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Values Validation RF-HPLC of Losartan Potassium

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ABSTRACT
Losartan is a class of oral antihypertensive drugs known as angiotensin II receptor antagonists. The RP-HPLC method developed in this study for estimating losartan potassium is simple, precise, and stability-indicating. Chromachemi (Puritas) C-18, 125 mm × 4.6 mm, 10 µm, was utilized for chromatographic separation, with a mobile phase consisting of 60:40 v/v acetonitrile and buffer at a flow rate of 1.0 mL/min. Detection occurred at a wavelength of 250 nm. The established procedure was verified in accordance with ICH requirements for various metrics, including robustness, linearity, precision, specificity, and accuracy. Recovery rates for losartan potassium ranged from 98 to 102%. The retention time was determined to be 1.6 minutes. A strong linear relationship was observed for the drug losartan potassium within the concentration range of 50–120 µ/mL, with a correlation coefficient of 0.999. Consequently, this method can be utilized for routine analysis.
KEY WORDS: Hypertension, RP-HPLC, Losartan Potassium, Validation.

I. INTRODUCTION

Several analytical methods have been applied to the analysis of Losartan potassium in pharmaceutical products that make use of high performance thin layer chromatography (HPTLC) [6], capillary electrophoresis (CE), capillary electrochromatography (CEC) [7], and spectrophotometry [8–10]. The literature reports many analytical methods for the quantitation of Losartan in tablets using HPLC [15]. All these reported methods either took a long time for analysis or employ mobile phases with pH adjustment of buffer solutions which is tedious and not suitable, especially for routine testing of quality control samples of dissolution study. Hence, this project was undertaken with an intention to develop a rapid analytical method for the estimation of Losartan potassium in dissolution samples to support product development and quality control efforts.

This paper described a new RP-HPLC method for the estimation of Losartan potassium in dissolution samples using simple mobile phase. The objective of the present study was to develop a simple, less time consuming and, economical analytical method for estimation of Losartan potassium in dissolution study of its formulation. The proposed method was validated as per ICH guidelines [18].

Nowadays, losartan is commonly prescribed to manage high blood pressure1, which is a significant issue for public health. It's a leading cause of cardiovascular disease2. Recognized by the World Health Organization (WHO) as a major risk factor for illness and death globally, hypertension claims around nine million lives each year3. It's prevalent worldwide, despite ongoing efforts to develop new drugs targeting various pathways involved in hypertension4,5. Losartan potassium, chemically describe as the 2-butyl-4-chloro -1-(p-(o-1H- tetrazol-5-ylphenyl) benzyl) imidazole-5-methanol mono-potassium salt, undergoes chemical modification shown in Figure.16. Losartan is the one of a novel type of oral antihypertensive drugs known as (ARB's) angiotensin II receptor antagonists.[18][1]

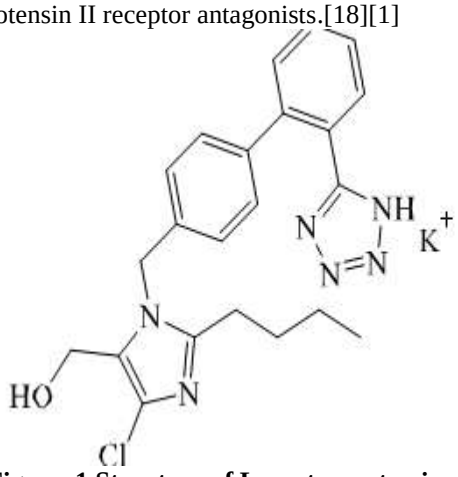

Figure. 1 Structure of Losartan potassium

After reviewing the literature, we found that only a few methods reported for our purpose. Our goal is to establish a straightforward, quick, cost-effective, validated, and sensitive method for analyzing the degradation product of losartan potassium. The current method recommended by

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the USP involves a gradient approach, which is time- consuming and expensive. While various analytical methods exist for analyzing losartan potassium using techniques like HPLC, capillary electrophoresis, electrophoresis, and spectrophotometry, they lack stability and precision because losartan potassium is the main degradation product. Therefore, for quantifying losartan potassium with a quick analysis time, following validation according to ICH guidelines. Our main target was to produce sensitive & selective High Performance Liquid Chromatography technique suited to routine analysis.[12]

