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Separation of Marketed Formulation containing Hydrochlorothiazide Amlodipine and Losartan through RP-HPLC Method

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Abstract

An attempt has been made to develop and validate an economical procedure for simultaneous estimation of hydrochlorothiazide, amlodipine and losartan in tablet dosage form. Chromatography was performed on a 25cm×4.6 mm i.d, 5µm particle, C18 column with Mixture of (A) Acetonitrile, methanol (65:35) (B) 0.4% triethylamine in 10 mM sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) buffer of the ratio of A:B is 60:40 v/v, adjusted to pH 3.0 with o-phosphoric acid (5%v/v) was used as a mobile phase at a flow rate of 1.5 ml/min. UV detection was performed at 230 nm. Total run time was less then 10 min; retention time for hydrochlorothiazide, amlodipine and losartan were 2.191, 3.780, and 5.450 minutes respectively. Limits of detection were 0.014, 0.10 and 0.0095 ng/mL limits of quantification were 0.043, 0.329 and 0.029 ng/mL for hydrochlorothiazide, amlodipine and losartan respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and without any interference by the excipients.

Key-Words: RP-HPLC, Simultaneous Estimation, Validation, hydrochlorothiazide, amlodipine and losartan

Introduction

Hydrochlorothiazide (HCTZ) belongs to the thiazide class of diuretics. It reduces blood volume by acting on kidneys to reduce sodium reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na^+-Cl^- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Amlodipine (AML), chemically, 2-[(2- aminoethoxy) methyl]- 4-(2-chlorophenyl) -1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester, is an anti-hypertensive and an antianginal agent. Losartan (LOS) is in a group of drugs called angiotensin II receptor antagonists. Losartan is used to treat high blood pressure (hypertension) and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes.

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Losartan is 2-Butyl-4-chloro-1-[[2-(1H-tetrazol-5-yl)[1,1-biphenyl]-4-yl]methyl]-1H-imidazole-5 methanol [1,2]. Simultaneous determi- nation of captopril and hydrochlorothiazide in human plasma by reverse-phase HPLC [3]. Simultaneous determination of amiloride HCl, hydrochlorothiazide and atenolol in combined formulations by derivative spectroscopy [4]. Stability indicating RP-HPLC method for simulatneus determination of amlodipine and benzapril hydrochloride from their combination drug product [5]. Validation of an UV derivative spectrophotometric determination of losartan potassium in tablets [6]. The objective of the present work is to develop and validate new analytical method for simultaneous determination of HCTZ, AML and LOS in tablet dosage form. This communication forms the new, simple, sensitive and reproducible methods for the simultaneous estimation of HCTZ, AML and LOS from combined dosage form.

Material and Methods

Chemicals and reagents

Pure samples of hydrochlorothiazide, amlodipine and losartan were obtained as gift sample from Plethico pharmaceuticals pvt. ltd., indore, M.P., India and tested for purity. The solid dosage form (trilopace tablets) was procured from local market (Label claim: 12.5 mg hydrochlorothiazide, 5 mg amlodipine and 50 mg

losartan). All the chemical and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India.

Equipment used

Shimadzu HPLC (LC-10 AT *VP*) system; LC system used consist of pump(Model SHIMADZU; LC- 10 AT VP) with universal loop injector(Rheodyne 7725 i) of injection capacity 20 µl. Detector consists of photodiode array detector SPD-10 AVP, SHIMADZU; the reverse phase column used was Luna C_{18} (5µM, 25cm×4.6mm i.d) phenomenex, USA, at ambient temperature.

Preparation of standard and sample solutions

The equivalent of 10 mg each of HCTZ, AML and LOS were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of methanol to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of HCTZ, AML and LOS.

20 Tablets of trilopace were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 50 mg of HCTZ was taken in 100 ml volumetric flask and dissolved in 75 ml of solvent (methanol) with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 100ml of volumetric flask through a whatman #41 filter paper. The residue was washed twice with methanol and the combined filtrate was made up to 100 ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent (mobile phase). Six replicates of sample solutions were prepared of required concentrations of all the three drugs. Then 20µL of each replicates were injected into the system. From the chromatograms it was observed that HCTZ, AML and LOS were eluted at 2.191, 3.780, and 5.450 minutes respectively. The concentrations of the all three drugs were extrapolated from their respective calibration curves by using the area.

