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Simultaneous Estimation and Validation of Rampiril, Losartan Potassium and Hydrochlorothiazide by RP-HPLC in Pure and Pharmaceutical Dosage Form

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A simple, accurate, fast and sensitive RP-HPLC method was developed for the estimation of ramipril (RAM), losartan potassium (LTP) and hydrochlorothiazide (HCTZ) in pure and pharmaceutical dosage form. The quantification was carried out using a C_{18} column 250 × 4.6 mm id, 5 µm particle size in isocratic mode, with mobile phase comprising of buffer and acetonitrile in the ratio of 60:40 (v/v) pH 2. The flow rate was 1 mL/min and the detection was carried out with UV detector at 210 nm. The retention times were 4.78 1.69 and 3.09 min for RAM, LTP and HCTZ, respectively. The method produced linear response in the concentration range of 10-50, 100-300 and 25-125 µg/mL for RAM, LTP and HCTZ, respectively. The percentage recovery was found to be 99.6, 99.8 and 98.9 for RAM, LTP and HCTZ, respectively. Clavunate potassium used as internal standard in the present study. The method was statistically validated for its linearity, accuracy and precision. The intraday and inter day variation was found to be showing less coefficient of variation having high precision of method.

Key Words: RP-HPLC, Ramipril, Losartan potassium, Hydrochlorothiazide.

INTRODUCTION

Ramipril (RAM)[1] is an angiotensin converting enzyme inhibitor. Chemically, it is [2s,3as,6as]-1-[(2s)-2-[[(1s)-1-(ethoxy-carbonyl)-3-phenyl-propyl]amino]-1-oxo propyl]octahydrocyc1openta[b]pyrrole-2-carboxylic acid (m.f. $C_{23}H_{32}N_2O_5$, m.w. 416.511 g/mol and m.p. 109°C). It is similar to benzapril an inactive pro drug, RAM is converted to ramiprilat in the liver and it is used to treat hypertension and heart failure to reduce proteinuria and renal disease in patients with nephropathies and to prevent stroke, myocardial infarction and cardiac death in high risk patients.

Losartan potassium (LTP)[2], chemically, it is [2-butyl-5-chloro-3-[(4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol (m.f. $C_{22}H_2ClN_6O$ K, m.w. 461.01). It is also used in the treatment of heart failure and hypertension.

Hydrochlorothiazide (HCTZ)[1] popular diuretic. Chemically, it is 6-chloro-3H-dihydro-2H-l, 2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (m.f. $C_7H_8ClN_3O_4S_2$, m.w. 297.73). It acts by inhibiting the kidney's ability to retain water, this reduces the volume of the blood, decreasing peripheral vascular resistance. It is used in treatment of hypertension, cardiac heart failure, symptomatic edema, diabetes insipidus and hyper calciuria.

The literature survey[3-11] indicates that RAM, L TP and HCTZ has been determined individually and with other antihypertensive drugs by using UV, HPLC and HPTLC, LC-MS in pharmaceutical and biological fluids preparations. There is no method has been reported for estimation of RAM, LTP and HCTZ in a single dosage form. In the present investigation, an attempt was made to develop a simple and economical validated RP-HPLC with greater precision, accuracy and sensitivity for the simultaneous estimation of ramipril, losartan potassium and hydrochlorothiazide.

EXPERIMENTAL

Pure standards of ramipril (RAM), losartan potassium (LTP), hydrochlorothiazide (HCTZ) were obtained as gift samples from ATOZ Pharmaceuticals, Chennai, India. The purities of these standards were 99.5, 99.3 and 98.6 %, respectively. HPLC grade acetonitrile (Qualigens), potassium dihydrogen phosphate AR grade (Ranchem), potassium hydroxide (Qualigens) and phosporic acid (Qualigens), Sartace-H tablets (Microlabs) was employed in the present study.

A isocratic, high performance liquid chromatograph with Shimadzu pump Lc-10 ATVp equipped with universal injector (Rheodyne) with injection volume 20 µL, ultra violet visible detector (UV-Vis) SPD-10AV_A-Shimadzu series and Shimadzu class Vp software. A thermo hypersil key stone C-18 ODS column 250 × 4.6 mm id with 5 mm particles. Detection was carried out by UV detection at 210 nm.

Chromatographic conditions: Freshly prepared 60:40 (v/v) buffer and acetonitrile used as mobile phase. These were filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was 1 mL/min. The column was maintained at ambient temperature. The detection was carried out by at 210 nm. The injection volume was 20 µL and run time was 10 min.

