Research Article

SIMULTANEOUS ESTIMATION OF ALISKIRAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL FORMULATION BY RP-LC-PDA

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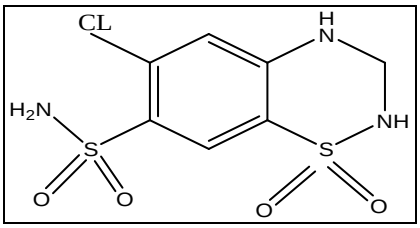
ABSTRACT

A simple, rapid, and precise reversed-phase liquid chromatographic method is developed for simultaneous determination of Aliskiren and Hydrochlorothiazide in their binary mixture of commercial pharmaceutical preparations. This method, reported first time for a binary mixture, uses a Kromasil C18, 250 × 4.6 mm, 5 µm analytical column. Analytes were estimated by isocratic elution with acetonitrile (ACN):tetrahydrofuran (THF):water (71: 13: 16 % v/v) mobile phase (pH adjusted to 3 with ortho phosperic acid) at flow rate of 0.5 mL/min; the column maintained at 50°C, and detector was set at 229 nm. The sample concentrations are measured on weight basis to avoid the internal standard. The method is validated and shown to be linear. The correlation coefficients for aliskiren and hydrochlorothiazide are 0.9998 and 0.9994, respectively. The recovery values for aliskiren and hydrochlorothiazide ranged from 99.25–99.54% and 98.84–100.01%, respectively. The relative standard deviation for six replicates is always less than 2%. The method is successfully applied for simultaneous quantitative analysis of the title drugs in combined tablets.

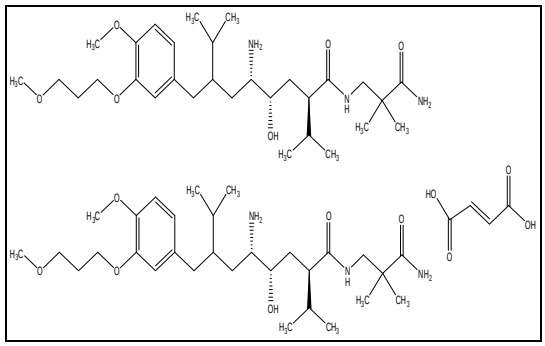
Keywords: Aliskiren, Hydrochlorothiazide, Isocratic elution, Reversed-Phase.

INTRODUCTION

Aliskiren (ALIS), (2S,4S,5S,7S)-5-Amino-N-(2-carbamoyl-2-methylpropyl)-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(3-methoxypropoxy) benzyl] -8-methylnonanamide hemifumarate (Figure 1a), the first oral direct renin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension.[1,2] Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II. A few UV Spectrophotometric and HPLC methods have been reported individually or in combination with other drugs for estimation of Aliskiren.[3] Hydrochlorothiazide (HTZ) is chemically known as 6-chloro-3,4-dihydro-7-sulfamoyl-2H-1,2,4-benzothiadiazine - 1,1-dioxide. It is a thiazide diuretic and increases sodium and chloride excretion in distal convoluted tubule. Hydrochlorothiazide is official in IP and various UV, HPLC, HPTLC and stability indicating methods have been reported for its quantitation, individually or in combination with other drugs.[6-24] To our knowledge there is no HPLC method reported for the combination of titled analytes, availability of an HPLC method with high sensitivity and selectivity will be very useful for the estimation of ALIS and HTZ in combined pharmaceutical dosage forms. Therefore the aim of the study was to develop and validate sensitive, precise, accurate and specific HPLC method for the determination of ALIS and HTZ simultaneously in tablet formulation. The present work describes a simple reverse phase LC method for the determination of ALIS and HTZ in tablets. During present study efforts were directed towards use of mobile phase without salt to increase column life and method was validated according to ICH guidelines.



a) Structure of Hydrochlorothiazide (HTZ)



b) Structure of Aliskiren (ALIS)

Figure 1: Structures of analytes a. HTZ b. ALIS

MATERIALS AND METHODS

Chemicals

ALIS (purity, 99.92%) was gifted by Vivan Life Sciences Pvt. Ltd. Mumbai and HTZ (purity, 99.93%) was obtained from Cipla Pvt. Ltd., Mumbai. Methanol (HPLC grade) was purchased from E. Merck (India) Ltd., Worli, Mumbai, India. Double distilled water prepared at lab scale was used throughout the experiment. Tablets were purchased from local market, containing ALIS 150 mg and HTZ 12.5 mg each per tablet. (Tablet RasilezTM, Lot: T0027, Novartis Healthcare Pvt. Ltd., Mumbai.

Instrumentation and chromatographic conditions

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Kromasil C–18 (250 mm × 4.6 mm, 5.0 µ) columns maintained at 50 0C using column oven. Isocratic elution with ACN:THF:Water (71: 13: 16 % v/v) mobile phase (pH adjusted to 3 with ortho phosperic acid) at the flow rate of 0.5 mL/min was carried out. The detection was monitored at 229 nm and injection volume was 10 µL. The peak purity was checked with the photodiode array detector. The mobile phase and sample solutions were filtered through 0.45 µ membrane filter and was degassed by ultrasonication for 15 min prior to use.

