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DEVELOPMENT AND VALIDATION OF HPLC AND SPECTROSCOPIC METHOD OF ESTIMATION OF ANTIHYPERTENSIVE DRUG

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Abstract:

The aim of this research was to develop and validate a stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Lisinopril and Atenolol in pharmaceutical topical dosage forms. The method was designed to be robust, precise, and capable of distinguishing the active pharmaceutical ingredients (APIs) from their degradation products under various stress conditions. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of Acetonitrile: Phosphate Buffer (pH 3.00) (60:40). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 231 nm using a UV detector. The retention time of drug was found to be 5.241 and 7.724 for LSP and ANL respectively. The method was validated according to ICH guidelines for linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. Linearity was observed in the concentration range of 25-150 µg/mL for Lisinopril and 10-60 µg/mL for Atenolol, with correlation coefficients (r^2) of 0.998 and 0.999 respectively. The method demonstrated good accuracy, with recovery rates between 98.0% and 102.0% for both drugs. Precision, both intra-day and inter-day, showed relative standard deviation (RSD) values of less than 2%. The developed RP-HPLC method is reliable, efficient, and suitable for the simultaneous estimation of Lisinopril and Atenolol in pharmaceutical oral dosage forms. It can be successfully applied for routine quality control and stability testing of these agents.

Keywords: *Lisinopril, Atenolol UV Spectrophotometry, RP-HPLC, Simultaneous Estimation, Method Validation.*

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INTRODUCTION:

Hypertension is one of the most prevalent chronic health disorders worldwide, affecting millions of individuals across both developed and developing nations. Characterized by consistently elevated arterial blood pressure, it significantly increases the risk of cardiovascular events such as myocardial infarction, stroke, heart failure, and renal dysfunction. Effective pharmacological management of hypertension is critical to prevent these complications, and this necessitates the use of well-established antihypertensive agents. Among these, angiotensin-converting enzyme (ACE) inhibitors and beta-adrenergic blockers are frequently prescribed, either alone or in combination, to achieve optimal therapeutic outcomes.[1-4]

Lisinopril is a widely used long-acting ACE inhibitor that plays a crucial role in the management of hypertension, heart failure, and post-myocardial infarction conditions. It functions by inhibiting the angiotensin-converting enzyme, thereby preventing the conversion of angiotensin I to angiotensin II—a potent vasoconstrictor. This leads to vasodilation, decreased systemic vascular resistance, and ultimately, reduced blood pressure. In addition, Lisinopril decreases aldosterone secretion, resulting in reduced sodium and water retention, which further supports its antihypertensive effects. The drug is well-absorbed orally and is typically administered once daily due to its prolonged duration of action. Due to its mechanism of action and favorable pharmacokinetics, Lisinopril is an integral component in antihypertensive therapy, especially for patients with concurrent diabetes or chronic kidney disease.[5-8]

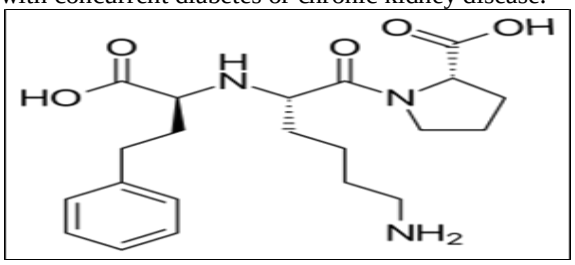

Figure 1: Structure of Lisinopril

Atenolol, on the other hand, is a cardioselective beta-1 adrenergic receptor blocker that exerts its antihypertensive effect primarily through the reduction of heart rate, cardiac output, and renin release from the kidneys. It is especially effective in patients with high sympathetic nervous system activity and is commonly used in the treatment of hypertension, angina pectoris, and certain arrhythmias. Atenolol has a longer half-life compared to non-selective beta-blockers and is generally well-tolerated, with a lower risk of bronchospasm in comparison to non-selective agents. Its selectivity makes it a drug of choice in hypertensive patients with co-existing respiratory disorders such as mild to moderate asthma or chronic obstructive pulmonary disease (COPD), where non-selective beta-blockers are contraindicated.

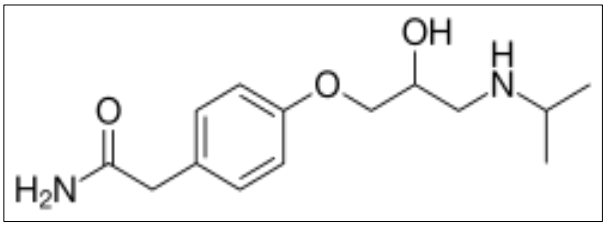

Figure 2: Structure of Atenolol

The combination of Lisinopril and Atenolol offers a synergistic approach in blood pressure control by targeting two different physiological pathways — the renin-angiotensin system and the sympathetic nervous system. For effective therapeutic monitoring and quality control, it is essential to develop reliable, sensitive, and validated analytical methods capable of simultaneously estimating both drugs in pharmaceutical formulations.

