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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND LOSARTAN POTASSIUM IN PHARMACEUTICAL FORMULATION

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Abstract

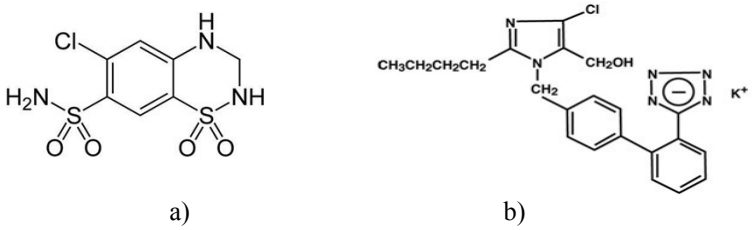
A new, simple and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the separation and quantification of Hydrochlorothiazide (HCTZ) and Losartan Potassium (LOS) in tablet dosage form. The determination was carried out using GRACE C18 [4.6 x 250 mm] column as a stationary phase and mobile phase comprised of Acetonitrile: Phosphate Buffer (50:50) pH 3.1 in proportion of 50:50(v/v); the pH of phosphate buffer adjusted to (3.1) using orthophosphoric acid. The flow rate was maintained at 1.0ml/min and the eluent was monitored at 226nm. The retention time of HCTZ and LOS were 4.250 min and 8.30 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity and robustness. The method was linear and for precision studies; RSD for HCTZ and LOS were 0.02 and 0.04 respectively. The percentage recoveries for both drugs from their tablets were 100.80 and 99.76 respectively

Keywords*:* Hydrochlorothiazide; Losartan Potassium; RP-HPLC; Tablets

1. Introduction

Hydrochlorothiazide is chemically 6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide is first-line diuretic drug of the thiazide class that acts by inhibiting the kidneys ability to retain water. It is calcium-sparing diuretic; frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones. It is official in Indian Pharmacopoeia, British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopeia. Losartan potassium is chemically ([2-butyl-4-chloro-1-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazol-5-yl] methanol monopotassium. It is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). It is official in United States Pharmacopeia, British Pharmacopoeia and Japanese Pharmacopoeia. A literature survey revealed spectrophotometry and Ion Pair Chromatographic Technique[1,2,3], HPTLC[4] and RP-HPLC[5-10]. Hydrochlorothiazide 12.5mg and Losartan 50mg in combination is available in market by brand name Losartas-HT which is an antihypertensive formulation. The structure of a) Hydrochlorothiazide and b) Losartan Potassium is shown in Figure 1.

Figure 1: Structure of a) Hydrochlorothiazide and b) Losartan Potassium



a) b)

A literature survey reveals that there are few analytical methods reported for the estimation of Hydrochlorothiazide alone and in combination with Losartan Potassium[11,12] or in combination with other antihypertensive drugs. However the reported methods have several limitations. In one of the reported method retention time for Losartan potassium was not found to be significant which limits its use and in another; flow rate for separation of both the drugs found to be >1ml/min which means excess of solvent is required throughout the analysis compared to usual flow rates (1±0.2ml/min) which is ideal for good column performance. Therefore in order to overcome the drawbacks of the reported methods;

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need arised to develop a new method which should be suitable for routine analysis of these drugs in combination. The present study is able to overcome the drawbacks in the sense of being economical and with significant retention time for both the drugs which proves that present method is perfect compared to reported methods.

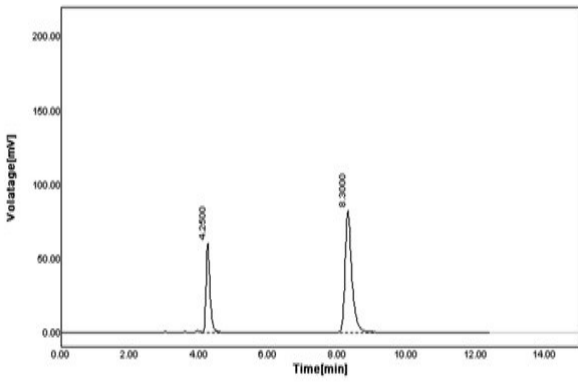
2. Experimental

2.1 Materials: HCTZ and LOS were kindly obtained from Glenmark pharmaceuticals limited. Losartas-HT tablets 50mg were purchased from the local market. HPLC grade methanol, acetonitrile and phosphate buffer were used. Deionized was used throughout the experiment.

