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Research Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LOSARTAN, HYDROCHLOROTHIAZIDE AND AMLODIPINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The present study was designed with an objective of a simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of Losartan, Hydrochlorothiazide and Amlodipine from bulk and formulations.

Methods: Reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for rapid analysis of simultaneous determination of Losartan, Hydrochlorothiazide and Amlodipine. The separation of these three drugs was achieved on a Hypersil Gold column (250 mm X 4.6 mm, 5 µ) as stationary phase with a mobile phase consisting of methanol: water in the ratio of 95:5% v/v at a flow rate of 0.8 mL/min and UV detection at 230 nm.

Results: The retention times were observed to be 2.850, 3.875 and 7.333 minutes for Losartan, Hydrochlorothiazide and Amlodipine, respectively. The method was statistically validated for linearity, recovery, limit of detection, limit of quantification, accuracy and precision.

Conclusion: The method was successfully applied for analysis of combined dose tablet formulation containing Losartan, Hydrochlorothiazide and Amlodipine.

Keywords: Amlodipine, hydrochlorothiazide, losartan, reverse phase high performance liquid chromatography.

INTRODUCTION

Losartan potassium (LOS) is an angiotensin II receptor antagonist and chemically it is 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2'-(1H-tetrazol-5-yl) (biphenyl-4-yl) methyl] imidazole, a strong antihypertensive agent (Fig.1). Losartan was developed by DuPont-Merck laboratories as a potent non-peptide angiotensin II receptor (type AT1) antagonist for hypertension treatment [1]. It is administered in its active form and is partially converted into an active metabolite, which is responsible for the drug's prolonged pharmacological effect. The therapeutic efficacy of losartan, as well as its renal and antihypertensive effects, seems to be similar to those of angiotensin converting enzyme (ACE) inhibitors. Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide1,1-dioxide (Fig.2). It is the prototype of the thiazide group and antihypertensive drug [2]. Amlodipine (AMLO), chemically is [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-methyl-1-ihydropyridine-3,5-dicarboxylate benzenesulfonate (Fig.3). It is a long acting calcium channel blocker used as an antihypertensive agent [3].

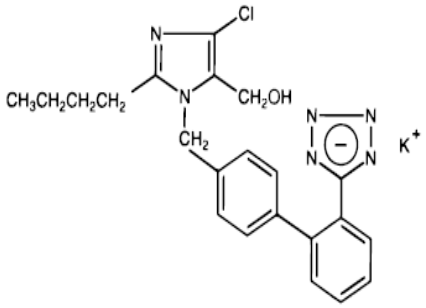

Fig.1: Chemical structure of Losartan potassium

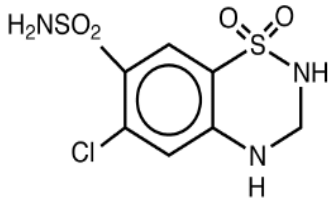

Fig.2: Chemical structure of HCTZ

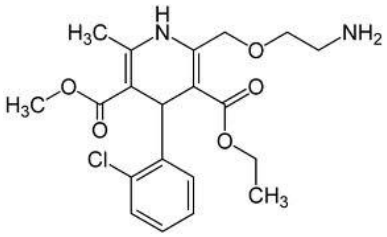

Fig. 3: Chemical structure of AMLO

A literature survey revealed that spectrophotometric, chromatographic methods have been reported for determination of LOS [4–7], HCTZ [8–9] and AMLO [10–15] in single and multicomponent pharmaceutical formulations or from biological fluids. However, there were few HPLC methods for simultaneous estimation of LOS, HCTZ and AMLO reported.

