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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD A FOR ESTIMATION OF LOSARTAN POTASSIUM UNDER STRESS CONDITION AND TABLET DOSAGE FORM

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Key words:
Losartan potassium, RP-HPLC, experimental design, Tablet, ICH guideline.

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ABSTRACT: Losartan potassium, a highly effective blood pressure lowering agent, has widely used for treatment of hypertension. Simple, economic, selective, and precise and stability indicating HPLC method has been developed and validated for analysis of losartan potassium in bulk drug and formulation dosage form. In order to optimize more one response at time, the chemo metric approach which includes 2^3 factorial design at two level with three factors was set up to standardize the chromatographic condition. The mobile phase was 40:60 ACN: Buffer consist of 0.05% of orthophosphoric acid and 0.05% Triethylamine at flow rate 1.0ml/minute the eluent was monitored at 225nm. The calibration plots constructed using the concentration of 6.4 to 9.6µg/ml (80 -120%) with r^2 =0.999, recovery =99.44% was indicative of accuracy of proposed method. The precision was calculated as repeatability, intraday (RSD) for the drug.

INTRODUCTION: Losartan potassium is chemically: 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-methanol monopotassium salt. Losartan is a phenyl tetrazole substituted imidazole compound which acts as a selective, angiotensin II receptor type I antagonist and is employed in the management of essential hypertension [1]. Method validation study include system suitability, linearity, precision, accuracy, specificity, robustness, limit of detection, limit of quantification and stability of sample, reagents, instruments.

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The aim of this investigation was to establishing a new simple and sensitive method that could be used in analysis degradation products of Losartan potassium.

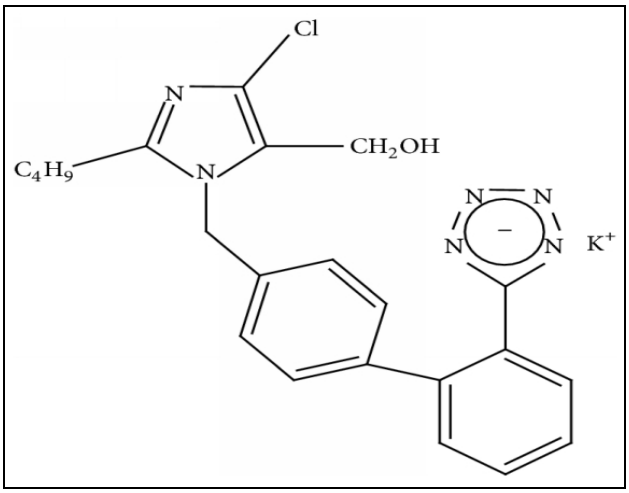


FIG.1: LOSARTAN POTASSIUM

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, capillary electrophoresis (CE), capillary electro chromatography (CEC and spectrophotometry. The literature reports many analytical methods for the quantitation of Losartan in tablets using HPLC.

The USP describes an RP-HPLC method for the determination of LOP in tablets

MATREIAL AND METHODS:

All chemicals and reagents used in the present study were Anal R grade and solvents were of HPLC. To attain high accuracy and reliability of results of the research work. The commercial tablets of losartan potassium are procured from local market, the details of procured materials are shown in **Table 1** and the details of instruments used are shown in **Table 2**.

TABLE1: DRUG AND CHEMICAL PROCURED AND USED FOR THE PRESENT STUDY

S.no	Material	Procured from
1	Losartan potassium working standard 99.86%	HUNHN Pharmaceutical CO.Ltd China
2	Acetonitrile HPLC grade	Merck pharmaceutical India
3	Methanol HPLC grade	Merck pharmaceutical India
4	Water HPLC grade	Merck pharmaceutical India
5	O-phosphoric acid	S.D fine Chem ltd India
6	Triethylamine HPLC grade	S.D fine Chem ltd India

TABLE 2: INSTRUMENT USED FOR PRESENT STUDY

S.no	Name of instrument	Model	Manufacturer
1	Digital balance sartorius	CP2245	Germany
2	Ultrasonic bath sonicator	M0109	INDIA
3	pH meter	MI180	U.K
4	Spectrophotometer	UV-1800	JAPAN
5	HPLC SYKAM	S3210	Germany
6	Stability Chamber		INDIA
7	Water bath	Type1083	Germany

Preparation of Standard:

Preparation of standard losartan potassium solution 109.26 mg of losartan potassium working standard 99.86% weighed and dissolve in dry clean 100ml volumetric flask, add 50 ml of methanol sounicate for 15 min in ultrasonic the volume completed to the mark to final obtained 1mg/ml stock solution. Series of five standard solution in the separate concentration range of 6.4 to 9.6 µg/ml were prepared by dilution with diluent.

