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Method Development and Validation of Losartan Potassium by RP-HPLC

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ABSTRACT

The Literature survey indicates several methods for the determination of Losartan potassium. So an attempt was made to develop and validate a simple, precise, accurate, and economical RP-HPLC method as per ICH guidelines for the estimation of Losartan potassium in bulk and pharmaceutical dosage forms. A simple reverse phase HPLC method was developed for the determination of Losartan potassium present in pharmaceutical dosage forms. Hypersil Silica (250 x 4.6 mm, packed with 5µm) in an isocratic mode with mobile phase Acetonitrile: Triethylamine buffer PH3 (50:50) was used. The flow rate was 1.0ml/ min and effluent was monitored at 254 nm. The retention time was 5.1min for Losartan potassium. The linearity ranges were found to be 40-120µg/ml

Keywords: losartan potassium**.**

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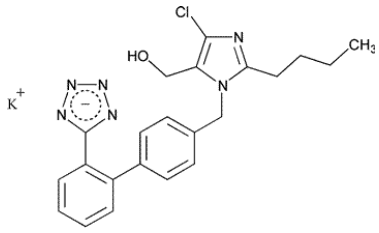
INTRIDUCTION

Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). Losartan was the first angiotensin II receptor antagonist to be marketed.



Losartan potassium is 2-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol monopotassium salt [1].

Losartan is a selective, competitive Angiotensin II receptor type 1 (AT_1) receptor antagonist, reducing the end organ responses to angiotensin II. Losartan administration results in a decrease in total peripheral resistance (after load) and cardiac venous return (preload) All of the physiological effects of angiotensin II, including stimulation of release of aldosterone, are antagonized in the presence of losartan. Reduction in blood pressure occurs independently of the status of the renin-angiotensin system. As a result of losartan dosing, plasma renin activity increases due to removal of the angiotensin II feedback.

Losartan is is estimated by various methods either by HPLC [2-4] or with the combination of HPLC with uv absorption densitometer or with uv spectrophotometric methods as per literature. The method involved in estimation of the losartan potassium uses HPLC Shimadzu Separation ModuleLC-20AT Prominence Liquid Chromatograph and Hypersil silica, C_{18} 250× 4.6 mm. 5µ. as column. In this method UV Visible Detector (SPD 20A Prominence) as an indicator. Detection was carried out using U.V detector set at 270 nm.

In the present work the method devolpment and validation [5] of the losartan potassium is carried out by using the RP-HPLC chromatographic method.

MATERIALS AND METHOD

Chemicals and reagents

Acetonitrile of HPLC grade is purchased from RANKEM, Triethylamine is a Laboratory reagent is brought from RANKEM ,Orthophosphoric acid is a Laboratory reagentof STANDARD REAGENT ,and Water of HPLC grade is used.

RP-HPLC apparatus and conditions

Single pan balance (Metler Toledo), a Control Dynamics pH meter (Metler Toledo),a HPLC Shimadzu Separation ModuleLC-20AT Prominence, a Liquid Chromatograph, a UV Visible Detector (SPD 20A Prominence), a Chromatographic data Software: a Spinchrom,Hypersil silica, a C_{18} 250× 4.6 mm. 5µ. Column, a Vacuum filter pump,(NUPORE filtrartion systems,0.45µ,47mm),a mobile phase reservoir,a Ultrasonicator.

Conditions

Instrument	:	HPLC Shimadzu Separation Module LC-20AT Prominence Liquid Chromatograph
Detector	:	U.V – Visible detector
Column	:	Hypersil silica, C_{18} 250× 4.6mm., 5µ column
Temperature	:	25^{o}C
Flow rate	:	1.0ml/min
Wave length	:	254nm
Runtime	:	7 min
Injection volume	:	20µl
Mobile Phase	:	Triethylamine buffer PH3: Acetonitrile (50:50)
Diluent	:	Mobile phase
Retention time	:	5.17 min

Stock solutions and standardsb [6]

Selection of Mobile phase

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug. The mobile phase containing Methanol: Water (50:50), Methanol: Phosphate buffer pH3 (50:50), Acetonitrile: Triethylamine buffer PH3 (50:50) was tried. Better peak resolution and adequate retention time were obtained with the ratio of Triethylamine Buffer (pH 3) : Acetonitrile (HPLC grade) (50:50).

Preparation of Mobile phase

The mobile phase was prepared by mixing Triethylamine buffer and Acetonitrile (pH adjusted 3.0 with Orthophosphoric acid) in the ratio of 50:50. The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of Triethylamine buffer: Dissolve 4.0 ml of triethylamine in 1000ml water to produce 0.02% buffer. P^{H} is adjusted to 3.0 with 10%v/v Orthophosphoric acid.

