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Dual Analytical Approach for Concurrent Quantification of Indapamide And Losartan Potassium Using Analytical Techniques

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ABSTRACT

Clinical studies have demonstrated that the combination of Indapamide with Losartan Potassium is used in the management of hypertension, especially when monotherapy is not sufficient. This combination offers the benefit of complementary mechanisms of action: diuresis from Indapamide and vasodilation from Losartan, leading to more effective blood pressure control and potential reduction in cardiovascular risk. Despite their therapeutic relevance, no validated analytical method has yet been reported for the simultaneous qualitative and quantitative estimation of these drugs in combination. Simple and sensitive ultraviolet visible (UV) spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods were developed and validated for the simultaneous estimation of Indapamide and Losartan Potassium in a synthetic mixture. From the **First Order Derivative overlain spectra**, multiple zero crossing points were observed for both drugs. The ZCP of **225 nm** was selected for **Indapamide**, and **248 nm** for **Losartan Potassium**. These two wavelengths 248 nm for determination of Indapamide and 225 nm for Losartan Potassium were used. Secondly, In the **UV-Visible Spectrophotometric Method**, Indapamide and Losartan Potassium were estimated by the **Simultaneous Equation Method**. UV detection was performed at 240 nm for Indapamide and 210 nm for Losartan Potassium. Both drugs exhibited absorbance at the λmax of the other, enabling simultaneous quantification. In RP-HPLC method, mobile phase Phosphate Buffer (pH 3.3 adjusted with 10% ortho phosphoric acid): Methanol: ACN (60:25: 15 % v/v/v) at 215 nm was chosen for high-resolution peaks. Retention time was found to be 3 min and 5.5 min for Indapamide and Losartan Potassium, respectively.

1. INTRODUCTION:

According to World Health Organization epidemiological data, the global prevalence of Hypertension has increased markedly over recent decades and continues to rise. Hypertension, or high blood pressure, is a chronic condition where the force of blood against artery walls is consistently too high, typically 130/80 mmHg or higher. Known as a "silent killer". The combination of Indapamide with Losartan Potassium is used in the management of hypertension, especially when monotherapy is not sufficient. This combination offers the benefit of complementary mechanisms of action: diuresis from Indapamide and vasodilation from Losartan, leading to more effective blood pressure control and potential reduction in cardiovascular risk. The combination of Indapamide and Losartan potassium has been evaluated in a Phase III clinical trial [1], demonstrating that the therapy is safe, well tolerated, and effective in Management of Hypertension. Furthermore Together, Indapamide with Losartan Potassium provide a synergistic effect by targeting different mechanisms of blood pressure regulation diuresis and vasodilation through indapamide, and renin-angiotensin-aldosterone system inhibition through losartan. This combination can also help in reducing cardiovascular risk and organ damage in long-term hypertensive patients [1, 2]. Indapamide(4-Chloro-N-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide) is an orally active, non-thiazide sulfonamide diuretic with antihypertensive properties. It belongs to a unique class of diuretics that exhibit both diuretic and vasodilatory effects, making it a valuable agent in the management of hypertension [3, 4], while Losartan potassium(**5-(4'-{[2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazol-1-yl] phenyl} phenyl) tetrazol-5-yl] methanol)** is an orally active, nonpeptide angiotensin II (AII) receptor antagonist. It is the first of a new class of drugs to be introduced for clinical use in hypertension [5]**.** Despite the availability of analytical methods [6] for the individual estimation of these drugs, no validated analytical method has yet been reported for their simultaneous estimation. Therefore, the present study aims to develop and validate simple, accurate, and reproducible UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Indapamide and Losartan potassium in a synthetic mixture. A literature survey reveals that several analytical methods have been reported for the estimation of Indapamide and Losartan potassium either individually or in combination with other active pharmaceutical ingredients. For Indapamide, reported methods include UV analysis in bulk and tablet dosage forms [7], UV spectrophotometric method [8] with amlodipine besylate in tablet dosage form [9], combination with Perindopril by absorbance correction method [10], and Multicomponent spectrophotometric method in combination with Delapril [11]. RP-HPLC method [12] for Amlodipine Besylate and Indapamide [13], RP-HPLC method [14] combination with Atenolol [15]. Similarly, various analytical methods have been reported for Losartan potassium, including UV methods [16], UV method in combination with Atenolol [17], HPLC method for Losartan potassium has also been developed [18, 19]. Additionally, review articles covering analytical methods for Hydrochlorthiazide and Losartan potassium in bulk and pharmaceutical formulations are available [20, 21], and RS HPLC method for simultaneous estimation of impurities in Losartan Potassium in pharmaceutical dosage form [22]. Furthermore, HPLC-UV Method for simultaneous estimation of Losartan and its active Metabolite EXP-3174 [23]. However, no analytical method has been reported for the simultaneous estimation of Indapamide and Losartan potassium in a synthetic mixture. Hence, the present study aims to develop and validate simple, economical, precise, and robust first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Indapamide and Losartan potassium, in accordance with ICH Q2 [R2] guideline [24] within all Validation Parameters.

