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A novel RP-HPLC method development and validation for quantitative determination of Ramipril and Hydrochlorothiazide in bulk and marketed solid dosage form

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Abstract
A simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of ramipril (RMP) and hydrochlorothiazide (HCT) in bulk and tablet dosage forms. Chromatographic separation was achieved using an Eclipse plus C18 column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of acetonitrile and phosphate buffer (40:60 v/v, pH 3.0) at a flow rate of 1.0 mL/min. Detection was carried out at 210 nm. The retention times for RMP and HCT were found to be approximately 3.31 min and 6.64 min, respectively, with a total analysis time of less than 10 minutes.The method exhibited good linearity over the concentration range of 2–12 µg/mL for RMP and 4–24 µg/mL for HCT, with correlation coefficients (r^2) of 0.999 for both drugs. The method was validated according to ICH guidelines and showed satisfactory accuracy, precision, robustness, and specificity. Recovery studies demonstrated accuracy within 99–100%, and %RSD values were less than 2 %, indicating high precision. The limits of detection (LOD) and quantification (LOQ) confirmed the sensitivity of the method. The validated method was successfully applied for the analysis of commercial tablet formulations, showing no interference from excipients. The results indicate that the proposed RP-HPLC method is suitable for routine quality control analysis of ramipril and hydrochlorothiazide in pharmaceutical formulations.

Keywords: RP-HPLC, method development, method validation, quantitative determination, ramipril, hydrochlorothiazide

Introduction

Ramipril and hydrochlorothiazide is used to treat hypertension, or elevated blood pressure. Reducing elevated blood pressure can help avoid heart attacks, strokes, and renal issues [1] Ramipril, often known as an ACE inhibitor, facilitates easier blood flow throughout the body by relaxing blood vessels [2]. Hydrochlorothiazide, often known as "the thiazide diuretic," causes your body to produce more urine, which eliminates excess water and salt. Additionally, it facilitates easier blood flow throughout the body by relaxing the blood vessels. Ramithiazide 5 mg/12.5 mg and Ramithiazide 10 mg/25 mg are two strengths of the commercial formulation [3-5]. Ramipril and hydrochlorothiazide, branded as RAMIPRES-H5 tablets, are one such medication combination that is used to treat hypertension.

Ramipril prodrug in the class of drugs known as angiotensin-converting enzyme (ACE) inhibitors is ramipril. It is broken down into ramiprilat in the kidneys and, to a lesser degree, the liver. Ramiprilat is a strong competitive inhibitor of ACE, the enzyme that converts angiotensin I (ATI) to angiotensin II (ATII). ATII is an essential part of the renin-angiotensin-aldosterone system (RAAS) and controls blood pressure [6-8]. In people who are at high risk of cardiovascular events, ramipril may be used to treat hypertension, congestive heart failure, nephropathy, and to lower the rate of death, myocardial infarction, and stroke.Ramiprilis chemically(1S,5S,7S)-8-[(2S)-2-[[(1S)-1-ethoxycarbonyl-3-phenyl-propyl]amino]propanoyl]-8-azabicyclo[3.3.0]octane-7-carboxylic acid is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure.[9,10] One thiazide diuretic that has long been regarded as the archetypal member of this class is hydrochlorothiazide. It decreases the electrolytes' reabsorption from the tubules of the kidneys [11-13]. Water and electrolytes like sodium, potassium, chloride, and magnesium are excreted more frequently as a result. Edema, hypertension, diabetes insipidus, and hypoparathyroidism are among the conditions it has been used to treat [14, 15]. Suddenly, hypertension, congestive heart failure, symptomatic edema, diabetic insipidus, and renal tubular acidosis are all treated with hydrochlorothiazide [16-20]. Additionally, it is used to avoid kidney stones in people with excessive urine calcium levels. Hydrochlorothiazide, sometimes abbreviated HCT, HCTZ, or HZT is chemically 6-chloro 3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide is a popular diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water, used as antihypertensive [21-23]

Materials and Method

Reagents and chemicals

Ramipril (RMP) and Hydrochlorothiazide (HCT) were supplied as a gift sample by Cipla Ltd (Goa, India) and Zydus cadila Healthcare Ltd. (Ahmedabad, India). These drugs were used as working standard. All the chemicals were used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

Selection of chromatographic Mode

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of stationary phase

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with

C_{18} bonded phase i.e. RP- Eclipse plus C_{18} (250 mm × 4.6 mm I.D.) with particle size 5 µm was selected.