Selection of column

Column trails were performed using BDS silica C_{18} 250 x4.6mm, 5µ and C_{18} 150x4.6 mm, 5µ. Better peak resolution with less tailing was observed with Hypersil silica C_{18} 250 x 4.6 mm, 5µ.

Preparation of standard stock solution

Stock solution of Losartan potassium (1 mg/ml) was prepared by dissolving 100 mg of Losartan potassium in 100 ml of volumetric flask containing mobile phase. The solution was sonicated for about 10 min and then made up to volume with mobile phase. Daily working standard solutions of Losartan potassium was prepared by suitable dilution of the stock solution with appropriate mobile phase.

Preparation of sample solution

20 tablets of Losartan potassium were weighed and powdered in glass mortar. The powder equivalent to 25mg of active ingredient present was transferred into a 100 ml volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 20 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent and the solution was filtered through 0.45 µm filter before injecting into the HPLC system.

Validation

1. Linearity

The linearity of the method was demonstrated over the concentration range of 60-140 µg / ml. Aliquots of 60, 80, 100,120, and 140 µg / ml were prepared from stock solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration v/s peak area and was given in the Fig 1. The results were discussed in Table 1

Table (1) Linearity of Losartan potassium by RP-HPLC

Concentraion (mcg)	Area
0	0
40	1767.729
60	2640.044
80	3160.615
100	4156.381
120	4871.855

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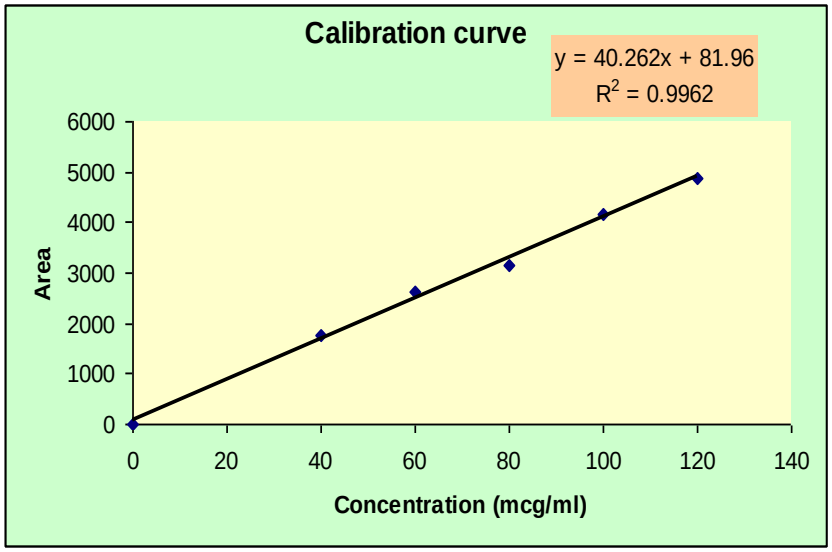

Figure 1: Calibration curve for linearity of Losartan potassium

Table (2)linearity parameters of Losartan potassium by RP-HPLC

intercept	81.6
Correlation coefficient(r^2)	o.9962
Slope	40.262

Acceptance criteria

Correlation Coefficient (r^2) should be not less than 0.9990.

2. Assay

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae. Recovery studies were carried out. The results were discussed in the Table 3.

Calculate the percentage purity of Losartan potassium present in tablet using the formula:

$$\text{Percentage purity} = \frac{\text{Spl}_{area} \times \text{Std}_{dil} \times \text{Avg wt} \times P}{\text{Std}_{area} \times \text{Spl}_{dil} \times \text{L.C} \times 100} \times 100$$

Where,

P = (%) potency of Losartan potassium working standard use
L.C = Label claim
Avg wt = Average weight of tablets

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Table (3): Assay of Losartan potassium by RP-HPLC

Sample id	Concentration	Peak area	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	80%	3183.388	99.58	99.41	0.106	0.120
2	80%	3193.328	99.75			
4	100%	4212.074	99.34	99.66	0.120	0.107
5	100%	4223.709	99.49			
7	120%	5164.659	99.46	99.37	0.127	0.128
8	120%	5169.752	99.28			

3. Accuracy

The accuracy of the method was evaluated by determination of recovery of losartan potassium at three levels of concentrations. The sample solutions were spiked with losaratan potassium standard solutions corresponding to 80, 100, and 120% of nominal analytical concentrations. (80µg/ml, 100µg/ml and 120µg/ml). The results showed good recovery within limits (98% – 102%). The results were discussed in the Table 4.