2. EXPERIMENTAL MATERIALS AND ANALYTICAL CONDITIONS

2.1 Chemicals and Reagents

Indapamide and Losartan Potassium were obtained as gift samples from **Jigs Chemical Limited, Ahmedabad** and Cadila Pharmaceuticals Ltd., Ahmedabad respectively. Methanol, acetonitrile (HPLC grade), potassium dihydrogen phosphate, and orthophosphoric acid were of analytical grade.

2.2 Instrumentation

The spectrophotometric measurements were performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. For chromatographic information acquisition and analysis, High-Performance Liquid Chromatography system Systronic RP-HPLC (SYS-LC-138) with UV Detector was utilized together. The pH of the buffer solution was observed utilizing the Chemi Line pH meter. The Scale-Tec analytical balance was utilized to weigh the samples. The HPLC mobile phase was subjected to sonication using an Sonicator- Digital Pro$^+$, PS-10A, (Broleo). Chromatographic separation was achieved using a Kromstar C_{18} column (250 × 4.6 mm, 5 µm).

2.3 *Analytical Condition*

In accordance with ICH Q2 (R2) [24] requirements, the analytical conditions for a simultaneous technique for the measurement of Indapamide and Losartan potassium in UV and HPLC were optimized and validated. For UV Spectroscopy Methanol was used as a Solvent. Detection wavelength (λmax) of INDA and LOSA were 240 nm and 210 nm, respectively. The first-order derivative UV spectra were derived from the zero-order spectra using methanol as the solvent. Quantitative analysis was performed at the zero-crossing point (ZCP) of Indapamide at 225 nm for the estimation of Losartan potassium, and at the ZCP of Losartan potassium at 248 nm for the estimation of Indapamide. For Simultaneous equation as Vierordt's method the absorption maxima (λmax) were established at 240 nm for Indapamide and 210 nm for Losartan potassium. This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated according to the mathematical expressions. For RP-HPLC, Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) was used in the procedure. The mobile phase consisted of Phosphate Buffer (pH 3.3 adjusted with 10% ortho phosphoric acid): Methanol: ACN (60:25: 15 % v/v/v) 215 nm wavelength was selected for RP-HPLC, with 1 mL/min flow rate.

2.4 Preparation of Solutions

2.4.1 Preparation of Stock Solution

Accurately weighed 10 mg of Indapamide (INDA) and 100 mg of Losartan potassium (LOSA) were individually transferred into separate 100 mL volumetric flasks and dissolved in methanol. The solutions were sonicated to ensure complete dissolution, and the volume was made up to the mark with methanol to obtain standard stock solutions having a concentration of 100 µg/mL of INDA and 1000 µg/mL of LOSA, respectively.

2.4.2 Preparation standard solution

Pipetted out 0.15 ml solution from stock solution of Indapamide (100 µg/ml) and 0.5 ml Losartan Potassium (1000 µg/ml) into different 10 ml volumetric flask and diluted upto mark with Methanol to get the 1.5 µg/ml of Indapamide and 50 µg/ml Losartan Potassium.

2.4.3 Preparation of standard working solution

The concentration ranges of 0.75-3.75 µg/mL of INDA and 25-125 µg/mL of LOSA produced from each stock solution, INDA (0.075, 0.150, 0.225, 0.300 and 0.375 ml) and LOSA (0.25, 0.50, 0.75, 1.00 and 1.25 ml) were pipetted out in ten different 10 ml volumetric flasks and made up to mark with Methanol to obtained 0.75, 1.5, 2.25, 3.00 and 3.75 µg/ml of INDA and 25, 50, 75, 100 and 125 µg/ml for LOSA, respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, the optimized chromatographic conditions, 20 µL of each standard working solution was injected into the RP-HPLC system.

2.4.5 Preparation of 10% Orthophosphoric acid

10% orthophosphoric acid was prepared by diluting 1.0 ml of concentrated ortho phosphoric acid in 10 ml HPLC grade water.

2.4.5 Preparation of 10mM Phosphate Buffer

Accurately weighed 0.272 gm potassium dihydrogen phosphate (KH_2PO_4) was transferred it in 200 ml HPLC grade water and allowed it to dissolve. It was filtered through 0.45 µm membrane filter and sonicated for about 10 min. Buffer pH was adjusted to 3.3 with 10% ortho phosphoric acid.

