International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

RP-HPLC-PDA-Based Simultaneous Estimation of Ramipril and Hydrochlorothiazide in Combined Dosage Forms: A Validated Approach

Amod. S. Patil[1,4], Bhushan J. Mali[2], Bhupendra L. Deore[3*], Divya Mahajan[4], Shital Chaudhari[4], Nitin G. Haswani[4]

[1]Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur Maharashtra-425405, MS India
[2]Department of Pharmaceutical Chemistry, Gangamai College of Pharmacy, Nagaon, Dhule
[3]Department of Quality Assurance, DCS's A. R. A. College of Pharmacy, Nagaon, Dhule
[4]Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmacy, Karwand Naka, Shirpur Maharashtra-425405, MS India

Abstract

A rapid, highly sensitive High-Performance Liquid Chromatographic method has been developed for the simultaneous determination of Ramipril (RMP) and Hydrochlorothiazide (HCT) in bulk drug and in tablets. Ramipril (RMP) and Hydrochlorothiazide (HCT) were eluted from an Eclipse plus C18 (250 mm × 4.6 mm I.D.) with particle size 5 µm reversed phase column with a mobile phase consisting of acetonitrile and potassium dihydrogen *ortho*-phosphate pH 3 (40: 60 *v/v*) at a flow rate of 1 mL/min with UV detection at 210 nm. The retention time for RMP and HCT was found to be 3.31 ± 0.02 min and 6.64 ± 0.02 min, respectively. The linear response (r^2 <0.99) was observed in the range of 2 - 12 µg/mL for RMP and 4 - 24 µg/mL of HCT with low values of limits of detection (LOD) and quantification (LOQ) for both drugs. The method shows good recoveries and intra and inter-day relative standard deviations were less than 1.0%. Validation parameters as specificity, accuracy, ruggedness and robustness were also determined. The proposed method provides accurate and precise quality control tool for routine simultaneous analysis of Ramipril and Hydrochlorothiazide in bulk and in tablet dosage form.

Keywords: Ramipril; Hydrochlorothiazide; RP-HPLC; Validation

Introduction

Ramipril (RMP) (Figure 1) is chemically (2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b]pyrrole-2-carboxylic acid [1]. It is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system

(RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events [2].

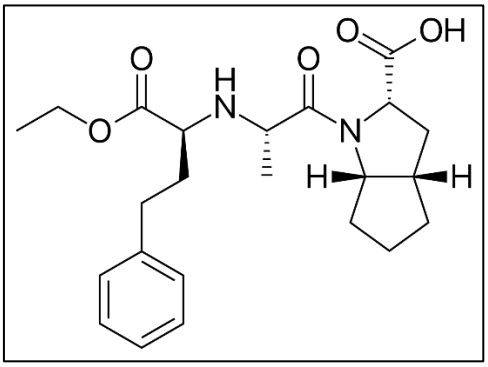

Figure 1: Chemical Structure of Ramipril

Hydrochlorothiazide (HCT) (Figure 2) is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. It is the most commonly prescribed thiazide diuretic. It is indicated to treat edema and hypertension. Hydrochlorothiazide use is common but declining in favour of angiotensin converting enzyme inhibitors. Many combination products are available containing hydrochlorothiazide and angiotensin converting enzyme inhibitors or angiotensin II receptor blockers [3, 4].

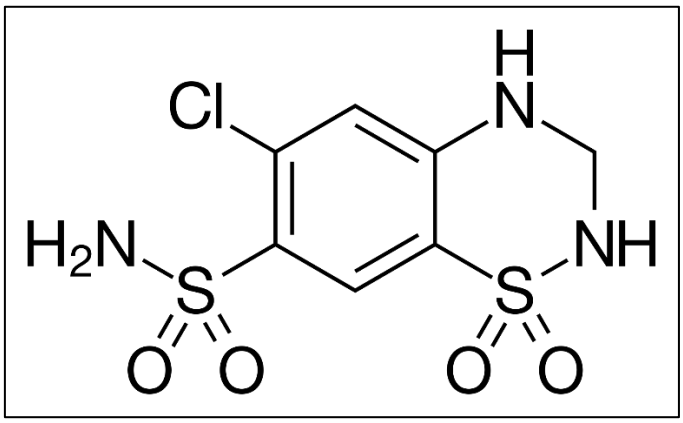

Figure 2: Chemical structure of Hydrochlorothiazide

In literature, several methods are reported for the quantification of Ramipril and Hydrochlorothiazide (alone or in combination) in bulk, pharmaceutical formulation and biological fluids. Such as UV-spectrophotometric [5-9], RP-HPLC [10-22], and HPTLC [23] methods are reported in the literature. Every analytical method is having several advantages and also limitations.

