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METHOD DEVELOPMENT AND VALIDATION OF LOSARTAN BY USING RP-HPLC IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

This review describes a strategy for systematically developing High-performance liquid chromatographic (HPLC) methods. HPLC is an analytical tool that is able to detect, separate and quantify the drug, its various impurities, and drug-related degradants that can form on synthesis or storage. A number of chromatographic parameters were evaluated in order to optimize the technique. An appropriate mobile phase, column, column temperature, wavelength, and gradient must be found that affords suitable compatibility and stability of the drug as well as degradants and impurities. Previously, the reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Losartan potassium. Analysis was performed on Losartan-25 and Losartan-50 C18 column (150 mm × 4.6 mm, 5 µm) with the mobile phase consisting of Methanol—Acetonitrile (50:50, v/v) at a flow rate of 1.0 mL/min. UV detection was performed at 208nm and the retention time for Losartan was about 3.0 minutes. The calibration curve was linear (correlation coefficient = 0.9991) in the selected range of analyte. The system suitability parameters, such as theoretical plate, tailing factor, and relative standard deviation (RSD) between five standard replicates, were well within limits.

Keywords: Losartan-25, Losartan-50, C-18 column and RP-HPLC.

INTRODUCTION

Drug Name: Losartan[7,8]
Brand Name : Cozaar,Hyzaar
Molecular Formula : C22H23CIN6O
Molecular Weight : 422.911
Boiling Point : 682°C.

Description

Losartan is used alone or together with other medicines to treat high bloodpressure (hypertension). High blood pressure adds to the workload of the heart and arteries. If it continues for a long time, the heart and arteries may not function properly. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. Lowering blood pressure may reduce the risk of strokes and heart attacks. Losartan is an angiotensin II receptor blocker (ARB). It works by blocking a substance in the body that causes blood vessels to tighten. As a result, losartan relaxes the blood vessels. Lower blood pressure will increase the supply of blood and oxygen to the heart. Losartan is also used to decrease the risk of stroke in patients with high blood pressure and heart enlargement. It is also used to treat kidney problems in patients with type 2 diabetes and a history of hypertension.

Mechanism of Action
Losartan reversibly and competitively prevents angiotensin II binding to the AT1 receptor in tissues like vascular smooth muscle and the adrenal gland. Losartan and its active metabolite bind to the AT1 receptor with 1000 times more affinity than they bind to the AT2 receptor. The active metabolite of losartan is 10-40 times more potent by weight than unmetabolized losartan as an inhibitor of AT1 and is a non-competitive inhibitor. Losartan's prevention of angiotensin II binding causes vascular smooth muscle relaxation, lowering blood pressure. Angiotensin II would otherwise bind to the AT1 receptor and induce vasoconstriction, raising blood pressure.

Absorption
Losartan is approximately 33% orally bioavailable. Losartan has a Tmax of 1hour and the active metabolite has a Tmax of 3-4 hours. Taking losartan with food decreases the Cmax but only results in a 10% decrease in the AUC of losartan and its active metabolite.A 50-80 mg oral dose of losartan leads to a Cmax of 200-250 ng/mL.

Volume of Distribution
The volume of distribution of losartan is 34.4±17.9L and 10.3±1.1L for theactive metabolite (E-3174).

Protein Binding
Losartan is 98.6-98.8% protein bound and the active metabolite (E-3174) is99.7% protein bound in serum.

Metabolism
Losartan is metabolized to an aldehyde intermediate, E-3179, which is further metabolized to a carboxylic acid, E-3174, by cytochrome P450s like CYP2C9. Losartan can also be hydroxylated to an inactive metabolite, P1. Approximately 14% of losartan is metabolized to E-3174. Losartan can be metabolized by CYP3A4, CYP2C9, and CYP2C10. Losartan can also be glucuronidated by UGT1A1, UGT1A3, UGT1A10, UGT2B7, and UGT2B17.

Route of Elimination
A single oral dose of losartan leads to 4% recovery in the urine as unchanged losartan and 6% in the urine as the active metabolite. Oral radiolabelled losartan is 35% recovered in urine and 60% in feces. Intravenous radiolabelled losartan is 45% recovered in urine and 50% in feces.

Half-Life
The terminal elimination half-life of losartan is 1.5-2.5 hours while the active metabolite has a half-life of 6-9 hours.

Food-Interactions
- Take it at the same time every day.
- Take it with or without food. Food delays absorption, but does not affect the extent of absorption.

