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Method Development and Validation of Ramipril and Telmisartan in Pharmaceutical Dosage Forms BY RP-HPLC

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ABSTRACT

The simple, sensitive, reliable and economically new method was developed for the estimation of Ramipril (RAM) and Telmisartan (TEL) by RP-HPLC in combined dosage form. After several trials with the different combinations and ratios of solvents, the present chromatographic parameters were optimized. It was found that potassium dihydrogenphosphate (pH 3.0): methanol: acetonitrile (30:20:50 v/v/v) was given satisfactory results. A C_{18} column (Agilent ODS UG 5 column) having dimensions of 4.5mmx250mm was used. The mobile phase was pumped at a flow rate of 1.0ml/min and the eluents were monitored at 210nm. System suitability was carried out by injecting six replicate injections of 100% standard concentration, number of theoretical plates, HETP(height equivalent Theoretical plate) and resolution were satisfactory. The optimized chromatograms confirm the presence of Ramipril and Telmisartan at Rt: 4.1 min and Rt: 5.11min respectively without any interference. The concentration range of 1-5µg/ml for RAM and 8-40µg/ml for TEL were linear with correlation coefficients 0.999 and 0.989 respectively. The percent recovery studies were found to be 99.5-99.88% and 99.93-99.99% w/w for RAM and TEL respectively which indicate method was accurate. The proposed method was precise and reproducible with %RSD of 0.93 for Ramipril and 0.41 for Telmisartan, respectively. The limits of detection and limits of quantification were 0.10µg/ml and 0.25µg/ml for Ramipril 0.32µg/ml and 0.78µg/ml for Telmisartan, respectively. The method was found to be robust and ruggedness and was well suitable for the estimation of commercial formulations of selected combinations.

Keywords: Validation, RP-HPLC, Ramipril, Telmisartan, RSD and HETP.

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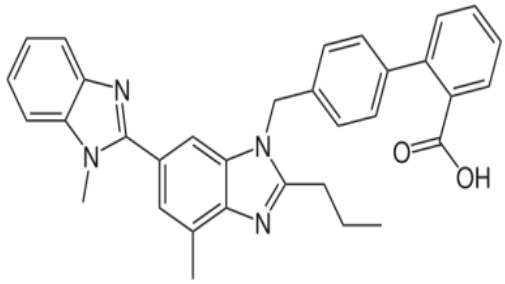
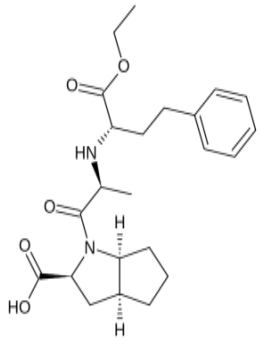
INTRODUCTION

Telmisartan (figure 1a) chemically, 2-(4-{[4-methyl-6-(1-methyl-1H-1, 3-benzodiazol -2-yl)-2-propyl-1H-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid. TEL is an angiotensin converting enzyme inhibitor and angiotensin II type I receptor blocker. It interferes with the binding of angiotensin II to angiotensin II AT1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland[1, 2]. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. TEL does not inhibit the angiotensin converting enzyme, other hormone receptors, or ion channels[3,4]. Ramipril (figure 1b) chemically, (2S, 3As, 6aS)-1-[(2S)-2-{[2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydrocyclopenta[b] pyrrole -2-carboxylic acid. RAM is an anti hypertensive and angiotensin converting enzyme inhibitor. It inhibits the actions of angiotensin converting enzyme (ACE), thereby lowering the production of angiotensin II and also decreasing the breakdown of bradykinin[5,6]. The decrease in angiotensin II results in relaxation of arteriole (arteriolar) smooth muscle leading to a decrease in total peripheral resistance, reducing blood pressure as the blood is pumped through larger diameter vessels. Its effect on bradykinin is responsible for the dry cough side effect[7]. Literature survey revealed that few analytical methods have been reported so far for the estimation of these two drugs simultaneously in combined dosage forms[8-14]. Hence, in the present study, a new reversed-phase high performance liquid chromatography method was developed and validated for simultaneous estimation of TEL and RAM. Method development for the estimation of RAM and TEL in combined tablet dosage form was initiated based on the method development guidelines[15] and literature review[16-22] several trails were conducted by changing the chromatographic parameters for optimization of method.



