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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND HYDROCHLORTHIAZIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

The day by day new combinations drugs are being launched in market. Then the multiple therapeutic agents which acts at diverse sites are used in the management of various diseases and disorders are done. Thus it is necessary to develop methods for analysis with the help of number of analytical methods which are available for the estimation of the drugs in combination. The analyst were estimate the rapid, selective, specific, simple, RP-HPLC method is developed and validated for simultaneous estimation of Losartan potassium and Hydrochlorthiazide in pharmaceutical tablet dosage form. RP-HPLC method was performed on the HPLC System with UV-VIS detector and Nucleodur,C8 (150 mm × 3.9 mm, 5 µm), using the mobile phase (Acetonitrile: Buffer B (7:93 v/v) pH 7.0 to 7.5 with 0.05% at a flow rate of 1.0 ml/min, injection volume 20 µl and UV detection at 280 nm. This method is validated according to BP, USP and ICH requirements for new methods, which include accuracy, precision, robustness, ruggedness, linearity and range. Linear relationships were obtained in the ranges of 100-300 µg/ml and 50-150 µg/ml with correlation coefficients of 0.9999 and 0.9996 at Rt value of 10.769 min and 21.633 min for Losartan potassium and hydrochlorthiazide respectively. According to ICH guidelines the developed method was validated. The proposed method can be used for estimation of these drugs in combined pharmaceutical dosage forms.

Keywords – *Losartan potassium, Hydrochlorthiazide, RP-HPLC, Development, Validation*

INTRODUCTION

The pharmaceutical formulations with combinations of drugs have shown an increasing trend to counteract the symptoms specific to one drug and formulation, and hence analytical chemist will have to accept the challenge of developing reliable and easy simultaneous methods because it does not require manual individual calculations and marginally give better results [1].

Theophylline (1,3 dimethyl, 2-3-6-7-tetrahydro-1-H Purine-2,6- dione. Theophylline is Xanthine dr. which is used for the treatment of asthma and bronchial obstructive diseases. It acts competitively inhibiting the Type 3 and Type 4 phosphodiesterase, the enzyme responsible for breaking down the cyclic AMP in smooth muscle cells, possibly resulting in bronchodilation.

Losartan potassium

The chemical name of Losarta potassium is: potassium;[2-butyl-5-chloro-3-[[4-[2-(1,2,4-triaza-3-azanidacyclopenta-1,4- dien-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol. It has a molecular formula of $C_{22}H_{22}ClKN_6O$ and the molecular weight of 461.0 gm/mol. Generously it is freely soluble in water and soluble in alcohols. It has the structural formula shown in Figure 1 [2-4].
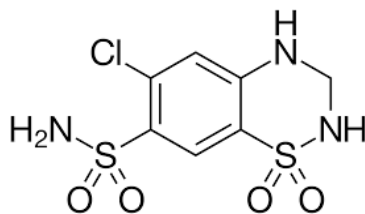
Fig. 1: Structural formula of Losartan potassium

Hydrochlorthiazide

The chemical name is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ6,2,4-benzothiadiazine-7-sulfonamide. It has the molecular formula of $C_7H_8ClN_3O_4S_2$ and molecular weight of 297.7 gm/mol. It is soluble in sodium hydroxide solution, in n-butyl amine, and in dimethylformamide. It has the structural formula shown in Figure 2.
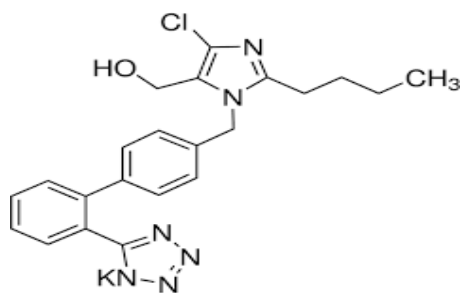

Fig. 2: Structural formula of Hydrochlorthiazide

Mechanism of action: 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)1-7.