MATERIALS AND METHOD

Table: Instruments used

S.No	INSTRUMENT	MODEL
1	HPLC	Waters, peak HPLC, Isocratic method, ADM
2	DETECTOR	Autochro 3000
3	WEIGHING MACHINE	Nivayo
4	PIPETTES AND BURETTES	Borosil
5	BEAKERS	Borosil

Table: Chemicals used

S.NO	Chemicals	Company name
1	Losartan Potassium	Losar-25
2	Losartan	Losar-50
3	Methanol for HPLC	Thermo Fisher Scientific Indi LTD
4	Water for HPLC	Thermo Fisher Scientific Indi LTD

HPLC METHOD DEVELOPMENT

Wavelength Selection

UV spectrum of 10 µg/ml Losartan in diluents (mobile phase composition) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength was selected as 240 nm. At this wavelength, both drugs show good absorbance.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Acetonitrile, Methanol: 0.1% OPA, Acetonitrile: Phosphate buffer, and Methanol: Phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile (pH 3.0) in proportion 50:50 v/v respectively.

Optimization of Column

The method was performed with various columns like the C18 column Phenomenex column, Inertsil ODS column, and YMC column. C18 Column was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with autosampler and UV detector.
Temperature : Ambient
Column : C18
Buffer : 0.1% TFA
pH : 3.0
Mobile phase : Methanol: Acetonitrile
Flow rate : 1 ml per min
Wavelength : 208 nm
Injection volume : 20 µg/ml
Run time : 3mins

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of 0.1% TFA buffer

Take 1 ml of TFA in 1000 ml of HPLC water, pH was adjusted with NaOH up to 3.0. Final the solution was filtered through 0.45 µ Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 50 ml of Methanol (50%) and 50 ml of Acetonitrile (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

ASSAY

Standard Solution Preparation

Accurately weigh and transfer 50 mg of Losartan working standard into a 10 ml clean dry volumetric flask add about 5 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to 10ml with diluent.(1000 microgram/ml) (Stock solution)

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and add 9ml diluent. (100 microgram/ml).

Sample Solution Preparation

Accurately weigh 1 tablet from 2 brands of Losartan crush in mortar and pestle and transfer sample into a 10 ml clean dry volumetric flask. Add about 10mL of diluent and sonicate it up to 3 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through a 0.45 micron injection filter. (Stock solution)

If any sample was found to be highly concentrated then further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to 5ml with the diluent.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Losartan peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSIONS

Method validation

Blank

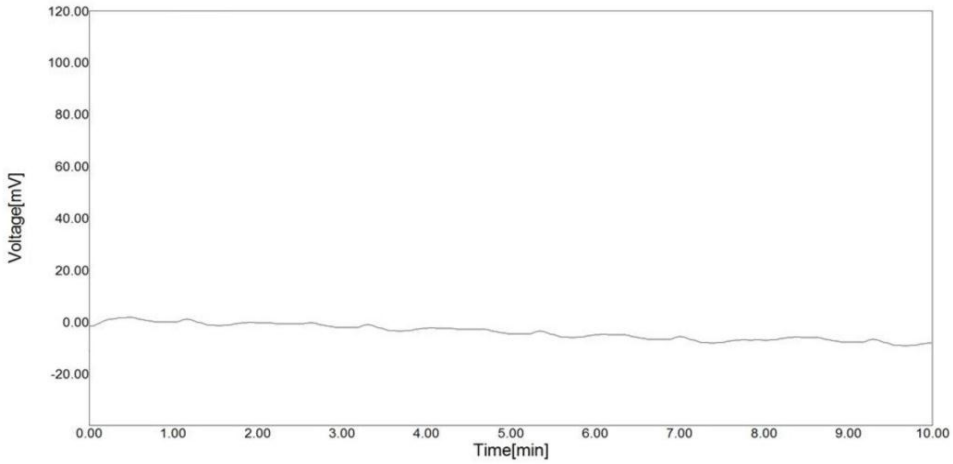

Fig. 1: Chromatogram for Blank

Table 1: Results of blank

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.4167	1011.8362	0.00	695.5	1.3246	0.0000
Sum			1011.8362				

Trail 1:

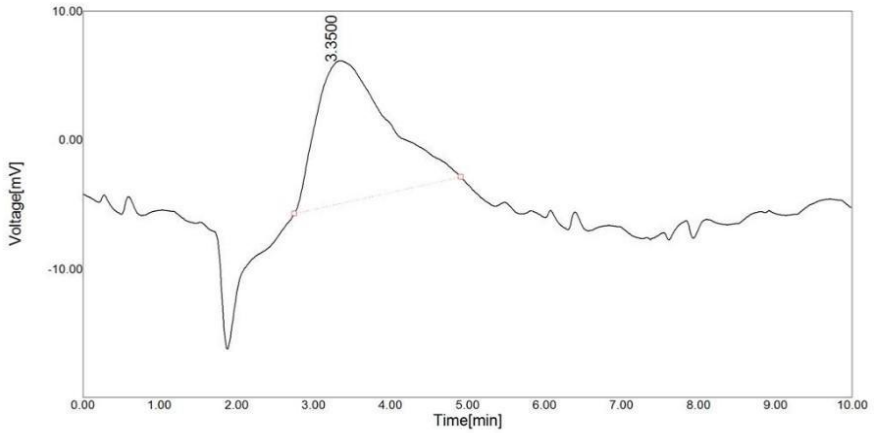

Fig. 2: Chromatogram for trial 1

Table 2: Results of trail 1

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	3.3500	712.1481	0.00	61.2	1.8262	0.0000
Sum			712.1481				

Observation

Spike of a Losartan Peak was observed, but the peak does not have symmetrical shape and does not meet system suitability conditions.

Trail 2:

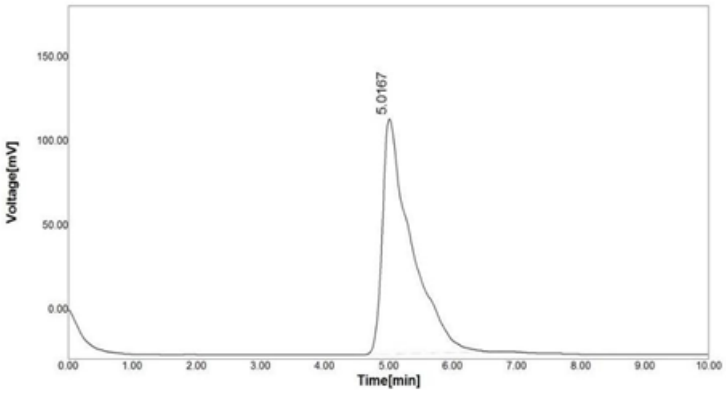

Fig. 3: Chromatogram for trial 2

Table 3: Results of trail 2

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount	TP	TF
1	Losartan	5.0167	4369.9507	139.8836	0.0000	476.8	2.5074
Sum			4369.9507	139.8836	0.0000		

Observation

No specific change was observed when compared with trail 1. Spike of a Losartan Peak was observed, but the peak does not have symmetrical shape and does not meet system suitability conditions.

Trail 3:

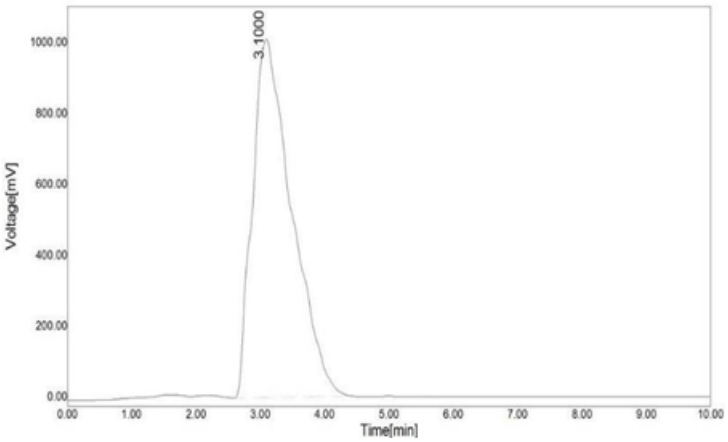

Fig. 4: Chromatogram for trial 3

Table 4: Results of trail 3

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount	TP	TF
1	Losartan	3.1000	8836.9507	139.4369	0.0000	476.8	2.5074
Sum			8836.9507	139.4369	0.0000		

Observation

Sharpening of peaks was observed with the previous trails.

Spike of a Losartan Peak was observed, in optimum condition the symmetrical shape is observed.