II. MATERIALS AND METHODS

02.01.METHOD DEVELOPMENT

To evolve a precise & effective method for quantifying Losartan potassium, we carefully selected the analytical conditions by testing various parameters like diluents, buffer types, buffer concentrations, and other factors. Different buffer solutions and mobile phase compositions were tested, and mobile phase, comprised of Buffer [pH 7.0] & CAN in a partition of 60:40 (%/%), was chosen to flow at rate of 1 mL per minute. A Chromachemi (Puritas) C-18, ,125mm × 4.6 mm,10µm column was used. Detection was tested at different wavelengths, but the drug maximal absorption was determined at 250 nm. Losartan potassium had a retention time of 1.6 minutes. The chromatography conditions are shown in Table.1.[Including Article Short Data]

Table . 1 Chromatographic conditions and system suitability parameters

Parameter	Condition
Column Dimension	C18,125mm×4.6 mm,10µm
Column Used	Chromachemi(Puritas)
Flow (mL/min)	1.0 mL/minutes
VolumeofInjection	10µL
Run Period	5 min
λ-max	250 nm
Mobile Phase	Buffer:Acetonitrile
System	Isocratic
ColumnTemp	25°C
Autosampler Temp	15°C
SystemSuitability Parameters	
Tailingfactor Acceptancelimit:NMT2.0	1.370
Theoreticalplates Acceptancelimit:NLT1000	1307
%Relativestandarddeviation (RSD)	0.10
Retentiontime	1. minutes

02.01.01.Instrumentation

Thermo Scientific (HPLC) with a separation module adjusted with a diode array and a UV-visible detector (Ultimate 3000), Chromeleon software 7.2, an analytical balance (Sartorius), an ultrasonic bath (precisonic), a vacuum oven (Thermolab), a centrifuge machine (REMI R-8M), and a pH metre (Labindia).[1]

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02.01.02.Chemicals and Reagents

The working standard grade of Losartan potassium was provided by Vasudha Pharma Chem LTD; the company also made and supplied the placebo. Chembio PVT.LTD. produced HPLC-grade acetonitrile, which was applied in the analysis. EMPARATA ACS made the monobasic potassium phosphate and the dibasic sodium phosphate anhydrous that were utilized.

02.01.03.Preparation of pH 7.0 Buffer solution

To prepare the solution, dissolve 1.25 gm of monobasic potassium phosphate and 1.5 gm of dibasic sodium phosphate anhydrous in one liter of water. Resulting solution should have a pH of approximately 7.0. Before use, ensure to run solution through a 0.45 µm nylon membrane filter & degas.

02.01.04.Diluent

The mobile phase is used to dilute the solution.

02.01.05.Preparation of Reference solution

Carefully weighed and transferred around 25.0 mg of Losartan potassium into 100 mL volumetric flask. Addition 70 mL diluent to the flask & sonicate the mixture until complete dissolution occurs. Cool, then dilute to volume with respect to diluent. Mix proper and inject. (Conc.: 250 ppm of Losartan potassium)

02.01.06.Preparation of Test stock solution

Precisely weigh twenty tablets and conclude their average weight. Then, weigh and transfer 10 intact tablets into a 500 mL VF. Added 250 mL of diluent into flask. Sonicate the mixture with intermittent shaking for a duration of 25 minutes. Cool, then dilute up to mark with diluent and thoroughly mix the solution. Pass an aliquot through a0.45 µm nylon syringe filter by discarding the first 2-3 mL of the filtrate. (Conc.: 2000 ppm of Losartan potassium)[Specification Methods].

02.01.07.Preparation of Test solution

Dispense 3 mL of the test solution into a 25 mL VF. Dilute into volume with diluent solution. Mixing properly and inject. (Conc.: 240 ppm of Losartan potassium)

02.02.METHOD VALIDATION

The purpose of validating the technique was to show that it is suited to its intended use while complying with ICH requirements. We validated the method according to these guidelines to understand its performance and ensure it meets the requirements for its intended application11.

02.02.01.System Suitability

In this process of developing an analytical method and conducting system suitability tests, it is crucial to ensure confidence in the selection of an appropriate mobile phase, flow rate, temperature, and column. This guarantees optimal performance of the system components, including the pump and detector. Retention time, efficiency, theoretical plates, and asymmetry factor must all fall within predetermined limitations, which adds to the dependability of the results. System suitability parameters reported in Table.1.