Table (4): Accuracy of Losartan potassium by RP-HPLC

Sample id	Concentration	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	80%	100.05	100.12	0.066	0.067
2	80%	100.14			
3	80%	100.18			
4	100%	100.15	100.09	0.055	0.055
5	100%	100.04			
6	100%	100.10			
7	120%	100.10	100.08	0.051	0.051
8	120%	100.03			
9	120%	100.13			

Acceptance criteria

The mean % recovery of the Losartan potassium at each level should be not less than 97.0% and not more than 103.0%.

4. Precision

a)Method precision

Five sample of 100 mcg per ml solutions were prepared and injected into the HPLC system as per test procedure. The results were discussed in Table below 5

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Acceptance criteria

% Relative standard deviation of results should not be more than 2.0 %.

Table (5): Method Precision of Losartan potassium by RP-HPLC

Injection number (100 mcg/ml)	Retention time	Area
1	5.177	4164.347
2	5.177	4191.593
3	5.18	4205.948
4	5.18	4206.838
5	5.18	4211.34
Avg	5.1788	4196.013
SD	0.001643	19.19032
%RSD	0.031729	0.457347

b)System precision

Five sample of 100 mcg per ml solutions were prepared and injected into the HPLC system as per test procedure. The results were discussed in Table below 6

Table (6): System precision of Losartan potassium by RP-HPLC

Injection number (100 mcg/ml)	Retention time	Area
1	5.177	4169.069
2	5.177	4164.785
3	5.18	4216.107
4	5.18	4226.08
5	5.18	4214.297
Avg	5.1788	4198.068
SD	0.001643	28.81908
%RSD	0.000317	0.6865

Acceptance criteria

% Relative standard deviation of results should not be more than 2.0 %.

c) Intraday Precision

Intraday precision of test method is demonstrated by 6 injections of the same batch (same conc) of samples at 0, 1, 2, 3, 4, and 5 hrs on same day. The results were discussed in Table 7.

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Table (7): Intraday precision of Losartan potassium by RP-HPLC

Injection number(100 mcg/ml)	Hours	Retention time	Area
1	0	5.177	4152.015
2	1	5.177	4161.469
3	2	5.18	4183.214
4	3	5.185	4201.61
5	4	5.18	4194.349
6	5	5.179	4188.185
Avg		5.179667	4180.14
SD		0.002944	19.37344
%RSD		0.0568	0.4635

Acceptance criteria

%RSD of 6 replicate injections should be not more than 2.0%.

d)Interday Precision

Interday precision of test method is demonstrated by 5 injections of the same batch(same conc) of samples on 5 successive days The results were discussed in Table 8.

Table (8): Interday precision of Losartan potassium by RP-HPLC

Injection number (100 mcg/ml)	Retention time	Area
1	5.177	4166.875
2	5.177	4188.8
3	5.18	4198.728
4	5.183	4204.411
5	5.18	4206.097
Avg	5.1794	4192.982
SD	0.00251	16.08343
%RSD	0.0485	0.3836

Acceptence criteria

%RSD of 6 replicate injections should be not more than 2.0%.

5. System Suitability

A Standard solution of losartan potassium working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Acceptance criteria

1. The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %
2. The number of theoretical plates (N) for the Losartan potassium peaks should be not less than 2000.
3. The Tailing factor (T) for the Losartan potassium peaks should be not more than 2.0.

From the system suitability studies it was observed that all the parameters were within limit.

Hence it was concluded that the Instrument, Reagents and Column were suitable to perform the assay. The results were discussed in Table below 9 .the chromatogram was shown in fig. 6

Table (9): System Suitability of Losartan potassium by RP-HPLC

Parameter	Observed value	Acceptance criteria
USP tailing	1.056	In between 0.5 to 2.0
USP theoretical plates	2730.334	Above 2000

6. Specificity

a) Losartan potassium Identification

Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria

Chromatogram of standard and sample should be identical with near Retention time.

b) Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria

Chromatogram of blank should not show any peak at the retention time of analyte peak.

There is no interference due to blank at the retention time of analyte.

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c) Stress degradation studies by treating with alkali and acid:

Losartan potassium was treated with 0.1M NaOH and 0.1M HCl. Solutions of sample was prepared as per test procedure and injected into the HPLC system.

