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Der Pharmacia Sinica, 2014, 5(6):79-85

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Development and validation of an analytical method for simultaneous estimation of telmisartan and ramipril using reverse phase HPLC in bulk and dosage form

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ABSTRACT

*The present work is focused to develop and validate a simple, rapid, accurate, sensitive and specific, linear Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for the estimation of Telmisartan and Ramipril in tablet dosage form. The elution was carried out through INTERSIL C18 column (250*4.6mm and 5 µm) in isocratic mode, with mobile phase containing mixed phosphate buffer(pH3.0):ACN :Methanol (20:50:30 v/v/v%) at a flow rate of 1.0ml / min and monitored at 220 nm. Chromatogram showed peaks at a retention time of 3.033 for Telmisartan& 4.003 for Ramipril. The method is validated for system suitability, linearity, precision, accuracy specificity, robustness, LOD and LOQ. Recovery of Telmisartan and Ramipril is found to be in the range of 98.8%-100.50% respectively. The LOD and LOQ for estimation of Telmisartan & Ramipril are found to be 1.63µg/ml and 0.399µg/ml, and 4.9µg/ml and 1.2µg/ml respectively. Proposed method can be successfully applied for the quantitative determination of Telmisartan and Ramipril in Bulk drug and Pharmaceutical dosage form.*

Key words: Telimasartan, Ramipril, RP-HPLC

INTRODUCTION

Telmisartan

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. Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1 (AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2. In addition Telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Telmisartan activates PPARδ receptors in several tissues. Telmisartan has a molecular weight $C_{33}H_{30}N_4O_2$ [1].

Ramipril

(2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydro cyclopenta[b] pyrrole-2-carboxylic acid. For the management of mild to severe hypertension used to reduce cardiovascular mortality following myocardial infarction in hemodynamically stable individuals who develop clinical signs of congestive heart failure within a few days following myocardial infarction. To reduce the rate of death, myocardial

infarction and stroke in individuals at high risk of cardiovascular events used to slow the progression of renal disease in individuals with hypertension, diabetes mellitus and micro alubinuria or overt nephropathy. Ramipril has a molecular weight $C_{23}H_{32}N_2O_5$ [2]

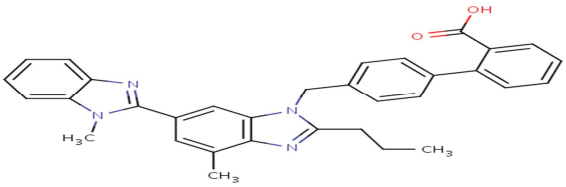

Fig -1: structure of Telimasartan

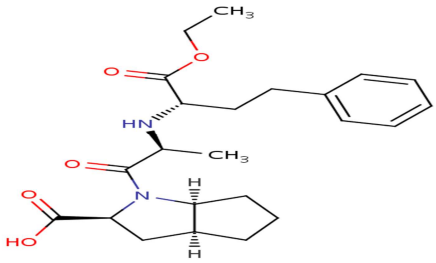

Fig-2: structure of Ramipril

The literature survey shows that spectroscopic and chromatographic methods for individual drugs and in combination with other drugs. However a few methods are available for the simultaneous quantification of Telimasartan and ramipri[3-15]l. The present work is aimed to develop a sensitive, accurate, precise and rapid method for routine analysis of this combination in pharmaceutical dosage form successfully which is economical than the existing ones.

MATERIALS AND METHODS

Instrumentation:
The present work was executed on a Shimadzu-Prominence HPLC system with PDA detector. A reverse phase HPLC column Intersil ODS C18 column (250 mm, 4.6mm and 5µm) was used for elution. The signal output was recorded and interpreted through LC solution software.

Chemicals and Solvents:
Water, Methanol Acetonitrile (HPLC grade), Orthophosphoric acid, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate of AR grade were procured from Merck (India) Ltd.

Buffer preparation:
1.625gm of potassium di hydrogen phosphate and 0.300gm of potassium hydrogen phosphate was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 4.0 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Determination of maximum wave length (λ max)
Solutions of 100µg/ml of Telmisartan and Ramipril were prepared separately and scanned from 190-400nm in UV-Visible spectrophotometer. The optimum response for both the analytes was observed at 220nm. Hence this wavelength was selected for further analysis

Preparation of standard:
80mg Telmisartan and 10mg Ramipril was dissolved in 100 ml of Diluent and was further diluted to get stock solution of Telmisartan and Ramipril. From this 1ml of the solution was transferred to 10 ml volumetric flask and made up with diluents to get 80µg/ml and 10 µg/ml respectively.