Selection of mobile Phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey; acetonitrile and buffer was selected.

Preparation of stock standard solution

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

Selection of Detector and Detection wavelength

From the overlain spectra 210 nm was selected for the estimation of both these drugs simultaneously.

Optimization of Chromatographic Parameters

Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimization of mobile phase strength

With a view to separate out both the drugs simultaneously, various mobile phases consisting of acetonitrile and water were tried, but splitting of the chromatogram was observed for RMP (Figure 1). Therefore, mobile phase consisting of acetonitrile and potassium dihydrogenortho-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.

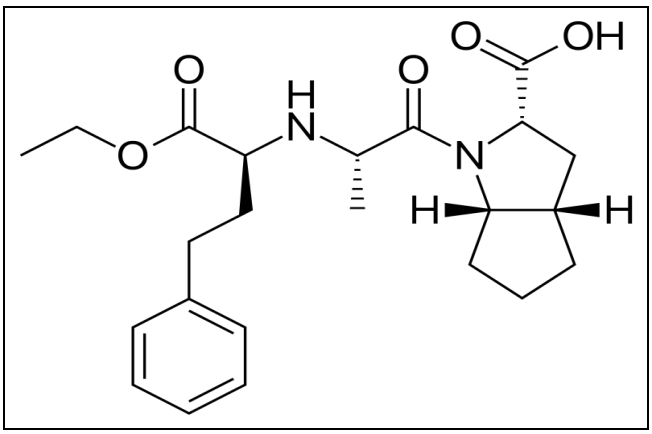

Fig 1a: Structure of Ramipril

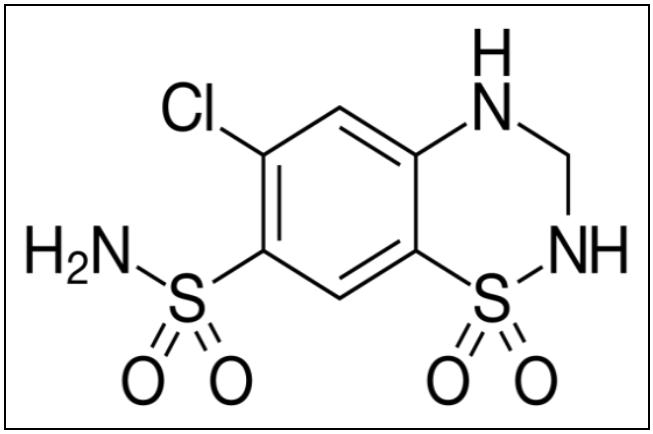

Fig 1b: Structure of Hydrochlorothiazide

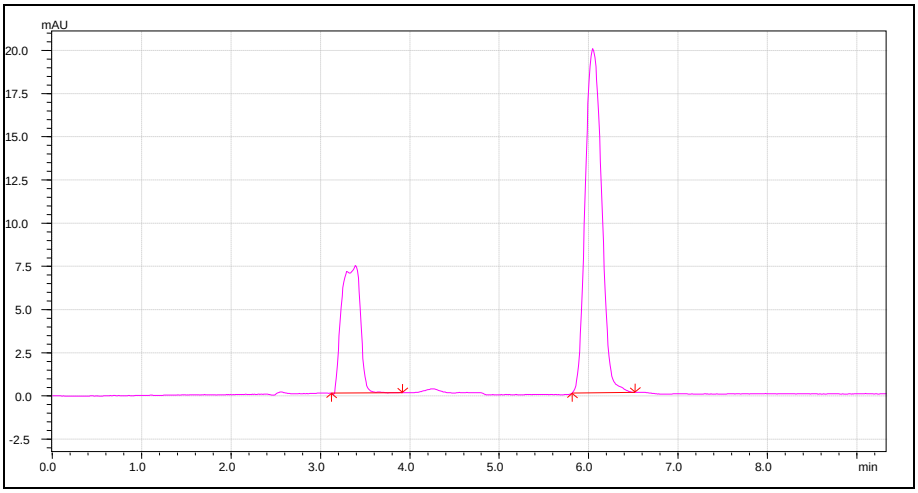

Fig 2: Chromatogram of RMP and HCT in (acetonitrile: water 65:35) pH adiusted to 5.0

Result and Discussion

Since the HPLC method has been developed, validation of method by using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications. Following parameters were performed for method validation:

- Linearity
- Accuracy
- Precision
- Robustness
- Sensitivity
- Specificity and Selectivity
- Ruggedness
- System suitability test

Linearity

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 observations are shown in Table 2, while calibration curves are shown in Figure 2and Figure 3.