02.02.02.Specificity

The method's specificity was validated by analyzing a sample containing analytes and a placebo. A distinct peak at a specific retention time was shown in the analyte sample, providing a definitive indicator for identifying the analyte. Conversely, when the placebo was subjected to the same chromatographic conditions, no peak was discernible.

02.02.03.Linearity and range

This method describes capacity to generate results within a specified range that correlate directly with the concentration of the drug in the sample. Linearity of Losartan potassium would be determined by evaluating dilutions of a standard solution of the working standard. Prepared and analyzed 5 concentrations (123.75, 197.87, 247.41, 271.95, and 296.49 ppm) of Losartan potassium, as outlined in Table.2. Subsequently, we calculatcorrelationcoefficient and %Y-axis intercept. Our findings indicated a high level of linearity, with an R2 value of 0.999 observed for the experimental data.

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Table . 2 Linearity Concentration ranges of Losartan Potassium

% Level	Vol.ofstocksolution (mL)	Dilutedinto (mL)	Final concentration in ppm
50%	3.0	25	123.75
80%	3.0	25	197.87
100%	3.0	25	247.41
110%	3.0	25	271.95
120%	3.0	25	296.49

02.02.04.Accuracy

The accuracy of procedure evaluated using a percent recovery analysis. Standard drug added to the pre-analyzed sample solution at concentrations of 50, 80, 100, 110, and 120 percent, with 5 percent of the standard drug solution added at each step. The recovery tests were done five times. % Mean and % Individual recovery were quantified. Refer to Table.3 for accurate preparation.[Estimation]

Table. 3 Accuracy of concentration % levels in Losartan potassium preparation

% level	**Weightof placebo (in mg)**	**Weightof API(mg)**	**Volume of Losartan Potassium stocksolution added (mL)**	**Diluted in(mL)**	**Losartan potassium's final concentration in ppm**
	2145.68	521.55			124.058
50%	2140.35	521.53	3.0	25	124.053
	2137.01	521.50			124.046
	2149.78	833.91			198.357
80%	2140.83	833.88	3.0	25	198.350
	2137.20	833.86			198.345
100%	2147.90	1042.66			248.011
	2145.87	1042.65	3.0	25	248.009
	2147.77	1042.61			247.999
	2144.98	1146.08			272.611
110%	2147.29	1145.93	3.0	25	272.576
	2142.88	1145.99			272.590
	2137.13	1249.53			297.218
120%	2140.54	1249.48	3.0	25	297.206
	2135.06	1249.60			297.235

02.02.05.Method Precision (Repeatability)

Precision in an analytical method shows how close the measurements are when you test the same thing multiple times. To check how consistent the method is, we did six separate tests of Losartan potassium. Then, calculated the percent (RSD). The percent RSD should be less than or equal to 2%.

02.02.06.Intermediate Precision

To see how consistent our assay method is over time, we did two separate tests on different days. First, we used data from the "repeatability" analysis done on the first day. Then, we did another set of experiments on a like day, either with another analyst or following different instrument. We calculated the standard deviation, %RSD (relative

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standard deviation), and mean difference for each day's results.

02.02.07.Robustness

The robustness of a method is about how it deals with small changes in its conditions while still giving accurate results when used regularly. We found that even with minor adjustments, such as changing the column oven temperature, the method remained effective.

02.02.08.Ruggedness

It ensures that the method can withstand these variations without compromising its accuracy and precision, thereby confirming its robustness and reliability for routine use.

02.02.09.Solution Stability

To prove solution stability of the standard, test the solution at a specified retention time, inject the respective solution at a different time interval from the initial to 13 hours, and calculate the difference in the result from the initial.

III. RESULTS AND DISCUSSION

Our study aimed to create a validated, easy-to-use, and trustworthy HPLC method using both a UV-visible detector and a Photodiode array detector to measure Losartan potassium accurately. We started by fine-tuning the analytical and instrument settings to ensure clear peaks for the Losartan potassium standard. Then, we tested and confirmed the method's reliability and accuracy by assessing its validation parameters following ICH guidelines.

03.01.SPECIFICITY

Peak of Chromatograms for the blank solution, reference, test, & placebo solution were compared, not peak interference was seen. Peaks were clearly separated. Refer to Figures. 2–5 for blank, placebo solution, reference solution, and test solution, respectively.