Preparation of diluent: 9:1 of Acetonitrile and water.

Preparation of Sample:

20 tablets of losartan potassium was poured in a mortar and thoroughly ground into fine powder. Out of this tablet powder equivalent to 100mg of losartan potassium was taken and dissolved in 100ml volumetric flask 50 ml of methanol added, sonicate for 15min complete the volume to the mark , filtrate through 0.45µm filter and the same solution was further diluted stepwise with diluent to have a concentration 8µg/ml.

Preparation of solutions for degradation studies

Acid and base degradation:

Accurately weight 54.63mg of losartan potassium and transferred into 100 volumetric flask, to it 10ml of methanol was added and sonicateted for 15 minutes with intermittent shaking. To it 5 ml of 0.1M of HCl was added and 5ml of 0.1M NaOH were added separately. The sample was heated on boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent the sample was neutralized to pH 7 by adding 0.1M HCl or 0.1 M NaOH, mixed well. The acidic degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradation effect of light. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to volume with diluent(9:1) acetonitrile

and water, mixed well and injected into the HPLC system.

Oxidation degradation:

Accurately weight 54.63mg of losartan potassium and transferred into 100 volumetric flask, to it 10ml of methanol was added and sonicateted for 15 minutes with intermittent shaking. To it 5 ml of 3% H_2O_2 was added. The sample was heated on boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent, mixed well. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to volume with diluent (9:1) acetonitrile and water, mixed well and injected into the HPLC system.

Thermal degradation:

Accurately weight 54.63mg of losartan potassium and transferred into 100 volumetric flask, to it 10ml of methanol was added and sonicateted for 15 minutes with intermittent shaking .The sample was heated on boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent, mixed well. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to volume with diluent(9:1) acetonitrile and water, mixed well and injected into the HPLC system.

Photolytic degradation:

Accurately weight 54.63mg of losartan potassium and transferred into 100 volumetric flask, to it 10ml of methanol was added and sonicateted for 15 minutes with intermittent shaking. Kept in UV 254nm (UV radiation for 16 hours), completed to the mark with diluent. This solution was filtered through 0.45um filter, 5ml of the filtrate was transferred to 25 ml volumetric flask, diluted to volume with diluent (9:1) acetonitrile and water, mixed well and injected into the HPLC system.

RESULTS:

Selection of detection wavelength:

To a chief goal of this study was to develop a rapid RP-HPLC method for the determination of losartan potassium in bulk drug and tablet formulation by utilizing most commonly column C_{18} with Ultra-violet detection at wavelength 225nm. By using UV spectrophotometer the losartan potassium was scanned in the region of 200-400 nm in spectrum mode, the outcome absorption 225nm **Fig.1**