Analytical methods such as High-Performance Liquid Chromatography (HPLC) and UV-Visible spectrophotometry have become indispensable tools in pharmaceutical analysis due to their accuracy, sensitivity, and reproducibility. However, there is a growing demand for the development of simpler, economical, and robust methods that can be employed in routine quality control laboratories. A simultaneous estimation method not only reduces analysis time and solvent usage but also ensures precision and consistency in combination dosage formulations.[9-12]

The present study is focused on the development and validation of a simple, precise, and accurate RP-HPLC method and a UV-spectrophotometric method for the simultaneous estimation of Lisinopril and Atenolol in bulk and tablet dosage forms. The methods are developed in accordance with International Conference on Harmonisation (ICH) Q2(R1) guidelines, and they are validated for parameters such as linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. The validated methods aim to serve as effective tools for routine analysis and quality assessment of these two essential antihypertensive agents in pharmaceutical industries.

MATERIALS AND METHODS:

MATERIALS:

Drug Acquisition:

The reference standards of the selected antihypertensive drugs Lisinopril and Atenolol were

procured from Trimurti Drug Supplier, located in Surat, Gujarat. These drugs were used as received without any further purification, and were of high purity suitable for analytical method development and validation purposes.

Reagents and Chemicals:

All reagents and solvents used in this study were of analytical or HPLC grade to ensure the accuracy and reliability of the developed methods. Milli-Q water was used throughout the analysis as the diluent and solvent where required. Methanol and Acetonitrile, both of HPLC grade, were obtained from Advent. These solvents were employed for mobile phase preparation and sample dilution. Buffer systems used in the method development included Potassium Dihydrogen Phosphate and Di-potassium Hydrogen Phosphate, both of European Pharmacopoeia (EP) grade, and were procured from Dipa Chemical Industry.

Characterization of Drugs

The procured drug samples, Lisinopril and Atenolol, were characterized by evaluating their organoleptic properties such as colour, odour, taste, and physical appearance through visual inspection. Physical parameters including melting point and solubility were also assessed. The melting point was determined using the open capillary method (uncorrected), while solubility studies were carried out in various solvents like water, phosphate buffer, ethanol, methanol, and acetonitrile to identify a suitable medium for further analysis.[13-15]

FTIR and DSC Studies

Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the identity and purity of Lisinopril and Atenolol. The spectra were recorded using the Jasco FTIR 4600 instrument (Japan), and the characteristic peaks of functional groups were analyzed. Differential Scanning Calorimetry (DSC) was performed using a calibrated MDSC 2920 model. Approximately 3–5 mg of each drug sample was weighed in sealed aluminium pans, and heated from 0 to 300°C at a rate of 5 K/min under a helium purge (30 cm^3/min) to study the thermal behavior and purity of the samples.[16-18]

Spectroscopic Analysis

Prior to HPLC method development, UV-spectroscopic analysis was performed to determine the absorption maxima (λ max) of Lisinopril and Atenolol. Standard drug solutions were scanned between 200–400 nm using a UV-Visible spectrophotometer (Lasany, Model LI-2702) equipped with 1 cm matched quartz cells. The λ max values were identified and used for method development.[19-20]

Calibration Curve and Instrumentation

Standard stock solutions were prepared by dissolving 10 mg of each drug in 10 ml of Water: Acetonitrile (50:50), sonicated, and filtered through a 0.45 µm membrane. Further dilutions were made with buffer to obtain concentrations ranging from 8–28 µg/ml for Lisinopril and 10–60 µg/ml for Atenolol. A digital analytical balance (Elder) was used for weighing, and an ultrasonic bath (Prama Instruments) was used for dissolving drug substances. The absorbance of each dilution was measured to construct calibration curves.[21-25]

UV Method Development

Solubility screening was initially conducted to identify a common solvent for both drugs. After testing various solvents and combinations, a mixture of Water: Acetonitrile (50:50) was finalized as the most suitable solvent system based on solubility and stability profiles. Using this solvent, standard solutions of both drugs were prepared and analyzed at their respective λ max values. Concentration ranges of 8–28 µg/ml for Lisinopril and 10–60 µg/ml for Atenolol were evaluated to establish the linearity of the method for spectrophotometric analysis.[26-28]

Method Validation

The UV method was validated as per ICH guidelines. Linearity was confirmed by preparing five serial dilutions of standard solutions. A calibration curve showed a consistent linear relationship. LOD and LOQ were estimated based on the calibration curve using statistical methods involving standard deviation and slope. Accuracy was determined through recovery studies at 80%, 100%, and 120% levels by spiking known amounts of standards into samples. Percent recovery was calculated to confirm accuracy. Precision was assessed by intra-day and inter-day analysis at three concentrations for both drugs. Results were expressed as % RSD to confirm reproducibility. Robustness was evaluated by changing solvents. Absorbance was recorded to assess the method's stability. Ruggedness was tested at different temperatures to ensure the method remains reliable under varied conditions.[29]

HPLC Analysis

A Shimadzu LC 2010 HPLC system with UV detection and C18 column was used. Software used was LC Solution. An ultrasonic bath and digital balance were employed for sample preparation. The mobile phase consisted of phosphate buffer (pH 3.5) and acetonitrile (60:40). Standard stock solutions of Lisinopril and Atenolol were prepared and diluted to various concentrations for method development. Multiple mobile phase combinations were tested. The selected phase provided optimal separation and resolution for both drugs.[30]

