International Journal of Multidisciplinary Research and Growth Evaluation.

RP-HPLC method development and validation for the simultaneous determination of losartan and hydrochlorothiazide in bulk and pharmaceutical formulations

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 02
March-April 2023
Received: 21-03-2023;
Accepted: 09-04-2023
Page No: 547-555

Abstract
A novel, simple, specific, accurate, precise method development and validated for the simultaneous estimation of Losartan and Hydrochlorothiazide by RP-HPLC in bulk and marketed formulation. A High Performance Liquid Chromatography WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector with Altima C18 (4.6×150mm, 5.0 µm) column, with mobile phase composition of Methanol and TEA Buffer pH 4.5: Acetonitrile taken in the ratio of 50:25:25% v/v/v was used. The flow rate of 1.0 ml min-1 and effluent was detected at 225 nm. The retention times of Losartan and Hydrochlorothiazide was 2.102 and 3.537minutes. Linearity was observed over concentration range of 5-25µg/ml and 12.5µg/ml for Losartan and Hydrochlorothiazide respectively. The Limit of detection and limit of quantification of Losartan and Hydrochlorothiazide was found to be 0.2ng ml-1 and 2.3ngml-1 & 0.8ng ml-1 and 7.04ngml-1 respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98% to 102%. Then method was validated in terms of linearity, accuracy, precision, (repeatability, intermediate precision) specificity (by assay), robustness and system suitability. Thus, the validated method is can be successfully applied to routine analysis for regulate the quality. It also should be used for analytical research purpose.

DOI: https://doi.org/10.54660/.IJMRGE.2023.4.2.547-555

Keywords: Losartan and Hydrochlorothiazide, RP-HPLC, Method Development, Validation

Introduction

Losartan is a non-peptide angiotensin II antagonist with antihypertensive activity. Upon administration, losartan [1] and its active metabolite selectively and competitively blocks the binding of angiotensin II to the angiotensin I (AT1) receptor. This blocks the vasoconstricting and aldosterone-secreting actions of angiotensin II, leading to a decrease in blood pressure. Angiotensin II, formed from angiotensin I by angiotensin-converting enzyme (ACE), stimulates the adrenal cortex to synthesize and secrete aldosterone, which decreases the excretion of sodium and increases the excretion of potassium. Angiotensin II also acts as a vasoconstrictor in vascular smooth muscle. Losartan [2] is indicated to treat hypertension in patients older than 6 years, reduce the risk of stroke in patients with hypertension and left ventricular hypertrophy (though this benefit may not extend to patients with African heritage), and to treat diabetic nephropathy with elevated serum creatinine and proteinuria in patients with type 2 diabetes and hypertension.3 Losartan [3] with hydrochlorothiazide is indicated to treat hypertension and to reduce the risk of stroke in patients with hypertension and left ventricular hypertrophy (though this benefit may not extend to patients with African heritage). Losartan is an angiotensin II receptor blocker used to treat hypertension, diabetic nephropathy, and to reduce the risk of stroke. Losartan has a long duration of action as it is given once daily. Patients taking losartan should be regularly monitored for hypotension, renal function, and potassium levels. The IUPAC Name of Losartan is [2-butyl-5-chloro-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazol-4-yl] methanol. The Chemical Structure of Losartan is as following.