Linearity 1

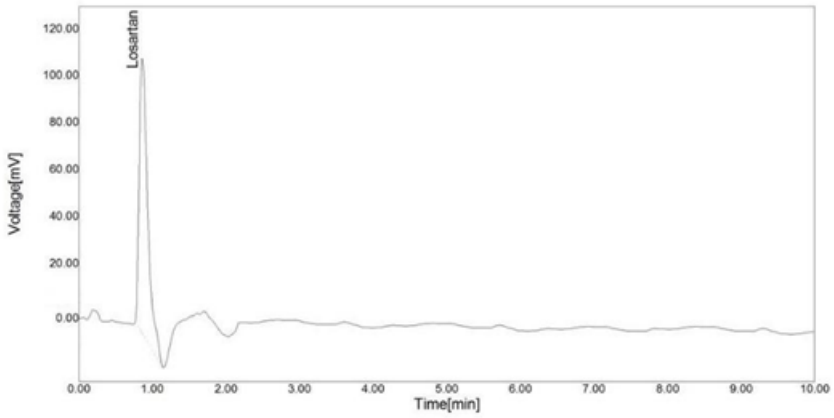

Fig. 5: chromatogram for Linearity 1

Table 5: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	0.9333	377	0.00	602.4	1.2767	0.0000
Sum			377				

Linearity 2

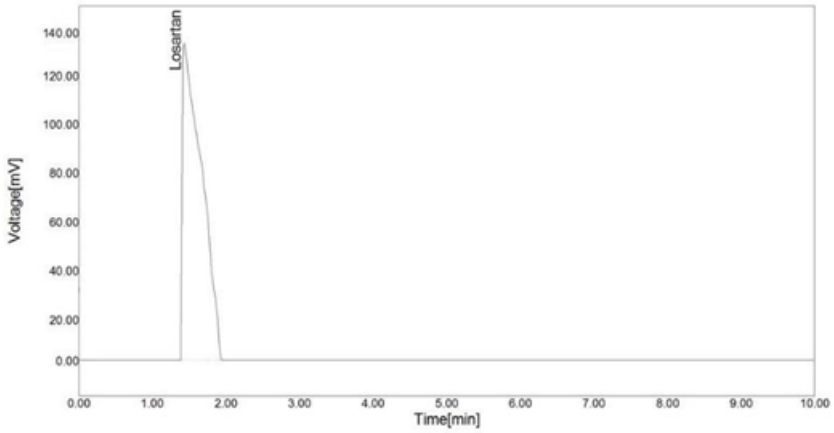

Fig. 6: chromatogram for Linearity 2

Table 6: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.4333	596	0.00	182.1	5.3783	0.0000
Sum			596				

Linearity 3

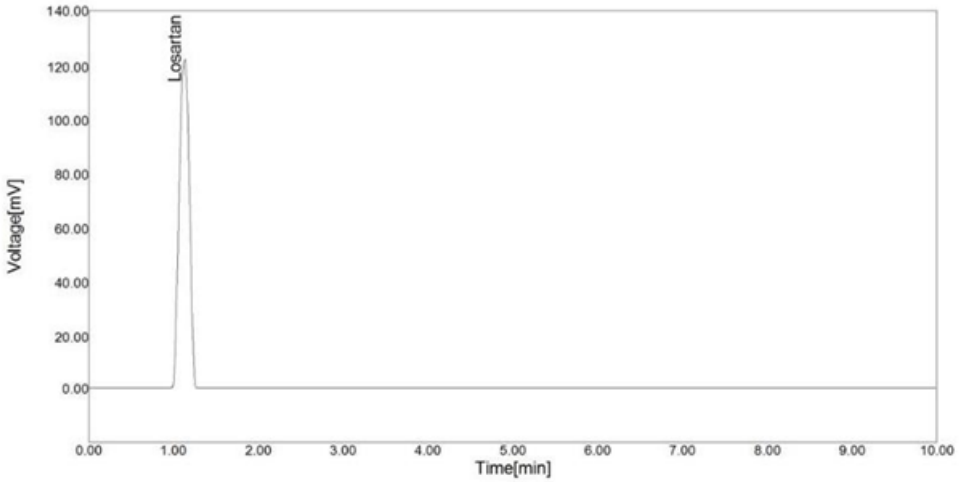

Fig. 7: chromatogram for Linearity 3

Table 7: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1333	1002	0.00	775.1	0.9271	0.0000
Sum			1002				