HPLC Method Validation

System suitability was confirmed by injecting standard solutions and evaluating retention time, peak

area, and other parameters. Specificity was verified by injecting blank, individual, and mixed solutions. No interference was observed at drug retention times. System and method precision were confirmed using six replicate injections. Results showed low variability. Intra- and inter-day precision studies were performed at three concentrations over different times and days. % RSD values were within acceptable limits. Accuracy was confirmed by recovery studies using the standard addition method at three concentration levels. Recovery was consistent with expected values. Linearity was established by injecting five concentration levels. Calibration curves for both drugs showed strong correlation. Stability of solutions was tested after 24 hours at room temperature and refrigerated conditions. The assay results remained stable. LOD and LOQ were determined statistically to identify the lowest detectable and quantifiable drug levels. Robustness was evaluated by varying flow rates. Results were consistent with method precision. Ruggedness was tested at different temperatures, showing reliable results under varying conditions.[31-35]

Analysis of Marketed Formulations[36-38]

Lisinopril Assay: Marketed Lisinopril tablets (Lipril 2.5, Lupin Ltd., 2.5 mg) were powdered and transferred to a 10 ml volumetric flask. The mobile phase was added, and the solution was sonicated for 5–10 minutes. The volume was made up, and appropriate dilutions were prepared. The final test solution was analyzed using RP-HPLC. The assay was calculated by comparing the sample peak area with that of the standard.

Atenolol Assay: Marketed Atenolol tablets (Atenova 25 mg, Lupin Ltd.) were similarly processed. The powdered sample was dissolved in the mobile phase, sonicated, and diluted to the required concentration. The solution was analyzed by RP-HPLC, and the percent assay was determined by comparing the sample and standard peak areas.

RESULTS AND DISCUSSIONS:

Organoleptic Properties

Lisinopril and Atenolol were both observed as white to off-white crystalline powders with a fine texture. Both drugs were odorless and exhibited a distinctly bitter taste.

Physical Parameters

Melting Point

The melting point of Lisinopril was found to be 170.37°C, aligning well with the reported range of 169.9–172.6°C. Atenolol showed a melting point of 152.75°C, consistent with the reported range of 152–155°C.

Solubility

Lisinopril was soluble in acetonitrile and water, and slightly soluble in methanol. Atenolol was soluble in acetonitrile and slightly soluble in phosphate buffer.

8.1 **FT-IR spectroscopy study:**

Identification of Lisinopril and Atenolol was confirmed by FTIR Spectra. All peaks was found in Lisinopril and Atenolol drugs.

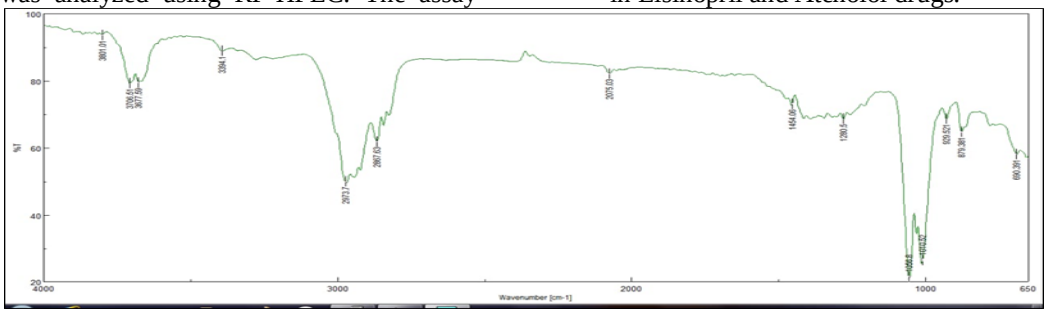

Figure 3: FTIR spectrum of Lisinopril

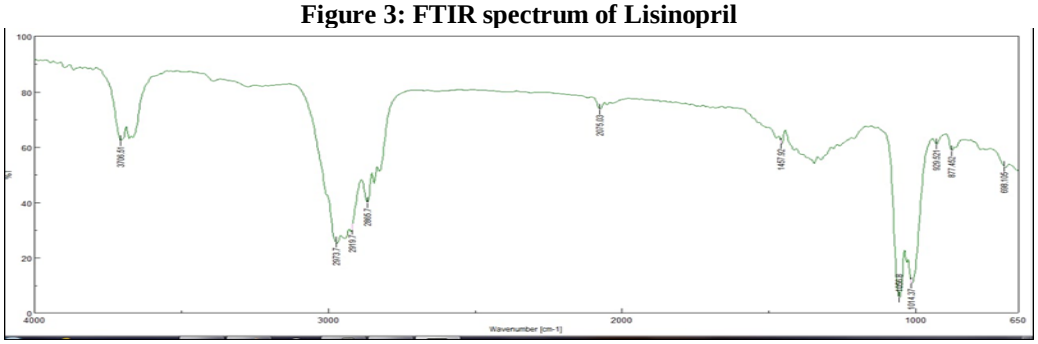

Figure 4: FTIR spectrum of Atenolol

DSC study of pure drug:

DSC profile of Lisinopril and Atenolol. Lisinopril showed a peak at 170.37^{0}C whereas, Atenolol showed a sharp endothermic peak at 152.75^{0}C corresponding to the melting transition temperature and decomposition of these drugs. Such sharp endothermic peak signifies that Lisinopril and Atenolol used was in pure crystalline state.