2.2 Instrumentation: The separation of HCTZ and LOS was achieved by using Younglin HPLC pump spectra ACME-9000 system with GRACE C18 [4.6 x 250 mm] 10mm column and UV-730D detector connected to AUTO-3000 Software was used for the study.

2.3Optimized Chromatographic Conditions: A GRACE C18 [4.6 x 250 mm] column was used for the separation of drugs. The mobile phase comprised of Acetonitrile: Buffer (50.50) pH 3.1 in proportion of 50:50(v/v) with pH of phosphate buffer adjusted to (3.1) using orthophosphoric acid. Injection volume was 20µl and run time was 15min and flow rate 1.0 ml/min. The column was maintained at ambient temperature and the eluent was detected at 226 nm. The separation of HCTZ and LOS under optimized condition is shown in Figure 2.

Figure 2: Typical HPLC chromatogram corresponding to mixed standard solution of HCTZ and LOS



2.4 Preparation of Standard solution:

HCTZ stock and working solution: Standard stock solution (100µg/ml) of HCTZ was prepared by dissolving in methanol. The working standard solutions were prepared to get various concentrations of HCTZ ranging from 2-10 µg/ml.

LOS stock and working solution: Standard stock solution (100µg/ml) of LOS was prepared by dissolving in methanol. The working standard solutions were prepared to get various concentrations of LOS ranging from 8-40 µg/ml.

2.5 Preparation of Sample solution: Twenty tablets were weighed and content emptied. The average weight determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 12.5 mg of HCTZ and 50 mg LOS was transferred in a 100 ml volumetric flask and methanol was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with methanol. The solution was filtered through whatman filter paper No.42. Further dilution was done with methanol to get concentration of 12.5 mg/ml of HCTZ and 50 mg/ml of LOS.

2.6 System suitability: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from 5 replicate injections of standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ±2% standard deviation range during performance of the method. Here tailing factor for peaks of HCTZ and LOS was less than 2% and resolution was satisfactory. The results of system suitability tests are shown in Table 1.

Table 1: System suitability test results

Sr. No.	Parameters	HCTZ	LOS
1.	Peak area	483.18	1167.70
2.	No. of theoretical plates	5456.06	8343.72
3.	Retention time (min)	4.18	8.04
4.	Asymmetry	1.47	1.29

3. Results and Discussion

The chromatographic conditions were optimized to develop RP-HPLC method for simultaneous determination of HCTZ and LOS with adequate resolution and rapid analysis time.

3.1 Method Validation: The developed chromatographic method for simultaneous estimation of MET and VILD was validated according ICH guidelines for linearity, accuracy, precision, specificity, robustness and ruggedness.

3.1.1 Linearity: According to USP; tablet powder equivalent to 60, 70, 80, 90, and 100% of label claim was taken and dissolved in acetonitrile, diluted appropriately with acetonitrile to obtain a concentration in the range of 60%-100% of the test concentration. Each of this concentration was injected to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study.

The results of the linearity studies are shown in Table 2.

Table 2: Linearity study data

Sr. No.	Drugs	Slope	Intercept	Correlation Coefficient (R^2)
1	HCTZ	19.10	8.556	0.998
2	LOS	29.58	15.36	0.998

3.1.2 Recovery: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out using standard addition method at 80, 100 and 120 % level; known amount of standards was added to reanalyzed sample and subjected them to the proposed HPLC method. Percentage recovery was calculated from the amount found and actual amount added. The mean recovery is within acceptable limits which indicate that the method is accurate .The results of recovery studies are shown in Table 3.

Table 3: Recovery study data

Sr. No.	Drug	% Recovery	%RSD
1	HCTZ	99.79	0.30
2	LOS	99.89	0.06

3.1.3 Precision: The precision of an analytical method is expressed in terms of SD or RSD of series of measurements. It was ascertained by replicate estimation of HCTZ and LOS by proposed method. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise. The results of precision study are shown in Table 4.

