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Development and Validation of Reverse Phase Liquid Chromatography Method for Estimation of Losartan in Bulk Drug.

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Abstract: *A simple, precise and reversed phase liquid chromatographic method was developed and it is validated for estimation of losartan in bulk drug. Losartan is use for treatment of hypertension. The separation was achieved on Acquity BEH C18 1.7µ, (2.1 X 100) mm, analytical column with mobile phase consisted of buffer (adjust pH 3.0 of water with dilute formic acid) : Acetonitrile (50:50 v/v) at isocratic flow of 0.3ml/min with UV detection wavelength was at 230 nm. The method was successfully validated in accordance to ICH guidelines for accuracy, precision, specificity, linearity. The linear regression analysis data for calibration plots showed good linear relationship in the concentration range 25-75µg/mL for losartan. The % Recovery/Accuracy was within the range between 98% and 102%. The percentage RSD for precision method was found to be less than 2%. The method was successfully applied for routine analysis of losartan in bulk samples.*
Keywords: *Assay, ICH, Isocratic, Liquid Chromatography, validation*

I. Introduction

Losartan is an angiotensin II receptor antagonist drug to be marketed. It is chemically described as a (2-butyl-4-chloro-1-{[2'-(1H-testrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazol-5-yl)methanol. Its empirical formula is $C_{22}H_{23}ClN_6O$, and the chemical structural is shown in fig-I. Losartan is a white crystalline powder with a molecular weight of 422.9. It is orally active non-peptide angiotensin II (Type AT1) receptor antagonists, losartan is indicated for the treatment of hypertension. It may also delay progression of diabetic nephropathy, and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes.[1-4]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The intended use of analytical methods is to assess product quality and validation is the process of generating experimental data that provides evidence that the performance of an analytical method is adequate for reliably assessing product quality.[5-8] Method validation acceptance criteria necessarily reflect what we believe is "adequate performance." The validation procedure has been performed by using ultra performance liquid chromatography. The method has been validated for linearity, precision (system repeatability, method repeatability, and method reproducibility), accuracy, range, specificity, and solution stability.[9-11] Literature survey indicates that there is no RP-LC short run time method available for assay determination of Losartan,[12-15] thus we aimed to develop it. Liquid chromatography is a new technique used in analytical chemistry for separating and analyzing substances. Chromatography depends on the distribution of the mixture between two phases, one of them is fixed and is called Stationary phase while the other is not fixed and is called the Mobile phase. The mixture is dissolved in the moving phase and passed over a stationary phase. When a mixture of components is introduced in to a LC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.[15-19] Use this separation technique and developed new very short run time method and validate as per USP and ICH guidelines.

II. Experimental

2.1 Methods and material

2.1.1 Chemicals and reagents Pure samples of losartan were obtained as gift. LC grade Acetonitrile, and formic acid were purchased from Merck Company Mumbai. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

2.1.2 LC instrumentation and conditions The analysis of the drug was carried out on a Waters Acquity UPLC (Ultra performance liquid chromatography) Binary Gradient System, 10µL injection loop column with auto injector. Column compartment having temperature control and for detection Ultraviolet Detector was employed throughout the analysis.

2.1.3 Chromatographic conditions Acquity BEH 1.7µ, (2.1 X 100) mm, Make: Waters, analytical column was used for separation. Column temperature was 40°C. Mobile phase consisted of buffer (Adjust pH 3.0 of water with dilute formic acid) : Acetonitrile (50:50 v/v). Mix well and filter through 0.22µm filter. The mobile phase was prepared freshly and degassed by sonicating for 5min before use. Water : Methanol (90:10 v/v) was used as diluent. The analysis was done on isocratic flow of 0.3ml/min with UV detection wavelength was performed at 230 nm at 15°C temperature using 3.0 µL injection volumes with auto injector.

2.1.4 Stock and working standard solutions Accurately weigh and transfer 25mg of losartan working standard into a 50mL volumetric flask, add about 30mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain solution concentration was 50µg/ml.

2.1.5 Assay of losartan sample Accurately weigh and transfer equivalent to 25mg of losartan sample into a 50mL volumetric flask, add about 30mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain solution concentration was 50µg/ml. This solution was injected into LC system. For the determination, Peak area of losartan was measured. Calculate % Losartan by following formulae.

$$\text{LOSARTAN (\%)} = \frac{A_1 \times C_2}{A_2 \times C_1} \times P \qquad \text{“Equation 1”}$$

Where,

A_1 = Area of Losartan in sample
A_2 = Area of Losartan in standard
C_1 = Concentration of Losartan in sample (mg/ml)
C_2 = Concentration of Losartan in Standard (mg/ml)
P = Potency of Standard

2.2 Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability.