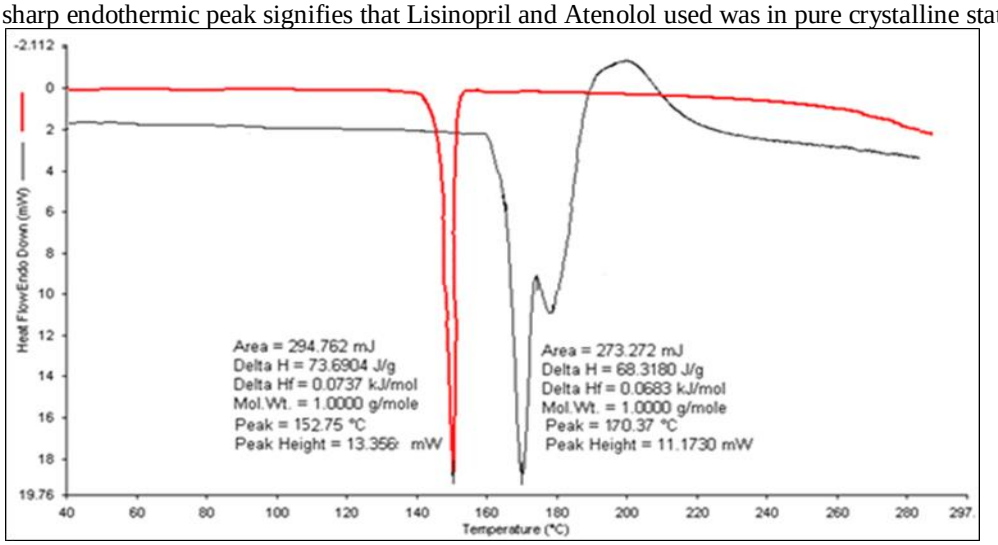

Figure 5: DSC Thermogram of Lisinopril and Atenolol

UV analysis:

Determination of λmax:

The standard solution of concentration 10µg/ml exhibited max absorbance at 218 nm for Lisinopril and 226 nm for Atenolol

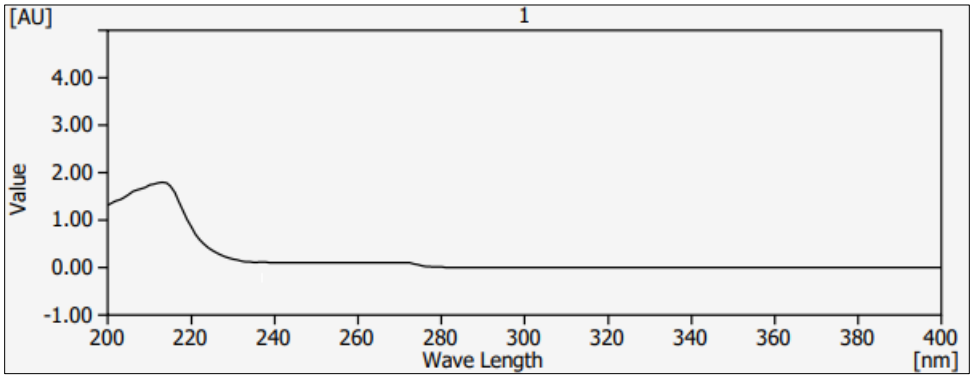

Figure 6: Maximum wavelength detection of Lisinopril

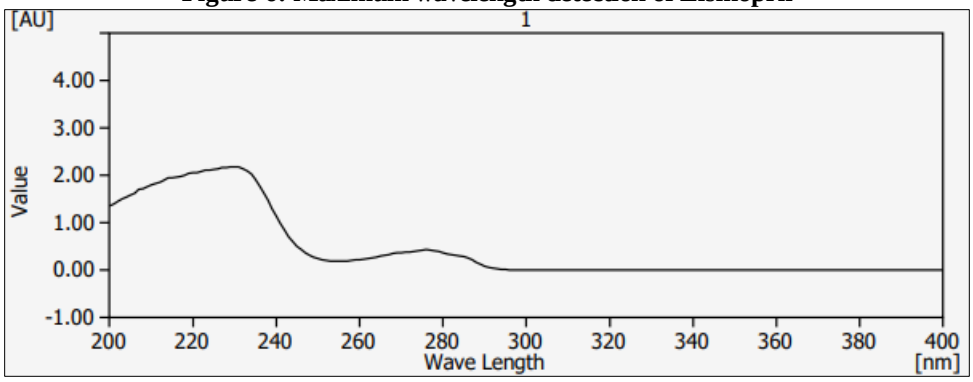

Figure 7: Maximum wavelength detection of Atenolol

Method Validation for UV method development

Linearity

For the linearity of the Lisinopril also six point calibrations curve were plotted in a concentration range of 8-28 (µg/ml). The equation was found to be y = 0.0408x-0.1426, with correlation coefficient of 0.9997 Whereas, Atenolol six point calibrations curve were plotted in a concentration range of 10-60 (µg/ml). From the linearity study it was observed that the drug was found to be linear in the concentration range and the linear regression equation was y = 0.0144x+0.0969 with correlation coefficient 0.9997.