Table 1: Linearity Study of RMP & HCT

Sr. No	Conc. of RMP [µg/mL]	Peak area [Mean ± SD; n = 5]	% RSD	Conc. of HCT [µg/mL]	Peak area [Mean ± SD; n = 5]	% RSD
1	2	163346.6 ± 1631.74	1.00	4	320451.4 ± 4165.87	1.30
2	4	307822.8 ± 4198.56	1.36	8	612354.5 ± 5449.96	0.89
3	6	444872.8 ± 4248.38	0.95	12	875241.3 ± 5776.59	0.66
4	8	603974.2 ± 4883.03	0.81	16	1192541.6 ± 7393.76	0.62
5	10	752744.6 ± 4401.88	0.58	20	1452354.4 ± 6245.12	0.43
6	12	906873.4 ± 4055.56	0.45	24	1756324.1 ± 11416.1	0.65

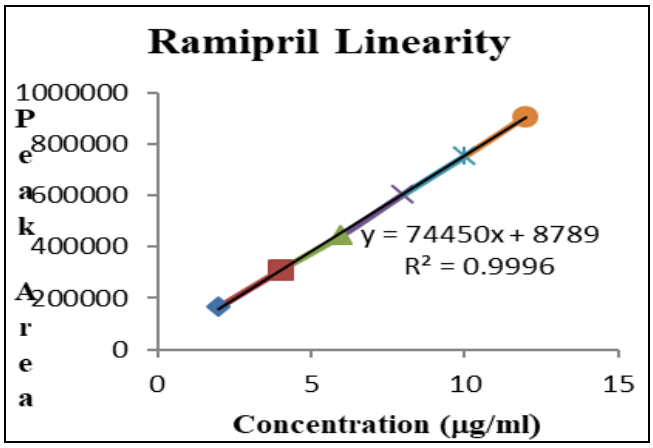

Fig 3: Calibration curve for RMP

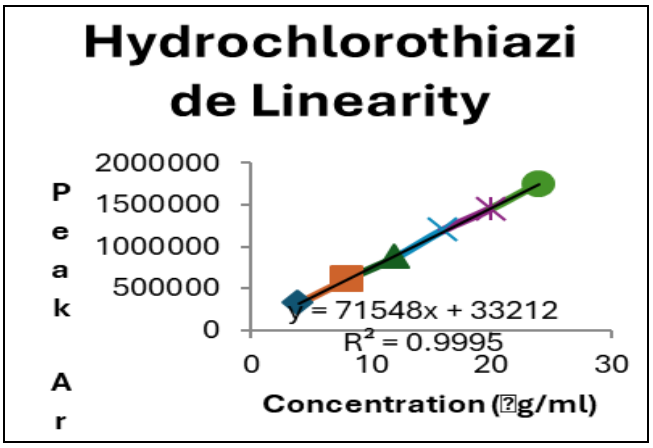

Fig 4: Calibration curve for HCT

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 20 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; results are shown in Table4 and chromatogram in Figure 4.

Table 2: Analysis of bulk material and Tablet formulation

Bulk material			Tablet formulation	
Parameters	**RMP**	**HCT**	**RMP**	**HCT**
Amount taken [µg/mL]	4.0	10.0	5.0	12.5
Amount found[µg/mL]	4.0	9.96	4.0	10.0
Drug content (%) ± SD, n =6	99.5± 0.82	99.62 ± 0.65	99.17 ±1.07	99.50 ± 1.25
%RSD	0.82	0.65	1.07	1.26