Linearity 4

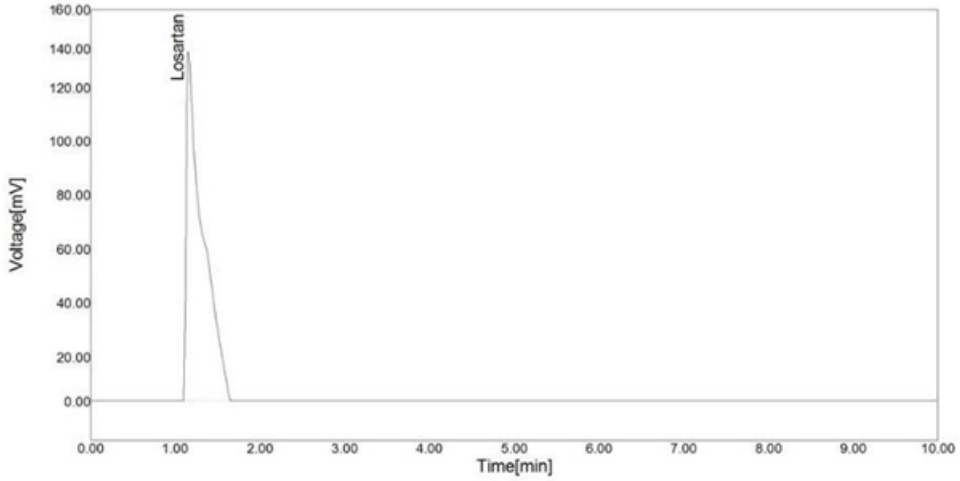

Fig. 8: chromatogram for Linearity 4

Table 8: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1500	1179	0.00	67.9	5.3457	0.0000
Sum			1179				

Linearity 5

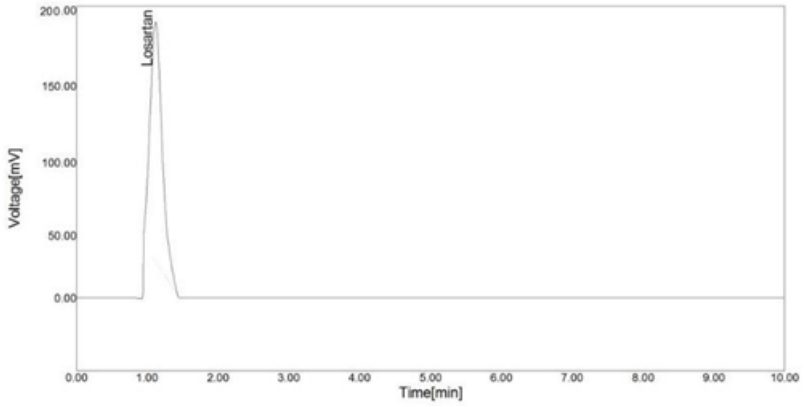

Fig. 9: chromatogram for Linearity 5

Table 9: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1167	1382	0.00	232.2	1.2802	0.0000
Sum			1382				

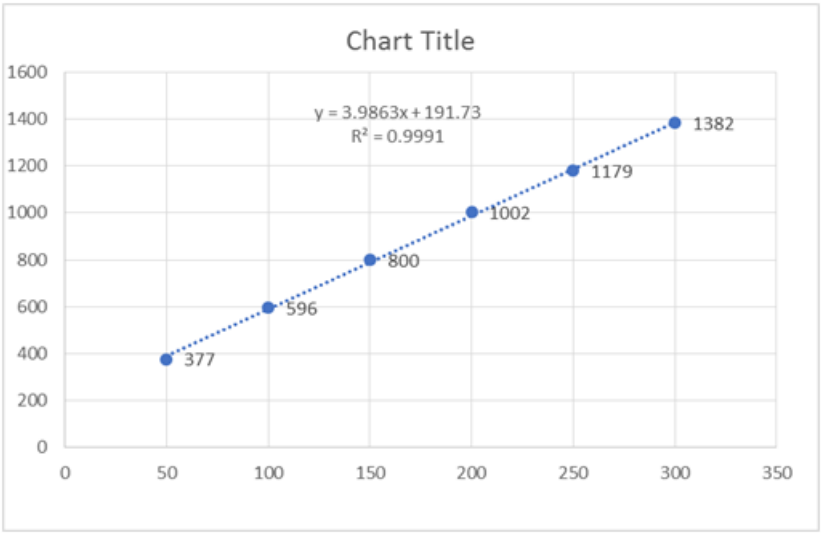

Fig. 10: Calibration Curve

Table: Analytical performance parameters of Losartan

Parameters	Losartan
Slope (m)	3.9863
Intercept (c)	191.73
Correlation coefficient (R^2)	0.9991