2.2.1 Linearity The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares).
Five standard solutions of Losartan were prepared from three stocks in the range of 50% to 150 % of the nominal concentration and injected once. Linearity regression analysis demonstrated the acceptability of the method for quantitative determination of Losartan over the concentration range of about 25ppm to 75ppm of the nominal concentration. Linearity graph was shown in fig-2 and slope, intercept correlation factor and Regression equation were shown in table-I.

2.2.2 Precision The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration, or a minimum of six determinations at 100% of the test concentration).

2.2.2.1 System Repeatability Standard solution is prepared 50ppm were injected in six times and RSD of areas and retention times were calculated. The percentage RSD of areas was less than 1.0%. Result are presented in table-2

2.2.2.2 Method repeatability Six preparation of Losartan sample was analyzed from sample preparation to final results by the same analyst and the percentage RSD of obtained results was less than 2% and obtained result were within given range 100 ± 2. Result are presented in table-3

2.2.3 System Reproducibility Three Losartan sample are analysed by this method in duplicate preparation and obtain result are in table-4

2.2.4 Accuracy It is defined as the closeness of test results obtained by the method to the true value. It may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method. The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). The three different concentrations of Losartan standard solutions were determined from three replicate injections, using the linear regression lines (linearity section). The deviations of the obtained results (expressed as percentage accuracy) were calculated from the true values were presented in table-5.

Each deviation should be calculated according to the following formula:

$$d = \frac{Calculated - Added}{Added} * 100$$ "Equation 2"

The average deviations from true value are less than 2.0 %.

2.2.5 Specificity The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients The specificity of the method was verified by testing the blank, standard and sample (un-spiked and spiked), determined the resolution factors between analyte peak (Losartan) and the nearest peak. Sample of Losartan sample spiked with Losartan diamide impurity.

No significant interfering peak appeared in the blank, System suitability and standard chromatogram at the retention times of the analyte peaks.

2.2.6 Range The range obtained from Linearity, Precision and Accuracy is summarized - ibesartan-25ppm to 75ppm (50% to 150% of nominal sample concentration)

III. Figures And Tables

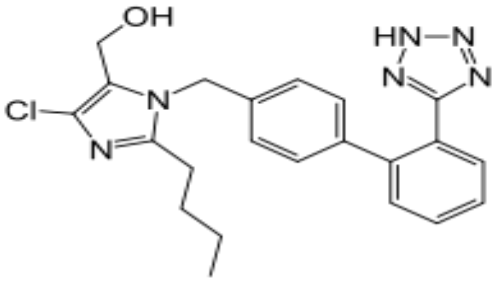


Fig-I losartan chemical structure

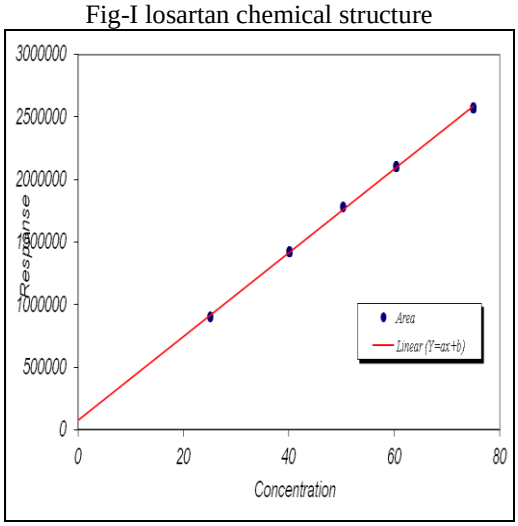


Fig-2 linearity graph

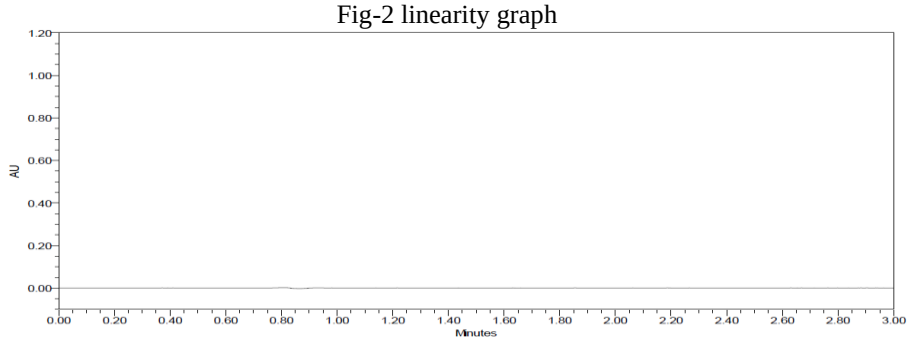
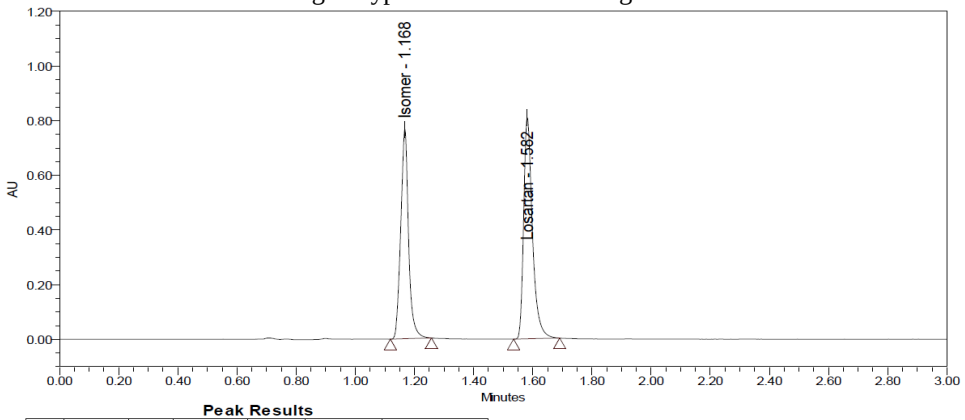