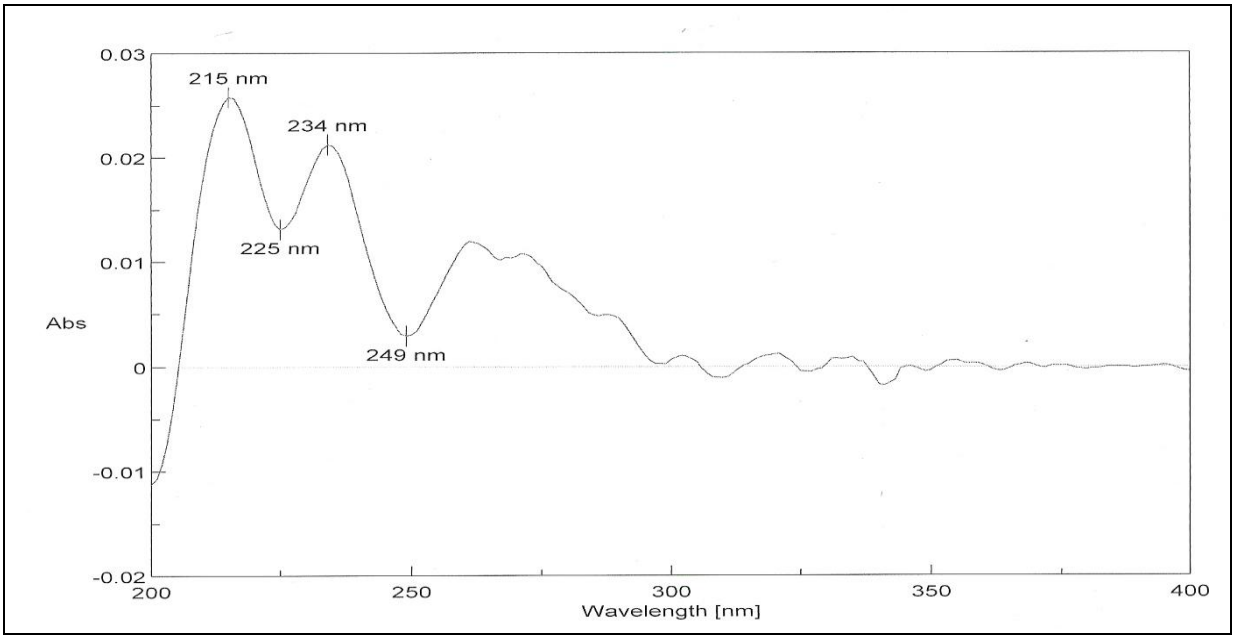


FIG.2: FIRST DERIVATIVE SPECTRUM OF LOSARTAN POTASSIUM.ID 225NM

Optimization and method development:

The main intention of method development is that all required chromatographic conditions are inevitably optimized.

The traditional approach to HPLC optimization is to perform an experiment by trial and error or by change one control variable at time; such method can frequently require a very large number of

experiments to identify the optimal condition. Recently computer assessed to HPLC separation has addressed the problem using factorial design strategies.

In this work a three factors with two level was applied to predict the retention behavior of Losartan potassium and optimize their isocratic elution using acetonitrile as organic modifier and buffer as mobile phase. The goal of this investigation was to establishing a new simple and sensitive method that could be used in analysis degradation products of Losartan potassium.

TABLE 3: MATRIX FOR THREE FACTORS (NUMBER OF RUNS =8)

Experiment	Factor X1	Factor X2	Factor X3
Run1	-1	-1	-1
Run2	1	-1	-1
Run3	-1	1	-1
Run4	1	1	-1
Run5	-1	-1	1
Run6	1	-1	1
Run7	-1	1	1
Run8	1	1	1

TABLE 4: CHROMATOGRAPHIC CONDITION EMPLOYED AS PER 2^3 FACTORIAL DESIGN

Run	Factors			Response			Peak. area
	X1	X2	X3	t_R	Total plate	asym5%	
Run1	40	35	1.0	4.40	4767	1.38	563.4
Run 2	60	35	1.0	3.316	4477	1.5	558.35
Run 3	40	40	10	9.45	5497	1.24	542.16
Run 4	60	40	1.0	9.533	5594	1.19	540.06
Run 5	40	35	1.4	6.816	4728	1.23	381.56
Run 6	60	35	1.4	2.383	2312	1.40	416.18
Run 7	40	40	1.4	3.10	2995	1.14	393.75
Run 8	60	40	1.4	6.766	5403	1.33	387.96

X2 and X3 factors are represent, Acetonitrile, Buffer and low rate (-) and (+) represent the low and high of acetonitrile, buffer and flow rate.

Standard regression curve analysis was performed by used IPM SPSS software version 22and minitab17 and prism 6.07.

The model summary indicate that the model was fit, R^2 =0.9961 and standard error of estimation was 0.1776%., this for dependent variable: retention time.

The obtained adjusted R^2 were within acceptable limit of $R^2 \geq 0.80$, indicating that the experimental data were a good fit to the equation:

$$Y = ß_0+\beta_1 X_1 +\beta_2X_2+\beta_3X_3+\beta_4X_1X_2 +\beta_5X_2X_3+ \beta_6X_1X_3+\beta_7X_1X_2X_3$$

Where Y is the level of the measured response, $ß_0$ is the intercept, $ß_1$ to $ß_7$ are the regression coefficients between,X1, X2 and X3 stand for the main effect, X1X2, X2X3 and X1X3 are the two – way interaction between the main effect and X1X2X3 is the three way interaction between the main effect [2, 3].

