CHAPTER-6

Losartan

6.1 A survey of literature for Losartan indicated the estimation reported by the following methods, Viz., High-performance liquid chromatography , Capillary isotachophoresis. Gas chromatography-mass spectrometry, UV Spectrophoto metric method , Micellar electrokinetic chromatography, Gas chromatography-mass spectrometry, HPTLC ,and High-performance liquid chromatography-electrospray ionization mass spectrometry,. The brief information on above analytical methods are follows.

Erk N. [184] described the analysis of binary mixtures of losartan potassium and hydrochlorothiazide by using high-performance liquid chromatography, ratio-derivative spectrophotometric and compensation technique.Lande NR. et. al [185] described simultaneous spectrophotometric estimation of losartan potassium and hydrochlorothiazide in tablet dosage form. Gonzalez L. et. al [186] described High-performance liquid-chromatographic method for screening angiotensin II receptor antagonists in human urine.Carlucci G. et. al [187] described Simultaneous determination of losartan and hydrochlorothiazide in tablets by high-performance liquid chromatography.

Jain HK et. al [188] described Estimation of losartan potassium from tablets. Argekar AP et. al [189] described A gradient reversed-phase high performance liquid chromatography method for simultaneous determination of hydro-chlorothiazide (HCT) and losartan potassium (LOS) from tablets. Williams,-RC et. al [190] described Analysis of potassium counter ion and inorganic cation impurities in pharmaceutical drug substances by capillary electrophoresis with conductivity detection. Kanumula GV et. al [191] described Simultaneous determination of hydrochloro thiazide and losartan potassium in pharmaceutical dosage by reverse phase high performance liquid chromatography. Iwasa T et. al [192] described Method for the simultaneous determination of losartan and its major metabolite, EXP-3174, in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. Zhao ZX. et. al [193] described Identification of losartan degradates in stressed tablets by LC-MS and LC-MS-MS.

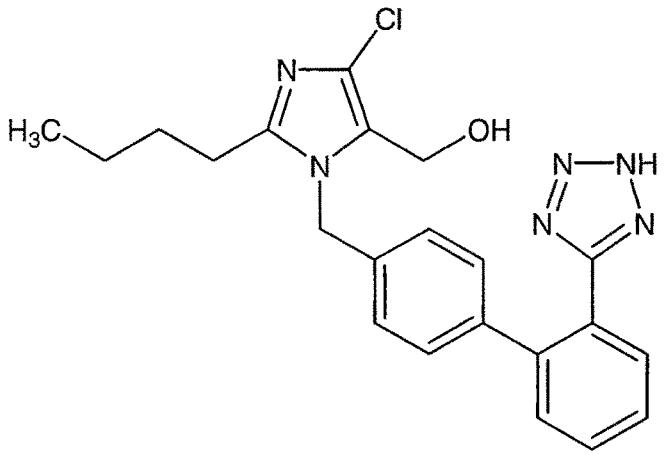
McCarthy KE. et. al [194] described Determination of losartan and its degradation products in COZAAR tablets by reversed-phase high-performance thin-layer chromatography. Soldner A. et. al [195] described A radioreceptor assay for the analysis of AT1-receptor antagonists. Correlation with complementary LC data reveals a potential contribution of active metabolites. Soldner A. et. al [196] described HPLC assays to simultaneously determine the angiotensin-AT antagonist losartan as well as its main and active metabolite EXP 3174 in biological material of humans and rats.Farthing D. et. al [197] described Simple high-performance liquid-chromatographic method for determination of losartan and E 3174 metabolite in human plasma, urine and dialysate.Ritter MA. et. al [198] described An improved method for the simultaneous determination of losartan and its major metabolite, EXP 3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection.

Williams RC. et al [199] described Comparison of liquid chromatography, capillary electrophoresis and supercritical-fluid chromatography in the determination of losartan potassium drug substance in Cozaar tablets. Lee H et al [200] described Simultaneous determination of losartan and active metabolite EXP3174 in rat plasma by HPLC with column switching.Furtek CI et. al [201] described Simultaneous determination of a novel angiotensin II receptor blocking agent,losartan, and its metabolite in human plasma and urine by high performance liquid chromatography

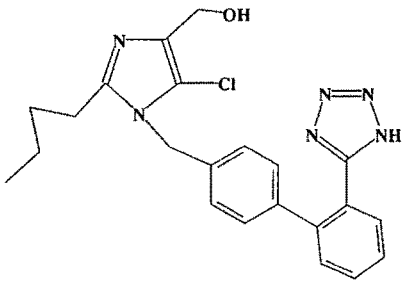
Even though there are several methods reported to estimate losartan from plasma samples as well as tablet matrices but no where losartan separations and estimations from its related compound and process impurities are reported. Hence we have under taken this problem for current work. Subsequently we have proposed to develop a simple and cost effective stability indicating assay method on HPLC, which will suit even small scale manufacturers keeping ICH guide lines and various regulatory requirements in mind. Developed HPLC methods are validated throughly to check the suitability and corrective ness as per ICH guidelines.

6.2 Development report for estimation of related substances in Losartan

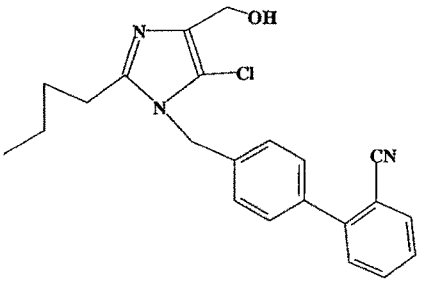
Losartan and its impurity structures are given below.