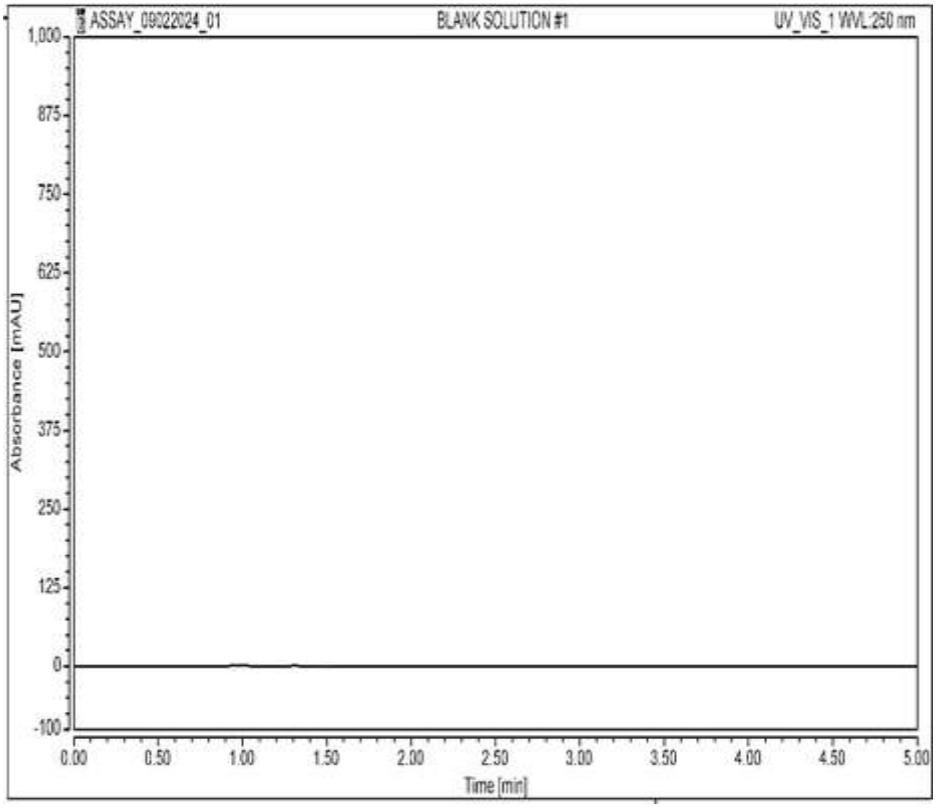

Figure . 2 Representative scans of Blank

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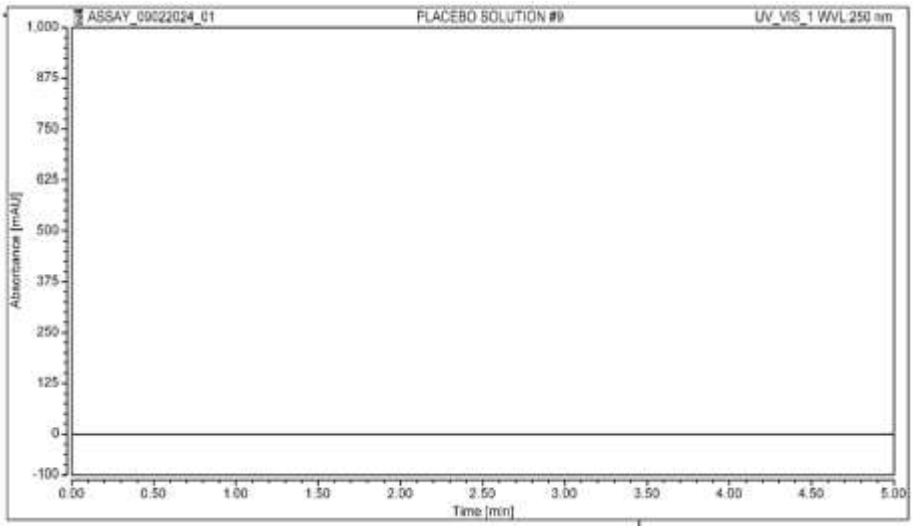

