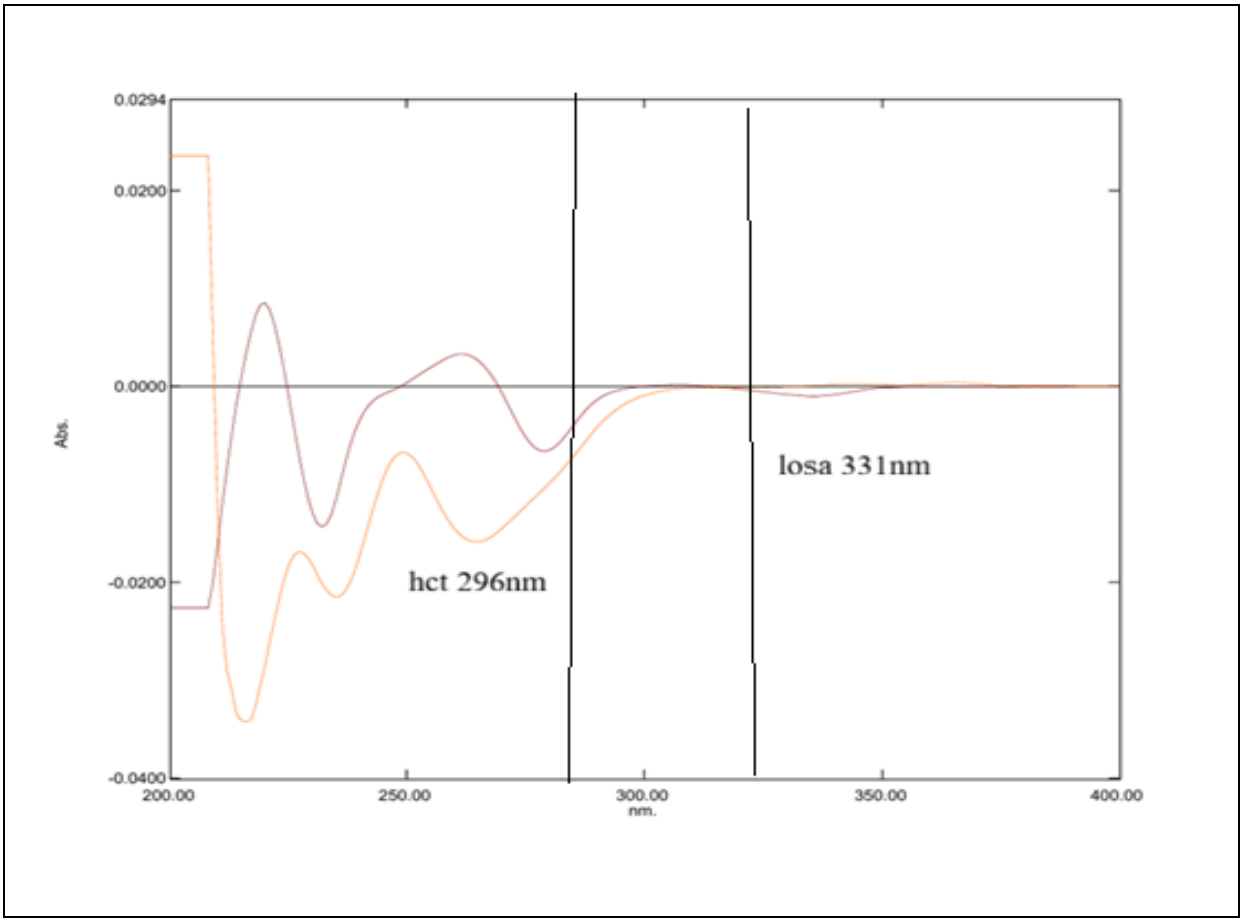

Fig 8 First Order Derivative Overlay Spectra of Losartan Potassium and Hydrochlorothiazide