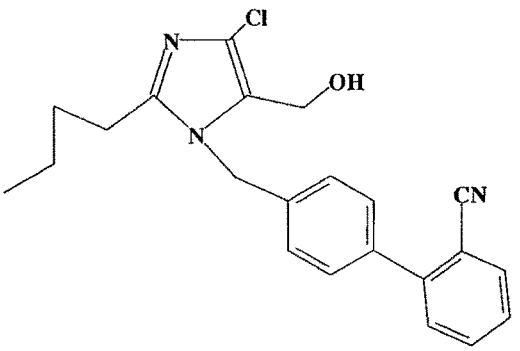
Losartan structure STR-6



Losartan Impurity A STR-7

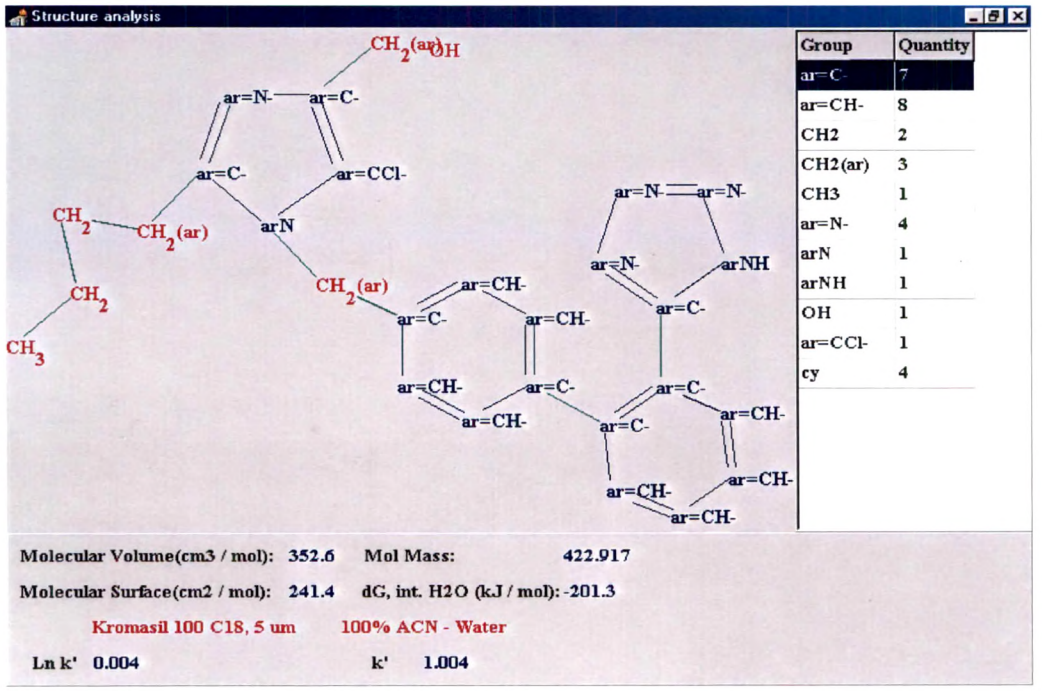


Losartan Impurity C STR-8

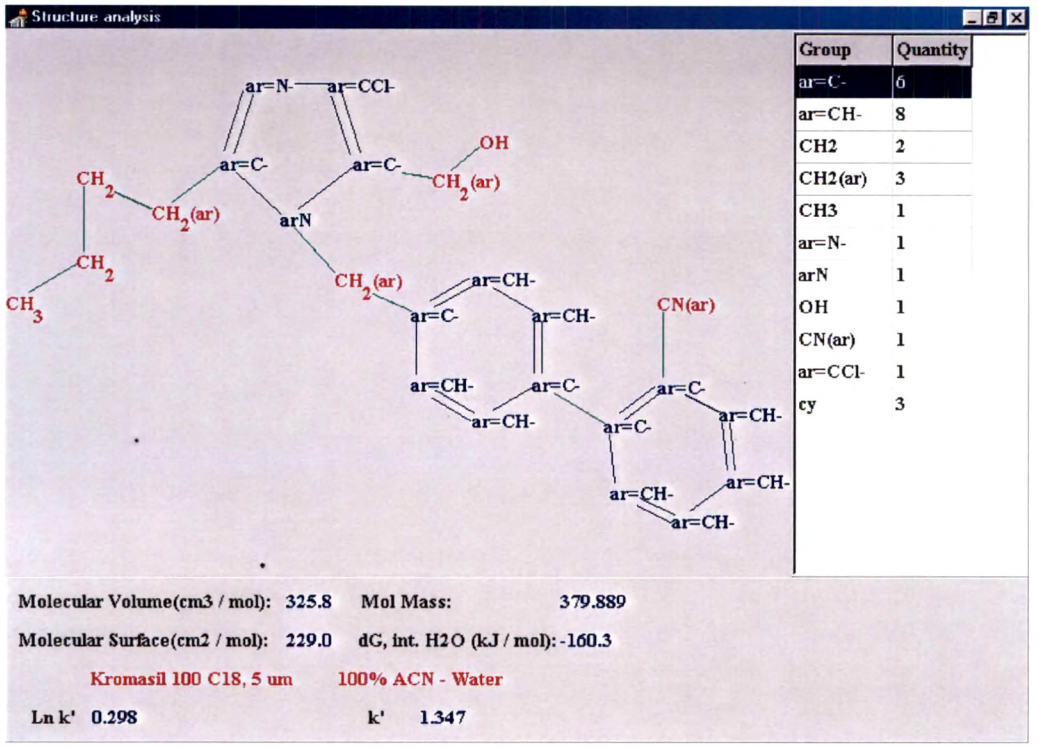


Losartan Impurity B STR-9

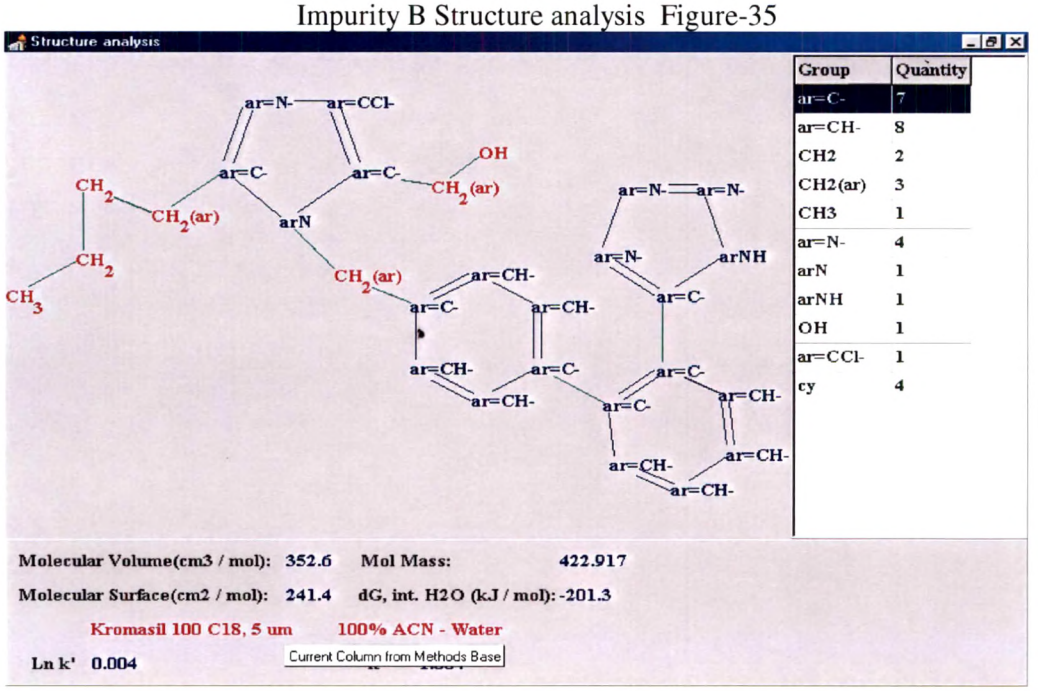
All structures are loaded into chromsword HPLC method development software to deduce structure analysis for method development. Figure-34 to 37 shows Losartan and its impurities structure analysis charts .