MATERIALS AND METHODS

Apparatus

HPLC-Waters Model NO.2690/5 series Compact System is Consisting of Inertsil-C8 column. Electronic balance (SARTORIOUS), Sonicator (FASTCLEAN), Mortar and pestle, 100 mL and 50 mL volumetric flasks, 100 mL beakers, 5 mL pipettes.

Materials

Losartan potassium and Hydrochlorthiazide were purchased from Verax Lifesciences pvt.Ltd, Solan, Himachal Pradesh, India. Their purity was found to be 100.14 and 99.36 respectively, according to company analytical certificate.

Market samples

The tablet of Losartan potassium (25 mg) and Hydrochlorthiazide (12.5 mg) was purchased in retail pharmacy, Coimbatore.

Reagents

Acetonitrile (AR Grade), Sodium dihydrogen orthophosphate (AR Grade) Dipotassium hydrogen ortho phosphate (AR Grade), Potassium Dihydrogen ortho phosphate (AR Grade), Water (HPLC Grade), Ortho phosphoric acid (HPLC Grade).

Preparation of standard stock solution

Standard solution: Weigh accurately 40 mg of Losartan Potassium WRS and 20 mg of Hydrochlorothiazide WRS into a 200 mL volumetric flask. Dissolve in 100 mL of diluent and dilute with Buffer A to volume. Pass a portion of the solution through a 0.45 µm nylon membrane filter discarding the first 2 mL.

Sample stock solution: Transfer 10 tablets into a 250 mL volumetric flask and add 210 mL of diluent. Mix well and mechanically shake or stir until the solid is dispersed. Dilute with Buffer A to volume and sonicate.

Sample solution: Transfer 5 mL of sample stock solution into a 25 mL volumetric flask, add 5 mL of Acetonitrile and then dilute to volume with Buffer A. Pass a portion of the solution through a 0.45 µm nylon membrane filter discarding the first 2 mL perform in duplicate.

Optimized chromatographic conditions:

Column: Nucleodur, C8, 150 × 3.9 mm, 5 µ

Detector wavelength: 280 nm

Flow rate: 1.0 mL/minute

Load: 20 µL

Mobile phase: Buffer: Acetonitrile (7: 93)

Method development

To saturate the column, mobile phase was pump for regarding 20 min so to induce the bottom line corrected. Standard calibration lines were created for each drug. A series of aliquots were prepared from the above stock solutions to get the concentrations 50-150 µg/ml for Hydrochlorthiazide, 100-300 µg/ml for Losartan potassium using diluents. Inject every concentration 6 times in to the chromatographic system. Every time peak area and retention time was recorded individually for each the medication. Calibration curves are established by taking average peak area on Y-axis and concentration on X-axis individually for both drugs. Regression equations were calculated from the calibration curves, these regression equations is used to calculate drug substance in formulation [5,6].

Method validation

The described method has been validated for linearity, accuracy, precision, and robustness, as per the ICH guidelines.

System suitability

The relative standard deviation of replicate injections of standard preparation is not more than 2.0% for both hydrochlorothiazide and losartan peaks and the tailing factor is not more than 2.5 for losartan peak [7].

Specificity

The term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other as per ICH. Solutions of standard and sample were prepared as per the test method are injected into chromatographic system [8].

Linearity

The linearity of the method was determined by preparing six different concentrations of both Losartan potassium and Hydrochlorthiazide in the concentration range of 100-300 µg/ml and 50-150 µg/ml. Each solution was prepared in triplicate. The calibration curves were obtained by plotting peak area versus concentration. Linearity was check over the same concentration range on three successive days and the results obtained. Plot a graph of concentration versus area and determine the correlation coefficient square and y-intercept [9].

Precision

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Intermediate precision: Analyst, instrument and day variability

Separately inject standard solution in 6 replicates and the sample solutions and record the peak area for major peaks. Calculate the content of Losartan potassium and Hydrochlorthiazide per tablet in all the six preparations [10].