Accuracy

Accuracy of the proposed UV method for Lisinopril and Atenolol was verified by conducting the recovery studies by using standard addition method. Standard drug concentration at three different percent levels was added to known amount of Lisinopril and Atenolol. The percent recovery of added standards was calculated. The results showed better % mean recovery for respective percent levels. The % mean recovery values are closer to 100% showed high accuracy of the proposed UV analytical method.

Table 1: Evaluation data of Accuracy study of Lisinopril and Atenolol

	Lisinopril				
Concentration (%)	**Origin level (µg/ml)**	**Amount added (µg /ml)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	8	6.4	101.37	99.70	1.469
80	8	6.4	99.10		
80	8	6.4	98.63		
100	20	20	100.46	100.16	0.431
100	20	20	100.37		
100	20	20	99.67		
120	28	33.6	100.48	101.06	0.546
120	28	33.6	101.58		
120	28	33.6	101.12		
	Atenolol				
Concentration (%)	**Origin level (µg/ml)**	**Amount added (µg/ml)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	10	8	99.64	99.99	0.323
80	10	8	100.27		
80	10	8	100.08		
100	40	40	101.67	100.47	1.125
100	40	40	99.42		
100	40	40	100.34		
120	60	72	99.84	100.69	0.775
120	60	72	101.38		
120	60	72	100.85		

Precision

Intra-day and inter-day precision study of drug were evaluated for the 8 µg/ml, 20 µg/ml and 28 µg/ml for Lisinopril and 10 µg/ml, 40 µg/ml and 60 µg/ml for Atenolol. Absorbance mean, percent assay and percent RSD were calculated for the intra-day as well as inter-day precision study.

Table 2: Evaluation data for Intra-day and Inter-day study of Lisinopril

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
8	0.184	99.84	0.863	0.178	100.20	0.653	0.185	100.27	0.486
20	0.678	101.02	0.756	0.682	100.47	0.572	0.672	101.13	0.597
28	0.982	101.31	0.861	0.980	100.3	0.639	0.973	100.85	0.537
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
8	0.189	101.02	0.728	0.188	101.78	0.547	0.186	99.63	0.537
20	0.681	100.57	0.561	0.671	100.21	0.354	0.683	100.27	0.674
28	0.981	100.91	0.579	0.983	99.37	0.567	0.972	100.48	0.638

Table 3: Evaluation data for Intra-day and Inter-day study of Atenolol

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
10	0.238	99.37	1.264	0.230	101.20	0.781	0.234	99.28	1.125
40	0.671	100.27	0.563	0.670	100.47	0.861	0.678	100.51	0.837
60	0.956	100.37	0.610	0.948	100.52	0.863	0.951	99.68	0.917
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
10	0.228	99.61	0.937	0.236	100.67	0.738	0.237	99.87	1.254
40	0.661	100.34	0.618	0.669	101.76	0.638	0.670	100.47	0.867
60	0.954	101.75	0.715	0.946	100.30	0.798	0.956	100.64	0.832

Robustness

Robustness study was evaluated by using three different solvent. The method was found to be robust as indicated by the % RSD values which are less than 2%.

Table 4: Evaluation data for Robustness of Lisinopril and Atenolol

Lisinopril			
Concentration (µg/ml)	**Solvents**	**Absorbance**	**% RSD**
20	Ethanol	0.681	0.948
20	Methanol	0.690	0.864
Atenolol			
Concentration (µg/ml)	**Solvents**	**Absorbance**	**% RSD**
40	Ethanol	0.674	0.480
40	Methanol	0.678	0.567
40	Phosphate Buffer: Methaol (50:50)	0.681	0.631

Ruggedness

Ruggedness study of drug was carried out at the three different temperature levels. From the results it was found that the method was rugged showing the % RSD value less than 2%.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

Form the results it was found that LOD & LOQ are in the sub-microgram level, which indicates the sensitivity of the method.

Table 5: Evaluation data for LOD & LOQ of Lisinopril and Atenolol

Lisinopril	
LOD	0.211 µg/ml
LOQ	1.289 µg/ml
Atenolol	
LOD	0.457 µg/ml
LOQ	1.354 µg/ml

Method Development by Reverse Phase High Performance Liquid Chromatography

Optimization of Chromatographic Conditions and Method Development

In order to achieve the optimized chromatographic conditions to separate and quantify Lisinopril and Atenolol one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. Various trials were carried out to finalize the optimized chromatographic conditions mentioned in the Table 6. Poor resolution, bad peak shapes, disturbances in base line were the few reasons of the rejections of the trials.