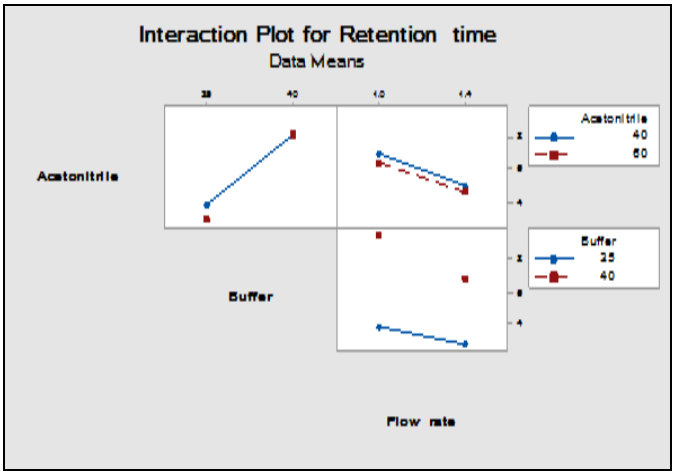


FIG.3: INTERACTION PLOT SHOWING THE INFLUENCE OF RETENTION TIME

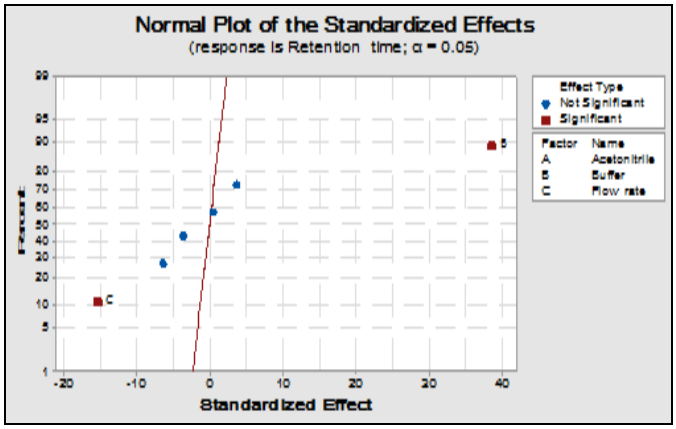


FIG. 4: STANDARDIZED EFFECT SHOWING THE INFLUENCE OF RETENTION TIME

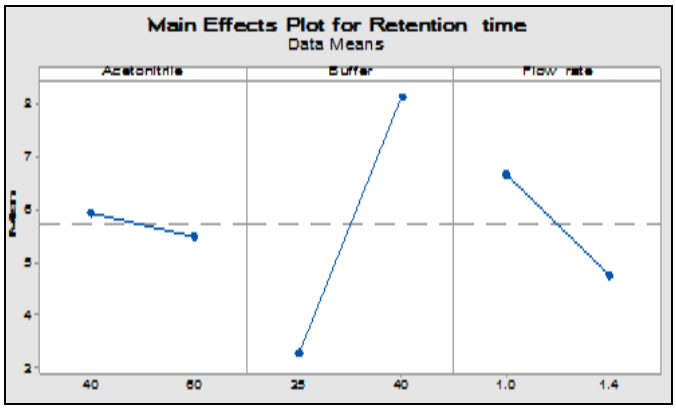


FIG.5: MAIN EFFECT SHOWING THE INFLUENCE OF RETENTION TIME

TABLE 5: OPTIMIZED CHROMATOGRAPHIC FOR THE PROPOSED METHOD

Parameter	Condition used for analysis
Mobile phase	Acetonitrile,0.05% orthophosphoric acid+0.05% triethylamine (40:60 v/v)
Flow rate	1.0 ml/min
Wavelength	225 nm
Injection volume	20µl loop
Column	C_{18} (250x4.6 mm,5µmm)
Column temperature	Ambient
Run time	20min
Retention time	10 min

After close observation of the concerned parameters based on the detailed results obtained and discussions of this part the following procedures were recommended for deciding losartan potassium in bulk samples as pharmaceutical formulation.