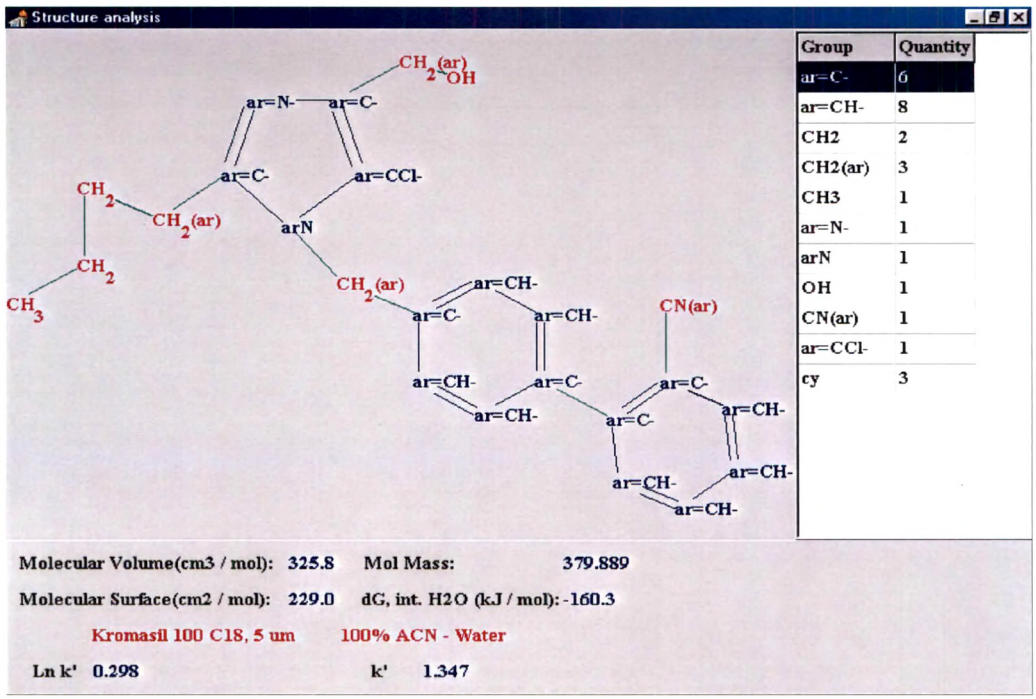
Impurity A Structure analysis Figure-34



Impurity B Structure analysis Figure-35

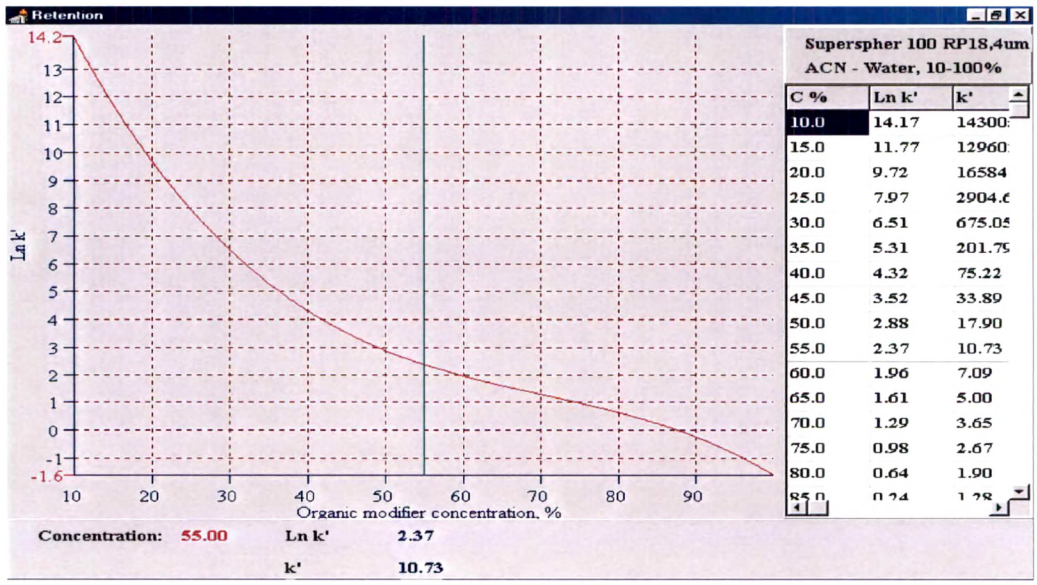


Losartan Structure analysis Figure-36

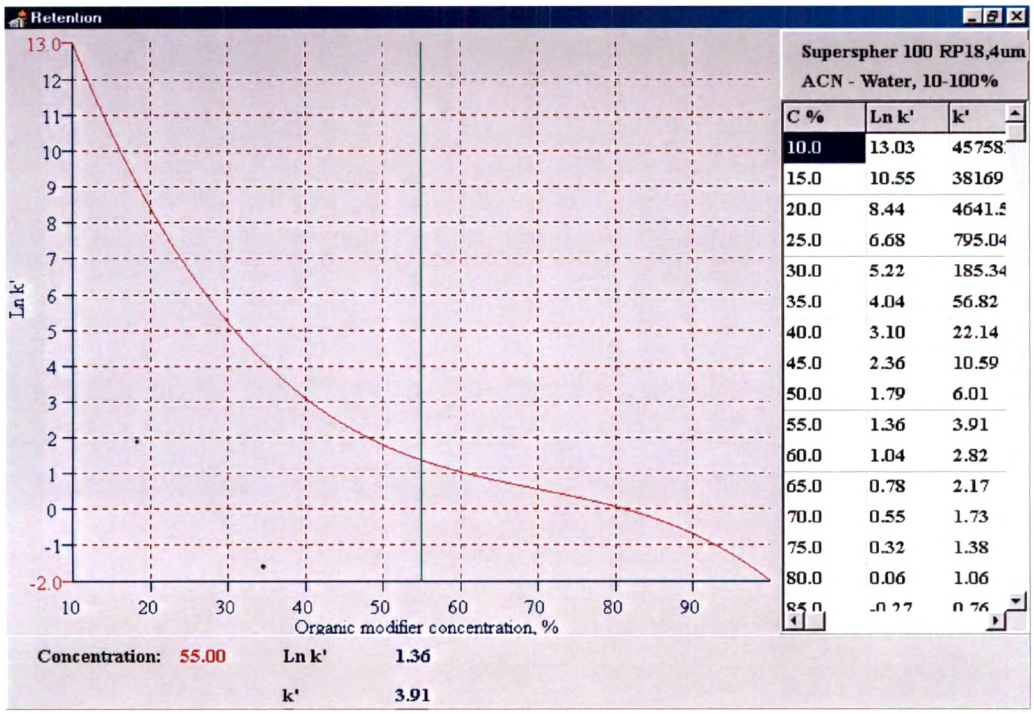


Impurity C Structure analysis Figure-37

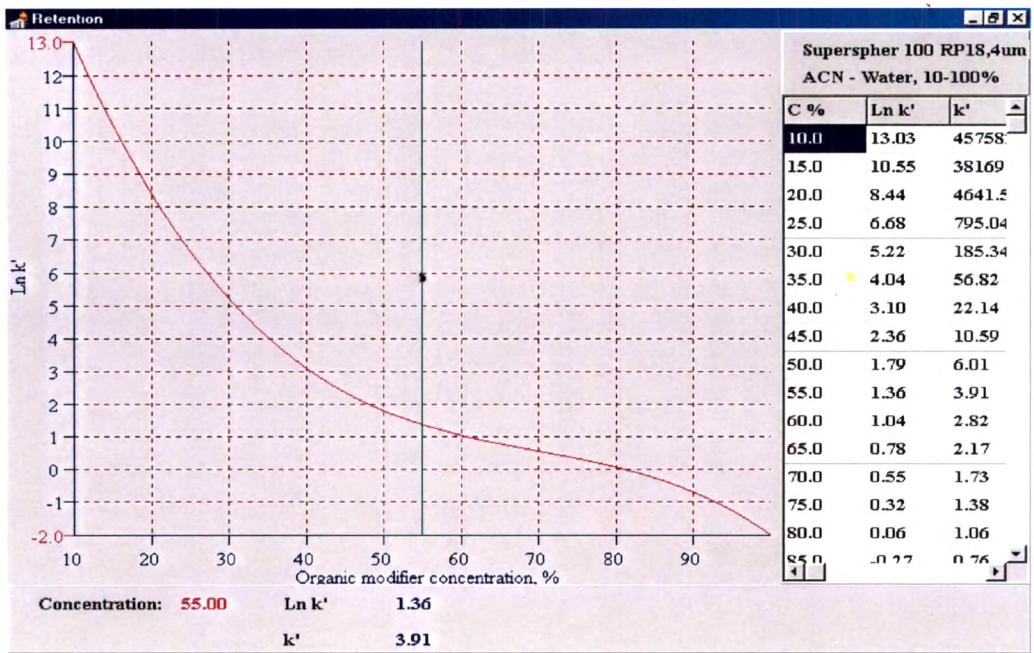
Figure-38 to 41 shows Losartan and its impurities retention analysis charts .



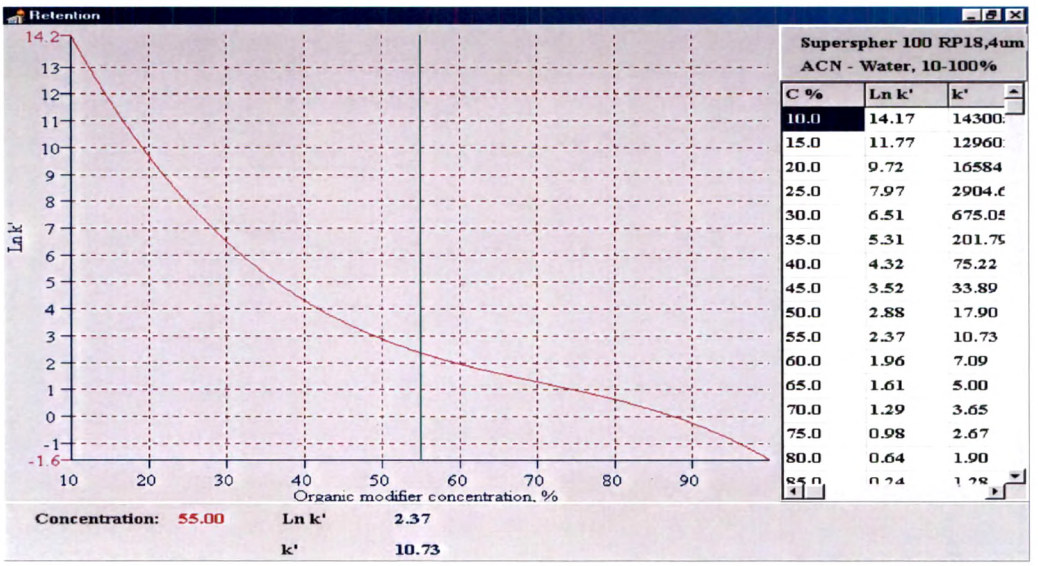
Impurity C retention analysis Figure-38



Losartan retention analysis Figure-39

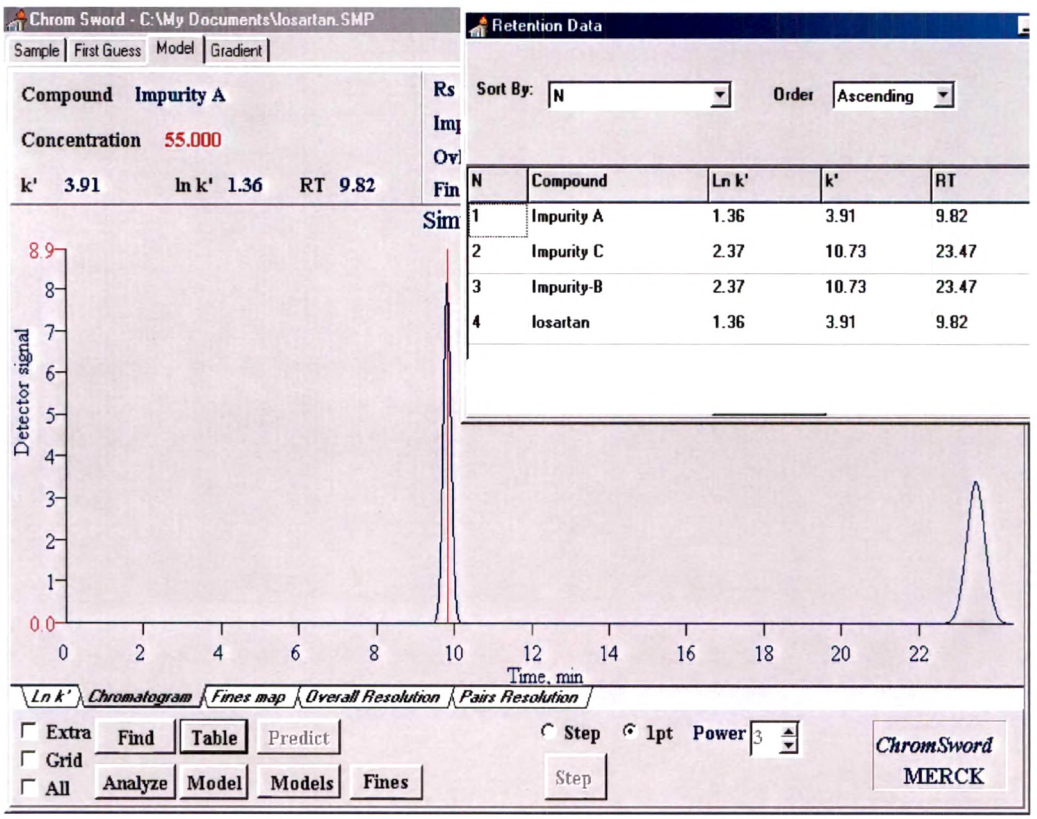


Impurity A retention analysis Figure-40



Impurity B retention analysis Figure-41

Figure 42 shows the best possible simulated chromatogram.



Simulated chromatogram Figure-42

Analysis: Software had predicted only two peaks are which are shown in Figure-42. Table mentioned in figure -42 indicating all four components but two components are at one place .These analysis were done based on at molecular volumes and molecular surfaces as given in structure analysis charts.