Preparation of Standard and Sample solutions and calibration graphs

The standard stock solutions of (ALIS) and (HTZ) were prepared separately by dissolving and diluting accurately about 50 mg analytes to 50 ml with methanol to obtain a final concentration of 1.0 mg/ml. Standard stock solutions were suitably diluted to have series of solution containing 1.2 - 480 µg/ml of ALIS and 0.1 - 40 µg/ml of HTZ and used to check linearity. Mix standard solutions of analysts in the same concentration were prepared and injected into column. A calibration curve was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. Sample solution was prepared by using tablet powder (form 20 tablet) equivalent to one tablet containing 150 µg/ml of ALIS and 12.5 µg/ml of HTZ and dissolved in the 30 mL of methanol with the aid of ultrasonication for 7 min and solution was filtered through Whatman filter paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with methanol. The solution was suitably diluted further with methanol to get required final concentration of ALIS (150 µg/ml) and HTZ (12.5 µg/mL), filtered through 0.45 µ membrane filter and used.

Method validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using seven-independent test solutions. The intermediate precision of the assay method was also evaluated. Assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to pre analyzed tablet solution. The mixtures were extracted and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in standard and sample solutions preparation. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 mL/min. Column temperature was varied by (±) 2°C and effect of column from different suppliers was studied. Measurement wavelength was varied by (±) 1 nm. The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing at 4°C for 30 days, auto-sampler stability was determined by storing the samples for 24 h in the auto-sampler.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Mobile phase containing water and methanol was initially used. In this view water and methanol were tried in different ratios as a mobile phase at a different pH (3-7) and along with change in column temperatures. All the times peak shape was unacceptable and HTZ retention time was too long (around 20 min) and both the peaks showed tailing. Mobile phase containing THF was tried, with addition of THF retention of HTZ reduced, but there was increase in baseline disturbance and tailing was reduced but it was still unacceptable. Two columns were used for performance investigations, including Kromasil C18 (5 micron 4.6 × 250 mm) and Qualisil C8 (5 micron 4.6 × 250 mm), the first column was the most suitable one since it produced symmetrical peaks with high resolution. To improve the peaks methanol was replaced by ACN and mobile phase with different (pH 3 - 4) was used, which showed reduced tailing and baseline disturbances when column was maintained at 50°C. Ultimately mobile phase ACN: THF: Water (71: 13: 16 % v/v) pH adjusted to 3 with ortho phosperic acid (OPA) and column was maintained at 50°C shown good resolution, peak shape and desired elution. Flow rate was selected to 0.5 ml/min for validation and short term stability studies. UV detection was carried out at 229 nm. Chromatogram showed symmetrical peaks with good shapes; tailing factor for ALIS and HTZ were within range and the resolution of the standard drugs was satisfactory. Retention time of ALIS and HTZ was 3.7 min and of 4.8 min, respectively.

Validation of method

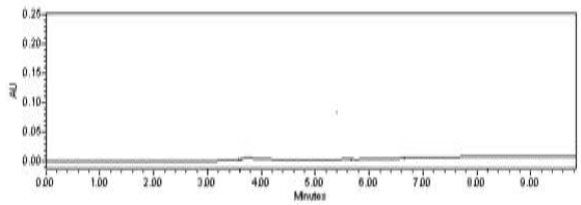
Linearity and Range

For the construction of calibration curves, seven calibration standard solutions were prepared over the concentration range. Linearity was determined for ALIS and HTZ in the range of 1.2 – 480 µg/mL and 0.1 - 40 µg/mL, respectively. The correlation coefficient ('r') values were > 0.9998 (n = 6). Typically, the regression equations for the calibration curve was found to be Y = 16404 X+ 33407 for ALIS and Y = 167323 X + 46291 for HTZ (Table 1).

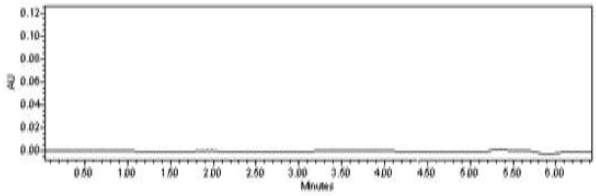
Application of the method to dosage forms

Marketed formulation was evaluated for the amount of the drugs present in the formulation. The amount of ALIS

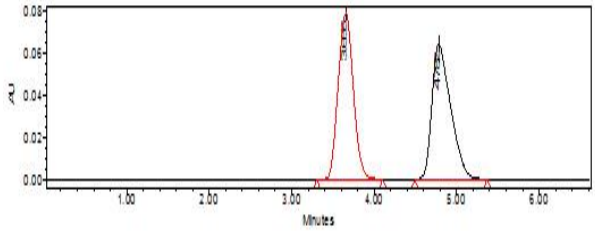
and HTZ estimated was in the range of 99.25-99.52 and 98.84-100.01%, respectively. None of the tablet ingredients interfered with the analyte peak (Table 1 & Figure 2).