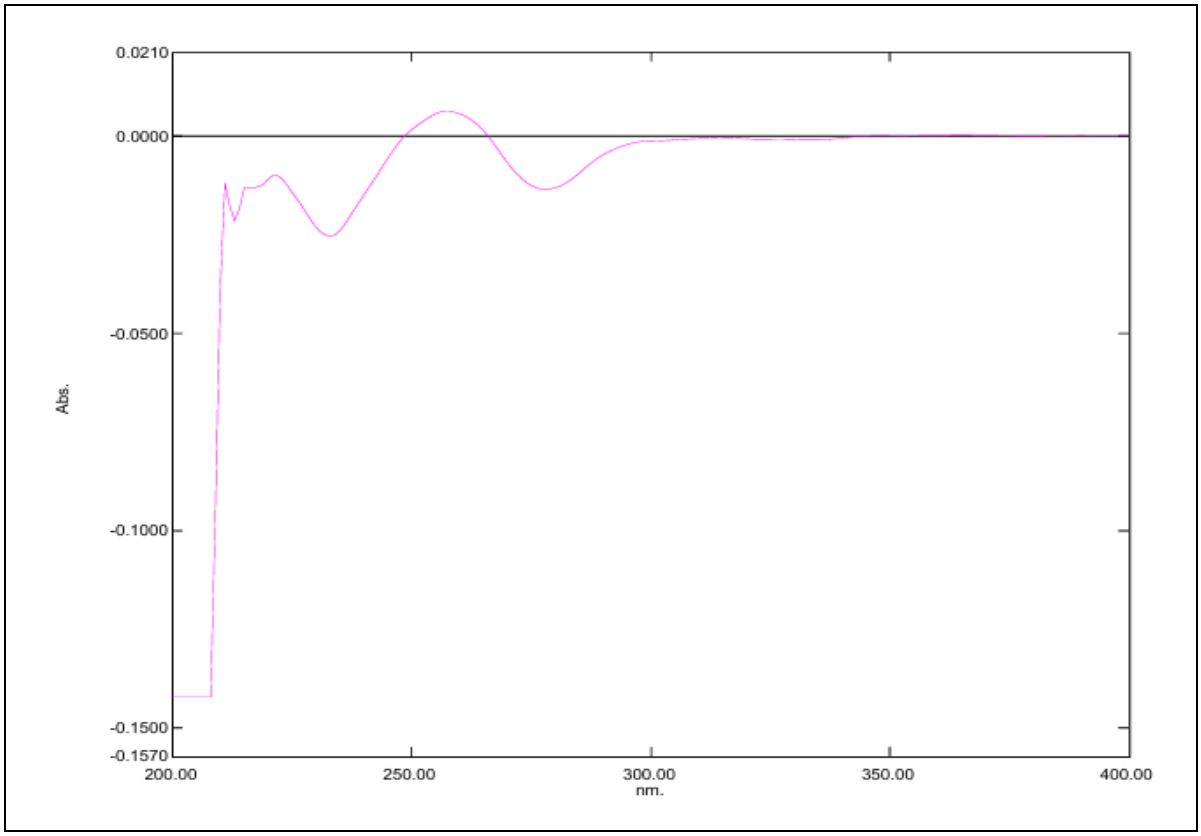

Fig 9 First Order Derivative Spectra of Mixture

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- *Selectin of Analytical Concentration Range*

Table 1 Calibration Table for Losartan and Hydrochlorothiazide

Sr. No.	For Losartan potassium		For Hydrochlorothiazide	
	Concentration (µg/ ml)	Absorbance at 331nm (λ_1)	Concentration (µg/ ml)	Absorbance at 296nm (λ_2)
1	2	0.00040	0.5	0.00132
2	4	0.00085	1	0.00182
3	6	0.00127	1.5	0.00222
4	8	0.00169	2	0.00272
5	10	0.00224	2.5	0.00322