At this point software failed to predict the correct simulated chromatogram due to closeness between the structures differing only some positions and can be called positional isomers. Hence software failed to predict the correct condition. Experiment was performed to verify the software prediction. Ammonium dihydrogen orthophosphate solution with pH to 3.0 ± 0.1 is selected as buffer and acetonitrile as organic modifier. Wavelength 254 is selected to detect all impurities.

Optimized buffer and organic modifier ration as 65:35 to resolve all impurities. Final conditions are given below.

Analytical Method :

Reagents and chemicals :

1.Ammonium dihydrogen orthophosphate (anhydrous)	AR grade
2.Acetonitrile	: HPLC grade
3.Water	: Milli Q Water
4. Orthophosphoric acid	: AR grade

Buffer solution : Transfer 8.62g of Ammonium dihydrogen orthophosphate (anhydrous) into a 1000ml volumetric flask. Dissolve in and dilute upto the mark with water. Adjust the pH to 3.0 ± 0.1 with 10%(v/v) orthophosphoric acid.

Mobile phase : Prepare sufficient quantity by mixing 65 volumes of buffer and 35 volumes of acetonitrile. Filter and degas prior to use.

Diluent : Prepare a mixture of 700 ml acetonitrile and 300 ml water.

System suitability: Transfer about 10 mg, accurately weighed, each of Losartan WRS, Losartan/Imp-A, Losartan/Imp-B and Losartan/Imp-C into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (100 µg/ml).

Sample preparation :Transfer about 50mg accurately weighed Losartan sample in to a 50ml volumetric flask. Dissolve in and dilute upto mark with diluent (1000 µg/ml).

Chromatographic system :Use a suitable high pressure liquid chromatography system equipped with a UV detector set to 254 nm and a column of 250 mm x 4.6mm containing 5µ C18 packing material (suggested column - Hypersil C18 BDS) The flow rate is about 1 5 ml/min

Procedure :Inject 20µl of the system suitability solution in duplicate in to the system and record the chromatogram upto the 50 min. The retention time are about 9 min. for Losartan, 4 min. for Losartan/Imp-A, 36min for Losartan/Imp-B and 16 min. for Losartan/Imp-C. Calculate the resolution between Losartan and Losartan/Imp-C is not less than 5 0

Inject 20µl of the sample preparation in duplicate into the system and record the chromatograms upto the 50 min. Calculate the % impurities in Losartan/II sample by area normalisation method.

Calculations :Calculate the Impurity by area normalization.

Limits (Proposed for method validation)

1.2-n-Butyl-4-chloro]-5-(hydroxymethyl)-1-2[(2'-(1H-tetrazol-5-yl)-biphenyl-4-yl) methyl] imidazole (Impurity-A) . NMT 0.2%

2 2-n-Butyl-4-chloro]-1-[(2'cyano-biphenyl-4-yl)methyl]-5-(hydroxymethyl) imidazole (Impurity-B) : NMT 0 2%

3.2-n-Butyl-5-chloro]-1-[(2'cyano-biphenyl-4-yl)methyl]-4-(hydroxymethyl) imidazole (Impurity-C) : NMT 0 2%

4 Any other impurity NMT 0 2%

5 Total impurities : NMT 1.0%

6.3 VALIDATION PROTOCOL

Purpose : The purpose of this document is to establish the precision, accuracy, linearity of detector response and ruggedness of the analytical method through number of scientific studies and discussions of the data.

Scope : This method, upon validation, can be used for the analysis of related substances in Losartan.

Analytical method: As per section 6.2

6.3.1 The experiments are designed to study

1 System suitability

2..Identification of individual component

3 Instrument precision

4.Method precision

5.Linearity and range

6.Accuracy

7.Minimum detection limit

8 Minimum quantitation limit

9 Ruggedness of the method

6.3.2 Stock Solutions :

Solution 1 : Transfer about 25mg.accurately weighed Losartan/Imp-A into a 25ml volumetric flask. Dissolve in & dilute upto mark with diluent (1000µg/ml).

Solution 2 : Transfer about 25mg, accurately weighed, Losartan/Imp-B into a 25ml volumetric flask. Dissolve in and dilute upto mark with diluent (1000µg/ml).

Solution 3 . Transfer about 25mg, accurately weighed, Losartan/Imp-C into a 25ml volumetric flask. Dissolve in and dilute upto mark with diluent (1000µg/ml)

Solution 4 Transfer about 25mg, accurately weighed, Losartan into a 25ml volumetric flask Dissolve in and dilute upto mark with diluent (1000µg/ml)

Solution 5 : Pipette out 5.0 ml each of solution 1, 2, 3 in to 100ml volumetric flask. Dilute upto the mark with diluent (50µg/ml each of Losartan/Imp-A, Losartan/Imp-B and Losartan/Imp-C).

6.3.3 System suitability :

System Suitability solution :Transfer about 10mg, accurately weighed, each of Losartan WRS, Losartan/Imp-A, Losartan/Imp-B and Losartan/Imp-C into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (100µg / ml).

Set the chromatographic system as mentioned in the analytical method.Inject 20 µL of the system suitability solution in duplicate and record the chromatograms upto 50 min. Calculate the resolution between the Losartan/Imp-C and Losartan peaks.

Acceptance criteria : Resolution factor *R* is not less than 5

6.3.4 Identification

i) Pipette out 1.0 ml of *Solution 1* into a 10 ml volumetric flask, dilute to volume with diluent (about 100 µg/ml of Losartan/Imp-A).

ii) Pipette out 1.0 ml of *Solution 2* into a 10 ml volumetric flask, dilute to volume with diluent (about 100 µg/ml of Imp-B).

iii) Pipette out 1.0ml of *Solution 3* into a 10 ml volumetric flask, dilute to volume with diluent (about 100 µg/ml of Imp-C).

iv) Pipette out 1.0ml of *Solution 4* into a 10 ml volumetric flask, dilute to volume with diluent(about 100 µg/ml of Losartan)

Inject 20 µL each of the above diluted solutions (about 100µg/ml) individually, in duplicate and record the chromatograms upto 50 min. Note the retention time of each for identification.

6.3.5 Instrument precision

System precision solution :Transfer about 50 mg, accurately weighed, Losartan standard into a 50ml volumetric flask. Pipette out 2 0ml *Solution5* into it. Dissolve by adding 20ml diluent and dilute to volume with diluent. (2µg/ml each of Losartan/Imp-A,

Losartan/Imp-B and Losartan/Imp-C corresponding to 0.2% of the Losartan concentration of 1000 µg/ml).

Set up the system as mentioned under the chromatographic conditions.Inject 20 µL each of the system suitability solution (about 100µg/ml) individually, in duplicate and record the chromatograms upto 50 min.

Inject 20 µL of the system precision solution six times and record the chromatograms upto 50 min. Calculate the relative standard deviation of the detector response for each component.

Acceptance criteria:

RSD of detector response for each component is ≤ 5.0 %

6.3.6 Method precision

i) Prepare a sample solution as directed under the procedure (about 1000µg/ml).

ii) Set the chromatograhic conditions as mentioned under the method, inject 20 µL of the system suitability solution in duplicate and record the chromatograms upto 50 min.

iii) Inject 20 µL of the sample solution in duplicate and record the chromatograms upto 50 min.

iv) Calculate the amounts of the Impurity present in the sample.

v) Prepare six sets of this sample as directed under the method.
Spike impurities upto the target level in each of the sample preparation.

vi) Inject 20 µL of each sample preparation in duplicate into the chromatography set to the condition mentioned under the method and record the chromatograms upto 50 min

vii) Calculate the % of impurity and its RSD for each.

Acceptance criteria : RSD of the calculated impurities in the six sets ≤ 5.0 %.

6.3.7 Linearity and range :

i) Prepare five sets for five levels (50, 75, 100, 125 and 150% of target condition). Linearity study by transferring 50 mg accurately weighed Losartan standard in 50 ml volumetric flask.

ii) Use solution-5 (mentioned under section 6.3.2) for preparing following solution.

Linearity solutions.

Sr. No.	Level % of target	Wt. Of Losartan *mg*	Vol. Of *Solution 5* *ml*	Final Dilution *ml*	Final Concentrations		
					Impurity- A *µg/ml*	*Losartan/ Imp-B* *µg/ml*	*Losartan/ Imp-C* *µg/ml*
L1	50	50	1.0	50	1.000	1.000	1.000
L2	75	50	1.5	50	1.500	1.500	1.500
L3	100	50	2.0	50	2.000	2.000	2.000
L4	125	50	2.5	50	2.500	2.500	2.500
L5	150	50	3.0	50	3.000	3.000	3.000

iii) Inject 20 µL each of the linearity solution in triplicate into the chromatographic system set to the conditions mentioned under the method and record the chromatograms upto 50 min

iv) Calculate the mean and RSD of the detector responses for each linearity level individually for each component.

v) Plot a graph of the concentration versus mean area count and perform mathematical regression for each component individually.

Acceptance criteria: RSD of area counts for individual component at each level is ≤ 5 0% Plot of concentration versus detector response for each component is linear. The regression correlation coefficient (r^2) ≥ 0.99

6.3.8 Accuracy :

Use Solution 5 [50µg/ml each of Impurity] for preparing the following solutions.

i) *Standard Solution :* Pipette out 2.0ml of *solution 5* in to a 50ml volumetric flask , mix and dilute upto mark with diluent.

ii) Prepare five sets for five level (70, 85, 100, 115 and 130% of target concentration) recovery study by transferring about 50 mg, accurately weighed, Losartan standard into five 50 ml volumetric flasks. Pipette out appropriate volumes of *Solution 5* as shown in the table below and dilute to volume with diluent.