Acceptance criteria

Chromatogram of degradants should not show any peak at the retention time of analyte peak. There is no interference due to degradants at the retention time of analyte. The chromatograms were shown in fig 7, 8

7. Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition which may differ but the responses were still within the specified limits of the assay.

a) Effect of variation of mobile phase composition

A study was conducted to determine the effect of variation in mobile phase ratio by Changing the ratio of mobile phase i.e. Acetonitrile : Buffer from 50: 50 to 45: 55 and 55: 45. Standard solution was prepared and injected into the HPLC system . The retention time values were measured and are given in Table 10

Table (10): Robustness of Losartan potassium by RP-HPLC

Proposed variations		Asymmetry factor of losartan potassium peak in standard	Acceptance criteria
Variation in mobile phase composition	**45% organic phase**	1.056	**In between 0.5 to 2.0**
	50% organic phase	1.055	
	55% organic phase	1.057	
Variation in buffer pH	**PH 2.5**	1.058	
	PH 3.5	1.057	
	PH 3.0	1.055	
Variation in flow rate	**1.0ml/min**	1.052	
	0.8ml/min	1.057	
	1.2ml/min	1.059	
Variation in temperature	**25˚c**	1.057	
	27˚c	1.055	
	30˚c	1.054	

Acceptance criteria

1. Tailing Factor of Losartan potassium standard should not be more than 2.0 for Variation in composition of mobile phase.
2. The % RSD of Losartan potassium standard should be not more than 2.0 for Variation in composition of mobile phase.

b) Effect of variation in Buffer PH

A study was conducted to determine the effect of variation in Buffer PH. Standard solution was prepared and injected into the HPLC system by maintaining PH 2.5 and 3.5. The effect of variation in Buffer PH was evaluated. The results were discussed in the Table 10

Acceptance criteria

1. The tailing factor of standard should be not more than 2.0 for Variation in flow.
2. The % RSD of Asymmetry and t_R of Losartan potassium standard should be not more than 2.0 % for variation in flow.

c) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates 0.8 ml / min and 1.2 ml / min. The effect of variation of flow rate was evaluated. The results were discussed in the Table 10

Acceptance criteria

1. The tailing factor of standard should be not more than 2.0 for Variation in flow.
2. The % RSD of Asymmetry and t_R of Losartan potassium standard should be not more than 2.0 % for variation in flow.

d) Effect of variation in temperature

A study was conducted to determine the effect of variation in temperature. Standard solution was prepared and injected into the HPLC system by keeping temperature 30˚c, 27˚c and 25˚c. The effect of variation of flow rate was evaluated. The results were discussed in the Table 10

Acceptance criteria

1. The tailing factor of standard should be not more than 2.0 for Variation in flow.
2. The % RSD of Asymmetry and t_R of Losartan potassium standard should be not more than 2.0 % for variation in flow.

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8. Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments. And hence the proposed method was found to be rugged. The Chromatograms were shown in Fig 9, 10.

9. Limit Of Detection (LOD)

The parameter LOD was determined on the basis of response and slope of the regression equation.

10. Limit Of Quantification (LOQ)

The parameter LOQ was determined on the basis of response and slope of the regression equation.

RESULTS AND DISCUSSIONS

Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). A simple reverse phase HPLC method was developed for the determination of Losartan potassium. Hypersil Silica (250 x 4.6 mm, packed with 5µm) in an isocratic mode with mobile phase Acetonitrile:Triethylamine buffer PH3 (50:50) was used. The flow rate was 1.0ml/ min and effluent was monitored at 254 nm. The retention time was 5.1min for Losartan potassium.