[a]

Figure 1[a]: Structure of telmisartan



[b]

Figure 1[b] Structure of ramipril

MATERIALS AND METHOD

Analytically, pure telmisartan and Ramipril were obtained as gift samples from M/s Dr. Reddy's Pvt.Ltd, Quality control department, Hyderabad and M/s Mylon Laboratories Pvt. Ltd., Hyderabad respectively. All the chemicals used were HPLC grade. Tablet formulation containing labeled amount of 20mg ramipril (ACOVIL) and 20mg of telmisartan (MICARDIS) and the combination formulation containing 5mg of ramipril and 40mg of telmisartan (TERAM* 5) was procured from local pharmacy.

Preparation of mobile phase

The mobile phase composition selected for the chromatographic separation of Ramipril and Telmisartan was Potassium dihydrogen phosphate buffer (pH 3.0): Methanol and Acetonitrile in the ratio of 30:20: 50v/v. The mobile phase was prepared using Potassium dihydrogen phosphate buffer 210 mL (pH 3.0 is maintained using orthophosphoric acid) (30%), 140ml Methanol (20%) and 350 mL Acetonitrile (500%) were mixed well and sonicated using 1.5L Ultrasonic bath sonicator.

Preparation of standard solution

Standard stock solutions were prepared by accurately weighed quantities of RAM and TEL dissolved separately in 10ml of methanol. Further dilution was made to obtain 100µg/ml with mobile phase. 1ml of RAM and 2.5ml of TEL pipette into 10 ml and 25ml volumetric flasks and diluted up to the mark with mobile phase.

Preparation of Sample Solution

20 tablets were accurately weighed and average weight of tablet was determined. The tablets were crushed to fine powder using mortar and pestle and transferred 0.0673mg powder equivalent to 10 mg of TEL into a 10 mL volumetric flask. 5 mL of diluent was added, sonicated for 10 min to dissolve, diluted up to the mark with and filtered through 0.45 µ Millipore Nylon filter. Further 1 mL of the above solution was diluted to 10 mL with diluent. The solutions were injected under above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

HPLC Method Development

The method was validated for the system suitability, linearity, detection limit, quantitation limit, accuracy, precision, robustness and ruggedness as per 'ICH' guidelines.

Linearity

The linearity of the method was demonstrated over the concentration range of 1 - 5µg/ml of Ramipril and 8-40µg/ml of RAM and TEL. Different level solutions were used for determining the linearity. 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of sample were taken in different volumetric flask of 10ml and diluted up to the mark with mobile phase. All the above solutions were injected separately in to the chromatographic system and chromatograms were recorded. Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration vs. peak area were constructed.

Limit of Detection and Quantitation

The Detection limit was determined based on the standard deviation of the response and slope. The detection limit may be expressed as 3.3σ/s, where σ is the standard deviation (SD) of the response'(s) is the slope of the calibration curve. The residual SD of a regression line or the SD of y-intercepts regression lines may be used as SD. Limit of quantization may be expressed as 10σ/s. 20 µL of the standard solution, 5 and 40 µg/ml of Ramipril and Telmisartan solutions were injected into the chromatographic system, chromatograms were recorded and peak areas were measured for the system suitability.

Accuracy and Precision

Accuracy was performed by following standard addition method. In this, standard was added to pre-analyzed sample solution. Accuracy studied by calculating recovery studies of the samples. The precision of the method was verified by performing repeatability and intermediate precision studies. 0.5 and 4ml of RAM and TEL standard stock solutions were transferred into each of six 10ml volumetric flasks and the volume was made up with the mobile phase. Injections from these solutions were given to the chromatographic system on the first day and the peak areas were recorded for repeatability. %RSD was calculated statistically from the obtained peak areas. For the Intermediate Precision, 6 injections from the solutions prepared on the first day were given to the system on the third day and peak areas were recorded and %RSD was calculated statistically.