Table 6: Various Trials and Optimization of Chromatographic Conditions

Trial No	HPLC System	Chromatographic Conditions	Observations	Remarks
1	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-**Acetonitrile:Water 80:20 **Column -** Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 232 nm	Peaks were not fully separated. Broad peaks were obtained.	**Rejected**
2	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Acetonitrile: Water (50:50) **Column -** Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 232 nm	Peaks were separated but the peak shapes was not acceptable.	**Rejected**
3	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-**Acetonitrile: Phosphate Buffer (pH 5.00) (50:50) **Column -** Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min **Injection**	Peaks were separated but the peak shapes was not acceptable by using pH 5.00	**Rejected**

		Volume- 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 232 nm		
4	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-**Acetonitrile: Phosphate Buffer (pH 3.00) (60:40) **Column -** Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 232 nm	Peaks shape were good, with good resolution and intensity at pH 3.00	**Accepted**

Blank Chromatogram

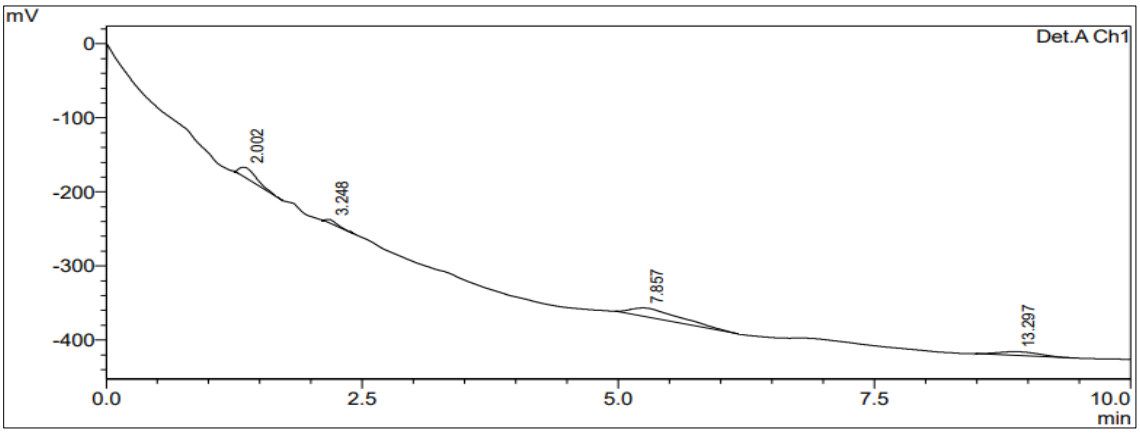

Figure 8: Blank Chromatogram

Trial 4

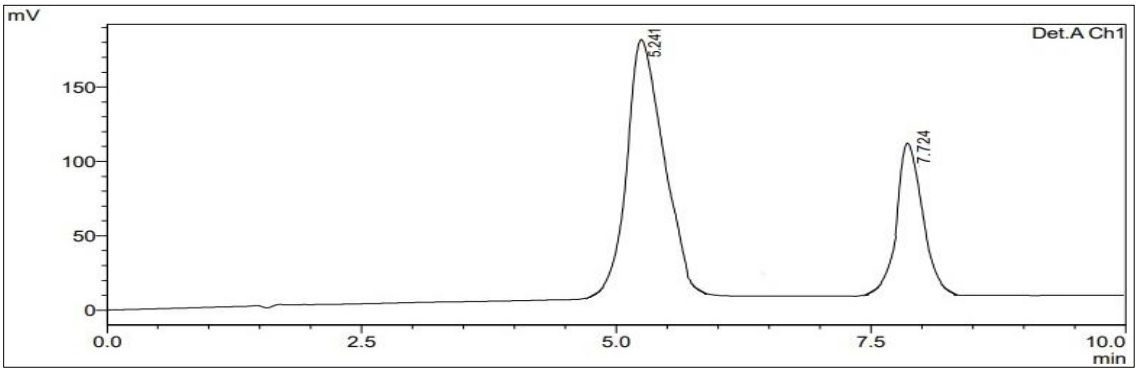

Figure 9: HPLC Fingerprinting of Optimized Lisinopril and Atenolol

Table 7: Evaluation parameter of Optimized Lisinopril and Atenolol

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Lisinopril	5.241	3397263	165923
2	Atenolol	7.724	2196436	148846

Method Validation
System Suitability

The HPLC method has been developed for the determination of the percentage assay of Lisinopril and Atenolol in Topical dosage forms. Parameters like Retention time, Peak area, tailing factor, and theoretical plates were found to be within acceptable limit.

Table 8: System Suitability Parameters of Lisinopril and Atenolol analysis

Replicates		Retention time	Peak area	Tailing Factor	Theoretical Plates
1	Lisinopril	5.124	3248165	1.154	2458
	Atenolol	7.702	2185672	0.986	2267
2	Lisinopril	5.102	3314586	1.027	2384
	Atenolol	7.720	2148567	1.031	2260
3	Lisinopril	5.201	3368472	1.161	2473
	Atenolol	7.742	2256841	0.963	2217
4	Lisinopril	5.224	3298454	1.125	2346
	Atenolol	7.741	2136458	1.067	2162
5	Lisinopril	5.274	3351487	1.163	2476
	Atenolol	7.752	2164150	0.942	2256

8.8.1.2 Specificity

The absence of additional peaks in the chromatogram indicates non- interference of excipients. There was no interference from the blank at the retention time of analyte peaks. The chromatograms of standard drugs alone and in their mixture are shown in figure 23, 24 and 25. The Retention time for Lisinopril and Atenolol was found to be 5.274 & 7.751 min respectively.