Analysis of LOS, HCTZ and AMLO has been carried out by gradient HPLC with flow rate of 1mL/min [16] while the proposed method employs isocratic elution and hence is simpler. Other methods reported for analysis of LOS, HCTZ and AMLO required more buffer solution and pH adjustment and also the flow rate changes during the process that is the 1mL/min to 1.3mL/min [17]. The proposed method is relatively better or comparable in terms of sensitivity, accuracy, and precision to the methods reported for analysis of LOS, HCTZ and AMLO. Rapid simultaneous estimation of LOS, HCTZ and

AMLO using a simple isocratic HPLC system with high sensitivity of estimation indicates easy application for analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Losartan (LOS), Hydrochlorothiazide (HCTZ) and Amlodipine (AMLO) were kindly supplied by Emcure Pharmaceuticals Ltd, Pune and Cipla Ltd Goa, India. Marketed sample of LOS, HCTZ and AMLO (Losagem-AH) in their combined tablet dosage form. Each tablet contained 50mg of LOS, 12.5mg of HCTZ and 5mg of AMLO. For HPLC work double distilled water was prepared in the laboratory. Methanol used was of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 1580). The solutions were injected into the chromatograph through a Rheodyne valve, with a 20 µL loop with auto sampler (AS 1555). The detector consisted of a UV/ VIS (Jasco UV 1575). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. Hypersil Gold C18 column (250 mm × 4.6 mm i.d., 5 µ) as a stationary phase and methanol: water (95:5% v/v) as mobile phase was used. The mobile phase flow rate was 0.8 mL/min a detection wavelength of 230nm was selected for analysis (Fig.4). An ultrasonic bath was used to remove the air from the mobile phases, operating at ambient temperature.

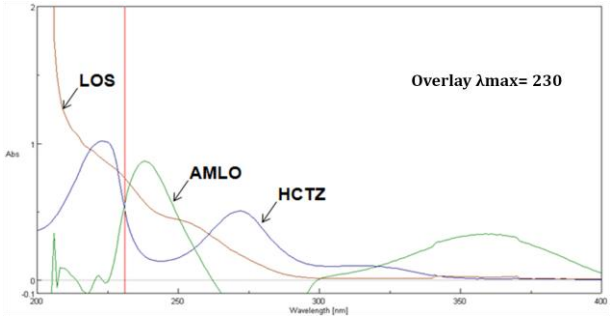

Fig. 4: Overlay Spectrum for LOS, HCTZ and AMLO (λmax = 230nm)

Standard Stock Solutions and Sample solution

Accurately weighed LOS (50 mg), HCTZ (12.5 mg) and AMLO (5 mg) were transferred to 50 mL volumetric flask and dissolved in, and then diluted to the mark with methanol. The stock solution was further diluted with methanol to obtain a solution of LOS (10µg/ml), HCTZ (2.5 µg/ml) and AMLO (1 µg/ml), respectively.

To determine the content of LOS, HCTZ and AMLO simultaneously in tablets (label claim: LOS (50 mg), HCTZ (12.5 mg) and AMLO (5 mg) per tablet), twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 50mg of LOS, 12.5 mg of HCTZ, and 5mg of AMLO was weighed. Then equivalent weight of the drug was transferred into a 50 ml volumetric flask containing 20 ml methanol, sonicated for 10 min and diluted to 50 ml with methanol to obtain solution of LOS (10µg/ml), HCTZ (2.5 µg/ml) and AMLO (1 µg/ml), respectively. The mixture was filtered using whatmann filter.

Validation of the method

Validation was done as per ICH guideline Q2 (R1) [18]. The developed method was validated with respect to parameters such as linearity, LOD and LOQ, precision, accuracy and specificity

System suitability

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their capacity factor, resolution (Rs), retention time, theoretical plates number (N) and tailing factors (T).

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved all the drugs very efficiently, as shown in Fig. 5. The identities of the peak for LOS, HCTZ and AMLO were confirmed by comparing the Rt with those of standards.