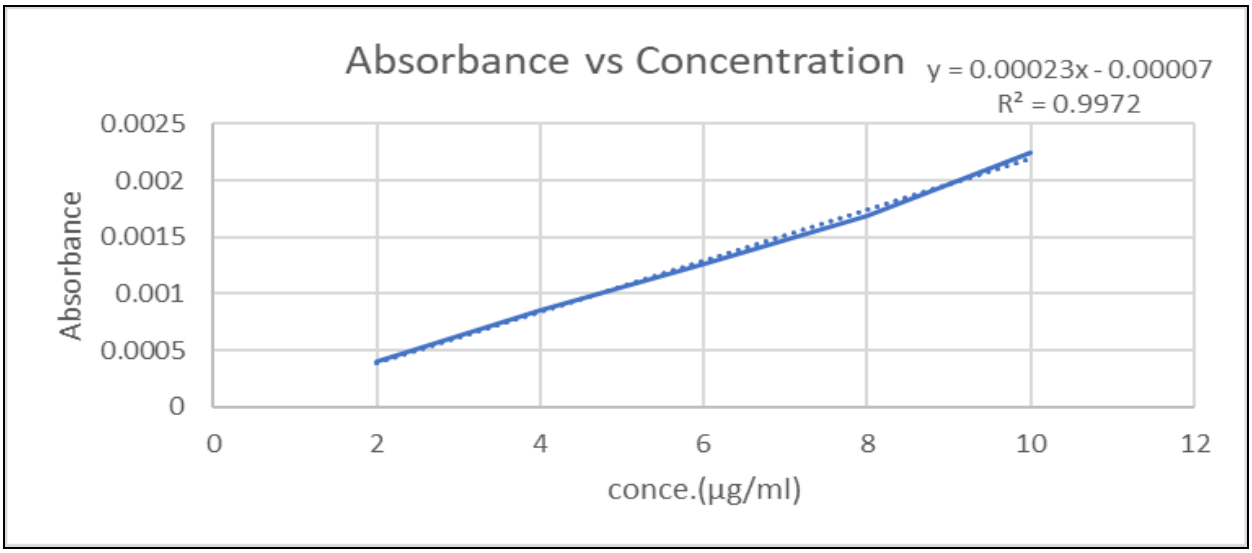

Fig 10 Calibration Curve for Losartan Potassium at 331nm

The calibration curve for Losartan potassium at 331 nm confirms a strong linear relationship within the concentration range of 2–10 µg/ml. This linearity is essential for ensuring that the absorbance measured by the spectrophotometer directly and predictably correlates to the amount of drug present in a sample. The mathematical backbone of this relationship is represented by the working curve equation: y = 0.00023x - 0.00007 In this equation, y represents the measured absorbance (or derivative response) and x represents the concentration of Losartan. The correlation coefficient (R2) of 0.9972 is particularly significant; being so close to 1.0, it indicates an excellent fit for the data points and proves that the method is highly reliable for quantitative analysis in pharmaceutical quality control.

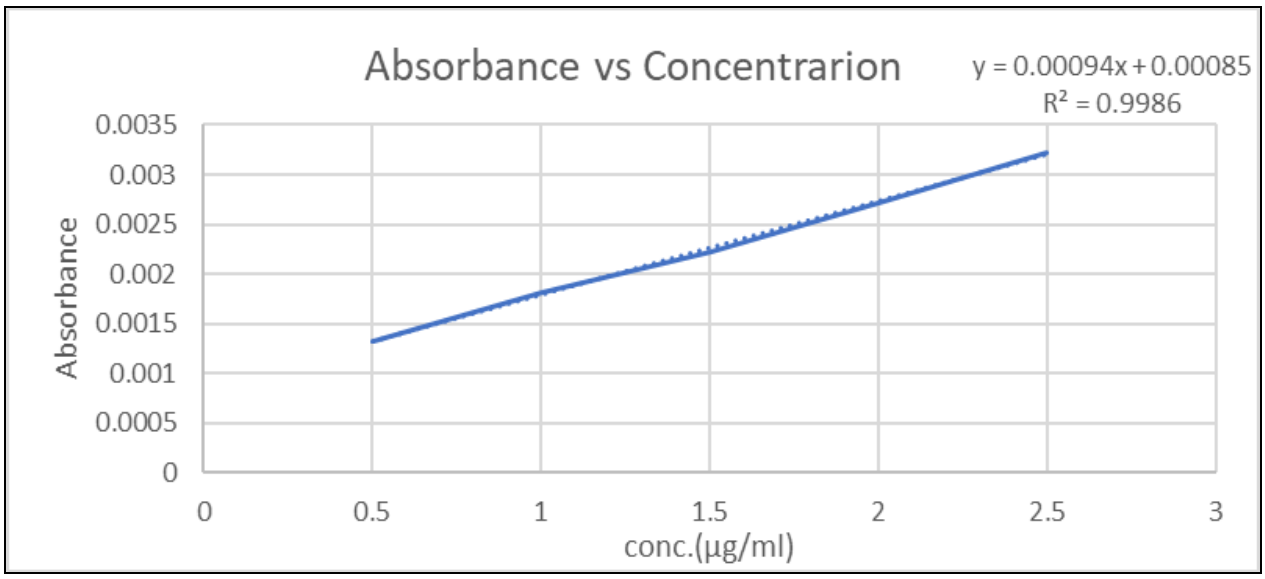

Fig 11 Calibration Curve for Hydrochlorothiazide at 296nm

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The calibration curve for Hydrochlorothiazide (HCT) at 296 nm demonstrates excellent linearity within the concentration range of 0.5–2.5 µg/ml. This specific range is crucial for capturing the drug's presence accurately, especially when it is used in smaller proportions within a mixture. The data follows a precise linear path defined by the regression equation: y = 0.00094x + 0.00085 With a correlation coefficient (R2) of 0.9986, the results show a very high degree of statistical confidence. An R2 value this close to 1 indicates that nearly all the variation in absorbance is directly explained by the change in concentration, making this wavelength a highly dependable choice for the quantitative analysis of Hydrochlorothiazide

- *Limit of Detection and Limit of Quantitation Using First Derivative Method*

Table 2 Data for LOD and LOQ of Losartan potassium and Hydrochlorothiazide

Drug	LOD (µg/ml)	LOQ(µg/ml)
Losartan	0.191	0.589
Hydrochlorothiazide	0.068	0.206

- *Accuracy Data*

Table 3 Result for Recovery Studies.