Sr. No.	Level % of target	Vol. of *Solution 5* *ml*	Final Dilution *ml*	Final Concentrations		
				Losartan/ Imp-A *µg/ml*	*Losartan/ Imp-C* *µg/ml*	*Impurity-B* *µg/ml*
R1	70	1.4	50	1.400	1.400	1.400
R2	85	1.7	50	1.700	1.700	1.700
R3	100	2.0	50	2 000	2.000	2.000
R4	115	2.3	50	2.300	2.300	2.300
R5	130	2.6	50	2.600	2.600	2.600

iii) Inject 20µL of standard solution, prepared as mentioned above, in triplicate and record the chromatograms upto 50 min. Calculate the mean area counts of the standard

iv) Inject 20 µL each of the recovery solution R1, R2, R3, R4 and R5 into the chromatography in triplicate and record the chromatograms upto 50 min.

v)Calculate the mean and RSD of the detector responses for individual impurity in each recovery set and standard solution.

vi) Calculate the amount of each impurity for each set of the recovery sample and calculate the percentage of recovery.

Acceptance criteria :Percentage recovery is 95.0 to 105.0 %.

RSD of detector response for each component is ≤ 5.0%

6.3.9 Minimum quantitation level (MQL)and Minimum detection level (MDL)

i) Prepare a MDL stock solution for this study by pipetting out 1.0 ml of Solution 5 *into* a 50 ml volumetric flask. Dilute to volume with diluent [1 µg/ml each of Impurity] - MDL Stock solution.

ii) Prepare subsequent diluted solutions as shown in the table below and inject them in triplicate and record the chromatograms up to 50 min.

iii) Calculate the RSD of the triplicate injections for each level.

Level	Vol. of MDL stock soln (ml)	Final Dilution (ml)	Concentrations of components in µg/ml		
			Losartan/ IMP-A	*Losartan/ Imp-C*	*Losartan /Imp-B*
D1	---		1.000	1 000	1.000
D2	5.0	10	0.500	0.500	0 500

D3	2 5	10	0.250	0.250	0.250
D4	1.2	10	0.120	0.120	0 120
D5	0 6	10	0.060	0.060	0.060
D6	0 3	10	0.030	0.030	0.030
D7	0.1	10	0.010	0.010	0.010
D8	0.1	25	0.004	0 004	0.004
D9	0.1	50	0.002	0.002	0.002

Acceptance criteria :

The MQL of each component is the lowest concentration at which the RSD of the triplicate injections ≤ 5.0 %.

The MDL of each component is that concentration at which the detector shows a positive response.

6.3.10 Ruggedness of the method:

A sample previously analysed for related substances is reanalysed by another analyst by this method and the results are compared.

6.4 Experimental

6.4.1 Reagents and chemicals :

1) Ammonium dihydrogen orthophosphate : AR grade (S D Fine chem)
2) Acetonitrile · HPLC grade
3) Water : Milli Q Water
4) Orthophosphoric acid · AR grade (S D. Fine Chemical)

6.4.2 Working standards and sample :

Losartan Reference standard : Losartan/III/188/13
Losartan/Imp-A : Losartan/II/172/24
Losartan/Imp-B · Losartan/I/172/14
Losartan/Imp-C : Losartan/I/165/44
Losartan sample : Losartan/III/188/22

6.4.3 Chromatographic system .

Column · 4.6 mm x 25 cm, 5 µm, Hypersil - C18 BDS
Detector : UV-254 nm
Flow rate · 1.5ml

Injection volume : 20 µl

Instrument: Shimadzu LC-10AT solvent delivery pump with SIL-10AVP Autoinjector Shimadzu SPD-10A UV detector with VP-SERIES computer software.

6.4.4 Buffer solution :32.8g of Ammonium dihydrogen orthophosphate was transferred in to a 5.0 lit. beaker. 4000 ml of water, measured with a measuring cylinder, was added to dissolve. pH adjusted to 3.0 with 10% (v/v) orthophosphoric acid.

6.4.5 Mobile phase :5.0lit. mobile phase is made by mixing 3250 ml of buffer solution and 1750 ml of acetonitrile. The entire mixture is filtered and degassed.

6.4.6 Diluent :2 Lit. of diluent was made by mixing 1400 ml of acetonitrile and 600ml of water. The entire mixture is filtered and degassed.

6.4.7 Stock Solutions :

Solution 1 : 25.2 mg of Losartan/Imp-A was dissolved and diluted to 25 ml in diluent (1008 µg/ml of Losartan/Imp-A).

Solution 2 :26.1 mg of Losartan/Imp-B was dissolved and diluted to 25 ml in diluent (1044 µg/ml of Losartan/Imp-B)

Solution 3 : 25.07mg of Losartan/Imp-C was dissolved and diluted to 25 ml in Diluent µg/ml of Losartan/Imp-C).

Solution 4 : 25.4mg of Losartan (RS) was dissolved and diluted to 25 ml in diluent (1016 µg/ml Losartan).

Solution 5 : 5.0ml each of stock solution 1, solution 2,and solution 3 was pipetted out into a 100ml volumetric flask and diluted to mark with diluent (50.4µg/ml of Losartan/Imp-A, 52.2µg/ml of Losartan/Imp-B, and 50.14 µg/ml of Losartan/Imp-C).

6.4.8 System suitability solution :1.0ml each of solution 1, solution 2, solution 3, and solution 4 pipetted out in to a 10ml volumetric flask, dissolve in and diluted to mark with diluent (100.8 µg/ml of Losartan/Imp-A, 104 4 µg/ml Losartan/Imp-B, 100.28µg/ml Losartan/Imp-C and 101 6 µg/ml of Losartan).

6.5 Results And Discussions :

6.5.1 Identification :1.0 ml of solution 1, solution 2, solution 3 and solution 4 are individually pipetted out in 4 separate 10 ml volumetric flasks and diluted to volume with diluent. 20 µl each of this solution is injected individually and the chromatograms recorded upto 50 min.

Results & discussion :Figure-106 to 109 shows typical chromatograms of individual components.

Retention time .	Losartan/Imp-A		RT about 4.6 min.
	Losartan	.	RT about 9 min.
	Losartan/Imp-C	.	RT about 17.5 min.
	Losartan/Imp-B	.	RT about 36 min.

The above results show that all the components are clearly separated and identifiable.

6.5.2 System suitability : Before starting a set of analysis, the system suitability solution is injected in duplicate The resolution between the Losartan/Imp-A and SUN-Losartan is calculated

Results & discussion : Figure-105 shows a typical system suitability chromatogram Figure-104 shows diluent blank chromatogram

The Resolution factor R between Losartan/Imp-C and SUN-Losartan = 15.22

[Limit NLT 5]

As the resolution meets the system suitability requirements the chromatographic system was used for further studies

6.5.3 Instrument precision

System Precision Solution :

50.50 mg Losartan standard was transferred into a 50ml volumetric flask. 2.0ml of solution 5 is pipette out into it. Dissolve in and diluted to mark with diluent (2.016 µg/ml Losartan/Imp-A, 2 088 µg/ml Losartan/Imp-B, 2 006 µg/ml Losartan/Imp-C and 1010µg/ml Losartan)

Individual area counts and % RSD values are shown in Table 6 5.3.1:

Results and Discussion :

Table 6.5.3.1 : Instrument precision

Injection	Detector Response (Area counts)			
	Losartan/Imp-A (2.016 µg/ml)	Losartan/Imp-B (2.088 µg/ml)	Losartan/Imp-C (2.006 µg/ml)	Losartan (1010 µg/ml)
1	71709	66370	69459	29926692
2	71263	67307	68969	29784792
3	72424	68915	69989	30160931
4	73584	69678	71292	30724122
5	73390	69764	71159	30726839
6	68555	68110	70266	30289558
Mean	71821	68357	70189	30268822
SD	1839	1353	919	395091
RSD (%)	2 56	1.98	1.31	1.30

[**Acceptance criteria** : RSD NMT 5.0 %]

Figure –103 shows typical chromatogram of system precision. The above results are well within the acceptance limits and indicates instrument precision.

6.5.4 Method Precision : The Table 4 2 shows the weights of Losartan sample (Batch No. Losartan/III/188/22) taken into 50 ml volumetric flask separately. 2.0 ml of *Solution 5 is* pipetted into each flask, dissolved by adding about 20 ml of diluent and made to volume with diluent. Each flask contains impurities at about target levels 0.2% each of, Losartan/Imp-A, Losartan/Imp-B and Losartan/Imp-C).

Results and discussion :

Table 6.5.4.1 : Sample analysis

Batch No.	Losartan/Imp-A	Losartan/Imp-B	Losartan/Imp-C	Any other unknown impurity
Losartan/III/188/22	Not detected	Not detected	Not detected	Not detected

Table 6.5.4.2 : Method Precision

Set	Wt. of Sample (mg)	Final Dilution (ml)	Conc. of Sample (µg/ml)	Observed Results in percentage		
				Losartan/ Imp-A	*Losartan /Imp-B*	*Losartan/ Imp-C*
T1	50.43	50	1008.6	0.2390	0.2065	0.2235
T2	50.10	50	1002.0	0.2360	0.2045	0.2220
T3	50.21	50	1004.2	0.2345	0.1990	0.2205
T4	50.15	50	1003.0	0.2340	0.1990	0.2190
T5	50.05	50	1001.0	0.2340	0.1970	0.2195
T6	50.23	50	1004.6	0.2340	0.1965	0.2195
Mean				0.2352	0.2004	0.2207
RSD %				0.85%	0.79%	2.05%

[**Acceptance criteria** . RSD NMT 5.0 %]

The above results are well within the acceptance limits and indicates method precision.