Precision 1

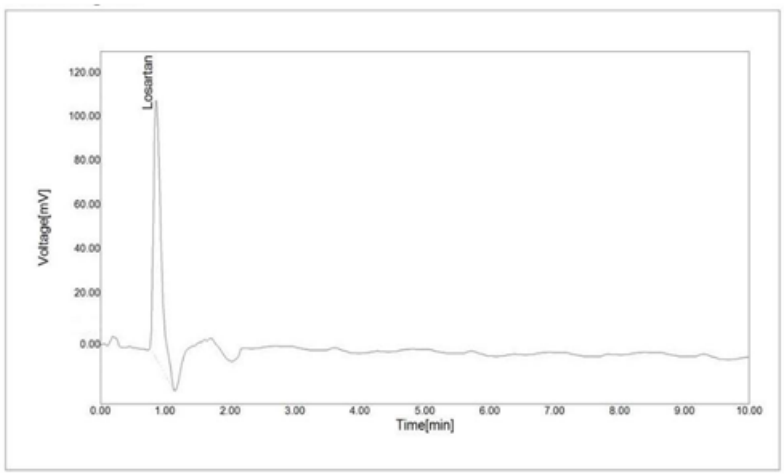

Fig. 11: Chromatogram for precision 1

Table 10: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	0.9333	1391.5435	0.00	602.4	1.2767	0.0000
Sum			1391.5435				

Precision 2

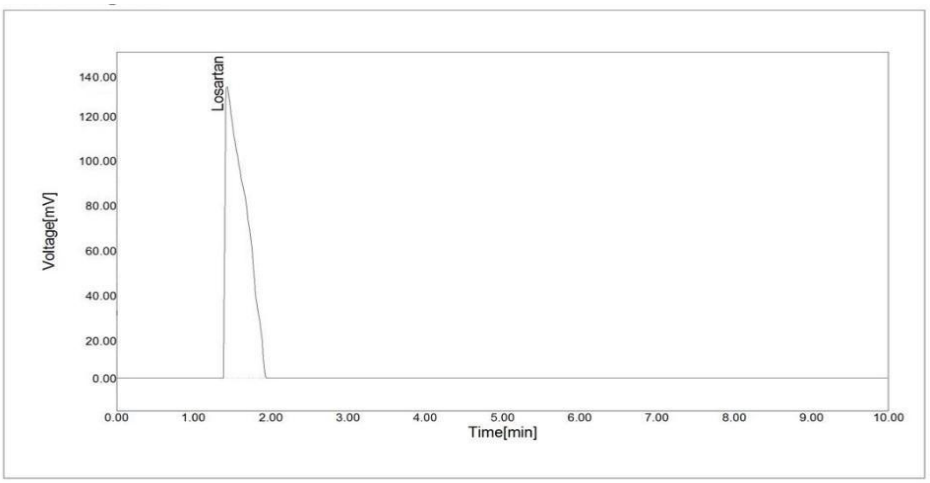

Fig. 12: Chromatogram for precision 2

Table 11: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.4333	1719.5782	0.00	182.1	5.3783	0.0000
Sum			1719.5782				

Precision 3

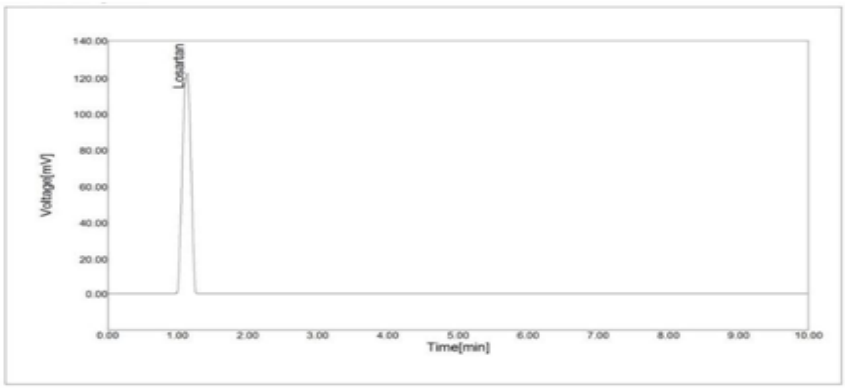

Fig. 13: Chromatogram for precision 3

Table 12: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1333	1222.7214	0.00	775.1	0.9271	0.0000
Sum			1222.7214				