Level of Recovery	Amount Present (mg)		Amount of Standard Adding (mg)		Total Amount Recovered		Absorbance		% Recovery	
	LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT
50	4	1	2	0.5	6.070	1.502	0.00105	0.00126	101.75	100.2
50	4	1	2	0.5	5.979	1.513	0.00169	0.00128	99.47	101.3
50	4	1	2	0.5	5.985	1.483	0.00201	0.00130	99.62	98.3
100	4	1	4	1	8.089	1.996	0.00158	0.00171	102.2	99.6
100	4	1	4	1	8.101	2.021	0.00160	0.00174	102.5	102.1
100	4	1	4	1	7.972	2.003	0.00161	0.00176	99.3	100.3
150	4	1	6	1.5	9.993	2.503	0.00217	0.00215	99.82	100.3
150	4	1	6	1.5	9.959	2.498	0.00249	0.00218	98.97	99.8
150	4	1	6	1.5	9.982	2.496	0.00282	0.00221	99.55	99.6

- *Precision Data*

Table 4 Intra-Day Precision

Sr. No.	Label Claim (mg)		Amount Found (mg)		% of Label Claim	
	LOS	**HCT**	**LOS**	**HCT**	**LOS**	**HCT**
1	10	10	9.896	10.001	98.96	100.01
2	10	10	9.968	9.998	99.68	99.98
3	10	10	9.665	10.002	96.65	100.02

Table 5 Inter-Day Precision

Sr. No.	Label Claim (mg)		Amount Found (mg)		% of Label Claim	
	LOS	**HCT**	**LOS**	**HCT**	**LOS**	**HCT**
1	10	10	9.956	9.865	99.56	98.65
2	10	10	9.856	1.006	98.56	100.06
3	10	10	10.001	9.910	100.01	99.10

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➢ *Simultaneous Spectrophotometric Determination of Losartan and Hydrochlorothiazide by Second Derivative Spectroscopy.*