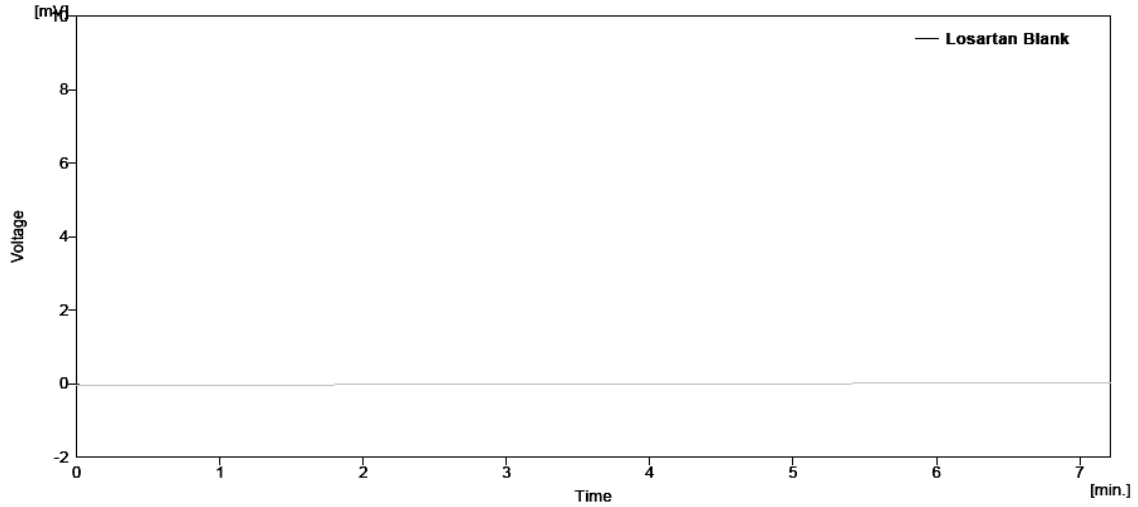

Figure 2: chromatogram of blank

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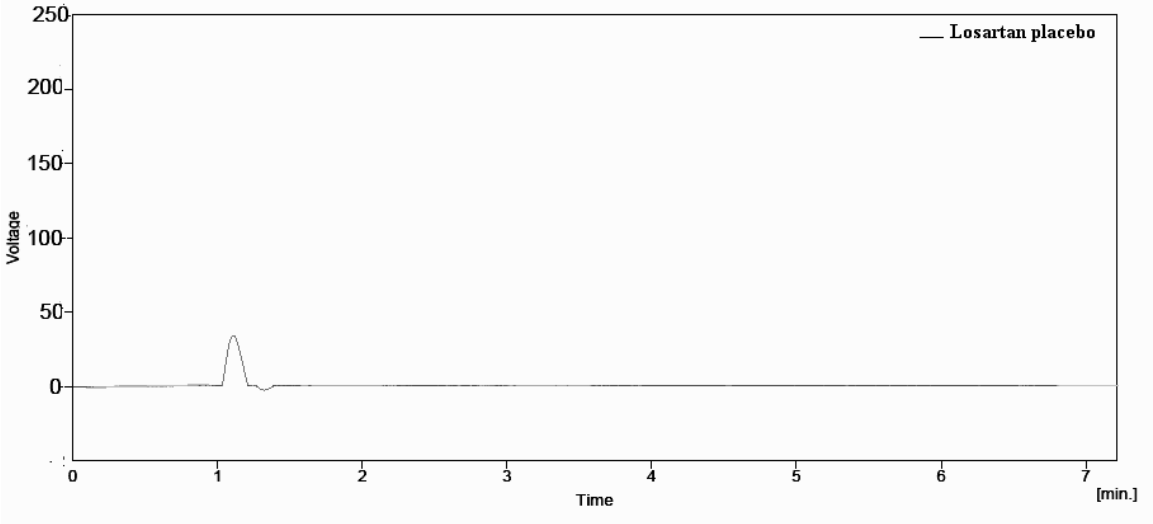

