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Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Telmisartan and Ramipril in Tablet Dosage Forms

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ABSTRACT: The present research work deals with the development of RP-HPLC method for the determination of Telmisartan and Ramipril in bulk and in formulation using UV detector. Selected mobile phase was a combination of Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 70: 30 pH 3.4 (adjusted with Orthophosphoric acid) and the wavelength selected was 221nm. The flow rate was kept at 2.0 ml/min, and the injection volume was 20µl. The sepration was performed at ambient temperature. Retention time of Telmisartan and Ramipril was found to be 4.90 and 6.14 minutes respectively. Telmisartan and Ramipril which were found to be linear in the range of 40-240 µg/ml and 10-60 µg/ml respectively. The correlation co-efficient of Telmisartan was found to be 1 and the correlation co-efficient of Ramipril was found to be 1. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.92-100.02 for Telmisartan and 99.93-100.96 for Ramipril respectively The system suitability parameters such as theoretical plates and tailing factor were found to be 5708, 1.24 and 2460, 1.2 respectively for Telmisartan and Ramipril. This method was validated according to ICH guidelines.

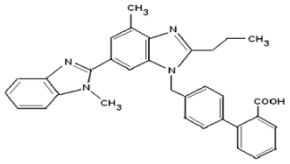
Keywords: Telmisartan, Ramipril, RP-HPLC, development, validation, simultaneous estimation

INTRODUCTION

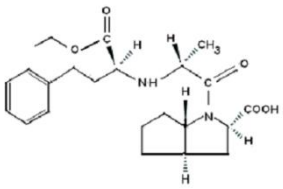
Telmisartan is a non peptide molecule. It is chemically described as 4' – [(1, 4'-dimethyl-2' propyl [2, 6' bi-1H benzimidazole] – 1'-yl) methyl] – [1, 1' biphenyl] 2-carboxylic acid. Telmisartan is a non peptide angiotensin II receptor antagonist which selectively and insurmountably inhibits angiotensin II AT1 receptor subtype without affecting other systems involved in cardiovascular regulation. Telmisartan blocks the vasoconstrictor and aldosterone secretion effect of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. AT2 receptor is found in many tissues. But AT2 is not known to be associated with cardio vascular homeostasis. Telmisartan has greater affinity (>3,000 fold) for AT1 receptor than for the AT2 receptor. Thus it is used for the treatment of hypertension [1].

Ramipril 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 used as anti- diuretic. There are two isoforms of ACE: the somatic isoform, which exists as a glycoprotein comprised of a single polypeptide chain of 1277; and the testicular isoform, which has a lower molecular mass and is thought to play a role in sperm maturation and binding of sperm to the oviduct epithelium. Somatic ACE has two functionally active domains, N and C, which arise from tandem gene duplication. Although the two domains have high sequence similarity, they play distinct physiological roles. The C-domain is predominantly involved in blood pressure regulation while the N-domain plays a role in hematopoietic stem cell differentiation and proliferation. ACE inhibitors bind to and inhibit the activity of both domains, but have much greater affinity for and inhibitory activity against the C-domain. Ramiprilat, the principle active metabolite of Ramipril, competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII. Decreasing ATII levels in the body decreases blood pressure by inhibiting the pressor effects of ATII. Ramipril also causes an increase in plasma renin activity likely due to a loss of

feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors. Ramipril is indicated for the treatment of mild to moderate hypertension[1], Congestive heart failure, myocardial infarction in patients with clinical evidence of heart failure [2]. For the present study the combination of Telmisartan and Ramipril was selected. The extensive literature survey carried out revealed that some methods for simultaneous estimation of by HPLC[3-6], HPTLC[7], Spectrophotometry[8] and LC[9] are available. Hence present study aims to develop a specific, precise, accurate, linear, simple, rapid, validated and cost effective RP-HPLC method for the simultaneous estimation of these drugs in combined dosage forms. Development and validation studies were carried out according to ICH guidelines [9].