Figure. 3 Representative scans of Placebo Solution

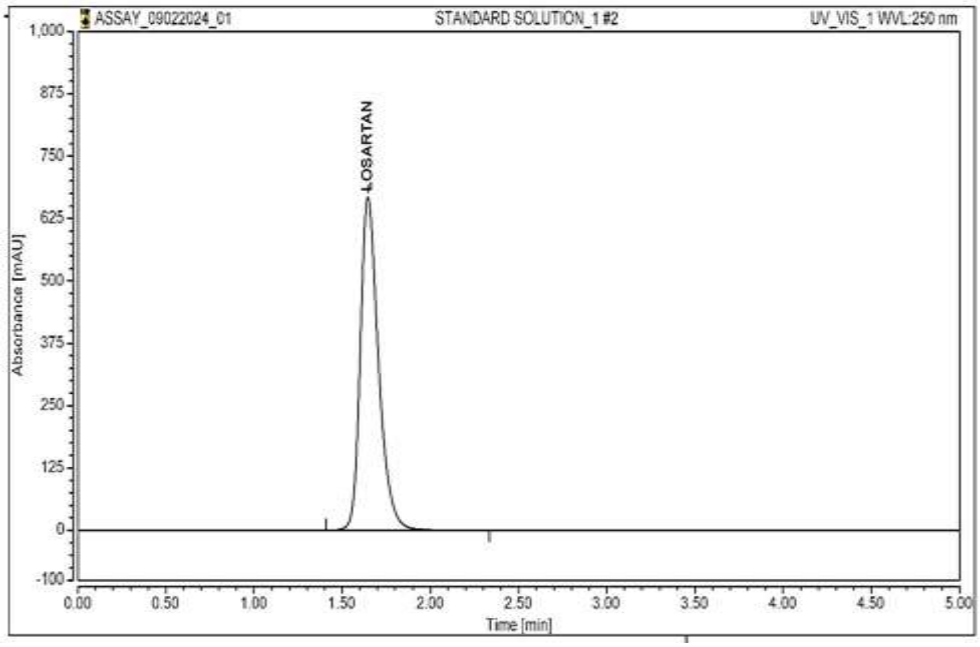

Figure. 4 Representative scans of Standard Solution

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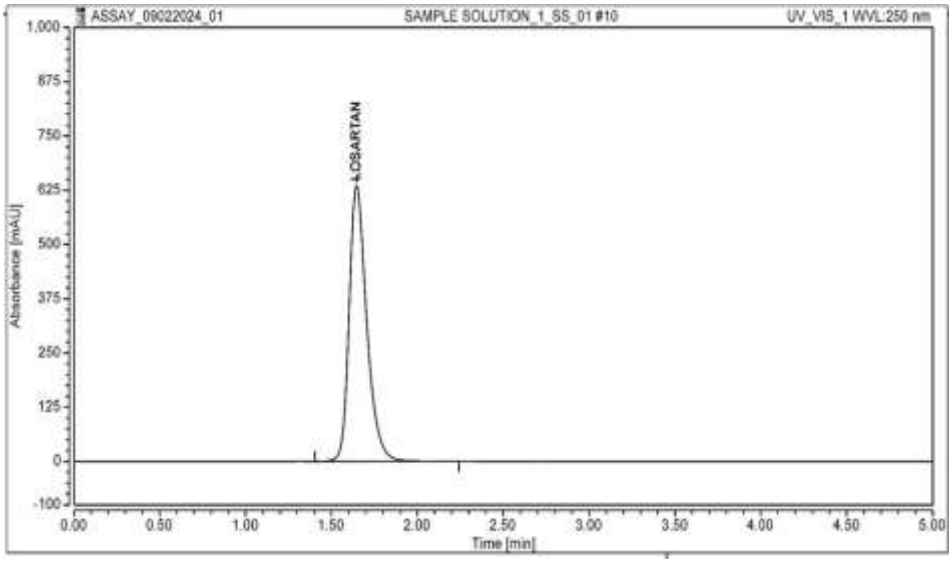

Figure. 5 Representative scans of Test solution

03.02.LINEARITY AND RANGE

Prepared solutions with different concentrations of Losartan potassium range from 123.75 to 296.49 µg/mL. Then, create a graph showing absorbance versus concentration, as seen in Figure.6 was obtained within the limit shown in Table. 4,displayed residuals against concentration. It was found that there was a straight-line relationship between the absorbance and concentration in the range of 50 to 120% (123.75–296.49 ppm for Losartan potassium). Table.5 shows (R2) & %y-axis intercept achieved needed conditions. method proved to be linear within the range of 123.75–296.49 µg/mL for Losartan potassium, meeting the specified limits for correlation coefficient and Y-axis intercept.