3. METHODOLOGY:

3.1 UV methods

3.1.1 (Method I) First Order Derivative Method

Pipetted out 0.15 ml solution from stock solution of Indapamide (100 µg/ml) and 0.5 ml Losartan Potassium (1000 µg/ml) into different 10 ml volumetric flask and diluted upto mark with Methanol to get the 1.5 µg/ml of Indapamide and 50 µg/ml Losartan Potassium. Each solution was scanned in the range of 200-400 nm. In zero order UV spectra, Indapamide exhibited an absorption maximum at 240 nm while Losartan potassium showed an absorption maximum at 210 nm in Figure 1. INDA and LOSA standard stock solutions were prepared in Methanol at concentrations of 100 µg/mL and 1000 µg/mL, respectively. A small amount of each stock solution was taken and placed into 10 mL volumetric flasks. Methanol was used to adjust the volumes to the mark, resulting in final concentrations of INDA ranging from 0.75 to 3.75 µg/mL and LOSA ranging from 25 to 125 µg/mL. All zero-order absorption UV spectra were converted to first-order derivative UV spectra. Calibration functions were established by plotting first-order derivative absorbance against corresponding concentrations for each analyte. Appropriate volume, 0.15 mL of INDA and 0.5 ml LOSA standard stock solution was transferred to two separate 10 mL volumetric flasks and the volume was adjusted to mark with methanol to get concentration 1.5 and 50 µg/mL, respectively. The solutions were scanned separately in the UV-region i.e., 400-200 nm. The zero-order UV absorption spectra of INDA and LOSA in Methanol shown in Figure 1. The zero-order spectrum was processed to obtain first-derivative spectrum. The two first derivative spectra were overlaid which showed that INDA showed zero crossing at 248 nm, while LOSA showed zero crossing at 225 nm which showed in Figure 2. The determinations were made at 248 nm for Indapamide (ZCP of Losartan potassium) and 225 nm for Losartan potassium (ZCP of Indapamide).

3.1.2 (Method II) Simultaneous equation as Vierordt's method

Solutions of Indapamide (1.5 µg/ml) and Losartan potassium (50 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, the absorption maxima (λmax) were established at 240 nm for Indapamide and 210 nm for Losartan potassium. This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated according to the following mathematical expressions.

Standard Stock solutions of Indapamide and Losartan potassium in the concentration range 0.75-3.75 µg/mL and 25-125 µg/ml were made in the methanol and absorbance of these solutions was measured at 240 nm and 210 nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).
At λ1 A1=ax1bCx+ay1bCy……………….(1)
At λ2A2= ax2bCx+ay2 bCy……………… (2)
For measurements in 1 cm cells b=1,
Rearrange eq. (2),
Cy= A2− ax2Cx/ ay2
Substituting for Cy in eq (1) and rearranging
Cx = A2ay1−A1 ay2/ax2 ay1−ax1 ay2………………..(3)
Cy = A1ax2−A2 ax1/ax2 ay1−ax1 ay2………………..(4)
Where Cx and Cy are the concentration of Indapamide and Losartan potassium, respectively, A1 and A2 are absorbance at 240 nm and 210 nm, respectively, ax1 and ax2 are absorptivity of Indapamide at 240 nm and 210nm, respectively; ay1 and ay2 are absorptivity of Losartan potassium at 210 nm and 240 nm, respectively. By solving the two simultaneous equations, the concentrations of Indapamide and Losartan potassium in sample solutions were obtained.

3.2 Method III: Reverse Phase High Performance Liquid Chromatography Method

For RP-HPLC, the analysis was carried out using an isocratic elution technique using a mobile phase comprised of different mobile phases such as Phosphate Buffer (pH 3.3): Methanol: ACN (60:25: 15 % v/v/v) (pH 3.3 adjusted with 10% ortho phosphoric acid) at a flow rate of 1 mL/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µm filter and sonicated for 30 min. The stationary phase was a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector from 200 to 400 nm, alongside chromatograms extracted at 215 nm (figure 1). The calibration curves were prepared by measuring the peak areas of INDA and LOSA and plotted their values against the pertinent concentrations. In accordance, the equations for linear regression were calculated.

3.3 Method Validation

The analytical procedures employed in this study were validated in accordance with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH Q2 (R2): *Validation of Analytical Procedures* [18]. Validation parameters evaluated included specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness

3.3.1 Specificity

Specificity denotes the capacity of an analytical procedure to accurately and distinctly quantify the target analyte despite the potential interference of co-existing substances. Within a complex sample, these extraneous components commonly encompass synthesis impurities, degradation products, or various matrix constituents that could otherwise confound the measurement.

3.3.2 Linearity and Range: (n=6)

The linearity of Indapamide and Losartan potassium was found to be in the range of 0.75-3.75 µg/mL and 25-125 µg/mL, respectively. Plot the calibration curve of peak area vs. concentration (µg/mL). Linearity of both the drugs were checked in term of slope, intercept and correlation coefficient.

3.3.3 Precision

The Intraday and Interday precisions also referred to as repeatability and intermediate accuracy, respectively were used to assess the precision of Methods I and II. The experiment was conducted on the same day and for the next three days for both Intraday and Interday precision, analysing freshly made solutions at concentrations of 0.75, 1.50, and 2.25 µg/mL of INDA and 25, 50, and 75 µg/mL of LOSA. To assess intermediate precision, the mean absorbance (UV) and peak area (HPLC) were recorded for each set of experiments. For repeatability, 1.5 µg/mL of INDA and 50 µg/mL of LOSA were used. The results were represented as a percentage Relative Standard Deviation (RSD), with a value of less than two considered acceptable. This meticulous approach ensures a comprehensive evaluation of the precision of the analytical methods, providing confidence in the reliability and consistency of the results obtained for the concentrations of INDA & LOSA in the tested solutions.