Telmisartan



Ramipril

MATERIALS AND METHODS

Working standards of Telmisartan and Ramipril were received as gift samples from Dr. Ceeal Analytical Lab, Chennai. Tablets were purchased at Service Medicals. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from E-Merck (Mumbai, India). Sodium acetate and o-phosphoric acid were obtained from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade. HPLC grade Water was used. Shimadzu LC-20 AT HPLC with UV visible (SPD 20A) detector was used for the study. Column used was C18 inertsil 5µ, 250cm x 4.6 mm id.

CHROMATOGRAPHIC SYSTEM AND CONDITIONS

Stationary phase: INERTSIL C18 column (250x4.6mm id, 5µm)
Mobile phase: Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate)
pH: 3.4 (adjusted with Orthophosphoric acid)
Solvent ratio: 30:70
Detection wavelength: 221nm
Flow rate: 2.0ml/min
Temperature: Room temperature

PREPARATION OF MOBILE PHASE

Acetonitrile and buffer were mixed in the ratio of 30:70 and filtered through membrane filter and degassed in a sonicator for 10 minutes.

PREPARATION OF BUFFER (0.01M)

1.3609 gm of Potassium di-hydrogen phosphate in sufficient water to produce 1000ml, pH adjusted to 3.4 with orthophosphoric acid.

PREPARATION OF STANDARD SOLUTION

Standard Stock solutions of Telmisartan and Ramipril were prepared by accurate weighing of 100 mg for both the drugs in the separate 100 ml volumetric flask and dissolving in a methanol and then made up to mark with methanol. From the above stock solutions, suitable aliquots were transferred and standard mixture solution having concentration of 500 µg/mL of Ramipril and Telmisartan was prepared. For simultaneous quantitative studies of both drugs, a series of standard solutions containing both the drugs were prepared by appropriate dilution of mixture of workingstandard stock solutions.

PREPARATION OF SAMPLE SOLUTION

Twenty tablets were weighed and finely, powdered. Tablet powder equivalent to 5 mg of Ramipril and 40 mg of Telmisartan was accurately weighed and transferred to a 100 ml volumetric flask. To this was added about 50 ml of diluent and flask was sonicated for 10 min. Centrifuged this solution in a centrifuge tube with cap at 4000 RPM for 10 minutes. The flask was shaken, and the volume was made up to the mark with suitable diluent. Further dilutions were made.

METHOD DEVELOPMENT AND OPTIMISATION

The wavelength for the analysis of was selected from the UV spectrum of Telmisartan and ramipril by scanning in the range of 200-400nm. From this, the wavelength of 221nm was selected for the final

method as these drugs has shown good absorbances. 3D view of the combined spectra using PDA detector was observed, the maximum absorbance with good peak intensity, good peak shape and height was observed at 221nm (Figure 1).

For HPLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between Telmisartan and Ramipril. The mobile phase having a combination of acetonitrile and 0.01M potassium dihydrogen phosphate buffer of pH 3.4 in the ratio of 30:70 v/v. was found to be an appropriate mobile phase allowing adequate separation of two drugs using a C18 inertsil 5µ, 250cm x 4.6 mm id with flow rate of 2.0ml/min using PDA detection at 221nm. A typical chromatogram of separation of two compounds is shown in (Figure 2).