Preparation of mobile phase: Buffer and acetonitrile in the ratio of 60:40 (v/v) were used as a mobile phase for present study. Buffer was prepared by taking accurately weighed quantity of 6.8045 g of potassium

dihydrogen phosphate was dissolved in water and make up to 1000 mL to obtain 0.05 M of potassium dihydrogen phosphate solution. Finally, the pH of the solution was the adjusted to 2 by adding *ortho*-phosphoric acid.

Preparation of internal standard solution: Weigh accurately an amount equivalent to 50 mg of internal standard and transferred into 50 mL volumetric flask. It was dissolved in mobile phase solution and volume made up to 50 mL so as to give concentration about 1 mg/mL solution (stock). Take 5 ml of stock solution made up to the volume to 100 mL with mobile phase to get concentration about 50 µg/mL. Clavunate potassium is used as a internal standard in the present study.

Preparation of stock solution of RAM, LTP and HCTZ: About 25 mg of RAM, LTP and HCTZ were weighed accurately and transferred into 50 mL volumetric flask. These were dissolved in mobile phase solution by shaking for 5 min. The volume was made up to 50 mL with mobile phase solution to get final concentration about 500 µg/mL for RAM, LTP and HCTZ, respectively.

Procedure for assay: From that stock solution take suitable volume 5-25, 2-10 and 20-60 mL of drug solution were transferred into 100 mL volumetric flask. These solutions were spiked with and 5 mL of internal standard solution make up to volume to 100 mL with mobile phase to get 10-50, 100-300, 25-125 and 50 µg/mL of RAM, LTP, HCTZ and clavunate potassium (IS), respectively. Five sets of RAM, L TP and HCTZ solution were prepared to get required concentrations, with 50 µg/mL clavunate potassium as an internal standard. The solutions were prepared as above were filtered through 0.45 µm membrane filter and each of the samples were injected five times into the column and flow rate was 1 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression analysis of the drug content over the ratio of drug peak area to that of internal standard was obtained.

Estimation of RAM, LTP and HCTZ in tablet dosage form: 10 Tablets were pulverized and an accurately weighed sample of powdered tablets equivalent to 5, 50 and 12.5 mg of RAM, LTP and HCTZ was taken in 50 mL volumetric flask. Add 50 mL of methanol and extracted by sonication to ensure complete solubility of drug. The mixture was made up to volume 50 mL with mobile phase solution (stock). The insoluble portion was filtered through a 0.45 µm membrane filter. From each stock solution 5-25 mL of filtrate was taken and diluted to 50 mL get required dilutions. The solutions were spiked with suitable 2.5 mL of the internal standard solution, such that the concentration of internal standard in each was 50 µg/mL. Each of these solutions was then injected five times in to the column. From those peak areas, the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The present study carried out to develop a simple, rapid, accurate and precise RP-HPLC method for the analysis of RAM, LTP and HCTZ in pharmaceutical dosage forms. The retention times for RAM, LTP and HCTZ were found to be 4.78, 3.09 and 1.69 min, respectively.

The ratio of the peak area of the RAM, L TP and HCTZ to peak area of internal standard for different concentrations set up as above were calculated and the average values of five such determinations given in below Table-1. By using peak area ratios, LOD, LOQ of RAM, LTP and HCTZ were calculated.

TABLE-1
CONCENTRATION *VERSUS* MEAN PEAK AREA RATIO OF RAM, LTP AND HTCZ

Drug	Drug concentration (µg/mL)	Mean peak area ratio*	Coefficient of variation (%)
RAM	10	0.15	0.85
	20	0.31	0.36
	30	0.45	0.25
	40	0.61	0.26
	50	0.76	0.20
LTP	50	0.32	0.75
	100	0.63	0.36
	150	0.89	0.25
	200	0.13	0.20
	250	0.15	0.17
HCTZ	25	0.19	0.86
	50	0.38	0.32
	75	0.57	0.17
	100	0.77	0.13
	125	0.96	0.26

*Mean of five values.

A good linear relationship (r = 0.9998) was observed between the RAM with respect to peak area ratio. The calibration equation was found to be Y = 0.0299x - 0.02679 (where Y = ratio of peak area of drug to that of internal standard, x = concentration of RAM).