Accuracy (recovery)

A study of accuracy was conducted. Drug assay was performed in triplicate as per test method with equivalent amount of Losartan potassium and Hydrochlorthiazide into each volumetric flask for each spike level to get the concentration of Losartan potassium and Hydrochlorthiazide equivalent to 50%, 100% and 150% of the labeled amount as per the test method. The average % recovery of Losartan potassium and Hydrochlorthiazide were calculated.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Make slight variations in wavelength, flow rate, mobile phase composition, pH of the buffer and check the system suitability under each variable parameter [11].

Filter integrity

Filter integrity is carried out to determine the compatibility of sample solutions with various filters. Prepare the sample solution as given under the method of analysis. Centrifuge 10 mL of the solution and inject the supernatant liquid. Filter the solution through 0.45 μm nylon membrane filter, discarding the first few mL. Inject the filtered sample. Similarly filter the solution through 0.45 μm PTFE and PVDF membrane filters, discarding the first few mL. Inject the filtered samples. Compare the areas obtained for the filtered samples against the centrifuged sample.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Reversed phase liquid chromatography in isocratic mode with mobile phase of methanol and water, acetonitrile and water are used in different combinations could not retain HCT satisfactorily (retention time about 4 min). Many attempts to retain HCT for longer time by increasing the aqueous phase of mobile phase resulted in very retention time greater than 30 minutes of LOS.

Further study was carried out using mobile phase of increased aqueous portion and heating the column to bring down the retention time of LOS, but this resulted in lower retention time (1.2 minutes) of HCT. Thus adequate separation of HCT and LOS became critical [12].

So it was go for gradient elution with mobile phase of higher aqueous phase in the beginning to retain HCT for longer time and gradually decreasing the aqueous portion to bring down the retention time of LOS. Different combinations of time and mobile phase composition were tried during optimization of gradient method and the one which is given in chromatogram was found to resolve HCT and LOS satisfactorily [13-15].

Most of all reported HPLC methods till date use C-8 or C-18 columns. Most of these use Complex mobile phase compositions. Hence, attempts were directed towards development of a Simple and better method on commonly used Nucleodur-C8, column with good resolution. Different logical Modifications were tried to get good separation among the drugs and the degraded products. These changes included change in mobile phase composition in gradient modes on different HPLC columns. The best peak shape and maximum separation was achieved with mobile phase composition of Acetonitrile:buffer (7:93), peak symmetry and reproducibility were obtained on Nucleodur-C8, 150 × 3.9 mm, 5 μ Column. The optimum wave-length for detecting the analyte was found to be

280 nm, a flow rate of 1.0 ml/min yielded optimum separation and peak symmetry. Chromatogram of Losartan potassium and Hydrochlorthiazide is shown in Figure 3 and optimized chromatographic condition is shown in Table 1
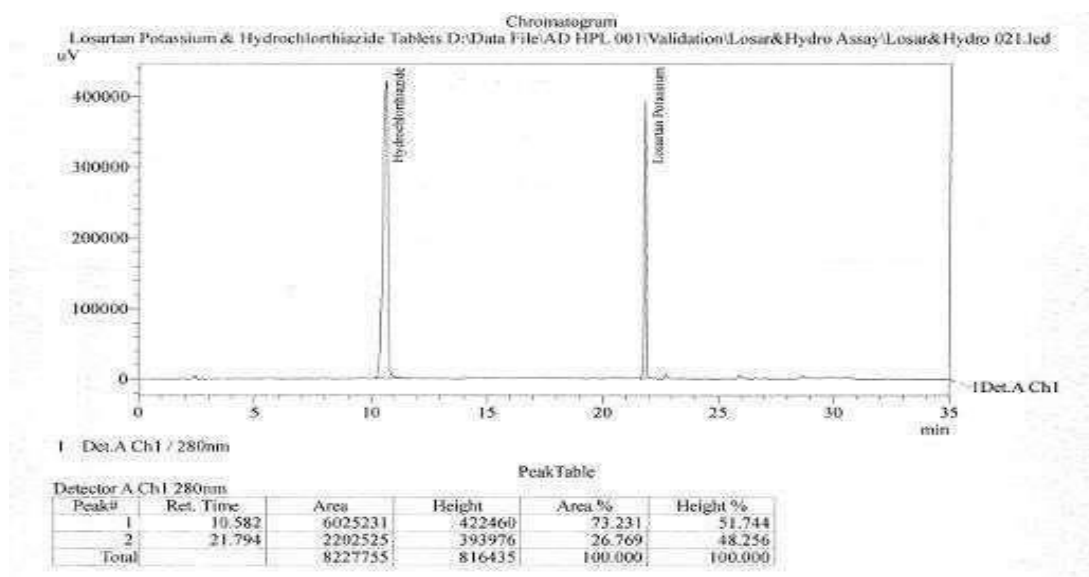