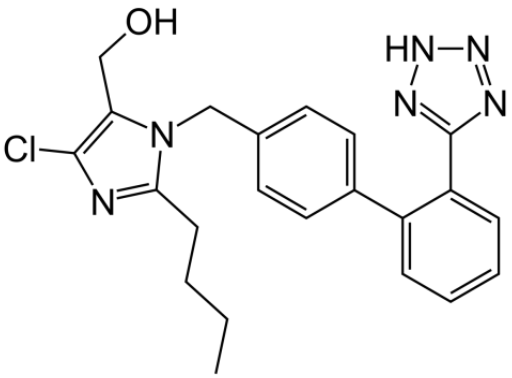

Fig 1: Chemical Structure of Losartan

Hydrochlorothiazide is the most commonly prescribed thiazide diuretic. It is indicated to treat edema and hypertension. Hydrochlorothiazide [4] use is common but declining in favour of angiotensin converting enzyme inhibitors. Many combination products are available containing hydrochlorothiazide and angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. Hydrochlorothiazide [5] is a short acting thiazide diuretic. Hydrochlorothiazide (HCTZ) is widely used to treat hypertension and edema. This agent's metabolite appears to preferentially bind to and accumulate in red blood cells. This agent is primarily excreted by the kidneys. Hydrochlorothiazide is indicated alone or in combination for the management of edema associated with congestive heart failure, hepatic cirrhosis, nephrotic syndrome, acute glomerulonephritis, chronic renal failure, and corticosteroid and estrogen therapy. Hydrochlorothiazide is also indicated alone or in combination for the management of hypertension. Hydrochlorothiazide[6] prevents the reabsorption of sodium and water from the distal convoluted tubule, allowing for the increased elimination of water in the urine. Hydrochlorothiazide has a wide therapeutic window as dosing is individualized and can range from 25-100mg. Hydrochlorothiazide should be used with caution in patients with reduced kidney or liver function. Hydrochlorothiazide is transported from the circulation into epithelial cells of the distal convoluted tubule by the organic anion transporters OAT1, OAT3, and OAT4. From these cells, hydrochlorothiazide is transported to the lumen of the tubule by multidrug resistance associated protein 4 (MRP4). Normally, sodium is reabsorbed into epithelial cells of the distal convoluted tubule and pumped into the basolateral interstitium by a sodium-potassium ATPase, creating a concentration gradient between the epithelial cell and the distal convoluted tubule that promotes the reabsorption of water. Hydrochlorothiazide acts on the proximal region of the distal convoluted tubule, inhibiting reabsorption by the sodium-chloride symporter, also known as Solute Carrier Family 12 Member 3 (SLC12A3). Inhibition of SLC12A3 reduces the magnitude of the concentration gradient between the epithelial cell and distal convoluted tubule, reducing the reabsorption of water. The IUPAC Name of Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1λ 6, 2, 4-benzothiadiazine-7-sulfonamide. The Chemical Structure of Hydrochlorothiazide is as following page.

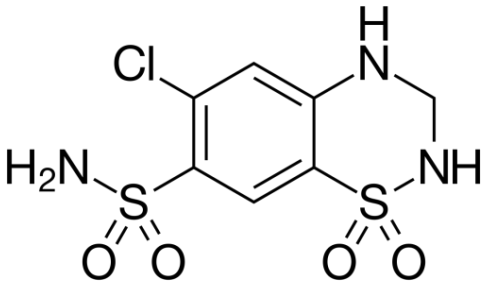

Fig 2: Chemical Structure of Hydrochlorothiazide

Materials and Methods

Table 1: Instruments Used

S. No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used

Table 2: Chemicals Used

S. No.	Chemical	Brand names
1	Losartan	Local Market
2	Hydrochlorothiazide	Local Market
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development

Trails for the method development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Losartan and Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions[7] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [35].

Mobile Phase Optimization

Initially the mobile phase[8] tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v

respectively.

Optimization of Column

The method was performed with various columns [9] like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Method Validation

Preparation of Buffer and Mobile Phase

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Filter and sonicate the solution by vaccum filtration[10] and ultrasonication.

Preparation of Mobile Phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent [11].

Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.1ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

The standard solution[12] was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Losartan and Hydrochlorothiazide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay[13] by using formula:

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (5 ppm of Losartan & 12.5ppm of Hydrochlorothiazide)

Pipette out 0.05ml of Losartan and 0.125ml of Hydrochlorothiazide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Losartan& 25ppm of Hydrochlorothiazide)

Pipette out 0.1ml of Losartan and 0.25ml of Hydrochlorothiazide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 ppm of Losartan& 37.5ppm of Hydrochlorothiazide)

Pipette out 0.15 ml of Losartan and 0.375ml of Hydrochlorothiazide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Losartan& 50ppm of Hydrochlorothiazide)

Pipette out 0.2 ml of Losartan and 0.5ml of Hydrochlorothiazide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Losartan& 62.5ppm of Hydrochlorothiazide)

Pipette out 0.25ml of Losartan and 0.625ml of Hydrochlorothiazide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system [14] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [15].

Precision

Repeatability

Preparation of Losartan and Hydrochlorothiazide

Product Solution for Precision

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [16].

Intermediate Precision

To evaluate the intermediate precision [17] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD[18] for the area of Six replicate injections was found to be within the specified limits.