A good linear relationship (r = 0.999) was observed between the LTP with respect to peak area ratio. The calibration equation was found to be Y = 0.0036x - 0.01512 (where Y = ratio of peak area of drug to that of internal standard, x = concentration of LTP).

A good linear relationship (r = 0.992) was observed between the HCTZ with respect to peak area ratio. The calibration equation was found to be Y = 0.0407x - 0.0028 (where Y = ratio of peak area of drug to that of internal standard, x = concentration of HCTZ).

The intraday and interday variations of the method were determined using five replicate injections of three difference concentrations which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed in Table-2.

TABLE-2
PRECISION OF METHOD

Drug	Concentration (µg/mL)	Observed concentration*			
		Intraday	CV (%)	Interday	CV (%)
RAM	10	10.09	0.17	10.14	1.42
	20	20.12	0.25	20.86	2.42
	30	29.98	0.55	30.12	0.47
LPT	50	50.06	0.43	50.55	1.21
	100	100.32	0.12	99.78	0.47
	150	150.45	0.19	150.89	1.52
HCTZ	25	25.59	0.47	25.02	0.92
	50	49.35	1.52	50.36	0.19
	75	75.02	1.21	75.14	0.25

*Mean of five values.

To ensure the reliability and accuracy of the method recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values were given in Table-3.

TABLE-3
RECOVERY STUDIES

Drug	Amount added (mg)	Amount present (mg)	Mean amount found* (mg)	Mean recovery (%)
RAM	10	110	119.89	99.57
	20	120	119.90	99.08
	30	130	129.56	99.68
LPT	50	150	149.06	98.40
	100	200	199.79	99.40
	150	250	249.90	99.50
HCTZ	25	125	123.99	98.32
	50	150	149.25	99.96
	75	175	175.92	101.79

*Mean of five values.

About 99.9 % of RAM, LTP and HCTZ could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of RAM, L TP and HCTZ estimated

and S.D was calculated. The values are given in Table-4 and system suitability parameters are given in Table-5.

TABLE-4
ESTIMATION OF AMOUNT PRESENT IN TABLET DOSAGE FORM

Brand name of tablet (Sartace-H)	Label claim (mg)	Amount estimated (mg)	Mean ± SD*	Mean ± SD % labeled amount*
RAM	5.0	4.97	4.95 ± 0.0125	99.42 ± 0.2848
LTP	50.0	52.3	52.06 ± 0.0004	104.89 ± 0.2891
HCTZ	12.5	12.9	12.8 ± 0.2356	103.76 ± 0.4613

*Mean of five values.

TABLE-5
SYSTEM SUITABILITY PARAMETERS

Parameter	RAM	LTP	HCTZ
Resolution factor	1.32	1.56	1.23
Theoretical plates	1015.1	1435.0	1297.0
Linearity range (µg/mL)	10-50	50-250	25-125
Limit of detection (µg/mL)	0.469	0.320	0.106
Limit of quantitation (µg/mL)	0.156	1.068	0.356
Relative standard deviation	0.796	0.865	0.746

It can be concluded that the proposed RP-HPLC method is simple, sensitive, rapid and reproducible for the analysis of RAM, L TP and HCTZ in pharmaceutical dosage form.

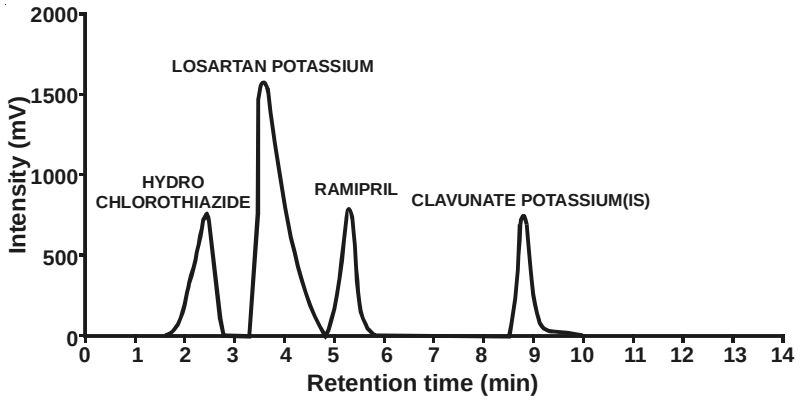

Fig. 1. A typical chromatogram of rampiril, losartan potassium and hydrochlorothiazide with internal standard

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