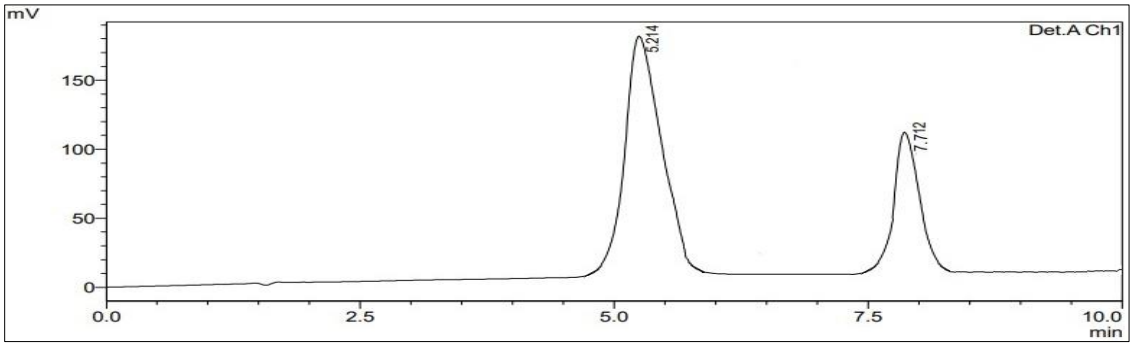

Figure 10: Standard Chromatogram of Mixture of Lisinopril and Atenolol

Table 9: Specificity Parameters for Lisinopril and Atenolol

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical PlateCount
1	Lisinopril	5.274	3315687	1.12	2452
2	Atenolol	7.751	2168422	0.987	2267
3	Lisinopril and Atenolol	5.214	3317612	1.11	2381
		7.712	2145683	0.968	2286

Precision

a) System Precision

The system precision was performed by measuring the peak response for standard drugs solutions in six replicates. Peak areas, mean, standard deviation and % relative standard deviation (%RSD) for Lisinopril and Atenolol was found to be 1.010% and 1.653 %. The results were found well within the acceptable criteria.

Table 10: System Precision Data of Lisinopril and Atenolol

Sr. No.	Peak areas of Lisinopril	Peak areas of Atenolol
1	4520191	3086715
2	4561762	3017518
3	4486271	3175642
4	4581678	3068947
5	4563821	3084671
6	4469657	3084692
Mean	**4530563**	**3086364**
SD (±)	**45762.783**	**51028.365**
RSD (%)	**1.010**	**1.653**
Acceptance criteria	**% RSD should not be more than 2**	

b) Method Precision

The method precision was performed by measuring the peak response for sample solutions in six replicates. The % assay for Lisinopril and Atenolol in six samples was calculated.

Table 11: Method Precision Data of Lisinopril and Atenolol

Sr. No.	% Assay of Lisinopril (w/w)	% Assay of Atenolol (w/w)
1	100.52	100.25
2	101.34	99.67
3	99.37	99.68
4	100.28	100.58
5	99.64	100.47
6	100.54	99.27
Mean	**100.28**	**99.98**
SD (±)	**0.705**	**0.522**
RSD (%)	**0.705**	**0.522**
Acceptance criteria	**% RSD should not be more than 2**	

c) Intraday and Inter-day Precision

The % RSD in intraday precision for Lisinopril (25, 75, 125 µg/ml) was found to be 0.021, 0.016, 0.688% and for Atenolol (10, 30, 50 µg/ml) was found to be 0.170, 0.957, 0.973% respectively. In inter-day precision % RSD for Lisinopril (25, 75, 125 µg/ml) was found to be 0.268, 0.101, 1.176 % and for Atenolol (10, 30, 50 µg/ml) was found to be 0.433, 1.265, 0.535 % respectively. % RSD in intraday and inter-day studies were found well within the acceptable limits.

Accuracy (Recovery Study)

The accuracy of the assay method was evaluated by standard addition method in triplicate at 100 % level of the labeled claim and the percentage recovery was calculated. The mean % recovery was found to be 100.00 % & 99.98 % for Lisinopril and Atenolol respectively.

Linearity and Range

Linearity for Lisinopril and Atenolol was found to be in the range of 25 - 150µg /ml and 10 – 60 µg/ml respectively with correlation coefficient value (r2) 0.9982 for Lisinopril and 0.9994 for Atenolol.

Table 12: Linearity and Range for Lisinopril and Atenolol

Concentrationin µg/ml for Lisinopril	Average PeakArea*	Concentrationin µg/ml for Atenolol	Average PeakArea*
25	3217035	10	1657861
50	4520191	20	3086715
75	5889052	30	4628435
100	6954711	40	6128467
125	8147719	50	7463849
150	9252753	60	9138471
Slope	**48125**	**Slope**	**148670**
CC	**24584**	**CC**	**147185**

Stability in Analytical Solution

No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The percentage assay is tabulated in table 13.

Table 13: Solution Stability Data of Lisinopril and Atenolol

Time level	Refrigerator (25°C)	Room Condition (37°C)
Time in hrs	% Assay of Lisinopril	% Assay of Atenolol
Initial	100.20 (±0.34)	99.38 (±0.032)
After 24 hrs	101.45 (±0.58)	100.02 (±0.046)

**Average of Six determination*

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

For Lisinopril LOD and LOQ were found to be 0.593µg/ml and 0.964 µg/ml respectively. Whereas, for Atenolol the LOD and LOQ were found to be 0.245µg/ml and 1.272 µg/ml respectively. These values indicate that the method is suitable for the determination of the lower concentration and confirms that proposed method is sensitive for the determination.