Day 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the above Losartan and 0.187ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.225ml of Losartan and 0.56ml of Hydrochlorothiazide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions [19]. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Losartan and Hydrochlorothiazide and calculate the individual recovery [20] and mean recovery values.

Robustness

The analysis was performed in different conditions[21] to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Losartan and 10mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Losartan and 0.375ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 40: 40:20, 60:10:30 instead (50:25:25), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Results and Discussion

Method Development

Optimised Chromatogram Condition

Mobile phase	:	Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)
Column	:	Altima C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	225 nm
Column temp	:	40ºC
Injection Volume	:	10 µl
Run time	:	7 minutes

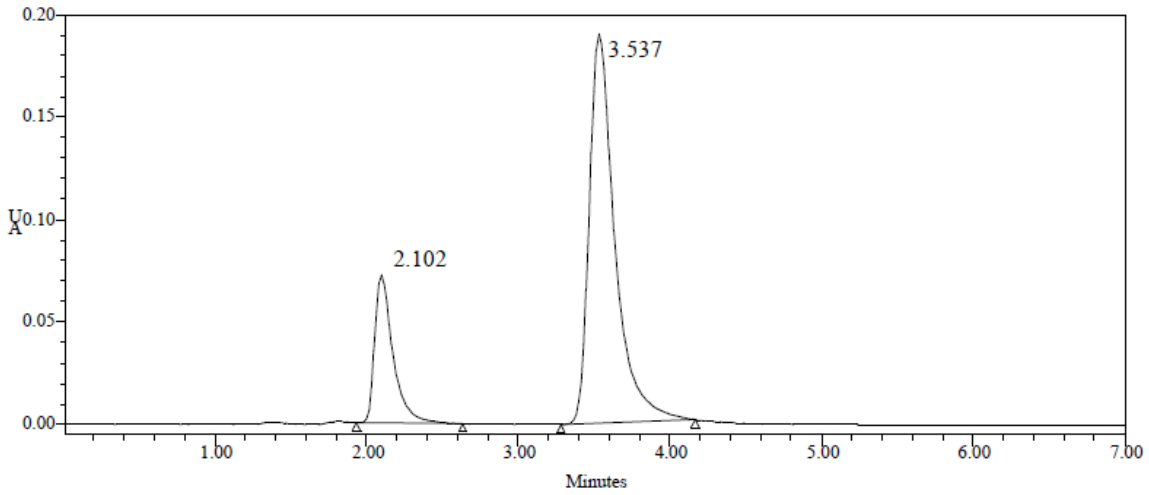

Fig 3: Optimized Chromatographic Condition

Observation: From the above chromatogram it was observed that the Losartan and Hydrochlorothiazide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimised trial.

Validation of Analytical Method

System Suitability

Before performing the main analysis, the system suitability[22] was evaluated. For this purpose, various parameters were calculated as per their standard procedure e.g. retention time (for bulk and tablet of Losartan and Hydrochlorothiazide), theoretical plates number of the column (for column efficiency), tailing factor, relative standard deviation of peak area and retention time. The Table 3 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000. The tailing factor was also within range. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 5 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suitable for routine analysis.

Table 3: Results of System Suitability for Losartan

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Losartan	2.117	608452	71498	5643	1.9
2	Losartan	2.118	606820	126412	5432	1.6
3	Losartan	2.116	608452	126471	5123	1.6
4	Losartan	2.109	595267	129859	5207	1.7
5	Losartan	2.102	596608	124691	5481	1.6
Mean			**603119.8**			
Std. Dev			**6607.31**			
% RSD			**1.09**			

Table 4: Results of System Suitability for Hydrochlorothiazide

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.547	2234724	188631	5043	1.2	2.07
2	Hydrochlorothiazide	3.539	2240080	2614821	5432	1.4	2.05
3	Hydrochlorothiazide	3.547	2234724	2321451	5987	1.5	2.0
4	Hydrochlorothiazide	3.565	2204466	2324710	5845	1.6	2.01
5	Hydrochlorothiazide	3.537	2209574	2531247	5371	1.6	2.01
Mean			**2224714**				
Std. Dev			**16399.05**				
% RSD			**0.73**				

Specificity

The ICH documents define specificity [23] 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. Analytical method was tested for specificity to measure accurately quantitates Losartan and Hydrochlorothiazide in drug product.

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity[24] of Losartan and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.6%.