(a) Placebo



(b) Mobile phase



(c) Formulation

Figure 2: Representative chromatograms obtained for (a) placebo, (b) mobile phase and (c) ALIS and HTZ extracted for formulation.

Table 1: Intraday and Inter day precision of ALIS (n=3)

ALIS	Measured concentration (µg/ml), % RSD	
Conc. (µg/ml)	Intra day	Inter day
180	180.05,0.39	181.09,1.35
240	241.08,0.75	240.22,0.75
300	300.51,0.45	300.12, 0.27

Specificity

The specificity of the HPLC method shows where complete separation of ALIS and HTZ was noticed in presence of tablet placebo (Fig 2). In addition there was no any interference at the retention time of ALIS and HTZ in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere to the analytes.

Precision and Accuracy

Precision

The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. (Table2)

Table 2: Intraday and Inter day precision of HTZ (n=3)

HTZ	Measured concentration (µg/ml), % RSD	
Conc. (µg/ml)	Intra day	Conc. (µg/ml)
15	15.19,0.26	15
20	20.05, 0.44	20
25	25.53,0.76	25

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out according to ICH guidelines by applying the standard addition method to known amount of ALIS and HTZ corresponding to 50, 100 and 150%. The recovery studies were performed three times at each level. (Table 3)

Table 3: Results of formulation analysis and accuracy studies

Drug Name (Label Claim)	Formulation Study (n=6)	Recovery Study (n=3)	
	% Assay Found, % RSD	Recovery Level	% Recovery, % RSD
ALIS (150 mg)	99.78, 0.86	50	99.54, 0.16
		100	99.25, 0.13
		150	99.52, 0.75
HTZ (12.5 mg)	99.41, 1.07	50	98.84, 0.61
		100	100.01,1.05
		150	99.37, 0.15

***Method Sensitivity* (Limit of detection; LOD and limit of quantitation; LOQ)**

LOD and LOQ for the procedure were performed on samples containing very low concentrations of analytes based on calibration curve method. Solutions of ALIS and HTZ were prepared in the range of 0.1 to 5 µg/ml and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using σ (standard deviation of the response) and b (slope of the calibration curve) and by using the equations LOD = (3.3 ×σ)/b and LOQ= (10.0 × σ)/b. The LOD and LOQ values were found to be 0.095, 0.29, µg/mL and 0.28, 0.89, µg/mL for ALIS and HTZ, respectively.

Method Stability

Solution stability as described in method validation under experimental section was studied. Result of short-term, long-term and the auto sampler stability of the ALIS and HTZ solutions were calculated form nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (98–102%).

Robustness

The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

Table 4: Result of robustness study

Factor	Level	% Assay, % RSD (n=3)	
		ALIS	HTZ
Flow rate (± 0.2 mL//min)	0.45	100.04, 0.29	99.97, 0.06
	0.55	98.78, 0.24	100.16, 0.22
Column oven temperature (± 2°C)	48	100.41, 0.49	99.98, 0.24
	32	98.54, 0.58	100.05, 0.77
Separation Column	Column I[a]	100.6, 0.92	100.12, 1.29
	Column II[b]	99.9, 0.83	100.04, 0.94
Measurement wavelength (±1 nm)	228.5	99.45, 0.53	100.15, 0.77
	229.5	99.8, 0.47	100.30, 0.28

System suitability parameters

To ascertain resolution and reproducibility of proposed chromatographic system for estimation of ALIS and HTZ in tablets, system suitability parameters like tailing factor (T), resolution (Rs) and column efficiency (number of theoretical plates, N) were studied. Standard stock solution containing ALIS (150 µg/ml) and HTZ (12.5 µg/ml) was used for analysis. The filtrate (10 µl) was injected into the column and chromatographed using optimized chromatographic conditions. The system suitability test was performed from six replicate injections of mixed standard solution. The corresponding chromatograms were recorded at 229 nm. System suitability parameters were calculated and given in Table 5.

Table 5: System suitability parameters (n=6)

Parameter		ALIS	HTZ
Retention time (t_R, min.)		3.7	4.8
USP Resolution[a] (R_S)		-	2.95
Tailing factor [a] (T_f)		1.02	1.24
No. of theoretical plates[a] (N)		2339	2449
k' (Capacity factor)		2.71	3.86
Peak Purity Data	Peak Angle	0.11	0.21
	Peak Threshold	0.23	0.27

CONCLUSION

A simple, specific, linear, precise, and accurate RP-HPLC-PDA method has been developed and validated for quantitative determination of aliskiren and hydrochlorothiazide in their binary mixture was carried out. The method is very simple and specific as all peaks are well separated and there is no interference by excipients peaks with total run time of 6 min, which makes it especially suitable for routine quality control analysis work. The method can be used for individual analysis of the titled drugs or their binary combinations.

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