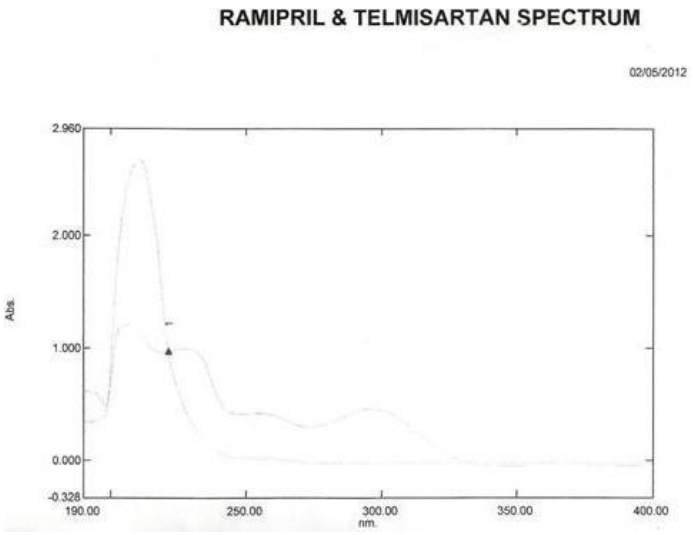


Figure 1: Overlay absorption spectra Telmisartan and Ramipril

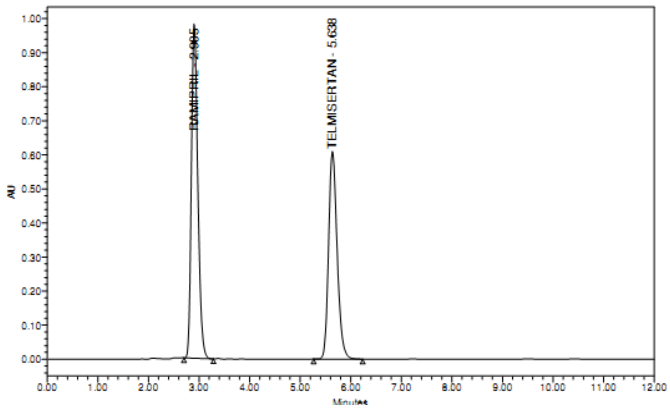


Figure 2: HPLC Chromatogram of Telmisartan and Ramipril.

METHOD VALIDATION

The developed method was validated for simultaneous assay determination of Telmisartan and Ramipril using following parameters.

Linearity

Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Telmisartan and Ramipril which were found to be linear in the range of 40-240 µg/ml and 10-60 µg/ml respectively. Coefficient of correlation was 0.9998 and 0.9999 (Figure 3).

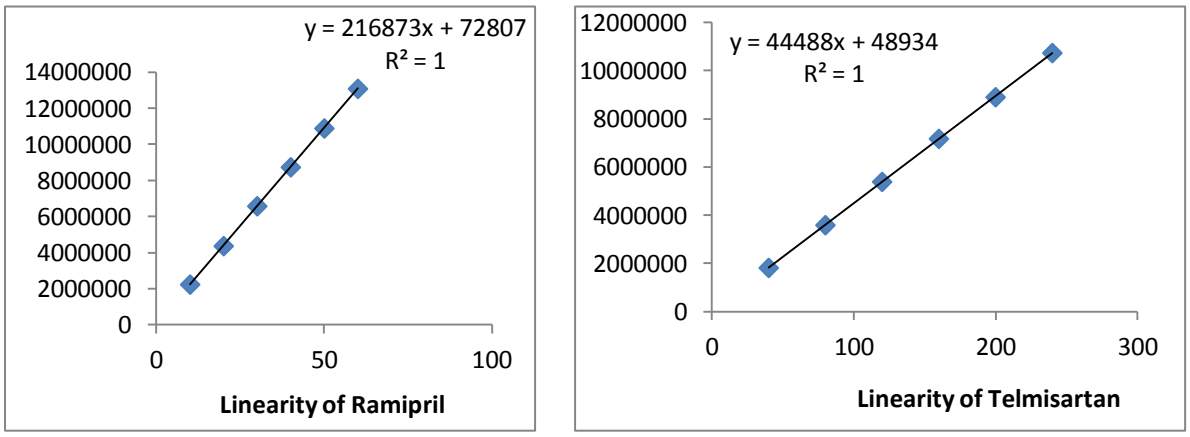


Figure 3: Linearty curve for Telmisartan and Ramipril.