Figure 3: chromatogram of Placebo

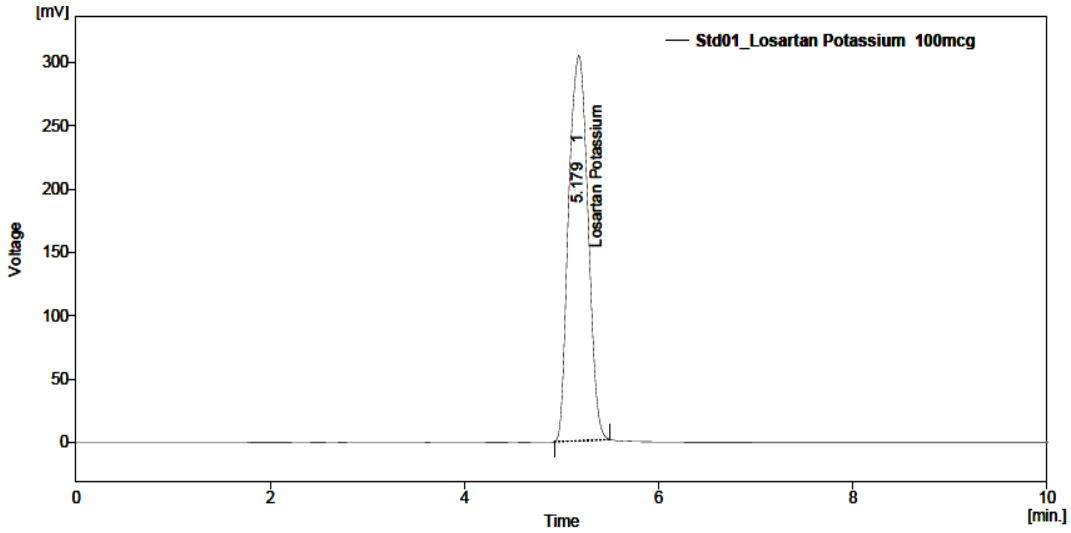

Figure 4: chromatogram of standard

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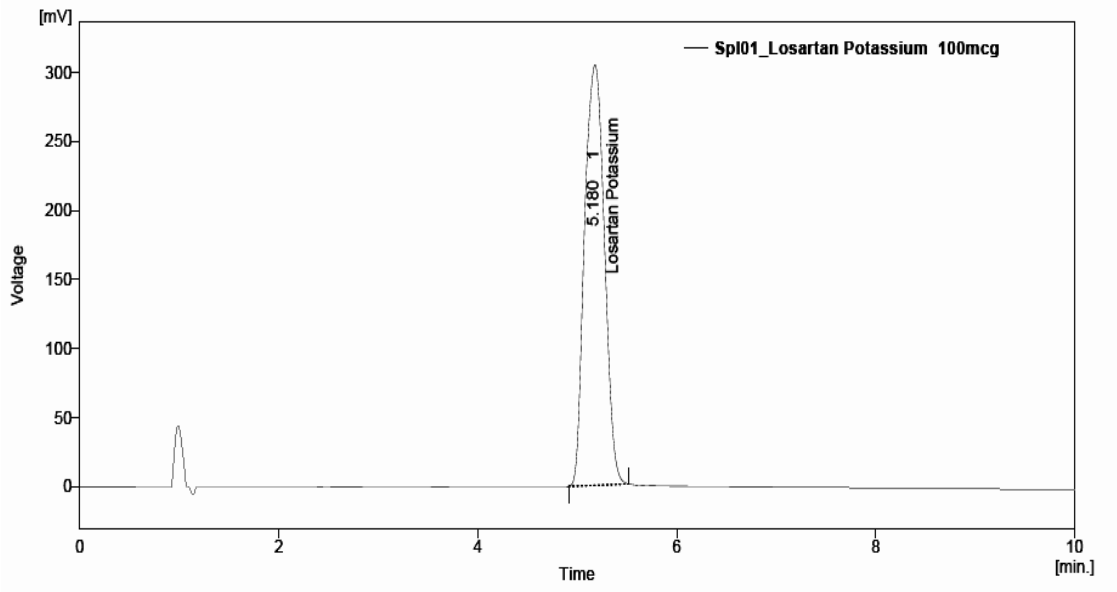
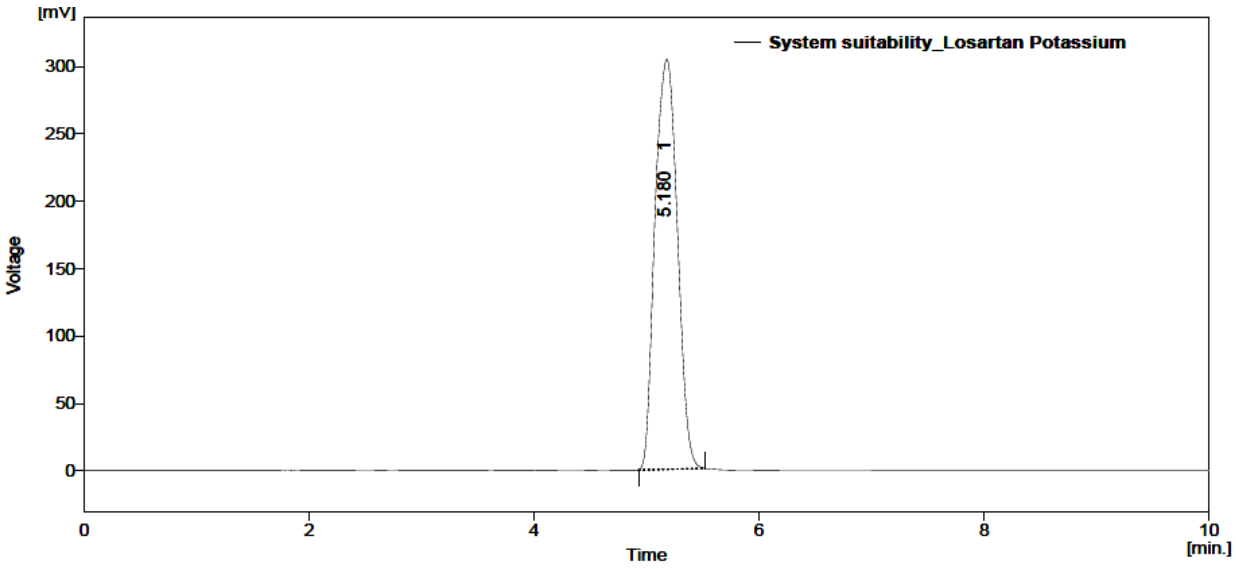