Therefore, an objective of the present work is to develop and validate RP-HPLC method for simultaneous estimation of ramipril and hydrochlorothiazide in bulk and pharmaceutical formulation as per ICH guidelines [24].

EXPERIMENTAL

Materials and reagents

Working reference standards of RMP and HCT were kindly supplied as gift samples by Ipca Laboratories Ltd., Mumbai, India. Marketed formulation RAMIPRES-H 5® (Label claim: RMP 5.0 mg and HCT 12.5 mg) were procured from the local pharmacy. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and double distilled R.O water was used for mobile phase preparation.

Equipment

The development and validation of the assay was performed on a UFLC-LC 20AD system (Shimadzu Corporation, Japan) provided with a LC-20AD solvent Deliver system (binary pump), SPD-M 20A Diode array detector, and LC solution software use as Data processor (version 1.25). SHIMADZU AUX 120 Weighing Balance was used for all weighing.

Preparation of mixed stock standard solution

Mixed stock standard solution of RMP (0.1 mg/mL) and HCT (0.2 mg/mL) was prepared by dissolving 10 mg of RMP and 20 mg of HCT in 100 mL methanol.

Chromatographic conditions

A stationary phase with C18 bonded phase i.e. Eclipse plus C18 (250 mm × 4.6 mm I.D.) with particle size 5 µm was selected with mobile phase consisting of acetonitrile and potassium dihydrogen *ortho*-phosphate (40:60 v/v) was tried and both these drugs were resolved properly. Well defined chromatograms were observed when the pH of the buffer was adjusted to 3.0 at flow rate of 1 ml/min; the retention time for RMP and HCT was found to be 3.31 ± 0.02 min and 6.64 ± 0.02 min, respectively. The total time of analysis was less than 10 min. The chromatogram of binary mixture is shown in Figure 3. Finalised chromatographic conditions are shown in Table 1

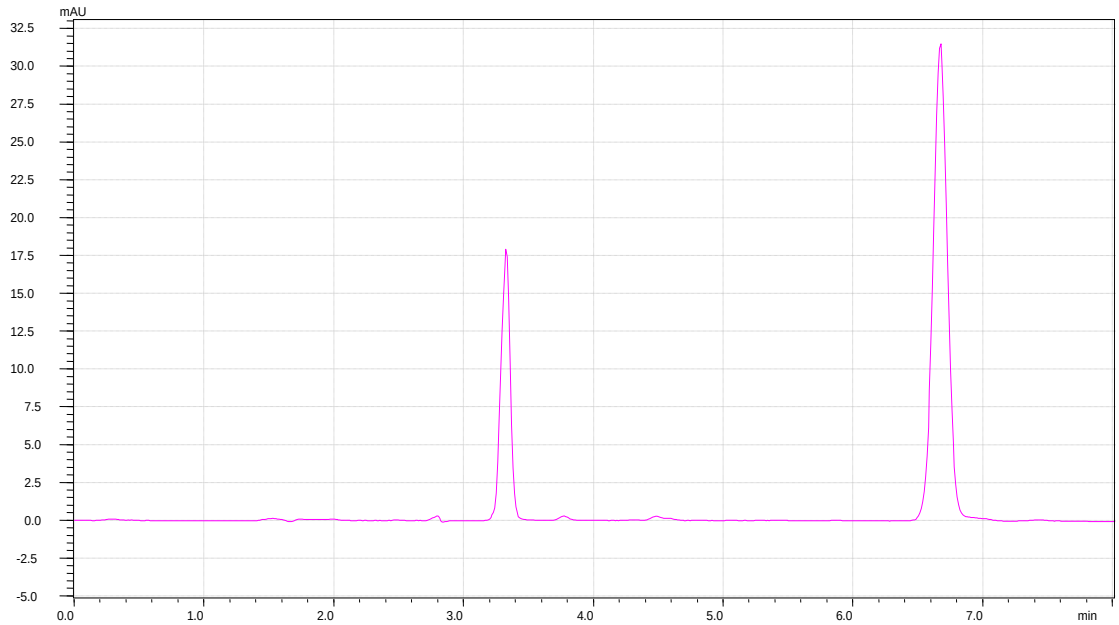

Figure 3: RP-HPLC Chromatogram of RMP and HCT in mixed stock standard solution

Table 1: Finalised chromatographic conditions

Chromatographic Mode	Chromatographic Condition
HPLC System	UFLC-LC 20AD system (Shimadzu Corporation, Japan)

Pump	LC-20AD solvent Deliver system (binary pump)
Detector	SPD-M 20A Diode array detector
Data processor	LC solution Data processor (version 1.25)
Stationary phase	Eclipse plus C_{18} column (250 mm × 4.6mm,5 µ)
Mobile phase	Acetonitrile : Buffer (40:60 %, *v/v*) pH 3
Detection wavelength	210 nm
Flow rate	1 mL/min
Sample size	20 µL

RESULTS AND DISCUSSION

Linearity studies

From the stock standard solution, aliquots portions (0.2 – 1.2 mL) were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of 2 - 12 µg/mL for RMP and 4 - 24 µg/mL of HCT. A constant volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration. The calibration curves are shown in Figure 3 and Figure 4.