Robustness and Ruggedness

As part of evaluation of robustness, deliberate changes were made in the flow rate and wavelength to evaluate the impact on the method. Standard solution prepared as per the test method was injected into the chromatographic system maintaining flow rates, less flow (0.5 ml/min), more flow (1.5ml/min) , actual flow (1 ml/min) and low wavelength (208nm), high wavelength (212nm) and actual wavelength (210nm). Ruggedness is a measure of reproducibility of the results under instrument to instrument and analyst to analyst variations. Injections of

working solutions of concentration 5µg/ml of RAM and 40µg/ml of TEL were given by the co-analyst and the peak areas were recorded. The %RSD was calculated statistically using standard tables.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, 0.01M potassium dihydrogen phosphate buffer (pH 3.0), Methanol and Acetonitrile 30:20:50v/v/v was selected as mobile phase because of better resolution and symmetric peaks. RAM and TEL were found to appreciable absorbance at 210nm when determined spectrophotometrically and hence it was selected as the detection wavelength. System suitability (table-1) was carried out by injecting 6 replicate injections of 100% standard concentration, number of theoretical plates, HETP and resolution were satisfactory.

Table 1: Data of System Suitability

Parameters	RAM	TEL
Retention time(min)	4.14	5.103
Tailing	1.34	1.5
Theoretical plates	1205	1643
%RSD*	0.781921	0.376

***%**Relative standard Deviation

The chromatograms confirm the presence of RAM and TEL at 4.1min and 5.11min respectively without any interference. Concentration range of 1-5 µg/ml for RAM and 8-40µg/ml for TEL were found to be linear with correlation coefficients 0.999 and 0.989 for RAM and TEL respectively (table 2).

Table 2: Data of Linearity

S.No	Levels	Peak name	Retention time(min)	Peak area
1	Level I	RAM	4.12	633926
		TEL	5.14	23343084
2	Level II	RAM	4.12	1197852
		TEL	5.16	42600615
3	Level III	RAM	4.13	1911778
		TEL	5.17	63229678
4	Level IV	RAM	4.12	2525704
		TEL	5.16	82920360
5	Level V	RAM	4.12	3119630
		TEL	5.14	102113452

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analyzed sample was calculated and found to be 99.5–99.88 % w/w, 99.93-99.99%w/w for RAM and TEL respectively, which

indicates that the method was accurate (table 4). The proposed method was found to be precise and reproducible with %RSD of 0.93 and 0.41 for RAM and TEL respectively (table 6). The limits of detection for RAM and TEL were found to be 0.10µg/ml and 0.25µg/ml respectively and the limits of quantitation were 0.32µg/ml and 0.78µg/ml. The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.5 ml). %RSD was calculated for each variation and reported. Ruggedness of the method was also verified by the change of the analyst. %RSD calculated was below 2.

Table 3: Assay of Ramipril and Telmisartan

Drug Substance	Label Claim (mg)		Percentage Purity (%w/w)
	Taken	Found	
Ram	5	5.09	101.8
Tel	40	39.7	99.3

Table 4: Summary Data of Accuracy

Drug	Recovery level	Peak area	Percent recovery
Ram	50	1197852	99.88
	50	1149222	
	50	1276454	
	100	1911778	99.5
	100	1907527	
	100	1890151	
	150	2525704	99.88
	150	2572071	
	150	2545255	
Tel	50	42865492	99.93
	50	42600615	
	50	42257489	
	100	63229678	99.99
	100	68929678	
	100	57769532	
	150	82920360	99.98
	150	83920360	
	150	82508484	

Table 5: Calibration Parameters of Ram and Tel

Parameters	Results of RAM	Results of TEL
Regression Equation	y = 62821x + 5714	y = 2535.6x – 1849.3
Slope	62821	2535.6
Intercept	5714	1849.3
Correlation Coefficient (R)	0.999	0.989

Acceptance Criteria: The percentage recovery for both RAM and TEL at each spiked level should be not less than 98% and not more than 102%.

Table 6: Intraday Precision Results

S.NO	Retention time (min)		Peak area	
	Ram	Tel	Ram	Tel
1	4.12	5.03	3119630	101399529
2	4.14	5.07	3063461	102213452
3	4.13	5.15	3135061	101896112
4	4.17	5.11	3106894	102413452
5	4.12	5.04	3118830	101379529
6	4.13	5.13	3133062	101789611
Mean			3112823	101848614
S.D*			29166.43	419429.19
%RSD**			0.93	0.411

*Standard deviation (average of six determinations).