Fig. 3: Chromatogram of losartan potassium and Hydrochlorthiazide

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Nucleodur-C8(150 × 3.9 mm, 5
Mobile phase	Acetonitrile: Buffer (7:93)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	35(°C)
Volume of injection loop (□l)	20
Detection wavelength (nm)	280 nm

System suitability and specificity

Relative standard deviation of the areas of losartan potassium is 0.09% and Hydrochlorthiazide is 0.10%. There is no interference of the placebo. Hence the method is specific.

Linearity

The correlation coefficient for hydrochlorthiazide was found to be 0.9996 and losartan potassium was found to be 0.9999, which complied with the prescribed limit of ICH guidelines (NLT 0.999). Thus the method is said to be linear in the concentration range of 50-150 µg/ml for hydrochlorhiazide and 100-300 µg/ml for losartan potassium (Table 2). The Regression results indicate that method was linear in the concentration range studied and can be used for detection and quantification of hydrochlorthiazide and losartanpotassium in very wide concentration range for Hydrochlorthiazide and losartanpotassium respectively (Figures 4 and 5).

Table 2: Linear regression data for calibration curves

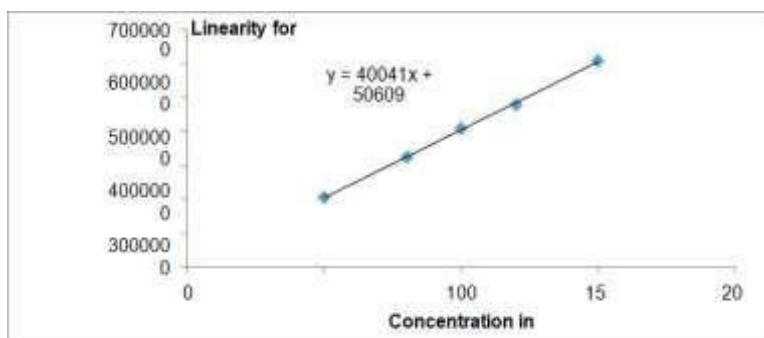| Drugs	LOS	HCT
Concentration range (mcg/ml)	100-300	50-150
Slope	7302	40041
Correlation coefficient	0.9999	0.9996
Correlation coefficient square	0.9999	0.9996

Fig. 4: Linearity of peak area at 280 nm to corresponding concentration of HCT
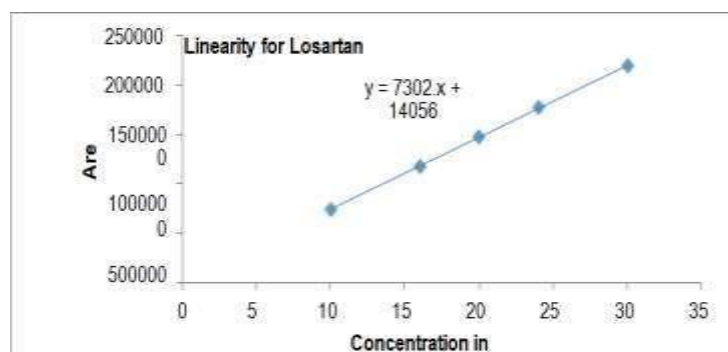

Fig. 5: Linearity of peak area at 280 nm to corresponding concentration of LOS

Precision

System precision: The relative standard deviation of the areas of Losartan potassium is 0.09% and Hydrochlorthiazide is 0.10% (Tables 3 and 4).