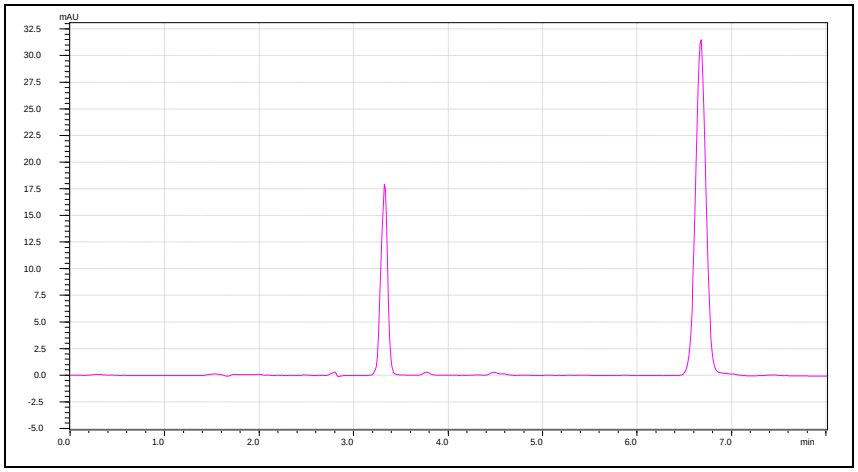

Fig 5: Chromatogram of RMP and HCT mixed stock standard solution

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 4 and Figure 5.

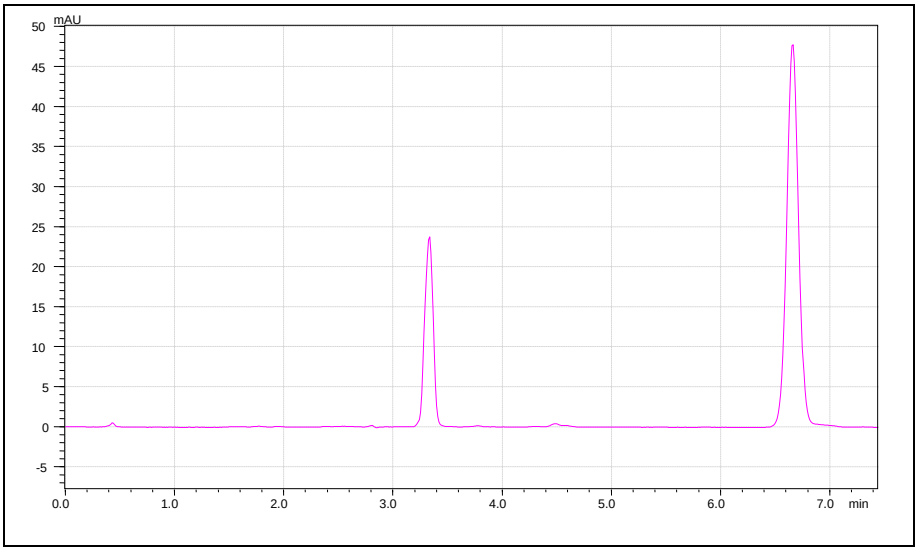

Fig 6: Chromatogram of RMP and HCT extracted from tablet formulation

Accuracy

It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard RMP and HCT were added to preanalyzed sample (4 µg/mL of RMP; 8 µg/mL of HCT) and subjected them to the proposed HPLC method. Results are shown in Table 5.

Table 3: 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 6µg/mL of RMP and 12µg/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.

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.

Table 4: Robustness Study

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

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 7.

Table 5: System Suitability parameters

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

Conclusion

A RP-HPLC method has been developed and validated for the simultaneous estimation of ramipril and hydrochlorothiazide in bulk and in tablet formulation. The HPLC analysis was performed on the Eclipse plus C_{18} column (250 mm × 4.6mm) 5µm particle size in gradient mode, at 30^0c using Acetonitrile: Buffer (40: 60 %, *v/v*)pH adjusted to 3 with ortho-phosphoric acid as mobile phase; flow rate was set at 1.0 mL/min. The detection was carried out 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.RMP and HCT followed linearity in the concentration range of 2-12 µg/mL (r^2 = 0.999) and4- 24 µg/mL (r^2 = 0.999) respectively. The method has successively been applied for the simultaneous determination of RMP and HCT in combined marketed formulation. There was no interference from the excipients routinely present in the tablet. The drug content for RMP and HCT was found to be 99.17 % ± 1.07 and 99.50 % ± 1.25. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within range of 99.38 – 99.95 %.The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The results were examined as % RSD values of concentration of drugs determined. The low value of % RSD (less than 2) indicates high precision of the method. The method proved to be adequately sensitivity as indicated by low values of LOD and LOQ

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