3.3.4 Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

3.3.6 Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Indapamide (1.5 µg/mL) and Losartan potassium (50 µg/mL). This performance was done in triplicate. The accuracy of the method was determined by calculating recovery of Indapamide and Losartan potassium by the standard addition method.

3.3.7 Assay as analysis of Synthetic Mixture

The synthetic mixture of Indapamide and Losartan potassium was prepared in the ratio of 1.5:50. A synthetic mixture equivalent to 100 mg was prepared by accurately weighing Indapamide(1.5mg) and Losartan potassium (50 mg). MCC (Micro Crystalline Cellulose) (8.5 mg), Lactose Monohydrate (20 mg), Magnesium Stearate (6 mg), Talc (1 mg), Starch (5 mg) and Croscarmellose (8 mg) were used as excipients. All the components were transferred into a mortar and blended thoroughly using a pestle to obtain a homogeneous synthetic mixture. This mixture was transferred in 100 ml volumetric flask and allowed to sonicate and made up to mark with Methanol. This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 15 µg/mL of Indapamide and 500 µg/mL of Losartan potassium.

3.3.7.1 Preparation of sample solution

Accurately 1 ml of the above [mixture solution of Indapamide (15 µg/ml) and Losartan Potassium (500 µg/ml)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Indapamide was 1.5 µg/ml and Losartan Potassium 50 µg/ml. Then analysed using the previously described UV-spectrophotometric and chromatographic conditions. The concentrations of INDA and LOSA were calculated using a regression equation.

3.3.8 Robustness

The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in approach situations. For the HPLC technique, samples have been subjected to evaluation below changed situations, which include adjustments inside the flow rate (± 0.1 mL/min), detection wavelength (± 2 nm), and natural content material (± 2 %) inside the mobile segment. The resulting results on machine suitability parameters have been intently monitored. In the times of Methods I and II, distinct analysts conducted sample analyses to evaluate the robustness of the strategies.

3.3.9 System Suitability Tests

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

4. RESULTS AND DISCUSSION

4.1 UV Methods

The first-order derivative UV spectrophotometric method provides distinct advantages over conventional zero-order UV techniques by enhancing spectral resolution and reducing baseline drift, thereby improving selectivity and accuracy. Measurement at zero-crossing wavelengths allows selective quantification of analytes in the presence of overlapping spectra without prior separation. In comparison with higher-order derivative methods, the first-order derivative approach offers a better signal-to-noise ratio, resulting in improved precision and reproducibility. Additionally, the method is simple, rapid, cost-effective, and requires minimal sample preparation, making it well suited for routine quality-control analysis of multicomponent pharmaceutical formulations.

4.1.1 Selection of wavelength for Indapamide and Losartan potassium

The remarkable absorbance of Indapamide exhibited an absorption maximum at 240 nm, while Losartan potassium showed an absorption maximum at 210 nm [figure 1]

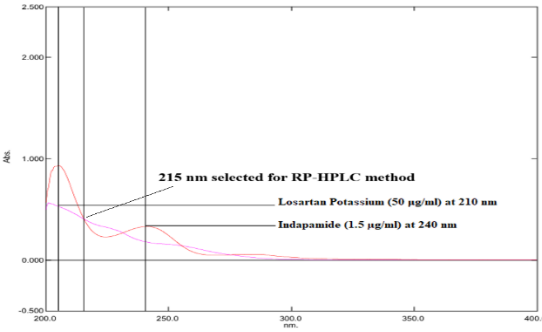


Figure 1: Overlain UV Spectra of Indapamide (1.5 µg/ml) and Losartan Potassium (50 µg/ml) at 240 nm and 210 nm in Methanol (Zero Order)

4.1.2 First order derivative UV Method Development

The INDA and LOSA overlapping absorption throughout the 200 - 400 nm range is shown by these spectra, which makes it more difficult to quantify the pharmaceuticals using traditional UV spectrophotometry without accounting for the overlap. The sum of the absorbances of the two compounds may be used to calculate the overall absorbance of a solution containing a combination of both at a certain wavelength. In situations where the levels of the two medicinal drugs overlap, the method entails figuring out the quantity of each drug using their zero-order spectra. The resulting absorbance spectra were derived to eliminate the interference of absorbing species. The first derivative corresponding to each absorption spectrum of each drug was recorded, using Δλ = 2 nm and scaling factor 4. The amplitude values were measured at 248 nm (λ1) (ZCP of LOSA) for Indapamide and 225 (λ2) (ZCP of INDA) for Losartan potassium showed in Figure 2. To determine the wavelength for measurement, Indapamide (1.5 µg/ml) and Losartan potassium (50 µg/ml) solutions were scanned between 200-400 nm.