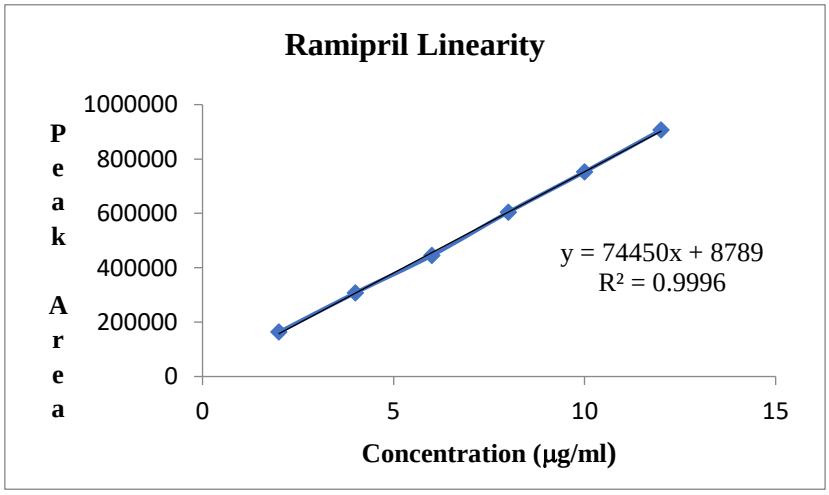

Figure 3: Calibration curve for RMP

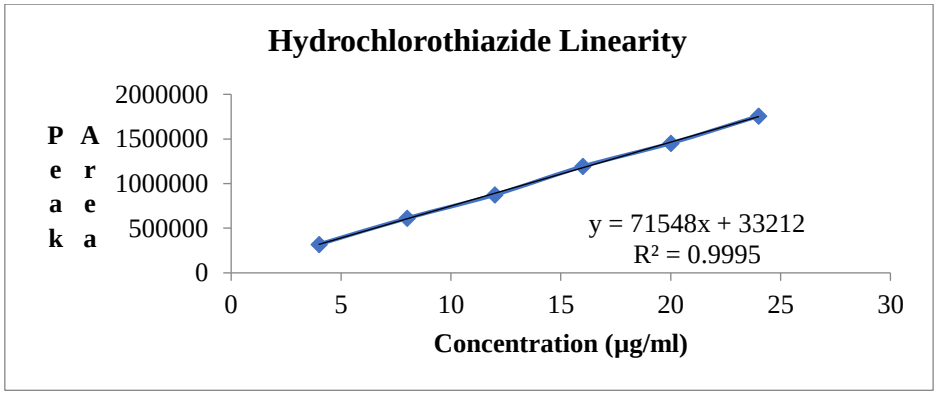

Figure 4: Calibration curve for HCT

Application of method for laboratory mixture

In order to see the feasibility of the method in the marketed formulation, it was first tried in physical laboratory mixture. Accurately weighed quantity of 10 mg (RMP) and 25 mg (HCT) were transferred to 100 mL volumetric flask containing 50 mL methanol and volume was adjusted up to mark. It was further diluted to get concentration 4 µg/mL of RMP and 10 µg/mL of HCT. Constant volume 20 µL was injected into column and peak area was recorded. The concentration of both these drugs were determined from their respective linearity curves. The procedure was repeated for six times. The percent amount ± SD of RMP and HCT was found to be 99.51± 0.82 and 99.62 ± 0.65 respectively.

Application of proposed method to tablet formulations

To determine the content of RMP and HCT in tablet formulation; twenty tablets RAMIPRES-H 5® (Label claim: RMP 5.0 mg and HCT 12.5 mg) were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of RMP and 25 mg of HCT was weighed and transferred into 100 mL volumetric flask containing about 50 mL methanol, sonicated for 15 min, and volume was made up to the mark with methanol. The solution was filtered through 0.45 µm membrane filter paper. The solution was further diluted with mobile phase to obtain concentration 4 µg/mL (RMP) and 10 µg/mL (HCT). The sample solutions were injected into column for six times. The concentrations of both these drugs were calculated from their linearity curve. Results are shown in Table 2.