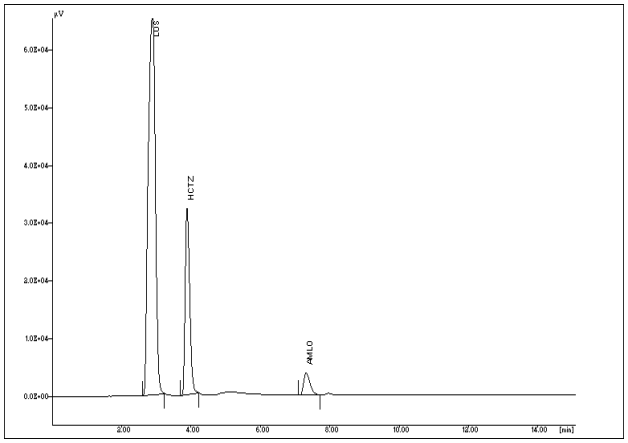

Fig.5: Chromatogram of LOS, HCTZ and AMLO

Linearity

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 10–60 µg/mL for LOS, 2.5-15 µg/mL for HCTZ and 1-6 µg/mL for AMLO, respectively. A 20 µL of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration.

Limit of detection and limit of quantitation:

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision

The precision of the proposed method was assessed as repeatability and intermediate precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated 3 different days over a period of a week to evaluate day-to-day variability (intermediate precision).

Accuracy

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation 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.

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized for simultaneous determination of LOS, HCTZ and AMLO. Good resolution of both the components was obtained with methanol: water at ratio 95: 5 v/v. The flow rate

of 0.8 mL/min was optimum. UV detection was made at 230 nm. At this wavelength LOS, HCTZ and AMLO can be quantified. Hence, 230 nm determined empirically has been found to be optimum. The average retention times for LOS, HCTZ and AMLO was found to be 2.850, 3.875 and 7.333 min, respectively.

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System Suitability Parameters of RP-HPLC for Tablet Analysis

Parameter	Recommended	LOS	HCTZ	AMLO
Capacity Factor (k)	≤ 2	0.941	1.255	1.161
Resolution (Rs)	> 2	0.00	4.359	14.339
Retention Time in min	--	2.850	3.875	7.333
Theoretical plates number (N)	> 2000	3272.51	6185.40	10318.15
Tailing Factor	≤ 2	1.071	1.255	1.320

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 10–60 µg/mL for LOS, 2.5-15 µg/mL for HCTZ and 1-6 µg/mL for AMLO, respectively. The linear regression equations were Y=15938X + 25057 (r^2= 0.9994) for LOS, Y= 19655X + 1857 (r^2= 0.9994) for HCTZ and Y= 8784X -201.9 (r2= 0.9997). The plots obtained from linear regression and residuals analysis are given in Fig 6a and 6b for LOS, 7a and 7b for HCTZ and 8a and 8b for AMLO, respectively.

Limits of Detection and Quantitation

The limits of detection and quantitation, calculated as described above, were 3 µg/mL and 8 µg/mL respectively, for LOS, 0.8 µg/mL and 1.2 µg/mL for HCTZ and 0.4 µg/mL and 0.8 µg/mL for AMLO. This indicates the method is sufficiently sensitive.