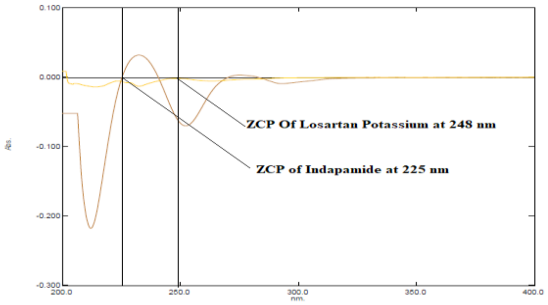
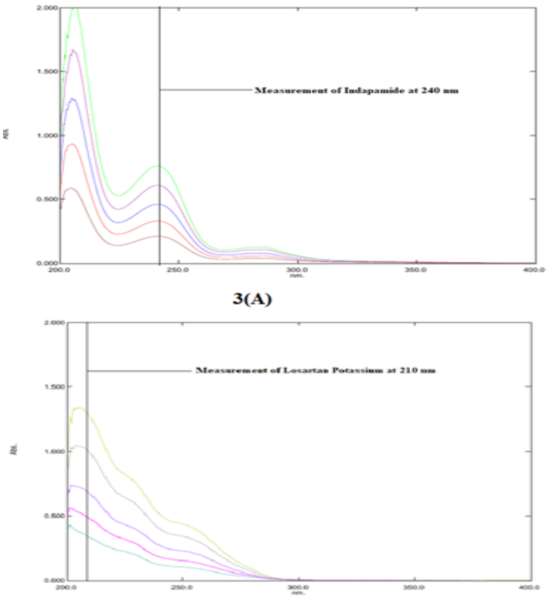


Figure 2: Overlain UV Spectra of Indapamide (1.5 µg/ml) and Losartan potassium (50 µg/ml) in Methanol (First Order)

4.1.2 Simultaneous equation (Vierordt's) Method

For the simultaneous equation method, standard solutions of Indapamide (1.5 µg/mL) and Losartan potassium (50 µg/mL) in methanol were subjected to spectral scanning between 200 and 400 nm at medium speed, with methanol employed as the blank solution. For the analytical determination, the absorption maxima (λmax) were established at 240 nm for Indapamide and 210 nm for Losartan potassium (Figure 1). This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated.

For multi-component UV analysis, Vierordt's method is named after the German scientist Karl Vierordt. UV Spectra of Indapamide (0.75-3.75 µg/mL) and Losartan potassium (25-125 µg/mL) over the linearity and range had been showed in Figure 3(A) and 3(B) respectively



3(A)

3(B)

Figure 3: Overlain UV Spectra of 3 (A) Indapamide (0.75-3.75) at 240 nm and 3(B) Losartan Potassium (25-125 µg/ml) at 210 nm

4.2 RP-HPLC Method

Reverse-phase high-performance liquid chromatography (RP-HPLC) was selected for the analysis due to its high resolution, sensitivity, and reproducibility in the separation and quantification of compounds with varying polarity. The technique offers excellent peak symmetry, shorter analysis time, and superior compatibility with aqueous and organic mobile phases, making it particularly suitable for routine quality control and analytical applications. In addition, RP-HPLC provides high method robustness and ease of method optimization, while requiring minimal sample preparation. Owing to these advantages and its wide regulatory acceptance, RP-HPLC is extensively employed in pharmaceutical and analytical research, making it an appropriate and reliable choice for the present study.

C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds.

4.2.1 Selection detection wavelength

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 215 nm both drugs give good peak height and shape. So, 215 nm was selected for simultaneous estimation of Indapamide and Losartan potassium in synthetic mixture. Overlain UV spectra of Indapamide (1.5 µg/ml) and Losartan potassium (50 µg/ml) in Methanol showed in figure 1.

4.2.2 Chromatography

The mobile phase Phosphate Buffer (pH 3.3 adjusted with 10% ortho phosphoric acid): Methanol: ACN (60:25: 15 % v/v/v) **was** selected because it was found to ideally resolve the peaks with retention time 3 min and 5.5 min for Indapamide and Losartan Potassium, respectively. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Indapamide and Losartan potassium with Flow rate of 1.0 ml/min. Figure 4 showed RP-HPLC Chromatogram of Indapamide (1.5 µg/ml) and Losartan potassium (50 µg/ml).

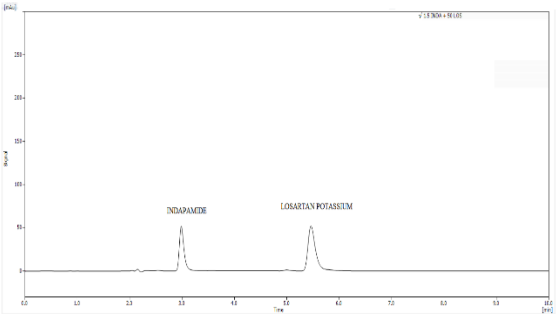


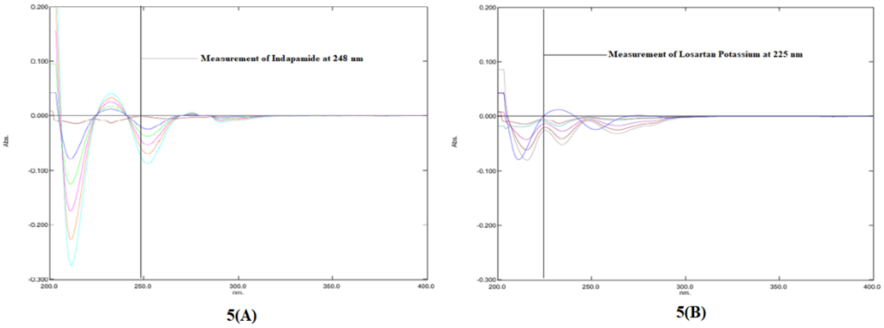
Figure 4: RP-HPLC Chromatogram of Indapamide (1.5 µg/ml) and Losartan Potassium (50 µg/ml) in Phosphate Buffer (pH 3.3 adjusted with 10% ortho phosphoric acid): Methanol: A (60:25:15 %v/v/v) at 215 nm {Run Time: 10 min, Flow Rate: 1.0 ml/min}