%RSD **(Percent Relative standard Deviation)

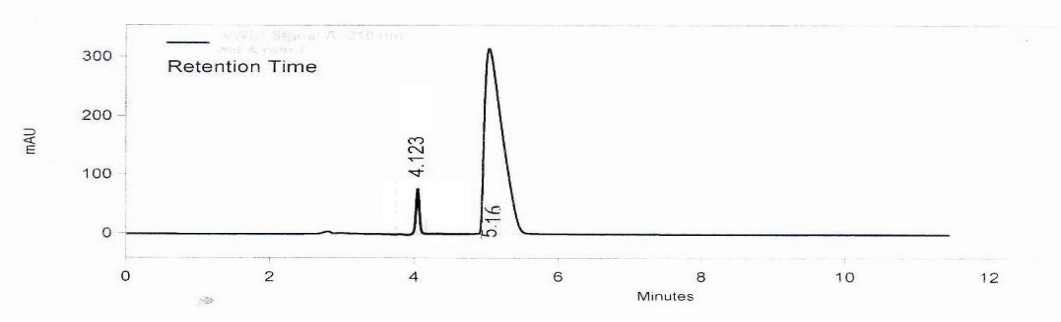


Figure 2: Optimized Chromatogram [Ramipril(rt:4.1mins) and Telmisartan(rt:5.1mins)]

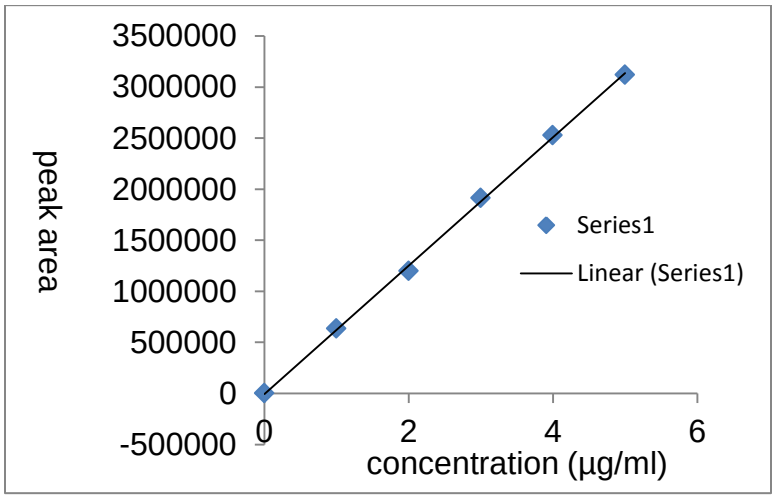


Figure 3: Calibration Curve Of Ramipril

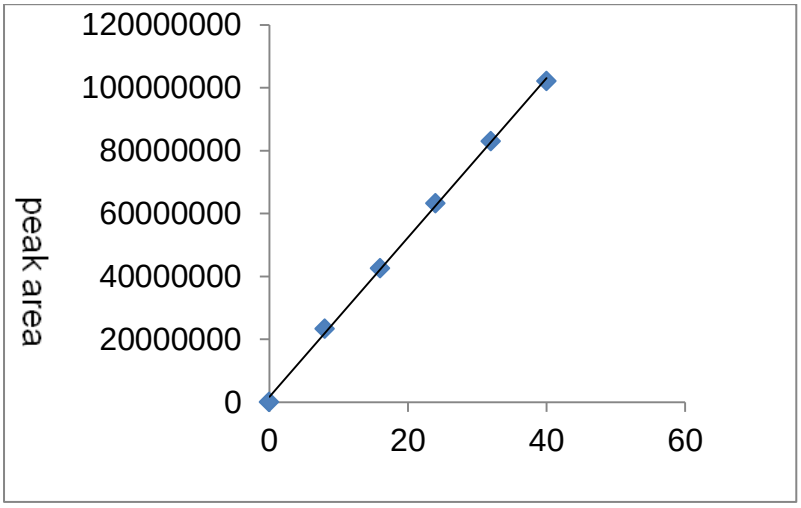


Figure 4: Calibration Curve of Telmisartan

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