For bulk samples:

To get a stable base line the HPLC system was stabilized for 40 minutes subject to the chromatographic conditions describe in **Table 2**. One blank followed by 6 replicates of a single standard solution 8µg/ml was injected to check system suitability.

For pharmaceutical formulation:

Twenty tablets of losartan potassium 50mg was put into mortar and crouched to smooth powder. From this grounded tablet powder; equivalent to 50mg of losartan potassium was accurately and diluted with methanol sonicated for 15 min then the Solution was filtered through 0.45 µm membrane filter to have a concentration 8µg/ml,. The results of assay in respect of the proposed method are mentioned in Tanle15.

Method validation: [4].

According to guideline of ICH Q2 (R1) all parameters as discussed below were analyzed and validated accurately following the procedure of proposed method.

System suitability:

During analytical method development system suitability test give an added level of confidence that the accurate mobile phase, flow rate, temperature, and column were used which ensures the system performance (pump and detector) where in parameters of system suitability such as retention time, resolution, efficiency (number of theoretical plate) and tailing factor are involved and they should be within the defined limits. The results of system suitability in respect to the proposed method are mentioned in **Table 6.**

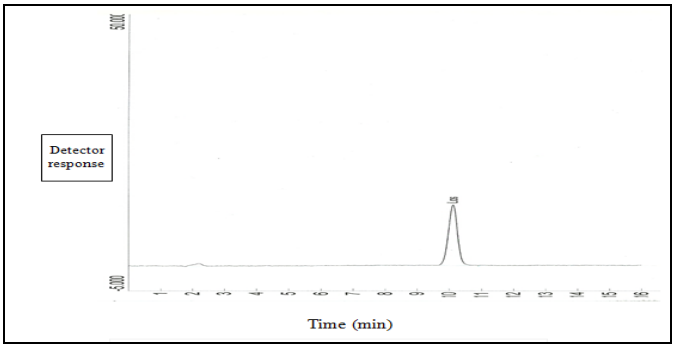
TABLE 6: SYSTEM SUITABILITY RESULTS FOR THE PROPOSED METHOD

S.no	Parameters	Results*
1	Retention time	9.98 minutes
2	Theoretical plate	3837
3	Theoretical plate per meter(t.p/m)	0.065
4	Asymmetry 5%	1.1
5	Resolution	-

* Results for triplicate value (n =3)

Specificity:

The excipients in tablets contained the following in active ingredients: Microcrystalline cellulose, lactose, maize starch and magnesium stearate as excipients, chromatograms showed that no excipients interfered with losartan potassium peak **Fig.8**.



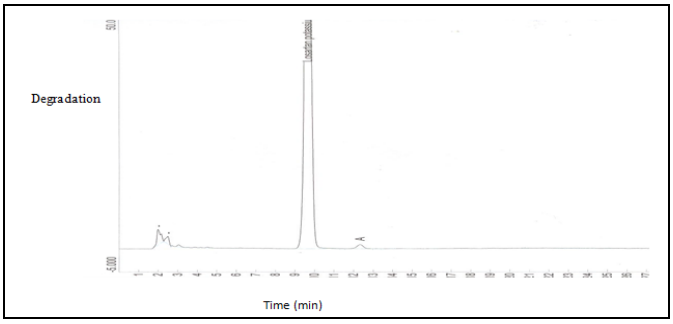


FIG. 7: ACID DEGRADATION OF LOSARTAN POTASSIUM

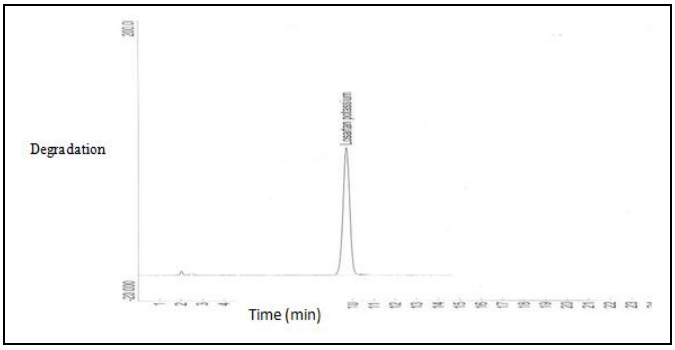


FIG.8: HPLC CHROMATOGRAM OF LOSARTAN POTASSIUM WORKING STANDARD

Stress testing of losartan potassium:

Under acidic condition losartan potassium was degradated up to 0.7 %.Under alkali stress losartan potassium was degradated up to 1.63 %. Only small percent of degradation occurred in UV radiation and oxidation, degradated up to 1.87 under thermal condition.