4.3 VALIDATION OF THE PROPOSED METHODS

4.3.1 Validation Parameters of the UV Method

4.3.1.1 Linearity and range

For INDA and LOSA, the absorbances ranged from 0.75-3.75 µg/mL at 248 nm and 25-125 µg/mL at 225 nm showed in Figure 5(A) and 5(B), respectively.



5(A) 5(B)

Figure 5: Overlain UV Spectra of 5(A)Indapamide (0.75-3.75 µg/ml) at 248 nm and 5(B)Losartan potassium (25-125 µg/ml) at 225 nm

A linear relationship was found and calibration curve was plotted for concentration vs. absorbance. For INDA, the calibration curve equation $y = 0.0635x + 0.0354$, while for LOSA, it was $y = 0.0002x - 0.0034$. Results showed that the correlation coefficient (R^2) was between 0.9992 and 0.9985 (Table 1).

For simultaneous Equation Method UV Spectra of Indapamide (0.75-3.75 µg/ml) and Losartan potassium (25-125 µg/ml) over the linearity and range had been showed in Figure 3(A) and 3(B), respectively. For UV, Indapamide exhibited a linear response in the concentration range of 0.75-3.75 µg/mL at 240 nm and 210 nm. The correlation coefficients (r^2) were found to be 0.9981 and 0.9999 at 240 nm and 210 nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 1.5%, demonstrating good precision and repeatability. Losartan potassium showed linearity over the concentration range of 25-125 µg/mL at 210 nm and 240 nm, with correlation coefficients (r^2) of 0.9996 and 0.9989, respectively. The Linearity Data are Summarized in Table 2.

Table 1: Linearity data of INDA and LOSA by UV (first order) and RP-HPLC

Parameters	UV Spectrophotometry		RP-HPLC	
	INDA at 248 nm	LOSA at 225 nm	INDA at 248 nm	LOSA at 225 nm
Linearity Range	0.75-3.75 µg/mL	25-125 µg/mL	0.75-3.75 µg/Ml	25-125 µg/mL
Correlation Coefficient	0.9992	0.9985	0.9995	0.9991
LOD	0.04	0.33	0.03	0.32
LOQ	0.12	1.00	0.10	0.96

Table 2: Linearity data of INDA and LOSA for Simultaneous equation UV method

Sr. No.	Parameters	Indapamide		Losartan Potassium	
1	Wavelength (nm)	240 nm	210 nm	210 nm	240 nm
2	Beer's Law Limit (µg/ml)	0.75-3.75	25-125	25-125	0.75-3.75
4	Correlation Coefficient (r^2)	0.9981	0.9999	0.9996	0.9989
9	LOD (µg/ml)	0.04	0.04	0.34	0.47
10	LOQ (µg/ml)	0.12	0.15	1.03	1.42

4.3.1.2 Calculation for Simultaneous Equation Method for Indapamide and Losartan potassium in Synthetic Mixture.

Indapamide (1.5 µg /ml) and Losartan Potassium (50 µg/ml) in Methanol, both the solutions were scanned over range of 200-400 nm against Methanol as blank, using medium scan speed. The sampling wavelength for analysis includes, Absorption maxima (λmax) of Indapamide was 240 nm, Absorption maxima (λmax) of Losartan Potassium was 210 nm. The method employs Simultaneous Equation as per Vierodt's method and the concentrations of drugs in sample solution were determined by using the following formula:

For Indapamide (X),

$$\mathrm{Cx} = \frac{\mathrm{A2\ ay1\text{-}\ A1\ ay2}}{\mathrm{a\ x2\ aY1\text{-}\ a\ x1ay2}}$$

For Losartan Potassium (Y),

$$\mathrm{Cy} = \frac{\mathrm{A1ax2\ -\ A2\ a\ x1}}{\mathrm{ax2\ aY1\text{-}\ a\ x1ay2}}$$

Where, ax_1 Where ax_1 and ax_2 represented the absorptivity of Indapamide at 240 nm and 210 nm, respectively; ay_1 and ay_2 denoted the absorptivity of Losartan potassium at 210 nm and 240 nm, respectively; and A_1 and A_2 corresponded to the absorbance of the sample measured at 240 nm and 210 nm, respectively