6.5.5 Linearity and range :The linearity of detector (UV) response for impurities was determined by preparing and injecting solutions in the concentration range of 50-150 % of limit conc. [0.2 %(2µg/ml) each of Losartan/Imp-C, Losartan/Imp-B, Losartan/Imp-A and Losartan/Imp-C].

Solution 5, prepared as under Section 6.3.2, is used for making the linearity solutions as shown in Table 6.5.5.1

Table 6.5.5.1 : Dilutions and Concentration for Linearity Study

Sr. No.	Level % of target	Wt. of Losartan WRS. mg.	Vol. of Solution 5 ml	Final Dilution ml	Final Concentrations		
					Losartan/ Imp-A µg/ml	Losartan/ Imp-B µg/ml	Losartan/ Imp-C µg/ml
L1	50	49.84	1.0	50.0	1.008	1.044	1.003
L2	75	50.20	1.5	50.0	1.504	1.566	1.504
L3	100	50.17	2.0	50.0	2.006	2.088	2.006
L4	125	50.10	2.5	50.0	2.507	2.610	2.507
L5	150	50.02	3.0	50.0	3.008	3.132	3.008

6.5.6 Results & discussion :

The results of individual impurities is shown in Table 6.5.6.1 to 6.5.6.3

Table 6.5.6.1 : Linearity of Losartan/Imp-A

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	24872	25616	25352	25288	1.49
L2	38120	37765	38305	38063	0.72
L3	51724	51069	50615	51136	1.09
L4	61975	61982	60177	61378	1.69
L5	69625	70961	70831	70472	1.05
Slope				22721	
Intercept				3675 8	
Correlation coefficient (r^2)				0.996	

Figure-111 shows typical linearity plot for Losartan impurity A

Table 6.5.6.2 : Linearity of Losartan/Imp-B

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	25230	24823	24441	24831	1.59
L2	35835	36049	34485	35456	2.39
L3	45571	45756	45486	45604	0.30
L4	59681	59266	61596	60181	2.07
L5	70621	70483	68994	70033	1.29
Slope				22055	
Intercept				1169.4	
Correlation coefficient (r^2)				0.9962	

Figure-112 shows typical linearity plot for Losartan impurity B

Table 6.5.6.3 : Linearity of Losartan/Imp-C

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	22412	22555	22846	22604	0.98
L2	34954	34357	34041	34451	1.35
L3	49741	48800	49860	49467	1 17

L4	60329	60198	63126	61218	2.70
L5	71300	71646	71669	71538	0.29
Slope				24863	
Intercept				-2009.3	
Correlation coefficient (r^2)				0.9962	

Acceptance criteria : RSD ≤ 5 0 %
Correlation coefficient (r^2) ≥ 0.99

Figure-53 shows typical linearity plot for Losartan impurity C

6.5.7 Accuracy :The five level (70,85,100,115and 130 % of 0.2 % Losartan/Imp-A, Losartan/Imp-B and Losartan/Imp-C each) recovery study is performed.

6 3.2 are used. The Losartan (# Losartan/III/188/22) was used for recovery study.

Table 6.5.7.1: Dilutions and Concentration for recovery study

Sr No	*% of target Level*	*Wt. Of Losartan mg*	*Vol. of Solution 5 ml*	*Final Dilution ml*	*Final Concentrations*		
					Losartan/Imp-A µg/ml	*Losartan/Imp-B µg/ml*	*Losartan/Imp-C µg/ml*
R1	70	49.4	1.4	50.0	1.4112	1.4616	1.4039
R2	85	50 0	1.7	50 0	1.7136	1.7748	1.7048
R3	100	50.1	2 0	50.0	2.0160	2.0880	2.0056
R4	115	50.1	2.3	50.0	2.3184	2.4012	2.3064
R5	130	50 1	2.6	50.0	3.0240	3.1320	3 0084

Results and discussion :

Table 6.5.7.2: Recovery of Losartan/Imp-A from Losartan

Sr. No.	*Level (%)*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.4112	1.4218	100.75 %
R2	85	1.7136	1.7115	99.88 %
R3	100	2.0160	2.0142	99.91 %
R4	115	2 3184	2.3244	100.26 %
R5	130	3 0240	3.0566	101.08 %
Mean				100.38 %
% RSD				0.53 %

Table 6.5.7.3: Recovery of Losartan/Imp-B from Losartan

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. Added*
R1	70	1.4616	1.4620	100.03 %
R2	85	1.7748	1.7627	99.32 %
R3	100	2.0880	2.0540	98.37 %
R4	115	2.4012	2.3769	98.99 %
R5	130	3 1320	3 1373	100.17 %
Mean				99.36 %
% RSD				0.75 %

Table 6.5.7.4.: Recovery of Losartan/Imp-C from Losartan

Sr. No.	*Level (%)*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.4039	1.4198	101.13 %
R2	85	1.7048	1 6939	99.36 %
R3	100	2.0056	1.9673	98.09 %
R4	115	2.3064	2.2623	98.85 %
R5	130	3.0084	2 9816	99.11 %
Mean				99.30 %
% RSD				1.13 %

[**Acceptance criteria**: Recovery - 95.0 % - 105 0 %]

Figure-110 shows typical recovery chromatogram.

6.5.8 Limit of Detection and Quantitation :The limit of detection is established by injecting a solution containing about 1.0 µg/ml of Losartan/Imp-A, Losartan/Imp-B, Losartan and Losartan/Imp-C impurity, further diluting the solution and injecting consecutively and recording the detector response. Table 6.5.8.1 shows the dilutions used. Table 6.5.8.2 to 6.5.8.4 shows the detector response for each impurity.

The Solution 5 prepared as mentioned under section 6.3.2 were used

Solution A (MDL Stock Solution) :
2.0 ml each of *Solution 5* were pipetted out into a 100 ml volumetric flask and diluted to volume with diluent.

Table 6.5.8.1: Dilutions and Concentration for Limit of Detection and Limit of Quantitation Study

Level	*Vol. of MDL stock soln (ml)*	*Final Dilution (ml)*	*Concentrations of components in µg/ml*		
			Losartan/ Imp-A	*Losartan/ Imp-B*	*Losartan/ Imp-C*
D1	-	-	1.008	1.044	1.003
D2	5.0	10	0.504	0.522	0.501
D3	2.5	10	0.252	0.261	0.251
D4	1.2	10	0.121	0.120	0.125
D5	0.6	10	0.060	0.0626	0.060
D6	0.3	10	0.030	0.0313	0.030
D7	0.10	10	0.010	0.010	0.010
D8	0.10	25	0.004	0.004	0.004

Results and Discussion :

Table 6.5.8.2: Limit of Detection and Limit of Quantitation Study (Losartan/Imp-A)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.008	23356	23561	23447	23455	0.44%
D2	0.504	10682	11172	11277	11044	2 87%
D3	0.252	5533	5623	5553	5569	0.85%
D4	0.121	2721	2268	3129	2706	15.92%
D5	0.060	1244	2430	1387	1687	-
D6	0.030	-	-	-	-	-

Table 6.5.8.3: Limit of Detection and Limit of Quantitation Study (Losartan/Imp-B)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.044	26834	27953	28066	27618	2.46%
D2	0.522	11490	12363	11856	11903	3.68%
D3	0.261	6092	5118	5328	5679	14.04%
D4	0.120	2251	2856	2253	2453	-
D5	0.0626	1367	2049	1212	1543	-
D6	0.0313	-	-	-	-	-

Table 6.5.8.4: Limit of Detection and Limit of Quantitation Study (Losartan/Imp-C)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.003	23012	22893	22964	22956	0.26%
D2	0.501	9061	9375	9597	9344	2.88%
D3	0.251	3041	6386	6321	5249	36.44
D4	0.125	2879	3654	3016	3183	-
D5	0.060	1454	1558	588	1445	-
D6	0.030	--	--	--	--	--

The limit of quantitation and detection for each impurity is summarized in below table 6.5.8.5:

Table 6.5.8.4: Summary of Limit of Detection and Limit of Quantitation Study

	Limit of quantitaion		*Limit of detection*	
	(µg/ml)	*(%)*	*(µg/ml)*	*(%)*
Losartan/Imp-A	0.253	0.025	0.03	0.003
Losartan/Imp-B	0.522	0.052	0.03	0.003
Losartan/Imp-C	0.501	0.050	0.03	0.003

6.5.9 Ruggedness :

Method ruggedness is established by a sample previously analyzed for related substances is reanalysed by another analyst independently by this method and the results are compared.