Table 3: Recovery result of Hydrochlorthiazide

S.No.	Recovery level	Amount added (mg)	Peak response	Amount Recovered (mg)	Recovery (98.0%-102.0%)	Mean and RSD
1	50	62.26	1985581	62.43	100.28	Mean=100.42% RSD (NMT 2.0%)=0.40%
2	50	61.86	1984587	62.40	100.88	
3	50	62.46	1988418	62.52	100.10	
4	100	125.11	3971147	124.87	99.81	Mean=99.23% RSD (NMT 2.0%)=0.61%
5	100	125.61	3954030	124.33	98.60	
6	100	125.31	3971989	124.89	99.28	
7	150	187.07	6032203	189.67	101.00	Mean=101.12% RSD (NMT 2.0%)=0.35%
8	150	187.37	6032585	189.69	100.84	
9	150	186.47	6044389	190.06	101.53	

Table 4: Recovery result of losartan potassium

S.No.	Recovery level	Amount added (mg)	Peak response	Amount recovered (mg)	Recovery (98.0%-102.0%)	Mean and RSD
1	50	125.36	734494	125.33	99.98	Mean=100.36% RSD(NMT 2.0%)= 0.37%
2	50	125.06	735580	125.52	100.37	
3	50	125.16	738782	126.06	100.72	
4	100	249.42	1442439	246.13	98.68	Mean=98.70% RSD(NMT 2.0%)= 0.05%
5	100	250.02	1445714	246.69	98.67	
6	100	248.93	1440693	245.84	98.76	
7	150	373.99	2193315	374.26	100.07	Mean=100.89% RSD(NMT 2.0%)= 0.80%
8	150	374.48	2214357	377.85	100.90	
9	150	377.87	2251938	384.27	101.69	

 Method precision: % RSD of Losartan potassium and hydrochlorthiazide 0.87% and 1.03% respectively. The RSD for the six Assay determinations is not more than 2.0% (Table 5).

Table 5: Data for repeatability (Method precision)

S. No.	Sample ID	Content of Hydrochlorothiazide (mg/tab)	Content of Losartan potassium (mg/tab)
1	Sample-1	12.50	25.25
2	Sample-2	12.43	25.19
3	Sample-3	12.53	25.14
4	Sample-4	12.60	25.35
5	Sample-5	12.80	25.71
6	Sample-6	12.63	25.55
Average		12.58	25.36
RSD		1.03%	0.87%

Solution stability and filter integrity

The deviation in areas for standard and test solutions passes the acceptance criteria after 48 hours. Hence the solutions are stable up to 48 hours**.**

Robustness

Robustness was studied for the variation of mobile phase flow rate 1.0 ± 0.1 mL/min and wavelength 280 nm. After change in the experimental condition there was no significant loss of sensitivity, selectivity and peak parameters. So the method is said to be robust for deliberate change of wavelength and flow rate (Table 6).

Table 6: Data for robustness

Parameters	Variation	RSD (NMT 2.0%)		Tailing factor of Losartan Peak NMT 2.5
		Hydrochlorothiazide	Losartan Potassium	
Change in wavelength	278 nm	0.63%	0.74%	1.29
	282 nm	0.70%	0.85%	1.29
Flow Rate	0.9 mL/min	0.23%	0.70%	1.40
	278 nm	0.63%	0.74%	1.29

CONCLUSION

The proposed method is precise, accurate for the simultaneous determination of HCT and LOS from tablet. It is also a stability indicating method and hence can be easily and conveniently adopted for routine quality control analysis and also bioassay.

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