= Mean Absorptivity of Indapamide at 240 nm (λ_1): 0.2244

ax_2= Mean Absorptivity of Indapamide at 210 nm (λ_2): 0.6208

ay_1= Mean Absorptivity of Losartan Potassium at 210 nm (λ_1): 0.0108

ay_2= Mean Absorptivity of Losartan Potassium at 240 nm (λ_2): 0.0201

A_1= Absorbance of Indapamide sample at 240 nm (λ_1): 0.211

A_2= Absorbance of Losartan Potassium sample at 210 nm (λ_2): 0.6148

$$\mathrm{Cx} = \frac{(0.6148)(0.0108)\text{-}\,(0.211)(0.0201)}{(0.6208)(0.0108)\text{-}\,(0.224)(0.0201)} = \frac{(0.00664)\text{-}\,(0.00424)}{(0.00671)\text{-}\,(0.00451)} = \frac{(0.0024)}{(0.0022)} = 1.09\ \mu\mathrm{g/ml}$$

$$\mathrm{Cy} = \frac{(0.211)(0.6208)\text{-}\,(0.285)(0.2244)}{(0.6208)(0.0108)\text{-}\,(0.2244)(0.0201)} = \frac{(0.13098)\text{-}\,(0.06395)}{(0.00671)\text{-}\,(0.00451)} = \frac{(0.06703)}{(0.0022)}$$

$$= 30.46\ \mu\mathrm{g/ml}$$

4.3.1.3 Precision

Methodological precision was evaluated through intraday, inter-day, and repeatability assessments using triplicate analyses of Indapamide (0.75, 1.50 & 2.25 µg/mL) and Losartan potassium (25, 50 and 75 µg/ml) across three consecutive days and within a single diurnal period. Absorbance values were recorded for these concentrations to establish intermediate precision, while repeatability was specifically determined using concentrations of 1.5 µg/ml for Indapamide and 50 µg/ml for Losartan potassium. The outcomes, expressed as Relative Standard Deviation (% RSD) for each precision parameters were less than two.

4.3.1.3 LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) are calculated using the standard deviation responses and slopes obtained from the calibration curves of each drug at their specific wavelengths. The results of LOD and LOQ were displayed in Table 1 & 2.

4.3.1.4 Accuracy

The accuracy of the technique recuperation accomplished by means of standard addition approach. To pre-analyzed pattern acknowledged quantity of general INDA and LOSA spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of INDA and LOSA. Accuracy was carried out by the Recovery Studies (standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. For first order UV spectrophotometric method, The % Recovery was obtained in range of 99.18%-99.84% for Indapamide and 99.18%-99.42% for Losartan potassium, were showed in Table 3. And For the Simultaneous Equation method, The % Recovery was obtained in range of 98.95%-99.62% for Indapamide and 99.41%-99.78% for Losartan potassium, were showed in Table 4.

Table 3: Recovery study data for UV (First order) and RP-HPLC Method

UV method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std Amt (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD(n=3)**
Indapamide	50	1.5	0.75	2.25	2.2240	98.84±0.6095
	100	1.5	1.5	3	2.9754	99.18±0.3472
	150	1.5	2.25	3.75	3.7404	99.74±0.2640
Losartan Potassium	50	50	25	75	74.385	99.18±0.6577
	100	50	50	100	99.230	99.23±0.3454
	150	50	75	125	124.28	99.42±0.2530
RP-HPLC Method						
Indapamide	50	1.5	0.75	2.25	2.24	99.55±0.5014
	100	1.5	1.5	3	2.991	99.70±0.6193
	150	1.5	2.25	3.75	3.742	99.78±0.7571
Losartan Potassium	50	50	25	75	74.91	99.88±0.3322
	100	50	50	100	99.93	99.93±0.4328
	150	50	75	125	124.98	99.98±0.5421

Table 4: Recovery study for simultaneous equation UV method

Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std. Amt (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD(n=3)**
Indapamide	50	1.5	0.75	2.25	2.2265	98.95±0.4905
	100	1.5	1.50	3.00	2.9886	99.62±0.5000
	150	1.5	2.25	3.75	3.7357	99.61±0.5303
Losartan Potassium	50	50	25	75	74.558	99.41±0.2585
	100	50	50	100	99.780	99.78±0.2923
	150	50	75	125	124.60	99.68±0.3489

4.3.1.5 Assay of synthetic mixture

From assay, Final concentration of Indapamide was 1.5 µg/mL and Losartan potassium 50 µg/mL were run into UV and RP-HPLC the Percentage assay of Indapamide and Losartan potassium were found to be 99.07 % and 99.64 %, respectively for UV(first order) For Simultaneous equation method The percentage Assay of Indapamide and Losartan potassium were found to be 99.60% & 99.35% Respectively. Its results showed in Table 5

Table 5: Analysis of synthetic mixture for UV and RP-HPLC Method

UV- Method (First order)				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± SD (n=3)**	**%RSD**
Indapamide	1.5	1.486	99.07±0.004	0.29
Losartan Potassium	50	49.82	99.64±0.1219	0.24
UV- Method (Simultaneous equation method)				
Indapamide	1.5	1.494	99.60±0.5581	0.56
Losartan Potassium	50	49.675	99.35±0.1255	0.13
RP-HPLC Method				
Indapamide	1.5	1.498	99.86±1.11	1.11
Losartan Potassium	50	49.98	99.96±1.06	1.07

4.3.2 Validation Parameters of the RP-HPLC Method

4.3.2.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks with peak of Indapamide and Losartan potassium.