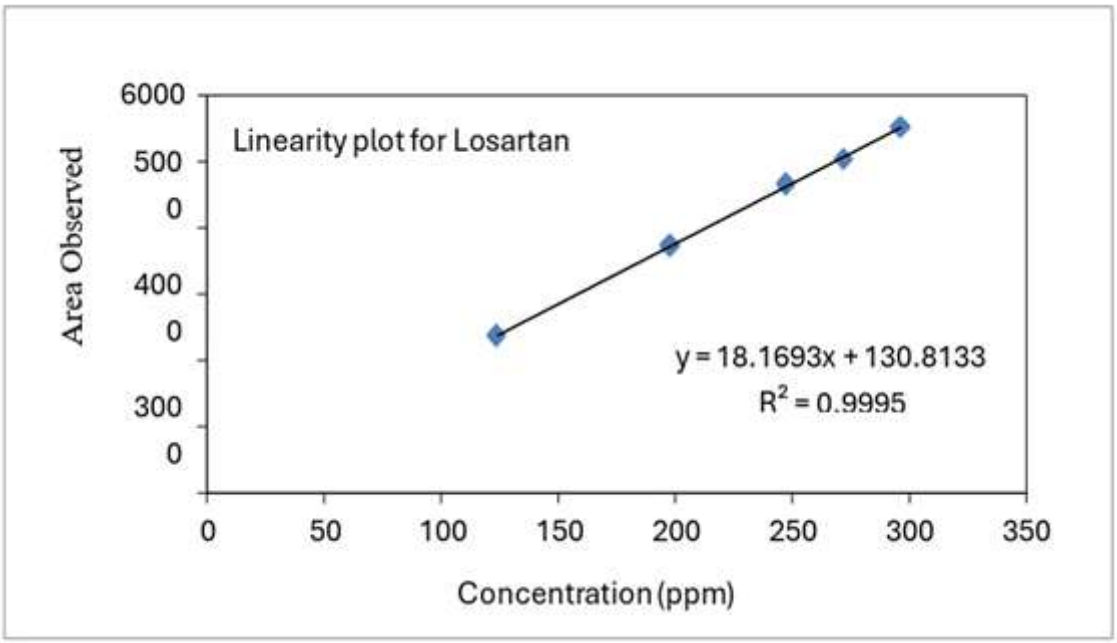

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Table. 4 Linearity concentration level preparation assay of losartan potassium

LosartanPotassium

Sr. No.	LinearityLevels	PercentLevel	Finalconcentration inPPM	PeakArea
1.	I	50%	123.75	2367.63
2.	II	80%	197.87	3738.89
3.	III	100%	247.41	4658.33
4.	IV	110%	271.95	5037.09
5.	V	120%	296.49	5518.92
	CorrelationCoefficient(R^2)			0.999

Table. 5 Losartan Potassium Linearity Observation Table

Parameter	**Values** **LosartanPotassium**	**Acceptancelimit**
Correlation coefficient(R^2)	0.999	>0.999
%Y–axis intercept	2.83	≤ ± 3 %
Theslopeofthe regression line	18.2	To be reported
Residualsumof squares	1785.3	To be reported

03.03.ACCURACY

The percent recovery of losartan potassium shown in Table. 6. The approach proved accurate because the individual recovery was between 97 and 103 percent, and the mean recovery was between 98 and 102%.

03.04.Method Precision (Repeatability) & Intermediate Precision

Method precision accurate, as evaluated by looking at how consistent the results were from six tests. They all fell within the accepted range of being close to each other, which is less than or equal to 2%. You can see the details in Table.7. We also checked how consistent the method was over different days by doing the same test twice on separate days. This tells us about the intermediate precision of the method. For details about how well the method measured the percentage of losartan potassium, you can check Table. 8. And to see how consistent the results were across different days, look at Table .9.

03.05.ROBUSTNESS

The robustness study was confirmed by changing the column temperature by ±2, and the results were within the limits. Refer Table.10.