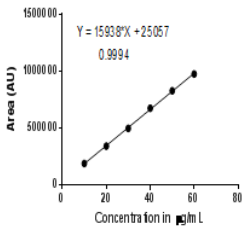

Fig. 6a linear regression for Losartan

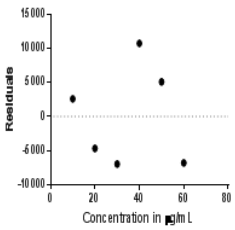

Fig. 6b Residuals plot for Losartan

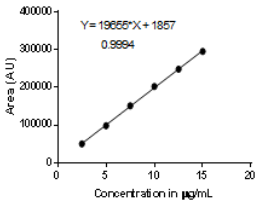

Fig. 7a linear regression for HCTZ

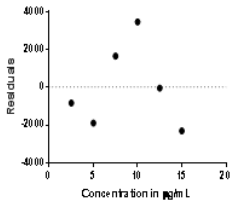

Fig. 7b Residuals plot for HCTZ

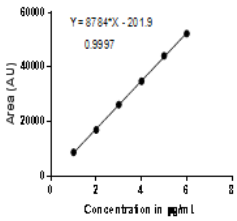

Fig. 8a linear regression for AMLO

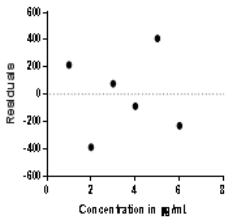

Fig. 8b Residuals plot for AMLO

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 2 reveal the high precision of the method.

Table 2: Precision studies of proposed HPLC method

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Measured Conc. ± SD	(%) RSD	Recovery[a] (%)	Measured Conc. ±SD	(%) RSD	Recovery[a] (%)
Losartan						
20	19.90 ± 0.183	0.92	99.50	19.87 ± 0.20	1.03	99.35
40	39.92 ± 0.41	1.03	99.80	39.80 ± 0.39	0.98	99.50
60	59.96 ± 0.64	1.07	99.93	59.82 ± 0.61	1.02	99.70
Hydrochlorothiazide						
5	4.94 ± 0.052	1.05	98.80	4.92 ± 0.054	1.10	98.40
10	9.94 ± 0.11	1.08	99.40	9.90 ± 0.10	1.04	99.00
15	14.85 ± 0.18	1.21	99.00	14.83 ± 0.14	0.97	98.86
Amlodipine						
2	1.98 ± 0.020	1.02	99.00	1.97 ± 0.020	1.03	98.50
4	3.97 ± 0.042	1.05	99.25	3.96 ± 0.040	1.02	99.00
6	5.95 ± 0.058	0.98	99.16	5.93 ± 0.060	1.01	98.83

[a]**Mean from three analyses**

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate. The results are presented in Table 3.

Table 3: Standard addition techniques for determination of LOS, HCTZ and AMLO (n=3)

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)
LOS	50	80	90	89.45	99.38
		100	100	99.89	99.89
		120	110	109.21	99.28
HCTZ	12.5	80	22.5	22.4	99.55
		100	25	24.92	99.68
		120	27.5	27.32	99.34
AMLO	5	80	9	8.92	99.11
		100	10	9.94	99.40
		120	11	10.89	99.00

Robustness

The standard deviation of peak the areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in Table 4 indicated robustness of the method.

Table 4: Robustness evaluation of LOS, HCTZ and AMLO

Chromatographic factors	Level	Chromatographic changes in t_R[a]		
		LOS	HCTZ	AMLO
A: Flow rate mL/min.				
0.7	-0.1	2.892	3.883	7.350
0.8	0.0	2.850	3.875	7.333
0.9	+0.1	2.817	3.867	7.308
Mean ± SD		2.853	3.875	7.330
		± 0.038	± 0.008	± 0.0211
B: % of methanol in the mobile phase (± 5%)				
90	-5.0	2.898	3.885	7.356
95	0.0	2.850	3.875	7.333
100	+5.0	2.810	3.850	7.292
Mean ± SD		2.853	3.870	7.327
		± 0.044	± 0.018	± 0.032

[a]**Mean from three estimates**

Sample Analysis

When the Losagem-AH tablets were analysed, sharp and well defined peaks for LOS, HCTZ and AMLO were obtained at Rt 2.850, 3.875 and 7.333 min, respectively, when scanned at 230 nm. The amount of the label claim measured were 100.7 ± 1.12% for LOS, 101.68 ± 1.25% for HCTZ and 100.23 ±1.52% for AMLO.

CONCLUSION

The proposed RP-HPLC method has been evaluated over the linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control of this pharmaceutical formulation. There are certain advantages associated with this method such as high selectivity, sensitivity, economic, less time consuming and low limit of detection. Moreover, the lower solvent consumption along with the short analytical run time leads to a cost effective procedure.

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