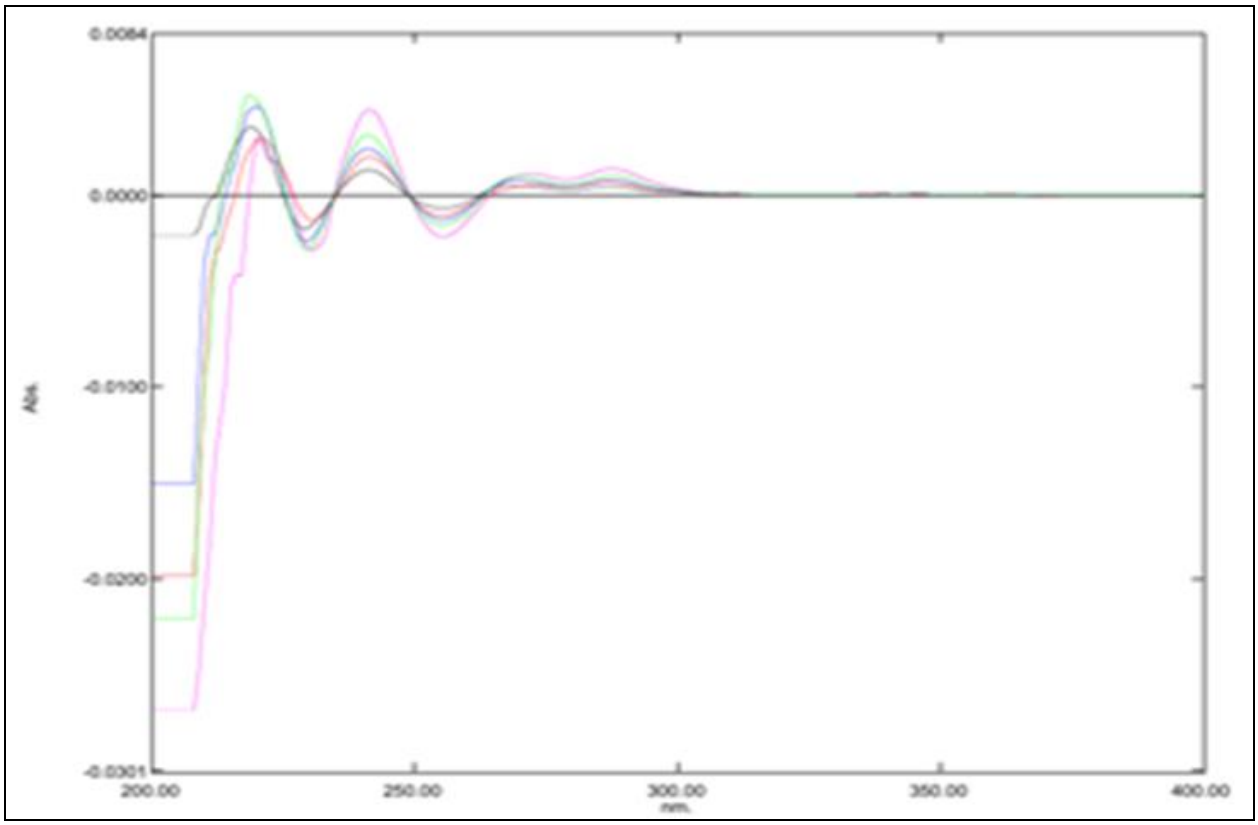

Fig 12 Second Order Spectrum of Losartan Potassium

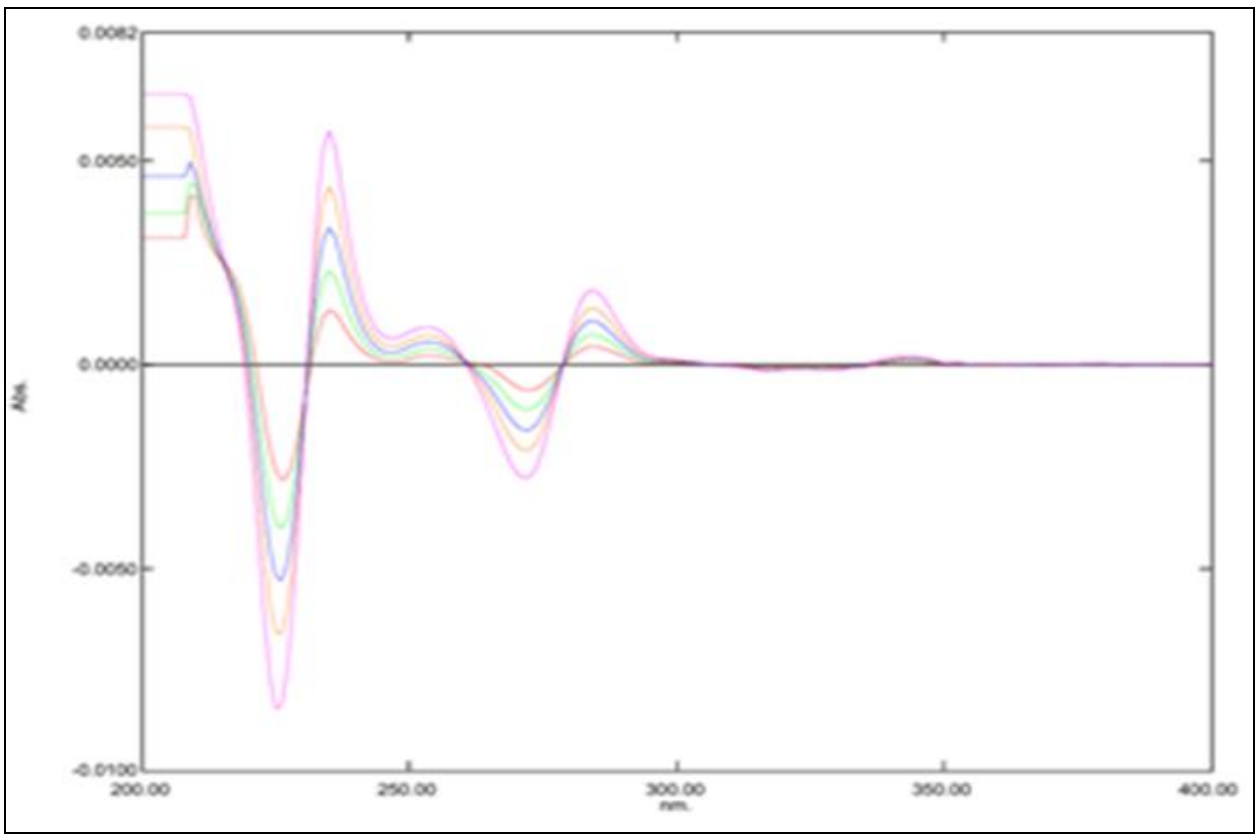

Fig 13 Second Order Spectrum of Hydrochlorothiazide