Ruggedness-I
Analyst : A.L.Prasad

Batch No.	*Losartan/ Imp-A*	*Losartan/ Imp-B*	*Losartan/ Imp-C*	*Any other unknown impurity*
Losartan/III/188/ 22	Not detected	Not detected	Not detected	Not detected

Analyst : RAVI

Batch No.	Losartan/ Imp-A	Losartan/ Imp-B	Losartan/ Imp-C	Any other unknown impurity
Losartan/III/188/ 22	Not detected	Not detected	Not detected	Not detected

6.5.10 Summary and Conclusions

	Accepta -nce criteria	*Actual results*		
System suitability Resolution between Losartan/Imp-C & Losartan	NLT 5	15.22		
		Losartan/Imp-A	**Losartan/Imp-B**	**Losartan/Imp-C**
Precision Instrument - RSD of Detector Response for each impurity	≤ 5.0 %	2.56 %	1.31 %	1 98 %
Method - RSD of each Impurity %	≤ 5.0 %	0.85 %	0.79 %	2.05 %
Linearity and Range Correlation coefficient (r^2)	≥ 0.99	0.994	0.996	0.996
RSD of detector responses	≤ 5.0 %			
Accuracy Percentage recovery	95.0 % - 105.0 %	100.38 %	99.36 %	99.30 %
Minimum quantitation level		0.252µg/ml	0.522µg/ml	0.501µg/ml
RSD at MQL	≤ 5 0 %	0.85 %	3.68 %	2.88 %

The results of the study indicates that this method for related substances in Losartan is precise, accurate, linear in detector response and rugged.

6.5.11 Recommendation And Limitations

1. This method is recommended for analysing Losartan related substances .
2. Though this method shows goods precision, linearity and accuracy, especially for known impurity like Losartan/Imp-A, Imp-B and Imp-C only. Any other impurity is done by area normalisation method.
3. Observed similar response factors for all known impurities and Losartan/ final Hence area normalisation method can be adopted for regular analysis.

6.6 Assay method development : Losartan related substances method was taken to for

assaying losartan and found suitable but run time and concentration of losartan reduced for optimum results.Optimised method is given below.

6.6.1 ANALYTICAL METHOD

Reagents :

1)	Ammonium dihydrogen orthophosphate	:	AR grade
2)	Acetonitrile	.	HPLC grade
3)	Orthophosphoric acid	:	AR grade

4) Milli Q water

Buffer solution .

Transfer 8 62 g of ammonium dihydrogen orthophosphate into a 1000 ml volumetric flask. To this add 500 ml of water and swirl to dissolve. Make up the volume to 1000 ml with water Adjust the pH of the solution to 3.0 with 10% v/v orthophosphoric acid Prepare filtered and degassed mixture of buffer and acetonitrile in the proportion of (65 35)

Diluent :Prepare filtered and degassed mixture of water and acetonitrile in the proportion of (30:70).

Standard preparation :Transfer about 20 mg of accurately weighed, losartan potassium WRS into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent

Test preparation : Transfer about 20 mg, accurately weighed, sample into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent.

Instrumental conditions .

Use a suitable High Performance Liquid Chromatograph (HPLC) with the following conditions.

Column	.	HYPERSIL C18 (25cmx4.6mm) 5 µ, BDS (Shandon, U.K)
Flow rate	·	1.5 ml/min
Detector	.	UV set at 254 nm
Attenuation	·	Set appropriately
Run time	:	About 20 min.
Injection volume		20 µl

System suitability and precision: Determine the instrument precision with six injections of losartan potassium standard preparation (200 µg/ml). RSD is not more than 2.0 % and retention time of losartan potassium is about 9.0 min. The tailing factor of losartan potassium peak should not be more than 1.6 and number of theoretical plates should not be less than 7000.

Procedure

(1) Set up chromatographic system as described under instrumental conditions

(2) Inject equal volume of the standard and test preparation in duplicate into the chromatograph and record the chromatograms

Calculation:

$$\%\ \text{Assay of losartan potassium (on dried basis)} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{DS} \times \frac{P \times 100}{100 - Q}$$

Where :

AT	=	Average area count of losartan potassium peak in test preparation
AS	=	Average area count of losartan potassium peak in standard preparation
WT	=	Weight of sample in mg
WS	=	Weight of losartan potassium standard in mg
DS	=	Dilution factor of standard preparation
DT	=	Dilution factor of test preparation
P	=	% purity of losartan potassium standard (as is basis)
Q	=	Loss on drying at 105°C for 3 hrs.

Acceptance criteria : 98 to 102% (on dried basis)

6.6.2 Validation protocol

Purpose :Purpose of this document is to generate supporting validation data for assay of losartan potassium by the HPLC method The validation data to demonstrate its specificity, stability indicating nature, accuracy, precision and linearity is described in the following sections.

General :

(a) For these validation studies, following equipments were used for all experiments unless specified otherwise.

- Shimadzu LC-10AS solvent delivery pump with SIL-10A Autoinjector
- Shimadzu SPD-10A UV detector
- CLASS LC-10 computer software
- HYPERSIL C18 (25cm x 4.6mm) 5 µ, BDS. (Shandon, UK)

(b) Reagents :

1)Ammonium dihydrogen orthophosphate : AR grade (S.D Fine Chem)

2)Acetonitrile : HPLC grade (Ranbaxy)

3)Orthophosphoric acid · AR grade (S.D. Fine Chem)

4) Milli Q water

(c) Working standard and sample :

1)Losartan potassium WRS · # losartan potassium /III/188/22

2)Losartan potassium sample : # losartan potassium /III/188/13

(d) System suitability parameters, i e. RSD of six replicate injections.

(e) The following limits are considered for acceptance criteria :

1 **System suitability and reproducibility** The relative standard deviation of response for losartan potassium standard solution with six replicate injection is not more than 2.0 % and retention time of losartan potassium is about 9.0 min

2 **Method Precision** .The relative standard deviation of the 6 sets of assay from the same losartan potassium sample is not be more than 2 0 %

3.**Specificity (degradation study)** :The peak purity index of losartan potassium peak as measured with a photodiode array detector from each degradation study is ≥ 0.999.

4 **Linearity and range** .The plot of detector responses for a concentration range of 50 to 150 % of the assay level (200 µg/ml) is linear and the regression correlation coefficient is ≥ 0 999.

5.**Accuracy (Recovery study)** :In the 5 level (70, 85, 100, 115 and 130 % of assay conc.) recovery study of drug from the sample, the recovery is between 98.0 and 102.0%. The relative standard deviation of recovery is not more than 2.0%.

6.**Ruggedness** :The assay of a sample carried out by deliberately changing some of the parameters should not differ by more than ± 0.5 %.

6.6.3 Experimental Data

6.6.3.1 System suitability and reproducibility :

The reproducibility of injection was checked by injecting the losartan potassium standard preparation 200.2 µg/ml (20.02 mg was dissolved in 100 ml diluent) six times. Individual area counts and % RSD values are shown in Table - 6.6.3.1 below :

[**Acceptance criteria** : RSD NMT 2.0 %]

Table - 6.6.3.1 : Results of reproducibility study

Injection	RT (min.)	Losartan potassium (200.02 µg/ml)
1	9.04	4409189
2	9.04	4413758
3	9.04	4410190
4	9.03	4414188
5	9.00	4405955
6	8.95	4393600
Mean		4407813
Standard deviation		± 7602.50
Relative standard deviation		0.17 %

The typical system suitability/ System precision chromatogram is shown in Figure-115

6.6.4 Method precision :

Six solutions of losartan potassium sample were prepared on same day for analysis by the HPLC method. Results of these analysis are shown in Table 6.6.4 1 .

Figure-117 shows typical method precision chromatogram for Losartan.

Table - 6.6.4.1 : Results of method precision

	Final conc. (µg/ml)	Area of individual injection				% Assay of losartan potassium (as is basis)
		1	*2*	*3*	*Mean Area*	
losartan potassium /III/ 88/22 (WRS)	200.2	4383074	4380760	4382573	4382136	
losartan potassium /III/188/13 (sample)						
1	199.3	4374761	4369497	4369645	4371301	99.70
2	203 6	4450491	4450215	4455058	4451921	99.04
3	201.9	4381863	4404013	4421981	4402619	99.32
4	201.0	4337318	4348744	4373200	4353087	99.57
5	199 9	4390586	4385211	4376797	4384198	99.69
6	203.4	4459999	4457252	4448665	4455305	99.57
Mean						99.54
Standard deviation						± 0.155
Relative standard deviation						0.156%

6.6.5 Specificity of the method :

Specificity of the method was established by demonstrating no interference from degradation products. This was demonstrated by carrying out forced degradation of the sample by adding 2.0M HCl, 2.0M NaOH, 30 % H_2O_2, 0.1M $KMNO_4$ separately. The sample was also subjected to exposure under UV light for 48 hrs, sunlight exposure for 16 hrs. and heat degradation by heating it in a oven at 105°C for 3 hrs. The samples were prepared as given below and were injected into HPLC with a Shimadzu SPD M-10A photodiode array detector. The chromatograms were recorded upto 20 min. to check for degraded peaks. In each case peak purity index of losartan potassium was determined to examine interference from degradation products.

6.6.5.1 Acid degradation :20.3 mg losartan potassium was transferred into a 100 ml volumetric flask containing 5 ml water Swirled to disperse. To this was added 5 ml of 2.0M HCl. The sample was kept for 48 hrs. After that, pH of the solution was adjusted to 7.0 with 2.0M NaOH and volume was made up with diluent. This solution was injected into the HPLC. Peak purity of losartan potassium peak as determined by diode array

detector was > 0.999. No significant degradation was found as determined by comparing area counts with the standard.