The mobile phase was a Mixture of (A) Acetonitrile, methanol (65:35) (B) 0.4% triethylamine in 10 mM sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) buffer of the ratio of A:B is 60:40 v/v, adjusted to pH 3.0 with o-phosphoric acid (5%v/v) was used as a mobile phase at a flow rate of 1.5 ml /min, was used to sharpen the peak. The run time was less then 10 min. Before analysis, both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-µm filter paper. The analytes were monitored at 230 nm.

Construction of Calibration curves

From the standard stock solutions of all the three drugs, different dilutions were prepared and chromatographed and the peak areas were measured. Calibration plot of concentration against peak area were then constructed for HCTZ, AML and LOS. From the calibration plots it was found that response to HCTZ, AML and LOS was a linear function of concentration in the range 1-25 µg/ml, 1-25 µg/ml and 1-25 µg/ml respectively. Unknown assay samples were quantified by reference to these calibration plots.

Analysis of pharmaceutical Formulation

Before assay of the formulations six replicates of the required dilutions were prepared from the stock solution and sonicated for 10 min. The solutions (20 µL) were then injected for quantitative analysis. The amounts of HCTZ, AML and LOS per tablets were calculated by extrapolating the peak area from the calibration curve. The results are reported in Table 1.

Under the optimum chromatographic conditions, the retention times obtained for HCTZ, AML and LOS were 2.191, 3.780, and 5.450 minutes respectively and their chromatograms were recorded and given in the Fig. 1. Capacity factors, tailing factors, and number of theoretical plates are reported in Table 2.

Recovery studies

To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method [7]. The results of the analysis are reported in Table 3.

Validation

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity. Accuracy was studied by measurement of recovery at three different levels 80, 100, and 120% of the amount expected in the formulation, in accordance with ICH guidelines [7,8]. Precision was measured both intra-day and inter-day. In the intra-day study the concentrations of all three drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the concentrations of all the three drugs were measured on three different days. The selectivity and specificity of the method were validated by injecting solutions containing all the three drugs; three sharp peaks were obtained for all the three drugs. The limits of detection and quantitation of the method were studied to detect the lowest amount of analyte and quantitative determination of analyte in a sample respectively. The results are reported in Table 4.

Results and Discussion

Different column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimised so that the components were not interfered with the solvent and excipients. Other criteria like time required for analysis, appropriate *k* range for eluted peaks, assay sensitivity, solvent noise and use of the same solvent system for extraction of drug from formulation matrices during drug analysis were also considered.

A series of aqueous mobile phases containing sodium dihydrogen phosphate buffer solutions of different pH in combination with different volume fractions of acetonitrile as modifiers were also tested. The best result was obtained at pH 3 for Sodium dihydrogen phosphate buffer (pH adjusted with o-phosphoric acid). From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasonable run time and other parameters by use of mixture of (A) Acetonitrile, methanol (65:35) (B) 0.4% triethylamine in 10 mM sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) buffer of the ratio of A:B is 60:40 v/v, adjusted to pH 3.0 with o-phosphoric acid (5%v/v) was used as a mobile phase for the formulations. The flow rate was determined by testing the effect of different flow rate on the peak area and resolution, flow rate of 1.5 ml/min found optimum.

All experiments were performed at ambient temperature. The values obtained for *k* and *R*S ($1 < k < 10$, $RS > 2$) show these chromatographic conditions are appropriate for separation and quantification of both the compounds. The number of plates (*N*) is a measure of column efficiency; which shows the high separation efficiency of the column used.

Conclusions

A new, reversed-phase HPLC method has been developed for simultaneous analysis of HCTZ, AML and LOS in a solid formulation. It was shown above that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is relatively short, i.e. 10 min, which enables rapid quantitation of many samples in routine and quality control analysis of formulations. The optimized solvent system was used throughout the experimental work and no interference from any excipient was observed. These results have shown that method could find practical application as a quality-control tool for simultaneous analysis of all three drugs from their combined dosage forms in quality-control laboratories.