Robustness

The robustness of an HPLC method is states about its capacity to remain unaffected by minor, deliberate alterations to its method parameters. This quality ensures the reliability of the method during routine usage. Percent (%) RSD at each condition was found less than 2. This indicates the robustness of the method.

Ruggedness

The ruggedness parameter was determined by analyzing the different concentration at different temperature.

Analysis of Marketed Formulation

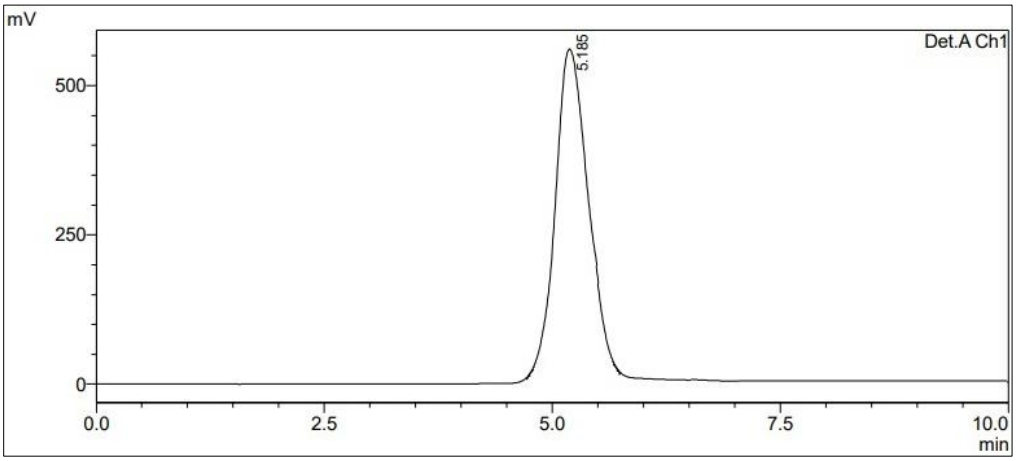

Figure 11: Chromatogram of Marketed Formulation of Lisinopril (Lipril 2.5, Lupin Ltd.)

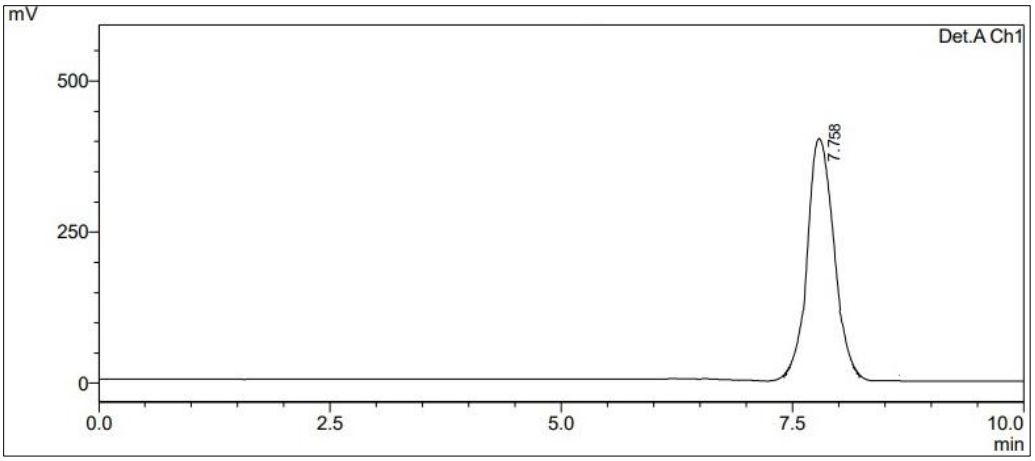

Figure 12: Chromatogram of Marketed Formulation of Atenolol (Atenova 25mg, Lupin Ltd.)

Table 14: Evaluation parameters of Marketed Formulation

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical PlateCount	% Assay
1	Marketed Formulation	5.185 (Lisinopril)	4352761 (Lisinopril)	1.12 (Lisinopril)	2451 (Lisinopril)	96.29
		7.758 (Atenolol)	2112376 (Atenolol)	0.943 (Atenolol)	2367 (Atenolol)	97.63

The results of Marketed formulation was found to be satisfactory. The percent assay of Lipril 2.5 Tablets IP was found to be 96.29 % for Lisinopril and Atenova Tablets IP 25mg was found to be 97.63% for Atenolol.

CONCLUSION:

A simple, accurate, and cost-effective UV and RP-HPLC method was successfully developed and validated for the simultaneous estimation of Lisinopril (LSP) and Atenolol (ANL) in pharmaceutical formulations. Both methods showed good linearity, precision, and accuracy without interference from other excipients. UV analysis used wavelengths of 218 nm for LSP and 226 nm for ANL, while HPLC used a mobile phase of Acetonitrile: Phosphate Buffer (60:40, pH 3.0) with retention times of 5.241 and 7.724 minutes. The methods complied with ICH guidelines and are suitable for routine quality control of LSP and ANL in combined dosage forms.

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AUTHORS CONTRIBUTIONS:
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CONFLICTS OF INTERESTS:
All authors have declared no conflict of interest.

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