Linearity calculation for Ramipril

Concentration Of Ramipril	Peak area	Statistical Analysis	
10	2254766	Slope	21687
20	4384905	y-Intercept	72807
30	6594695	Limit of detection	1.21700
40	8749662	Limit of quantification	3.79890
50	10900930	Correlation co efficient	1
60	13095293		

Linearity calculation for Telmisartan

Concentration of Telmisartan	Average area	Statistical Analysis	
40	1828792	Slope	44488
80	3600918	y-Intercept	48934
120	5397622	Limit of detection	1.21700
160	7183345	Limit of quantification	3.79870
200	8910487	Correlation coefficient	1
240	10742639		

Precision

It was demonstrated by the assay of six replicate samples. Six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.33and 0.10 for Telmisartan and Ramipril (Table 1).

Table 1: Assay of Ramipril and Telmisartan

Drug	Method Precision		
	Mean %	RSD (%)	Assay amount found
Telmisartan	97.94	0.10	0.23mg
Ramipril	98.3	0.33	0.28m

Ramipril

S.No.	RT	Area
1	2.888	8774269
2	2.88	8786169
3	2.875	8802689
4	2.871	8812564
5	2.868	8809470
6	2.861	8844757
Avg.	2.873833	8804986
stdev	0.009453	24339.9
%RSD	0.33	0.28

Telmisartan

S.No.	RT	Area
1	5.649	7209986
2	5.652	7214783
3	5.654	7209881
4	5.658	7225436
5	5.663	7225811
6	5.663	7253389
Avg.	5.6565	7223214
St. Dev	0.005822	16407.58
%RSD	0.10	0.23

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Telmisartan and Ramipril. The mobile phase combination of Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 30:70 PH 3.4(adjusted with Orthophosphoric acid) found to be satisfactory and gave two symmetric and well resolved peaks for Telmisartan and Ramipril .The retention time for Telmisartan and Ramipril were 4.90 and 6.14, respectively (figure 2). The calibration curve for Telmisartan was obtained by plotting the peak area of Telmisartan versus the concentrations of Telmisartan over the range of 10-60 µg/ml , and it was found to be linear with r2 =1. Similarly, the calibration curve for Ramipril was obtained over the range of 40-240 µg/ml and was found to be linear with r2 =1. The recoveries of Telmisartan and Ramipril were found to be in the range of 96.81%-97.94% and 97.48%-98.39% within precision RSD of 0.10 and 0.33 for Telmisartan and Ramipril. The system suitability parameters such as theoretical plates and tailing factor were found to be 5708, 1.24 and 2460, 1.20 respectively for Telmisartan and Ramipril. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The detection limit (LOD) was found to be 1.21 µg/ml for Telmisartan and 1.2170 µg/ml for Ramipril respectively.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The quantitation limit (LOQ) was found to be 3.798 µg/ml for Telmisartan and 3.798 µg/ml for Ramipril respectively. Proposed study describes a new RP-HPLC method for estimation of Telmisartan and Ramipril combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from interference of the excipients used in the formulation (Table 2). Therefore, the proposed method can be used for routine analysis of Telmisartan and Ramipril their combined dosage form.

Table 2: Validation and system suitability parameters

Parameters	Telmisartan	Ramipril
Linearity range µg/ml	40-240 µg/ml	10-60 µg/ml
Correlation Coefficient (r2) ± S.D	1	1
Retention time (min) ± S.D	4.90	6.14
Tailing factor	1.24	1.20
Theoretical Plate	5708	2460
Limit of detection (µg/ml)	1.21	1.21

Limit of Quantification (µg/ml)	3.798	3.798
Precision (RSD %) intraday (n=6)	0.10	0.33

CONCLUSION

In the current study a new RP-HPLC method for estimation of Telmisartan and Ramipril combination in mixture using simple mobile phase was developed, optimized and validated. The developed method is simple, sensitive, accurate and precise. The developed method can be used for routine analysis of Telmisartan and Ramipril in a combined dosage form.

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