Precision 4

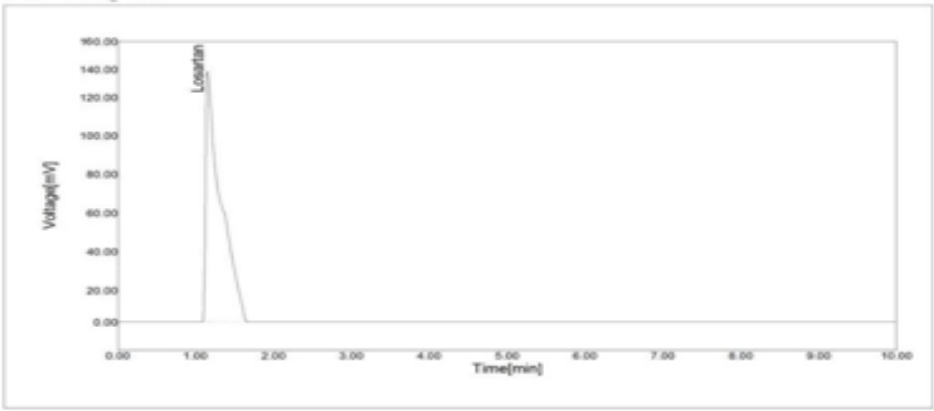

Fig. 14: chromatogram for precision 4

Table 13: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1500	1133.1427	0.00	67.9	5.3457	0.0000
Sum			1133.1427				

Precision 5

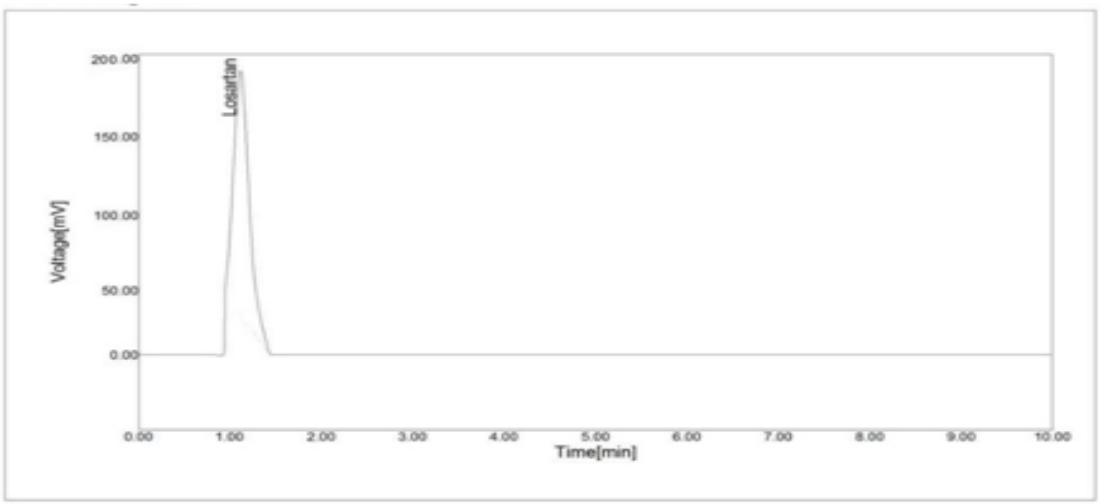

Fig. 15: Chromatogram for precision 5

Table 14: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	1.1167	1222.6973	0.00	232.2	1.2802	0.0000
Sum			1222.6973				