4.3.2.2 Linearity

The RP-HPLC chromatogram of Indapamide (0.75-3.75 µg/mL) and Losartan potassium (25-125 µg/mL) at 215 nm showed in figure 1. The Peak Area was found. Linearity was showed in figure 6. Calibration graphs were plotted between concentrations and peak areas. The regression equation of calibration curve was generated y = 130.88x + 17.469 for INDA and y = 6.8352x - 127.39 for LOSA. The correlation coefficient (R^2) values were observed to be 0.9995 and 0.9991. (Table 1).

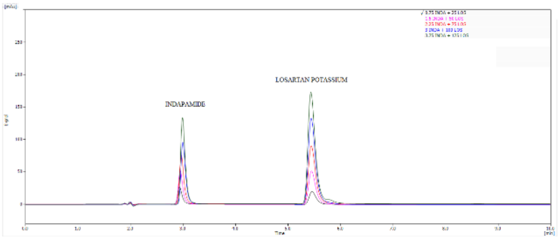


Figure 6: Overlain Chromatogram of Indapamide (0.75-3.75 µg/ml) and Losartan Potassium (25-125 µg/ml) at 215 nm {Run Time: 10 min, Flow Rate: 1.0 ml/min}

4.3.2.3 Precision

Concentrations of 0.75,1.5 and2.25 µg/mL for INDA and 25,50 and 75 µg/mL for LOSA were selected for precision studies. On the same day, the peak area of the prepared solutions was measured at three different time intervals at the selected wavelength. Similarly, on the first, second, and third days, the peak area of the same solutions was measured to evaluate inter-day precision. Each solution was prepared and analyzed in triplicate.

4.3.2.4 Accuracy

The accuracy of the technique recuperation was decided change into accomplished by means of standard addition approach. To pre-analyzed pattern acknowledged quantity of general INDA and LOSA spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of fashionable INDA and LOSA. The results were studied in triplicate and the accuracy changed into indicated by% recovery (Table 3). Accuracy was carried out by the Recovery Studies. For HPLC, The % Recovery was obtained in range of 99.55%-99.78% for Indapamide and 99.88%-99.98% for Losartan potassium were showed in Table 3. The mean percentage recovery values for both drugs were found to be within the ICH-accepted range of 98-102%, with low standard deviation. These results confirmed the accuracy, trueness, and reliability of the RP-HPLC method and indicated that excipients present in the synthetic mixture did not interfere with the estimation of either drug.

4.3.2.5 LOD and LOQ

LOD Values were found to be 0.03 and 0.32 µg/mL for Indapamide and for Losartan Potassium, respectively. LOQ Values were found to be 0.10 and 0.96 µg/mL, respectively for Indapamide and Losartan Potassium. These results showed in Table 1.

4.3.2.6 Assay

From assay, Final concentration of Indapamide was 1.5 µg/mL and Losartan potassium 50 µg/mL were injected into HPLC System and The Percentage assay of Indapamide and Losartan potassium were found to be 99.86 % and 99.96 %, respectively. Results showed in Table 5.

4.3.2.7 Robustness

Chromatographic analysis was used to analyze the effects of changes in analysts, and the results showed that there was no statistically significant difference in the% RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential as a result showed in Table 6.

Table 6: Robustness data

Condition	**Variation**	**Indapamide**	**Losartan Potassium**
		% Assay ± SD (n=3)	**% Assay ± SD (n=3)**
Flow rate (1 ml±0.2 ml/min)	0.8 ml/min	99.21±4.51	98.95±2.37
	1.0 ml/min	99.88±9.42	99.86±0.55
	1.2 ml/min	99.65±6.47	99.55±1.02
Detection wavelength (215 nm±2 nm)	213	98.74±1.12	99.25±9.45
	215	99.99±9.42	100.05±0.55
	217	99.18±2.04	99.65±3.76
Mobile Phase Phosphate Buffer (pH 3.3): Methanol: ACN (68:25: 15 ±2 %v/v)	66:23: 11	99.07±2.07	98.95±1.11
	68:25: 15	99.95±9.42	99.94±0.55
	70:27: 03	99.75±0.14	99.97±7.18

5. CONCLUSION:

The present study successfully demonstrates the development and validation of UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Indapamide and Losartan potassium in a synthetic mixture. Both methods were validated in accordance with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R2) and showed excellent linearity, accuracy, precision, sensitivity, and reproducibility. Both UV methods **Simultaneous Equation** and **First Order Derivative** method and RP-HPLC method were exhibited **excellent linearity, precision, and accuracy**, and were successfully validated according to **ICH Q2 (R2)** guideline. There was **no interference** from excipients, confirming the specificity of the methods. These methods can be reliably applied for **routine quality control** of combined **Indapamide and Losartan Potassium in synthetic mixture.** Furthermore, the proposed methods can be extended for the analysis of marketed formulations and stability studies, highlighting their potential applicability in regulatory and industrial quality-control settings.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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