Linearity

Linearity may be defined as the capacity of an analytical method to produce outcomes that are directly related to the concentration of an analyte. Linearity [25] was determined by taking 50 mg Losartan, and 12.5 mg of Hydrochlorothiazide working standards both the standards were transferred into a 100 mL volumetric flask and 7 mL of diluent was added and sonicated for 10 min and the volume was adjusted with the same solvent. The linearity results for Losartan & Hydrochlorothiazide are shown in Tables 5 & 6 and Fig. 4 & 5.

Chromatographic Data for Linearity Study

Table 5: Linearity Data of Losartan

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	205035
66.6	10	381239
100	15	561128
133.3	20	740162
166.6	25	909922

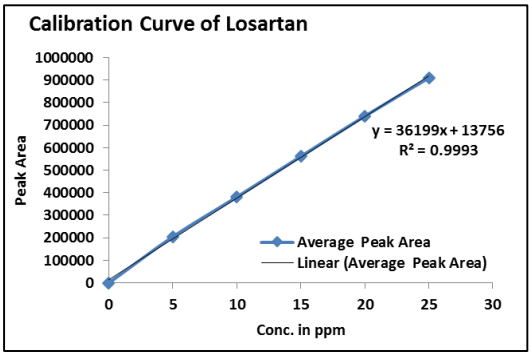

Fig 4: Calibration Graph for Losartan

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Losartan is a straight line.

Y = mx + c
Slope (m) = 36199
Intercept (c) = 13756
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient [26] is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 13756. These values meet the validation criteria.

Table 6: Linearity Data of Hydrochlorothiazide

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	12.5	757881
66	12.5	757881
100	25	1458941
133	37.5	2132457
166	50	2901811

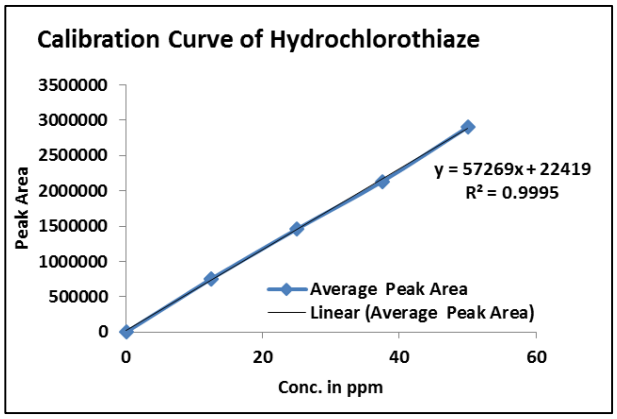

Fig 5: Calibration Graph for Hydrochlorothiazide

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Hydrochlorothiazide is a straight line.

Y = mx + c
Slope (m) = 56304
Intercept (c) = 33265
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 33265. These values meet the validation criteria.

Precision

The precision [27] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions [28]. Recorded the peak areas and calculated % RSD.

Table 7: Results of Repeatability for Losartan

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Losartan	2.108	602223	128898	2586	1.6
2	Losartan	2.105	607748	129233	2947	1.4
3	Losartan	2.113	607302	127409	2468	1.6
4	Losartan	2.109	608674	127047	2146	1.9
5	Losartan	2.109	607376	129859	2307	1.7
Mean			**606665**			
Std. Dev			**2542.3**			
% RSD			**0.42**			

Table 8: Results of Method Precision for Hydrochlorothiazide

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Hydrochlorothiazide	3.552	2220333	2231111	1.6	2371
2	Hydrochlorothiazide	3.550	2221573	2674210	1.6	2841
3	Hydrochlorothiazide	3.564	2215483	2231261	1.5	2816
4	Hydrochlorothiazide	3.564	2217379	2421301	1.5	2872
5	Hydrochlorothiazide	3.565	2211255	2324710	1.6	2845
Mean			**2217205**		**1.6**	**2841**
Std. Dev			**4100.8**			
% RSD			**0.18**			

Intermediate Precision

Day 1

Table 9: Results of Intermediate Precision for Losartan

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Losartan	2.108	596608	128898	2547	1.6
2	Losartan	2.105	598959	129233	2944	1.4
3	Losartan	2.113	595728	127409	2361	1.6
4	Losartan	2.109	594485	127047	2546	1.9
5	Losartan	2.109	595267	129859	2207	1.7
6	Losartan	2.102	596608	124691	2481	1.6
Mean			**596209**			
Std. Dev			**1718.7**			
% RSD			**0.29**			