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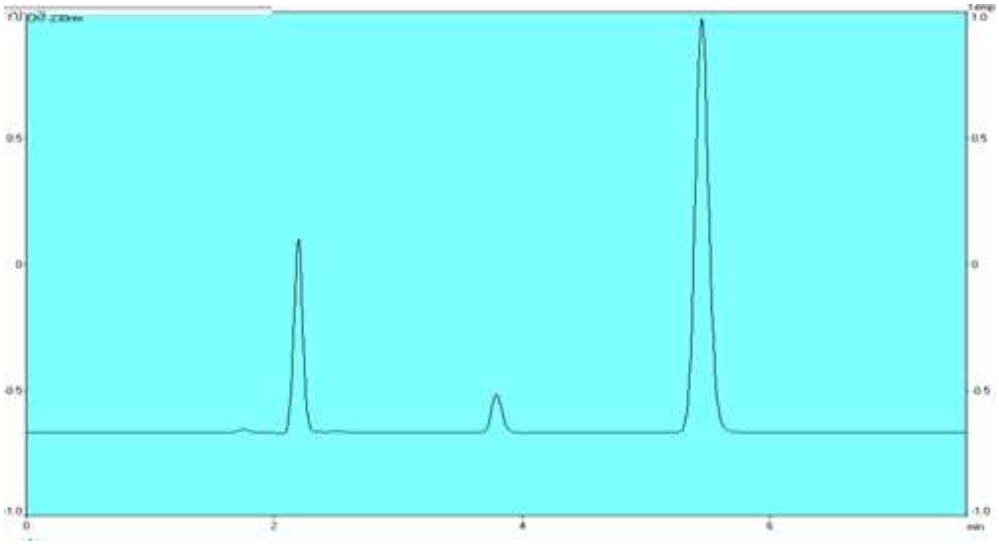

Fig.1. Chromatogram of HCTZ, AML and LOS in market preparation. From the chromatograms it was observed that HCTZ, AML and LOS were eluted at 2.191, 3.780, and 5.450 minutes respectively.

Table 1: Results from assay of the marketed formulation

Drug	Label claim (mg\Tablet) n=6	Amount Found in mg	Drug Concentration (%)	SD	COV (%)	SE
HCTZ	12.5	12.525	100.20	1.08	0.99	0.41
AML	5	4.985	100.98	0.41	0.41	0.19
LOS	50	50.650	101.30	0.72	0.71	0.32

S.D- standard deviation; COV- coefficient of variance; S.E- standard error;

Table 2: System suitability parameters

Property	HCTZ	AML	LOS
R^t	3.012	4.211	8.01
T_f	1.321	1.43	1.11
K'	0.612	0.34	2.22
N	61342	24432	48735
R_s	2.11	6.21	11.65

Rt: retention time; Tf: tailing factor; k′: capacity factor; N: number of theoretical plates Rs, resolution;

Table 3: Results of recovery studies

DRUG	Amount taken (µg/ mL)	Amount added %	µg/mL	Recovery (%, ±S.D).	COV (%)
HCTZ	12.5	80	10	100.32	0.985
		100	12.5	100.77	0.453
		120	15	100.00	0.852
AML	5	80	4	99.32	0.254
		100	5	99.65	0.284
		120	6	99.01	0.187
LOS	50	80	40	100.95	0.309
		100	50	99.88	0.213
		120	60	100.10	0.418

S.D: standard deviation; COV: coefficient of variance;

Table 4: Results from determination of intra-day and inter-day precision, and LOD and LOQ

Drug	Intra-day precision (COV, %)	Inter-day precision (COV, %)			LOD Ng/mL	LOQ Ng/mL
		Day 1	Day 2	Day 3		
HCTZ	1.242	1.400	1.321	0.401	0.014	0.043
AML	0.639	0.635	1.328	0.532	0.100	0.329
LOS	1.213	1.005	1.128	1.451	0.009	0.029

A Mean from six determinations COV: coefficient of variance; LOD: limit of detection; LOQ: limit of quantitation;

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