Table 4: Precision study data

Sr. No	Weight of sample	Peak area of std		Peak area of sample		% Label claim	
	(mg)	HCTZ	LOS	HCZ	LOS	HCTZ	LOS
1.	225	1905.23	2960	1905.31	2959.99	100.0	99.99
2.	225.10			1905.19	2960.17	99.98	99.94
3.	225.60			1904.34	2959.89	99.96	100.0
Mean						99.98	99.97
S.D.						0.02	0.03
%R.S.D.						0.02	0.03

3.1.4 Specificity of the method: Specificity was measured as ability of the proposed method to obtain well separated peak for HCTZ and LOS without any interference from component of matrix. The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution was injected into the column, under optimized chromatographic conditions to demonstrate the separation of both HCTZ and LOS from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of tablet formulation. The mean retention time for HCTZ and LOS was found to be 4.083 and 7.466 min respectively.

3.1.5 Robustness: Robustness of the method was determined by making slight changes in the experimental conditions such as the, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that, the RP-HPLC method developed, are rugged and robust. The results of robustness study are shown in Table 5.

Table 5: Robustness study data

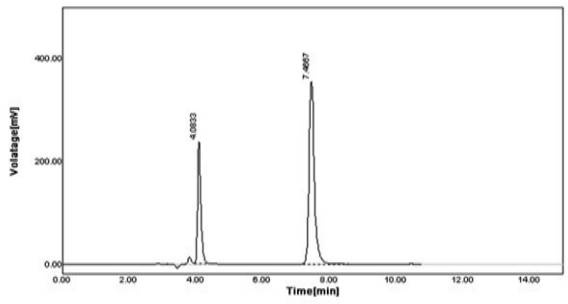
System suitability Parameters		% RSD of peak area response (n=3)		Mean tailing factor (n=3)		Mean retention time in min. (n=3)	
Variations		HCTZ	LOS	HCTZ	LOS	HCTZ	LOS
Change in flow Rate	+10	1.92	1.43	1.0	1.55	1.40	1.10
	0	1.90	1.27	0.23	0.05	0.66	1.13
	-10	1.93	1.85	0.56	0.06	1.2	1.12
Change in % Organic phase (Methanol)	+10	1.92	1.43	1.0	1.68	1.96	1.90
	0	1.90	1.27	0.23	0.05	0.66	1.13
	-10	2.0	1.75	0.56	0.08	1.2	1.18
Change in pH	+0.2	1.92	1.98	1.0	0.68	1.98	1.90
	0	1.90	1.27	0.23	0.05	0.66	1.13
	-0.2	2.0	1.89	1.23	0.66	1.6	1.18

3.2 Application of the method in tablets: Equal volume (20mL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The procedure was repeated three times, individually weighing the tablet powder each time. The responses from the standard and sample were used to calculate the amounts of the drug in the tablet. Results obtained are shown in Table 6. The chromatogram showing separation of HCTZ and LOS in tablet formulation is shown in Figure 3.

Table 6: Results of Tablet formulation study

Sr. No.	Weight of std.(mg)		Weight of sample (mg)	Peak area of std		Peak area of sample		% Label claim	
	HCTZ	LOS		HCTZ	LOS	HCTZ	LOS	HCTZ	LOS
1.	12.5	50	225	140.41	586.52	141.38	584.93	100.69	99.72
2.			224.95			141.30	584.84	100.63	99.71
3.			225.05			141.95	585.62	101.09	99.85
Mean								100.80	99.76
S.D.								0.25	0.07
%RSD								0.24	0.078

Figure 3: Typical HPLC Chromatogram corresponding to marketed formulation of HCTZ and LOS



4. Conclusion

The developed RP-HPLC method for simultaneous determination of HCTZ and LOS in tablet dosage form is new, simple, sensitive and reproducible. Therefore it can be used in routinely for simultaneous estimation of HCTZ and LOS in bulk as well as in pharmaceutical dosage form. The developed method was validated to find out the suitability of the method and it was found to be valid and suitable for intended purpose.

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