Table 10: Results of Intermediate Precision for Hydrochlorothiazide

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.552	2207732	2231134	8371	1.5	2.04
2	Hydrochlorothiazide	3.550	2202266	2674210	6841	1.6	2.03
3	Hydrochlorothiazide	3.564	2209375	2247461	7816	1.6	2.01
4	Hydrochlorothiazide	3.564	2204037	2454301	8872	1.6	2.05
5	Hydrochlorothiazide	3.565	2204466	2324710	4845	1.6	2.02
6	Hydrochlorothiazide	3.537	2209574	2531247	8371	1.6	2.03
Mean			**2205575**				
Std. Dev			**2899.8**				
% RSD			**0.13**				

Day 2

Table 11: Results of Intermediate Precision Day 2 for Losartan

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Losartan	2.102	602155	127998	5586	1.5
2	Losartan	2.105	603662	134844	5636	1.6
3	Losartan	2.112	603931	161103	5432	1.6
4	Losartan	2.113	607302	127409	5468	1.6
5	Losartan	2.109	608674	127047	5146	1.9
6	Losartan	2.109	607376	129859	5307	1.7
Mean			**605516.7**			
Std. Dev			**2602.622**			
% RSD			**0.42**			

Table-12: Results of Intermediate Precision for Hydrochlorothiazide

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.537	2241579	2263528	2371	1.6	7.98
2	Hydrochlorothiazide	3.552	2236409	2224418	2414	1.6	6.4
3	Hydrochlorothiazide	3.560	2239093	2233725	2384	1.6	8.9
4	Hydrochlorothiazide	3.564	2215483	2231261	2816	1.5	8.3
5	Hydrochlorothiazide	3.564	2217379	2421301	2872	1.5	7.5
6	Hydrochlorothiazide	3.565	2211255	2324710	2845	1.6	5.3
Mean			**2226866**				
Std. Dev			**13567.02**				
% RSD			**0.60**				

Accuracy

Accuracy [29] at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 13: The accuracy results for Losartan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table 14: The accuracy results for Hydrochlorothiazide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

Limit of Detection

The detection limit [30] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve [31]

Result

Losartan

=0.2µg/ml

Hydrochlorothiazide

=2.3µg/ml

Limit of Quantitation

The quantitation limit [32] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response [33]

S = Slope of the calibration curve

Result

Losartan

= 0.8µg/ml

Hydrochlorothiazide

= 7.04µg/ml

Robustness

The robustness [34] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Losartan and Hydrochlorothiazide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Losartan and Hydrochlorothiazide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 15: Results for Robustness for Losartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

Table 16: Results for Robustness for Hydrochlorothiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

Summary and Conclusion

Summary

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 225nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Altima C18 (4.6×150mm, 5.0 µm) because it was giving good peak.

40°C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: TEA Buffer pH 4.5: Acetonitrile was taken in the ratio of 50:25:25% v/v/v was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study.

Methanol and water were selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 7.0min because analyze gave peak around 2.102 and 3.537min and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range.

The analytical method was found linearity over the range of

5-25µg/ml of the Losartan and 12.5-50µg/ml of the Hydrochlorothiazide target concentration.
The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative simultaneous estimation of Losartan and Hydrochlorothiazide in bulk drug and pharmaceutical dosage forms.
This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.
Losartan (potassium salt) is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide. The solubility of losartan (potassium salt) in these solvents is approximately 20 mg/ml. Hydrochlorothiazide was found to be Freely soluble in sodium hydroxide solution, in n-butylamine and in dimethylformamide; sparingly soluble in methanol; insoluble in dilute mineral acids, Soluble in ethanol at approximately 750 g/L; soluble in acetone, dilute ammonia; freely soluble in sodium hydroxide solution, n-butylamine, dimethylformamide; sparingly soluble in alcohol; insoluble in ether, chloroform, dilute mineral acids, Soluble in sodium hydroxide solution.
Methanol and TEA Buffer (pH 4.5) and Acetonitrile in the ratio of 50:25:25% v/v/v was chosen as the mobile phase. The solvent system used in this method was economical.
The %RSD values were within 2 and the method was found to be precise.
The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.
This method can be used for the routine determination of Losartan and Hydrochlorothiazide in bulk drug and in Pharmaceutical dosage forms.

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