TABLE 7: PERCENTAGE OF DEGRADATION

S.no	Condition	% Assay of Losartan potassium	% degradation
1	No stress treatment(control sample)	99.88	Nil
2	Acid	99.17	0.70
3	Alkali	98.25	1.63
4	H_2O_2	99.31	0.57
5	UV	99.84	0.37
6	Thermal	98.01	1.87

The linearity for HPLC method was determined at five concentration levels ranging from 6.4-9.6 µg/mL for Losartan potassium the calibration curve was constructed by plotting response factor against respective concentration of Losartan potassium. The method of least square analysis was performed to obtain slope, intercept and correlation coefficient value. Figure. Shows the calibration curve of losartan potassium.

TABLE 8: LINEARITY DATA OF LOSARTAN POTASSIUM

Level method%	Peak area at 225nm	Actual %	RSD %
80	399.6630	79.30	
80	398.956	79.16	0.28
80	401.1640	79.60	
90	463.0825	91.89	
90	459.1520	91.11	0.74
90	456.3130	90.54	
100	504.380	100.07	
100	503.350	99.88	0.10
100	504.140	100.04	
110	556.816	110.48	
110	548.022	109.00	0.68
110	552.9085	110.00	
120	600.7550	119.2	
120	597.909	119.00	0.54
120	594.933	118.00	
			Limit NMT 2%

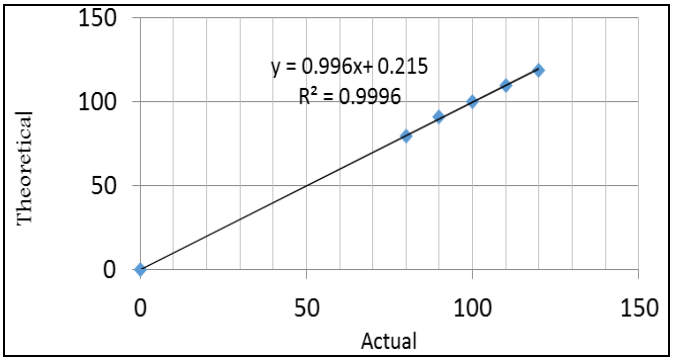


FIG.9: LINEARITY CURVE OF LOSARTAN POTASSIUM

TABLE 9: REGRESSION ANALYSIS DATA OF LOSARTAN POTASSIUM

Parameters	Results
Linear range	6.4-9.6µg/ml
Regression equation(y=a+bx)	y = 0.996x + 0.215
Correlation coefficient(r^2)	0.9996
Slope (b)	0.996
Intercept (a)	0.215
Standard deviation of slope(s_b)	0.009
Standard deviation of intercept(s_a)	0.845
Standard error of estimation	0.88509

The precision (system method) of the proposed method was evaluated by carrying out six

independent assays of test sample. RSD (%) of six assay value obtained was calculated.

The intermediate precision was carry out by analyzing the sample in different days.

TABLE 10: INTRA-DAY PRECISION RESULT OF LOSARTAN POTASSIUM

Repeatability precision		Injection	Peak area	Assay	% RSD
Sample	Concentration	no	(mV.s)		N=6
Losartan potassium	8µg/ml	1	237.66	100.22	0.44
		2	236.12	99.60	
		3	238.70	100.65	
		4	235.77	99.42	
		5	236.63	99.80	
		6	236.81	99.86	

TABLE 11: INTER-DAY PRECISION RESULT OF LOSARTAN POTASSIUM

Intermediate precision		Injection no	Peak area(mV.s)	Assay	% RSD
Sample	Concentration				N=6
Losartan potassium	8µg/ml	1	238.54	100.62	0.43
		2	236.66	99.83	
		3	237.26	100.10	
		4	236.19	99.63	
		5	235.94	99.52	
		6	235.78	99.46	