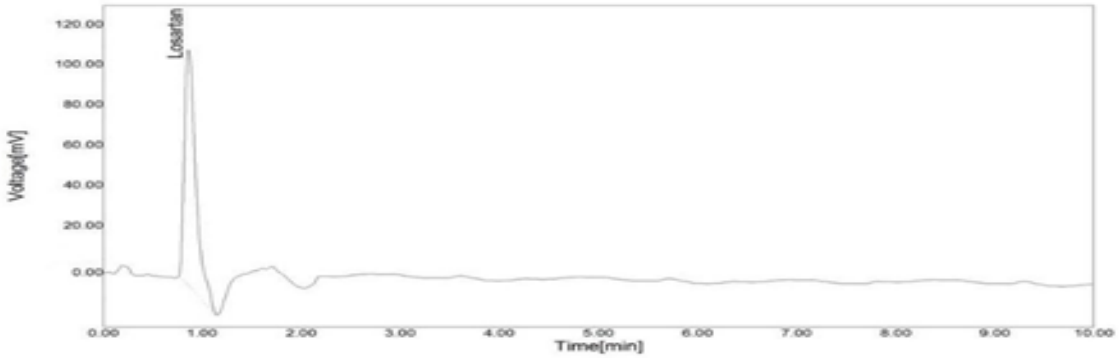

Fig. 16: Chromatogram for Formulation 1

Table 15: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	0.8500	1072.0896	0.00	381.4	2.1867	0.0000
Sum			1072.0896				

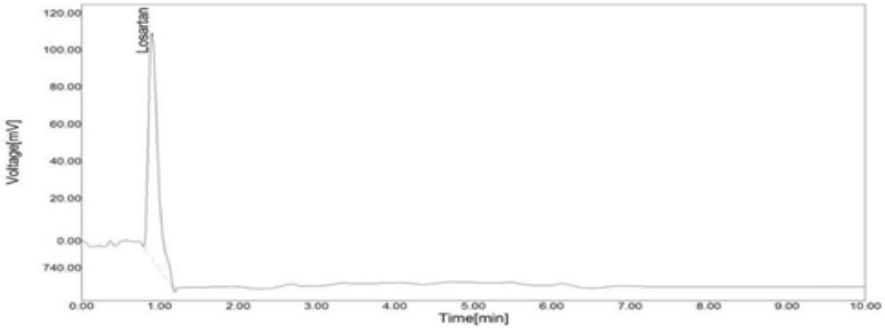

Fig. 17: Chromatogram for Formulation 2

Table 16: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	0.9000	1156.8859	0.00	389.6	1.8107	0.0000
Sum			1156.8859				

Calculation
Formulation assay

Formulation	Dosage	% Assay
Losartan	50mg	90.32%
Losartan Potassium	25mg	90.83%

Limit of Detection (LOD)
Limit of Detection was carried out as described under experimental work. The corresponding chromatograms and results are shown below.

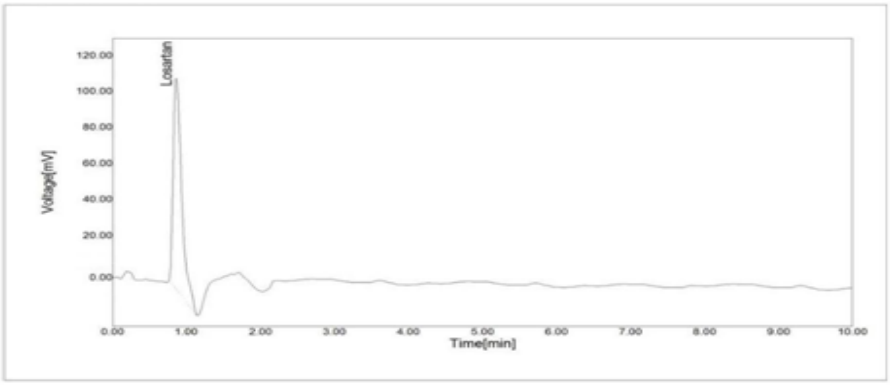

Fig. 18: chromatography for LOD

Table 19: Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Losartan	0.9300	391.5435	0.00	602.4	1.2767	0.0000
Sum			391.5435				

CONCLUSION

The estimation of Losartan was done by RP-HPLC.[19,20]

The assay of Losartan was performed with tablets and the % assay was found to be 98.99%which show that the method is useful for routine analysis.

The linearity of Losartan was found to be linear with a correlation coefficient of 0.9991, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision are RSD should be not more than 2.0% and the method shows a precision of 0.1667 for Losartan which shows that the method is precise.

The acceptance criteria of intermediate precision are RSD should be not more than 2.0% and the method shows a precision of 0.1667 for Losartan which shows that the method is repeatable when performed on different days also.

The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 98% for Losartan. The validation of the developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The robustness limit for mobile phase variation and flow rate variation is well within the limit, which shows that the method is having good system suitability and precision under a given set of conditions.

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