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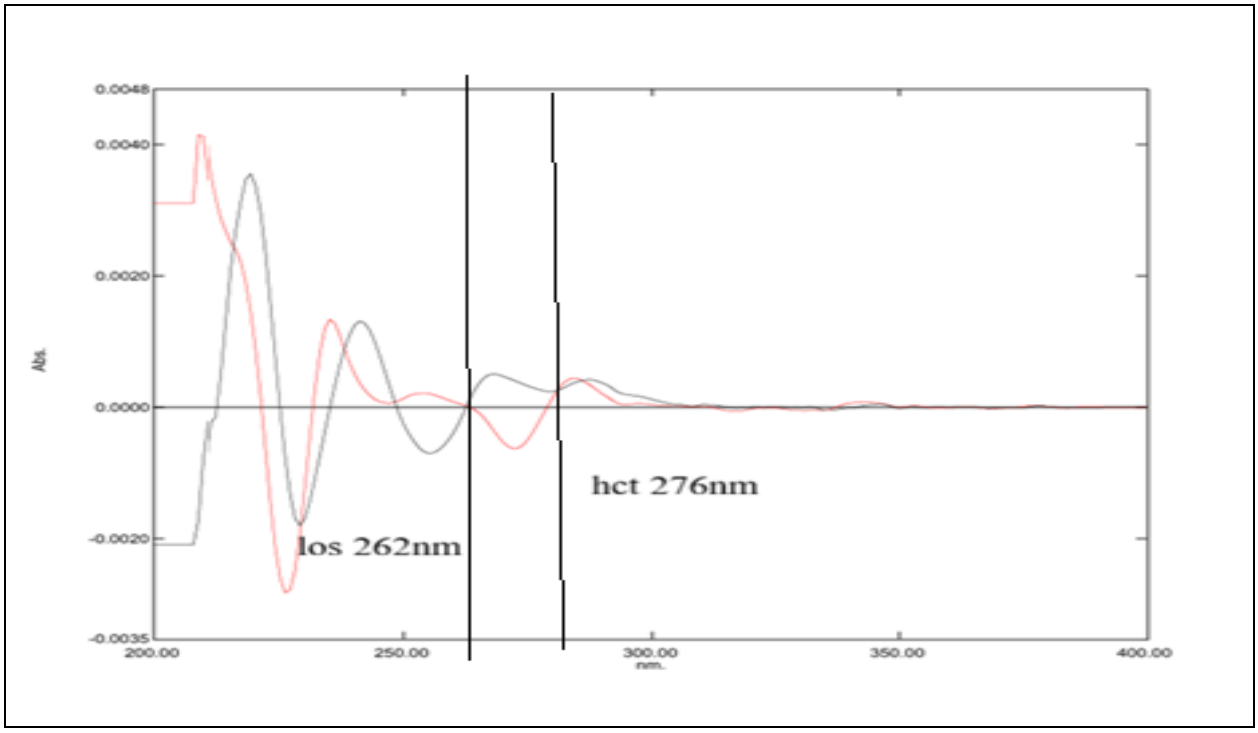

Fig 14 Second Order Derivative Overlay Spectra of Losartan Potassium and Hydrochlorothiazide