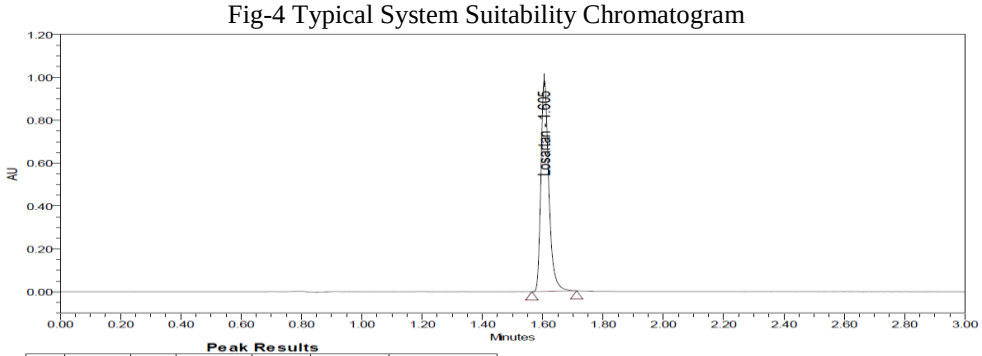
Fig-3 Typical Blank Chromatogram



Peak Results

	Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Isomer	1.168	1402147	45.81	1.08	9704
2	Losartan	1.582	1658641	54.19	1.44	14963
Sum			3060788.0			

Fig-4 Typical System Suitability Chromatogram



Peak Results

	Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Losartan	1.605	1782188	100.00	1.36	19732
Sum			1782188.1			

Fig-5 Typical Standard Chromatogram (50ppm)

Losartan Concentration(ppm)	Losartan Area
Slope	33447
Intercept	78179
Correlation factor	0.999

Table-1 Linearity Data

System Repeatability	
Concentration (ppm)	Area
50.60	1782188
50.60	1785896
50.60	1780652
50.60	1777835
50.60	1775957
50.60	1724026
Average	1771092
STDEV	23315.308
%RSD	1.316

Table-2 System Repeatability data

Method Repeatability			
Concentration (ppm)	Retention time (min)	Area	% Losartan
50.28	1.590	1769471	100.6
50.22	1.589	1766449	100.5
50.34	1.584	1769776	100.5
50.26	1.584	176821	100.5
50.30	1.585	1766448	100.4
50.22	1.587	1764657	100.4
Average	1.587		100.5
STDEV	0.003		0.085
%RSD	0.163		0.084

Table-3 Method Repeatability data

Method Reproducibility				
S No.	Concentration (ppm)	Area	% Losartan	% Losartan Average
Sample-I Pre-I	50.72	1765089	99.61	99.4
Sample-I Pre-II	49.62	1719240	99.14	
Sample-II Pre-I	50.00	1728259	98.92	99.2
Sample-II Pre-II	50.94	1768855	99.51	
Sample-III Pre-I	50.98	1772343	99.65	99.6
Sample-III Pre-II	51.12	1774123	99.48	

Table-4 Method Reproducibility data

Injection No	Level	Concentration (ppm)	Area	Calculated concentration (ppm)	Accuracy (%)
1	80 %	40.10	1417847	40.05	99.90
2			1420881	40..14	100.12
3			1411704	39.87	99.44
Average			1416811		99.8
1	100 %	50.28	1782188	50.95	101.33
2			1785896	51.06	101.55
3			1769776	50.58	100.59
Average			1779287		101.2
1	120 %	59..98	2103248	60.55	100.97
2			2100269	60.46	99.97
3			2106833	60.65	100.29
Average			2103450		101.1

Table-5 Accuracy test data of Losartan

IV. Conclusion

The finally method developed and validation demonstrated that The Method "Determination of Losartan Assay content by liquid Chromatography as per united state of pharmacopeia (USP) and international conference on harmonisation (ICH) guideline. it is selective, precise, linear, and accurate for performing the determination over the required concentration ranges of 50 to150 % of Losartan nominal sample concentration with very short analysis time that is 3 minutes.

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