Accuracy (recovery studies)

The accuracy of the HPLC method was confirmed by recovery studies by spiking 80,100 and 120% of pure drugs to the pre analysed samples and the samples after dilution injected into the system (n=3). The peak area of each drug was measured and recovery data for losartan potassium given in **Table 12.**

TABLE12: RECOVERY RESULTS OF LOSARTAN POTASSIUM

Amount added µg/ml	Amount found µg/ml	Recovery%	Statistical analysis of % recovery	
6.4	6.34	99.06	MEAN	99.15
6.4	6.33	99.0	SD	0.17
6.4	6.36	99.4	%RSD	0.22
8.0	8..05	100.62	MEAN	100.22
8.0	8.00	100.00	SD	0.28
8.0	8.004	100.05	%RSD	0.34
9.6	9.54	99.37	MEAN	98.96
9.6	9.52	99.17	SD	0.45
9.6	9.44	98.33	%RSD	0.55

Robustness:

Robustness of the method was determined by small deliberate changes in flow rate, and wavelength. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

TABLE 13: CHANGE OF FLOW RATE

Flow rate(ml/min)			Mean area ±SD	SEM	Mean t_R±SD	% Bias	% RSD
Original	Used	Level	N= 3				
1.0	0.95	-0.05	227.27±1.81	1.28	10.12±0.097	0.0173	0.097
	1.0	0	213.57±2.06	1.45	9.794±0.0098	0.00	0.10
	1.05	+0.05	230,84±0.87	0.61	9.777±0.0098	-0.0093	0.1003

TABLE 14: CHANGE OF WAVELENGTH

Wavelength			Mean area ±SD	SEM	Assay %	%Bias	% RSD
Original	used	level	N= 3				
225	220nm	-5	579.42±5.35	3.783	99.94	-0.022	0.923
	225nm	0	521.39±0.690	0.487	100.05	0.008	0.132
	230nm	+5	498.69±2.91	2.057	100.63	0.199	0.585

LOD and LOQ:

LOD and LOQ for losartan potassium were evaluated by injecting a series of solutions and diluted with known concentrations based on the peak response and the slope of the regression equation of the parameters of LOD and LOQ were decided. The LOD of drug noticed as 0.608µg/ml and LOQ was found 1.843µg/ml. By adopting the following formula LOD=3.3(SD)/S and LOQ = 10(SD)/S, where SD = standard deviation of response and S= slope of the calibration curve were computed.

Comparison with the reported UV spectrophotometric method:

The results obtained above were compared with that obtained from the reported HPLC method confirming similar accuracy and precision in the determination of losartan potassium by both methods **Table 8** [5].

TABLE 15: ASSAY RESULTS OF LOSARTAN POTASSIUM

Method	Dosage form	Declared value mg	Found value mg ±SD*	RSD	Recovery
Reference	LOSACAR	50	50.13±0.026	0.064	100.26
	ZYLTAN	50	50.07±0.0.024	0.061	100.14
	COZAL	50	49.99±.101	0.24	99.98
Proposed	LOSACAR	50	50.15±0.104	0.25	100.3
	ZYLTAN	50	49.99±0.106	0.26	99.98
	COZAL	50	50.11±0.024	0.060	100.22

*Average of three determination

DISCUSSION: The proposed method obeys linearity within the concentration range of 6.4 to 9.6µg/ml and coefficient correlation was found to be 0.999.The regression of the curve was Y=0.999x+0.215.The detection and quantization limit as LOD (K=3.3) and LOQ (K=10) were calculated and the these were found to be0.608µg/ml and 1.843µg/ml respectively. The precision measurement of intra-day and inter-day with percent relative standard deviation, 0.44% and 0.43%, which indicated that the method is highly précised. The main reason of study precision is to establish that promoted RP-HPLC is accurate for analyzing losartan potassium in pharmaceutical formulation as well as bulk forms. The percentage recovery value **Table 6**, bout % indicates the accuracy of the method. No excipients present or interference in the analysis market formulation tablets. The result of analysis of dosage form obtained were good agreement with label claim. Losartan potassium was stale drug toward stress testing.

CONCLUSION: The developed HPLC method is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of Losartan potassium in tablet dosage form.

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