Table 2: Analysis of tablet formulation

Parameters	RMP	HCT
Label Claim (mg)	5.0	12.5
Amount found[µg/mL]	4.0	10.0
Drug content(%) ± SD:n = 6	99.17 ± 1.07	99.50 ± 1.25
% RSD	1.07	1.26

Validation of Method

The objective of validation of an analytical procedure is to demonstrate that it is adequate for its intended purpose. To meet the pharmaceutical regulatory guidelines i.e., ICH guidelines [24] a number of parameters must be investigated in order to validate analytical methods such as accuracy, precision, sensitivity, specificity, ruggedness and robustness study.

Accuracy

It was performed by recovery study using standard addition method at 80, 100 and 120 % level; known amount of standard RMP and HCT was added to pre-analyzed sample 4 µg/mL of RMP; 8 µg/mL of HCT and subjected them to the proposed RP-HPLC method. Results are shown in Table 3.

Table 3: Results of Recovery studies

Drugs	Initial amount [µg/mL]	Excess drug added to the analyte (%)	Amount recovered ± S.D. [µg/mL, n = 3]	Recovery (%)	% RSD
	04	80	3.18 ± 0.03	99.38	0.94

RMP	04	100	3.98 ± 0.025	99.5	0.63
	04	120	4.78 ± 0.017	99.58	0.36
	08	80	6.38 ± 0.05	99.68	0.78
HCT	08	100	7.87 ± 0.04	99.37	0.51
	08	120	9.5 ± 0.03	98.95	0.32

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability was measured by multiple injections of a homogenous sample of 6 µg/mL of RMP and 12 µg/mL of HCT. Intra-day precision was studied by analyzing 4, 6 and 8 µg/mL of RMP and 8, 12, 16 µg/mL of HCT for three times on the same day. Inter-day precision was checked analyzing the same concentration for three different days over a period of week.

The results are shown in Table 4 and Table 5.

Table 4: Results for Precision studies

Drug	**Conc. [µg/mL]**	**Intra day Amount Found [%] [n = 3]**		**Inter day Amount Found [%] [n = 3]**	
		Mean	**% RSD**	**Mean**	**% RSD**
RMP	4	99.45	0.71	99.87	1.28
	6	98.13	0.84	98.34	0.56
	8	99.34	0.34	99.12	0.86
HCT	8	99.38	0.72	99.56	0.58
	12	98.81	0.65	99.62	0.83
	16	99.86	0.43	98.42	0.68

Table 5: Results for Repeatability studies

Drug	**Concentration [µg/mL]**	**Peak Area Mean ± SD:n=6**	**% RSD**
RMP	6	267467± 2836.16	1.11
HCT	12	884251 ± 5780.11	0.653

Robustness

Robustness of the method was studied by making deliberate changes in few parameters *viz*; change in flow rate, pH and mobile phase composition. The effects on the results were studied by injecting 6 µg/mL for RMP and 12 µg/mL for HCT; one factor was changed at one time to estimate the effect; the results are shown in Table 6.

Table 6: Results for Robustness Studies

Parameters	RMP		HCT	
	± SD of peak area [n=6]	% RSD	± SD of peak area [n=6]	% RSD
Change in pH of buffer				
2.8	3854.36	0.87	2684.55	1.22
3.2	3389.07	0.76	1625.50	0.73
Change in mobile phase composition				
(acetonitrile:buffer38:62v/v)	3936.29	0.89	4070.34	0.64
(acetonitrile:buffer42:58v/v)	3699.79	0.83	2542.75	1.15
Change in flow rate				
0.9	4780.56	1.09	2976.67	1.36
1.1	3794.10	0.85	1998.84	0.91

Sensitivity

The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae. LOD = 3.3(SD)/S; LOQ = 10 (SD)/S Where SD = Standard Deviation of response, S = the slope of the calibration curve. LOD and LOQ were found to be 0.32 µg and 0.96 µg for RMP and 0.63 µg and 1.89 µg for HCT, respectively.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of both the drugs; also the base line did not show any significant noise. The specificity of the HPLC method was determined by complete separation of RMP and HCT along with other parameters like retention time (t_R), capacity factor (k), tailing factor and asymmetric factor (T) etc.

Ruggedness

From stock solutions, sample solutions of RMP (4 µg/mL) and HCT (8 µg/mL) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times; the results are shown in Table 7.

Table 7: Results for Ruggedness study

Analyst	% Amount found (RMP)[%] [n = 3]	%RSD	% Amount found (HCT)[%] [n = 3]	%RSD
I	99.16	0.81	100.33	1.16
II	99.59	1.44	99.39	0.72

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Results are shown in Table 8.

Table 8: Results for System Suitability Test

System suitability parameters	RMP	HCT
Retention time (t_R)	3.31 min	6.64 min
Capacity factor (K')	0.8	2.2
Theoretical plate (N)	11489	7100
Tailing factor (T)	1.17	1.04

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