Figure 5: cromatogram of sample



Result Table (Uncal - System suitability_Losartan Potassium)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.180	4206.838	304.515	100.000
	Total	4206.838	304.515	100.000

Column Performance Table (From 50% - System suitability_Losartan Potassium)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Resolution [-]
1	5.180	0.233	1.056	0.00	2730.334	-

Figure 6: chromatogram indicating system suitability

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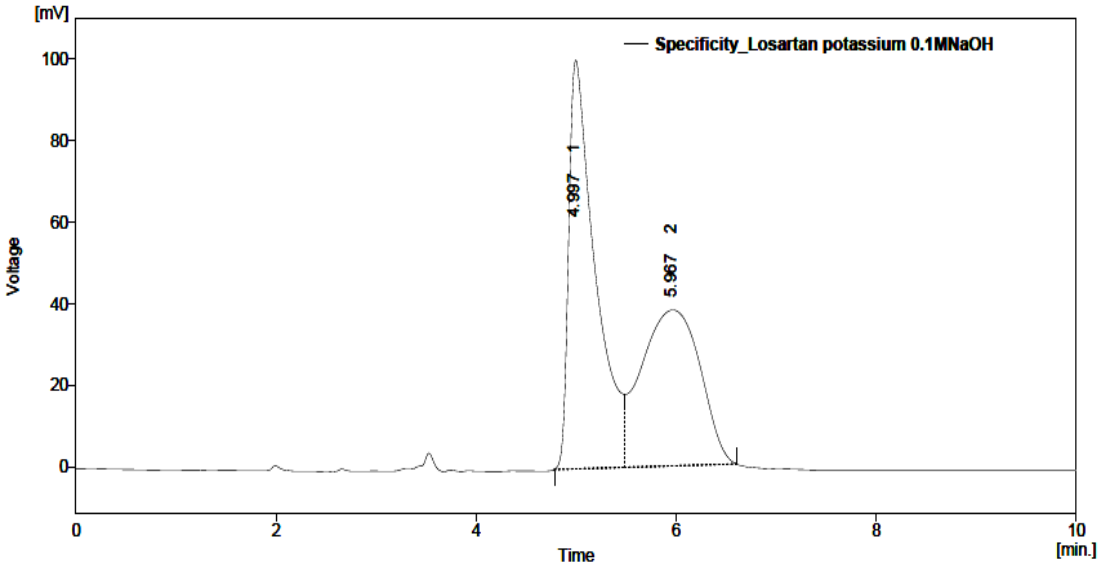

Figure 7:

Figure 7: chromatogram for stress degradation by alkali

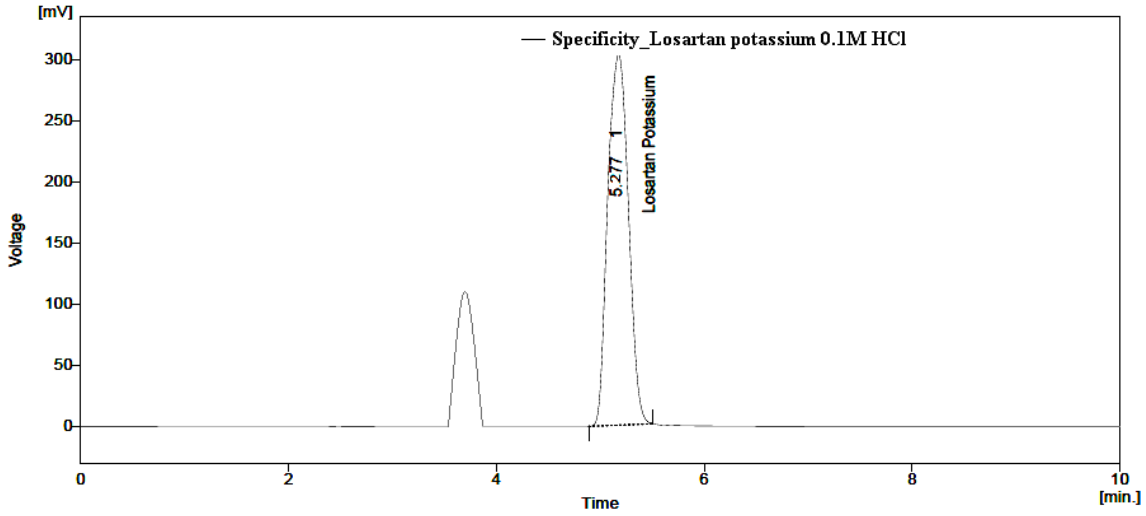

Figure 8: chromatogram for stress degradation by acid

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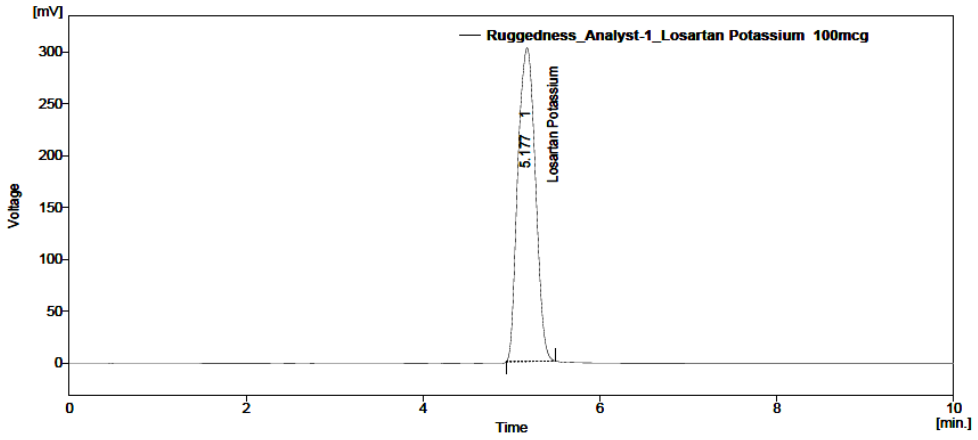


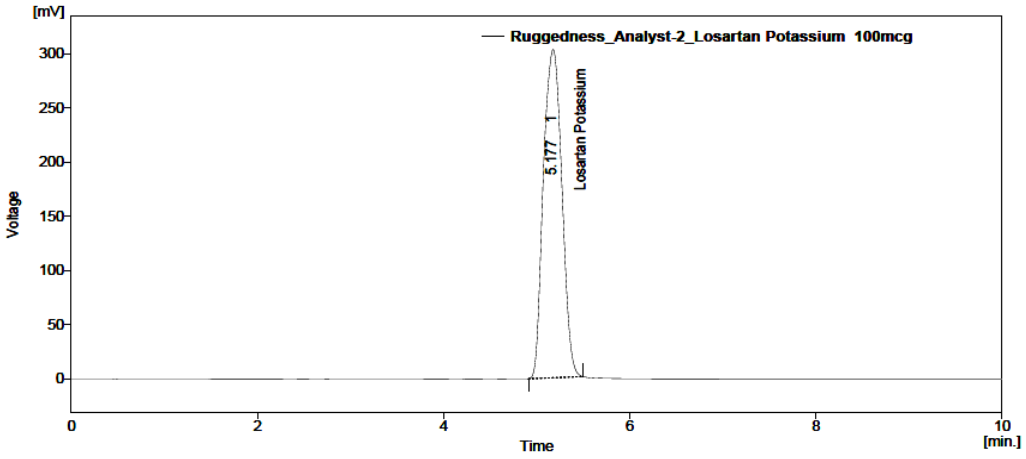
Result Table (Uncal - Ruggedness_Analyst-1_Losartan Potassium 100mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.177	4162.926	302.483	100.000
	Total	4162.926	302.483	100.000

Column Performance Table (From 50% - Ruggedness_Analyst-1_Losartan Potassium 100mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Resolution [-]
1	5.177	0.233	1.075	0.00	2726.821	-

Figure 9: cromatograph indicating ruggedness (analyst 1)



Result Table (Uncal - Ruggedness_Analyst-2_Losartan Potassium 100mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.177	4185.663	303.251	100.000
	Total	4185.663	303.251	100.000

Column Performance Table (From 50% - Ruggedness_Analyst-2_Losartan Potassium 100mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Resolution [-]
1	5.177	0.233	1.056	0.00	2726.821	-

Figure 10: chromatogram indicating ruggedness(analyst 2)

From the linearity Table 1, it was found that the drug obeys linearity within the concentration range of 40-120μg/ml for Losartan potassium. From the results shown in accuracy Table 4, it was found that the percentage recovery values of pure drug were in between 100.05 to 100.13, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in precision Tables 5,6,7,8, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

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