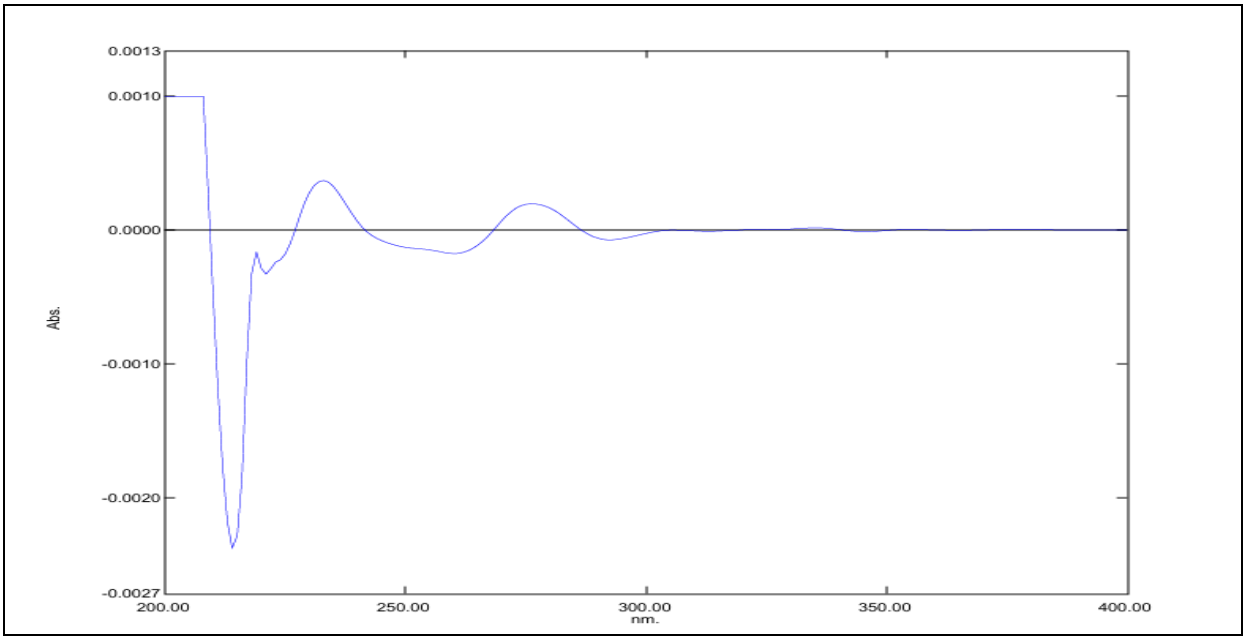

Fig 15 Second Derivative Spectrum of Mixture

- *Selectin of Analytical Concentration Range*

Table 6 Calibration Table for Losartan Potassium and Hydrochlorothiazide

Sr. No.	For Losartan Potassium		For Hydrochlorothiazide	
	Concentration (µg/ ml)	Absorbance at 262nm (λ_1)	Concentration (µg/ ml)	Absorbance at 276nm (λ_2)
1	2	-0.00021	0.5	-0.00039
2	4	-0.00034	1	-0.00062
3	6	-0.00044	1.5	-0.00092
4	8	-0.00055	2	-0.00122
5	10	-0.00067	2.5	-0.00157

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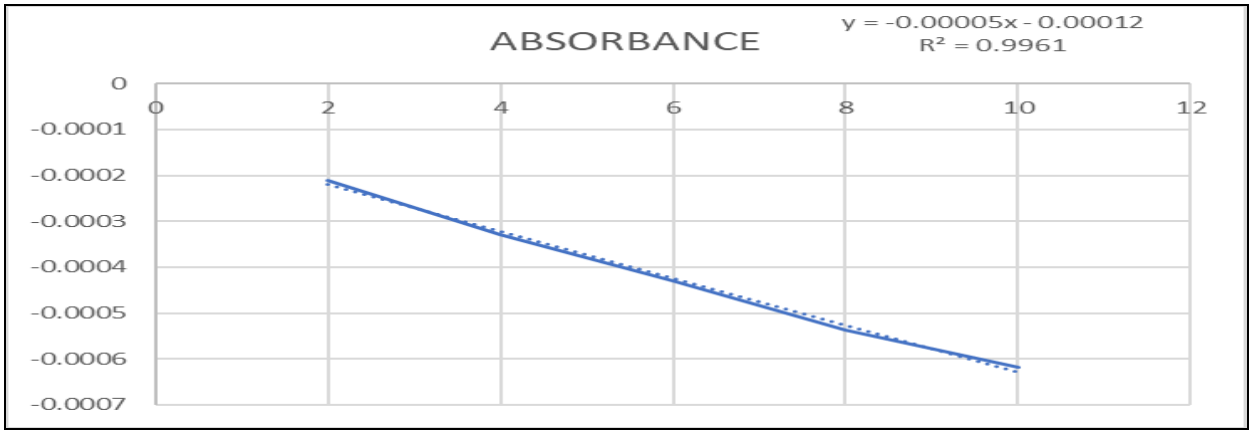

Figure 16 Calibration Curve for Losartan potassium at 262nm

The calibration curve for Losartan potassium at 262 nm—the wavelength selected for the second-derivative method—confirms that linearity is maintained within the 2–10 µg/ml range. Even at this different harmonic, the data remains consistent and predictable. The relationship is expressed through the following regression equation: y = -0.00005x - 0.00012 While the slope is negative, reflecting the nature of the derivative peaks in this region, the correlation coefficient (R2) of 0.9961 demonstrates a strong linear fit. This confirms that the second-derivative approach is just as mathematically sound as the first, providing a reliable alternative for quantifying Losartan with high statistical confidence.