Table .6 Recoveries of Losartan potassium at different concentration levels

Level	**%RecoveryofLosartan Potassium**	**AreaResponse**	**Acceptance Criteria**
	98.8	2350.557	
50%	100.1	2380.141	

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	99.7	2372.218	
	97.8	3719.362	
80%	98.8	3759.547	
	98.3	3737.767	
	97.4	4636.863	Individual Recovery= 97 %-103 %
100%	98.1	4666.702	
	98.2	4671.442	
	96.4	5040.984	
110%	96.4	5041.44	
	96.2	5028.865	
	97.1	5535.947	
120%	96.7	5511.159	
	96.7	5509.658	
Mean	**98%**		Mean Recovery 98 %– 102 %

Table . 7 Repeatability of Losartan potassium

SampleNo.	**Area Response**	**% Assay of LosartanPotassium**
Sample01	4541.37	98.9
Sample02	4473.977	97.4
Sample03	4473.487	97.5
Sample04	4450.548	97.0
Sample05	4443.638	96.8
Sample06	4560.149	99.4
Mean		**97.9**
Standard Deviation **(STD Deviation.)**		**1.036**
%RSD(relativestandard deviation)		**1.06**

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Table. 8 Intermediate Precision of Losartan potassium

SampleNo.	Area Response	% Assay of LosartanPotassium
Sample01	4643.735	100.2
Sample02	4567.734	98.6
Sample03	4640.645	100.2
Sample04	4574.507	98.9
Sample05	4664.552	100.7
Sample06	4565.787	98.6
Mean		**99.6**
STD Dev.		**0.9579**
%RSD(relativestandard deviation)		**0.96**

Table A. 9 Difference between two repeatability experiments for assay of Losartan Potassium

Parameter	LosartanPotassium 1st day Repeatability	2nd day Repeatability
Numberof determinations	6sampledeterminations	6sampledeterminations
Mean% Assay.	97.9	99.6
RSD (%)	1.06	0.96
Meanvaluedifference(%) limit: 2.0%	<**1.7**	

Table. 10 Robustness Observations

Nameof Drug	Method precision	Changeincolumn oven temperature	
		27ºC	**23 ºC**
% Assay			
LosartanPotassium	97.9	99.8	99.0

03.06.RUGGEDNESS

The ruggedness test was carried out by various analysts on separate days. The results were within the limit, refer Table .11.

03.06.FILTER STUDY

Inject three types of solutions into chromatographic system such as test solution unfiltered but centrifuged, Test solution filter through different makes of filters, Monitor the discard volume for 1 mL to 3 mL or more. Refer Table.12 and Table.1

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Table.11Observationtablefor ruggedness

Drug	Analysts-1 (Chromatogram Area)	Analyst-2 (Chromatogram Area)	%Assay Analyst1	Analyst2
Losartan potassium	4490.46	4609.49	97.9	99.6

Table.12FilterCompatibility

Filterused	Arearesponse	%Release Report	Comparison with Centrifugedsolution
Centrifuged	4508.467	97.6	NA
0.45µ Nylon Filter	4656.196	100.8	3.2
0.45µPVDF Filter	4545.710	98.37	0.8
0.45µPTFE Filter	4552.988	98.5	0.9
	Average:	**98.8**	
	% RSD:	**1.39**	

Filterused	Arearesponse	%Release Report	Comparison with Centrifugedsolution
Centrifuged	4508.467	97.6	NA
0.45µ Nylon 1 mL	4545.457	98.4	0.8
0.45µ Nylon 2 mL	4466.26	96.7	0.9
0.45µ Nylon 3 mL	4462.211	96.6	1.0
	Average:	**97.3**	
	% RSD	**0.87**	

Table.13FilterSaturation

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Table. 14Solutionstability ofTest solution

Component	Time (Hrs:Min)	Area Response	% Release	% Difference w.r.t. Initial
Losartan Potassium	Initial	4541.370	98.7	0.00
	1.40	4541.702	98.7	0.00
	3.46	4567.894	99.3	0.61
	10.48	4617.925	100.4	1.72

Table.15SolutionStability ofReferencesolution

Component	Time (Hrs:Min)	Area Response	% Release	% Difference w.r.t. Initial
Losartan Potassium	Initial	4818.279	100.00	0.00
	2:34	4861.804	100.90	0.90
	3:20	4856.451	100.79	0.79
	3:54	4895.334	101.60	1.60
	4:40	4852.318	100.71	0.71
	5:27	4873.048	101.14	1.14
	6:00	4865.763	100.99	0.99
	13:02	4899.701	101.69	1.69

IV. CONCLUSION

The developed RP-HPLC method demonstrated simplicity, precision, specificity, and accuracy in analyzing losartan potassium in tablet dosage form. Through statistical analysis, we confirmed its reproducibility and selectivity, indicating its reliability for routine analysis. The validation of our procedures, conducted according to ICH and USP norms, further affirmed the method's robustness.

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