6.6.5.2 Alkali degradation :20.6 mg losartan potassium was transferred into a 100 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 5 ml of 2.0M NaOH. The sample was kept for 48 hrs. After that, pH of the solution was adjusted to 7.0 with 2.0M HCl and volume was made up with diluent. This solution was injected into the HPLC Peak purity of losartan potassium peak as determined by diode array detector was > 0.999. However, no significant degradation was found in alkali.

6.6.5.3 Peroxide degradation :20.3 mg losartan potassium was transferred into a 100 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 1 ml of 30 % H_2O_2. The sample was kept for 48 hrs. After that, volume was made with diluent. This solution was injected into the HPLC Peak purity of losartan potassium peak as determined by diode array detector was > 0.999. Degradation was found as determined by comparing area counts with the standard. However, the degraded products do not interfere with losartan potassium peak as shown by peak purity index.

6.6.5.4 Degradation with $KMnO_4$:20.2 mg losartan potassium was transferred to 100 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 1 ml 0.1M $KMnO_4$. The sample was kept for 48 hrs. After that the volumes were made with diluent and filtered. This solutions were injected into the HPLC. Peak purity of losartan potassium as determined by diode array detector was > 0.999. However significant degradation was observed. However, the degraded products do not interfere with losartan potassium peak as shown by peak purity index.

6.6.5.5 Degradation under UV light :19.7 mg losartan potassium (previously kept under UV light at 254 nm for 24 hrs.) was transferred into a 50 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with diluent. This solution was injected into the HPLC. Peak purity of losartan potassium peak as determined by diode array detector was > 0.999. No significant degradation was observed.

6.6.5.6 Sunlight degradation :19.5 mg losartan potassium was transferred to 50 ml volumetric flask. Expose to sunlight for 24 hrs. Then 5 ml water was added. Swirled to disperse. After that volume was made with diluent, This solution was injected into the

HPLC. Peak purity of losartan potassium as determined by diode array detector was > 0.999. No significant degradation was observed.

6.6.5.7 In oven at 105°C for 3 hr. degradation :19.8 mg losartan potassium was transferred into a 50 ml beaker. The sample was heated in a oven at 105°C for 3 hr. After cooling sample was transferred into 100 ml volumetric flask and volume was made with diluent. This solution was injected into the HPLC. Peak purity of losartan potassium as determined by diode array detector was > 0.999. No significant degradation was observed.

Table - 6.6.5.1: Specificity of the method

Degradation	Condition	Retention time (min.)	Peak purity	Acceptance criteria (Peak purity)
Acid degradation	20.3 mg losartan potassium & 5 ml 2.0M HCl kept for 48 hrs.	9.1 9.1	Up 0.9995 Dn. 0.9994 Up 0.9994 Dn. 0.9993	NLT 0.999
Alkali degradation	20.6 mg losartan potassium & 5 ml 2.0M NaOH kept for 48 hrs.	9.1 9.1	Up 0.9992 Dn. 0.9991 Up 0.9993 Dn. 0.9991	NLT 0.999
Peroxide degradation	20.3mg losartan potassium & 1ml of 30% H_2O_2 water kept for 48 hrs.	9 1 9.1	Up 0.9994 Dn. 0.9992 Up 0.9994 Dn. 0.9992	NLT 0.999
$KMnO_4$ degradation	20.2 mg losartan potassium & 1 ml 0.1M $KMnO_4$ kept for 48 hrs.	9.1 9.1	Up 0.9999 Dn. 0.9999 Up 0.9998 Dn. 0.9999	NLT 0.999
Sunlight degradation	19.5 mg losartan potassium kept for 16 hrs. for sunlight exposure	9.1 9.1	Up 0.9995 Dn. 0.9995 Up 0.9995 Dn. 0.9995	NLT 0.999
UV degradation	19.7 mg losartan potassium kept under UV light at 254 nm for 48 hrs.	9.1 9.1	Up 0.9994 Dn. 0.9993 Up 0.9995 Dn. 0.9994	NLT 0.999
Oven (at 105°C) degradation	19.8 mg losartan potassium and heated in oven at 105°C for 3 hrs.	9.1 9.1	Up 0.9994 Dn. 0.9992 Up 0.9994 Dn. 0.9992	NLT 0.999

6.6.6 Linearity and range :

(i) The linearity of detector (UV) response for losartan potassium was determined by preparing and injecting solutions in the concentration range of 500 - 1500 µg/ml (50-150 % of assay conc.) for losartan potassium standard. Figure-119 shows linearity chromatogram , Figure-120 shows linearity graphs of losartan potassium and Table - 6.6.6.1 shows values of slope, intercept and correlation coefficient of linear plot.

Stock solution : 251.2 mg of drug was dissolved in 100 ml of diluent (2512 µg/ml)

Table – 6.6.6.1: Linearity study

Vol. of stock solution (ml)	Final dilution (ml)	Final conc. (µg/ml)	Area counts			Mean Area counts (n = 3)	RSD (%)
			1	2	3		
1.0	25	100.48	2239500	2222521	2216408	2226143	0.54
1.5	25	150 72	3382369	3404157	3417597	3401374	0 52
2.0	25	200.96	4380608	4416503	4418095	4405069	0.48
2.5	25	251.20	5516773	5545270	5547667	5536570	0 31
3.0	25	301.44	6590050	6622610	6618458	6610373	0.27
Slope						74443	
Intercept						21703	
Correlation coefficient (r^2)						0.9996	

[**Acceptance criteria** : Correlation coefficient (r^2) - NLT 0.999]

[**Acceptance criteria** : RSD of triplicate injections - NMT 2.0 %]

6.6.7 Accuracy (Recovery study) :

Recovery of drug from losartan potassium sample : The recovery of added drug from losartan potassium sample was performed at 70-130 % of assay concentration level (200 µg/ml). Recovery at each level was performed in triplicate.

Standard stock solution : 251.2 mg/100 ml with diluent (2512 µg/ml)

Sample stock solution : 250.9 mg/100 ml with diluent (2509 µg/ml)

Table -6.6.7.1 : Recovery study (details of dilutions)

Recovery level (%)	Amount of sample stock solution taken (ml)	Amount of standard stock solution added (ml)	Final dilution (ml)	Final conc. (µg)
70	2.0	1.4	25	341.39
85	2.0	1.7	25	371.54
100	2.0	2.0	25	401.68
115	2.0	2.3	25	431.82
130	2.0	2.6	25	461.97

Table - 6.6.7.2: Results of recovery study

Recovery level (200 µg/ml) (%)	Amount of losartan potassium sample (µg)	Amount of losartan potassium added (µg)	Amount of losartan potassium found (µg)	% Recovery = Amt found ---------- x 100 Amt. added
70	200.72	140.67	140.59	99.94
85	200.72	170.82	168.92	98.89
100	200.72	200.96	199.43	99.24
115	200.72	231.10	233.41	100.10
130	200.72	261.25	259.76	99.43
Average				99.70
% RSD				0.82%

[**Acceptance criteria**: 98.0 % - 102.0 %]

[**Acceptance criteria** : RSD for recovery levels - NMT 2 0 %]

6.6.8 Ruggedness : Method ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as instrument and analyst.

Parameter	:	**Normal condition**	**Changed condition**
Column	:	Hypersil C18 25cm x 4.6mm, 5 µ, BDS (Shandon, U.K.) S.No. · 1-16625	Hypersil C18 25cm x 4.6mm, 5 µ, ODS (Shandon, U.K) S No. : 1-17245

Flow rate	1.5 ml/min.		1.4 ml / min.
Mobile phase :	Buffer Acetonitrile	: 65 : 35	Buffer Acetonitrile	: 70 : 30
Pump :	LC-10AT		Waters - 510
Detector	SPD - 10A		Waters - 486
Software	Shimadzu Class LC-10		C-R6A Chromatopak Recorder
Injection volume	20µl		20µl
Analyst :	YDC		NRP
Assay	99.53%		99.30%

Conclusion :No significant change in assay was found even under the deliberately change conditions. Thus the ruggedness of the method is established

Figure-118 shows typical chromatogram for ruggedness experiment

6.6.9 Stability in analytical solution : Solution of standard (200.2 µg/ml) was injected at different time intervals and peak areas were recorded.

Table – 6.6.9.1: Stability of drug in analytical solution

Time (hrs.)	losartan potassium (peak area)
0.0	4385612
7.5	4397127
11.0	4380350
Mean	4387696
Relative standard deviation	0.196 %

No significant change was observed for losartan potassium peak areas upto 11.0 hrs.

6.6.10 Summary and conclusions :

Table - 6.6.10.1 : Validation results

Sr. No.	Acceptance criteria	Observed value	Limit
1.	System suitability and reproducibility	RSD = 0.17 %	RSD : NMT 2 0 %
2.	Accuracy	Recovery = 99.698 % RSD = 0.82 %	Recovery : 98.0 % - 102.0 % RSD : NMT 2.0 %
3	Linearity range	Correlation coefficient (r^2) = 0.9996	Correlation coefficient (r^2) NLT 0.999
4.	Precision	RSD : 0 156 %	RSD . NMT 2.0 %
5.	Ruggedness	Variation = - 0 23 %	Variation : ± 0.5 %

All these observations indicate that this method for assay of losartan potassium is specific, accurate, precise and is also stability indicating.