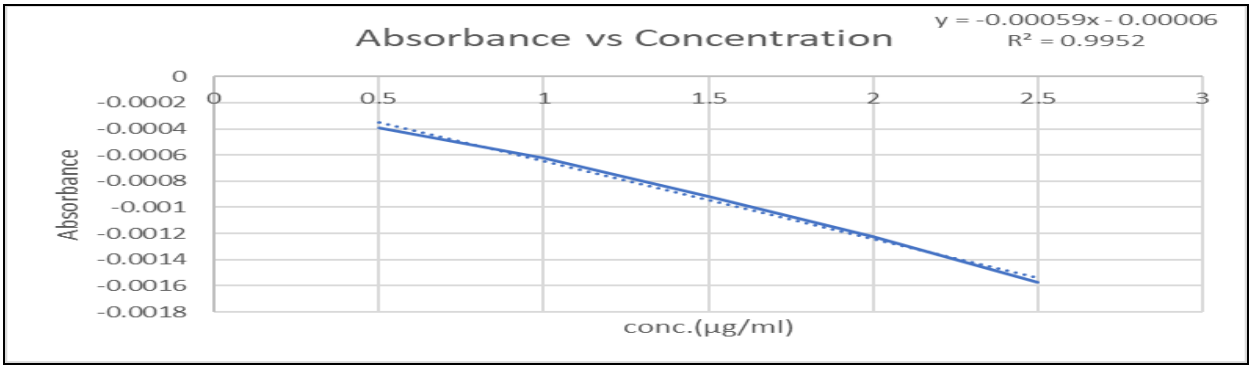

Fig 17 Calibration Curve for Hydrochlorothiazide at 276nm

The calibration curve for Hydrochlorothiazide (HCT) at 276 nm highlights the effectiveness of the second-derivative method within the low concentration range of 0.5–2.5 µg/ml. The results confirm that the method remains linear and highly sensitive even when focusing on these specific derivative peaks. The data follows the regression equation: y = -0.00059x - 0.00006 With a correlation coefficient of 0.9952, the calibration shows a strong degree of reliability. Although the R2 is slightly lower than the first-derivative measurement at 296 nm, it still comfortably meets the rigorous standards required for pharmaceutical validation. This ensures that HCT can be quantified accurately even in the presence of Losartan at this wavelength.

- *Limit of Detection and Limit of Quantitation Using First Derivative method*

Table 7 Data for LOD and LOQ of Losartan Potassium and Hydrochlorothiazide

Drug	LOD (µg/ml)	LOQ(µg/ml)
Losartan	0.9714	2.943
Hydrochlorothiazide	0.1041	0.3155

- *Recovery Data*

Table 8 Result for Recovery Studies.

Level of Recovery	Amount Present (mg)		Amount of Standard Adding (mg)		Total Amount Recovered		Absorbance		% Recovery	
	LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT
50	4	1	2	0.5	6.013	1.521	-0.00045	-0.00354	100.3	102.1
50	4	1	2	0.5	5.987	1.499	-0.00046	-0.00359	99.6	99.9
50	4	1	2	0.5	6.023	1.501	-0.00047	-0.00365	100.5	100.1

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100	4	1	4	1	7.984	2.023	-0.00037	-0.00470	99.6	102.3
100	4	1	4	1	8.045	2.012	-0.00045	-0.00476	101.1	101.2
100	4	1	4	1	8.102	1.997	-0.00058	-0.00480	102.5	99.7
150	4	1	6	1.5	10.023	2.501	-0.00069	-0.00587	100.5	100.01
150	4	1	6	1.5	10.042	2.497	-0.00070	-0.00592	101.0	99.7
150	4	1	6	1.5	9.994	2.499	-0.00072	-0.00604	99.8	99.9

- *Precision Data*

Table 9 Intra-Day Precision

Sr. No.	**Label Claim (mg)**		**Amount Found (mg)**		**% of Label Claim**	
	LOS	**HCT**	**LOS**	**HCT**	**LOS**	**HCT**
1	10	10	9.956	10.101	99.56	101.01
2	10	10	9.963	9.999	99.63	99.99
3	10	10	9.899	10.004	98.99	98.99

Table 10 Inter-Day Precision

Sr. No.	**Label Claim (mg)**		**Amount Found (mg)**		**% of Label Claim**	
	LOS	**HCT**	**LOS**	**HCT**	**LOS**	**HCT**
1	10	10	9.965	9.945	99.65	99.45
2	10	10	9.856	10.006	98.56	100.06
3	10	10	10.101	9.910	101.01	99.10

IV. SUMMARY AND CONCLUSION

The proposed derivative spectroscopic method offers a streamlined, accurate, and highly reproducible approach for the simultaneous estimation of Losartan potassium and Hydrochlorothiazide in combined samples. By utilizing established regression equations, the analytical process is simplified to basic absorbance measurements at pre-selected wavelengths followed by minimal calculations, eliminating the need for more complex and costly procedures. Statistical validation confirms the method's reliability, with low standard deviation and standard error values. Most notably, the relative standard deviation (%R.S.D.) consistently remains below 2%, ensuring full compliance with the rigorous quality standards set by USP and ICH guidelines.

The practical utility of this technique was successfully demonstrated through the precise quantification of a synthetic mixture containing 50 mg of Losartan and 12.5 mg of Hydrochlorothiazide. Because the method is specific, repeatable, and mathematically simple, it is an ideal candidate for routine laboratory quality control and dissolution testing. Its efficiency makes it a valuable tool for high-throughput pharmaceutical environments where maintaining accuracy while reducing analysis time is a top priority.

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