Preparation of sample solution
Ten tablets were weighed separately and powdered. Powder of tablets equivalent to 80 mg of Telmisartan and 10 mg of Ramipril were weighed and taken in a 100 ml volumetric flask, dissolved in diluent, shaken and sonicated for about 20 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted (1 to10ml) in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.

Preparation of placebo:
The inactive powder ingredient supposed to be present is accurately weighed and transferred in to 10ml volumetric flask, 7ml of diluent is added, sonicated for thirty minutes and was diluted to the mark with diluent and allowed to

stand. 0.8ml of the upper clear solution was transferred to a10ml volumetric flask and diluted with diluent up to the mark. The solution was filtered through 0.45µm filter before injecting into HPLC system.

Note: Mobile phase was used as diluent.

Chromatographic conditions:
A reverse phase HPLC column Intersil ODS C18 column (250 mm, 4.6mm and 5µm) was used for elution at ambient temperature. The mobile phase was pumped through the column at a flow rate of 1ml/min. The sample injection volume was 20µl. The detector was set to a wavelength of 220 nm and the chromatographic run time was set to 10 minutes.

Procedure:
20 µl of the standard, sample, Blank and placebo preparations in duplicate were injected separately into HPLC system and the peak responses for Telmisertan and Ramipril were measured. The developed RP-HPLC method for the simultaneous estimation of Telmisartan and Ramipril was carried out on Inertsil ODS 250*4.6mm in isocratic mode using mobile phase composition of Mixed phosphate buffer (pH3.0): ACN: Methanol (20:50:30 v/v/v %) with flow rate of 1.0 ml/min at 220 nm.

Method Development:
The method development was started with initial chromatographic conditions as stated above. Various compositions of phosphate buffer and acetonitrile were tested for better separation of the analytes. The first trial was initiated with composition Phosphate buffer (pH 2.5): ACN (20:80) at ph 3.5 .The retention time of Ramipril was more. Another trial was initiated with modification in mobile phase composition i.e. Mixed phosphate buffer (pH3.5): ACN:Methanol (20:60:20). Peak response of Telmisartan was less and efficiency less than 2000 was observed. Finally the method was optimized with the mobile phase composition mixed phosphate buffer (pH3.0): ACN: Methanol (20:50:30 v/v/v%).With this composition peaks for both the analytes were eluted with good resolution and the retention times and theoretical plates were also satisfactory. The chromatogram has passed the system suitability parameters and the retention times for Telmisartan and Ramipril were found to be 3.033 and 4.003 respectively. The chromatogram is shown **in figure-3.**

Method Validation:
The proposed method for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form is validated as per ICH guidelines by the following parameters.

System Suitability:
Sample solutions of Telmisartan and Ramipril were injected in replicates as per the procedure. From the standard chromatogram system suitability parameters like tailing factor, theoretical plates are recorded and peak areas were evaluated through %RSD. The results are given in **table-1&2**

Linearity:
Several aliquots of standard stock solutions of Telmisartan and Ramipril were transferred into 10ml volumetric flasks and diluted up to the mark by diluents to achieve the concentration level 50-150%. Each sample solution was injected into HPLC system in replicate and the peak areas were recorded. A graph of peak areas vs concentrations was plotted and the correlation coefficient was calculated. The results were shown in **table –3.**

Precision:
The standard solution was injected for six times and the peak areas for all six injections were measured in HPLC. The % RSD for the area of six replicate injections results were reported in terms of % RSD. The results are given in tables **-4**.

Accuracy
The accuracy of the proposed method was evaluated in triplicates by recovery studies at various concentrations of Telmisartan and Ramipril equivalent to 50,100&150%. The percentage recovery values were calculated and reported in **tables –5&6.**

Specificity:
It is the ability of the method to measure the analyte of interest specifically in presence of matrix and other components. Samples of blank and placebo were injected as per the test procedure. The chromatograms of blank and placebo were represented as **Fig no: 3&4.**

Limit of Detection and Limit of Quantification:
The detection limit of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. Limit of quantification is the lowest concentration of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy.

$$DL = \frac{3.3\,\sigma}{S}$$

$$QL = \frac{10\sigma}{S}$$

σ = standard deviation of the response

S= slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

Robustness:
It is the capacity of the analytical method to remain unaffected by small but deliberate variations in method parameters. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, wave length etc.

Effect of variation of flow rate:
The flow rate is varied between 0.8-1.2ml/min and the chromatograms are recorded.

Effect of variation of wave length:
Standard solution was prepared and injected into HPLC system and the chromatograms were recorded at three different wavelengths. The results are summarized in **table-7.**

Method validation:
The proposed liquid chromatographic method for the quantification of Telmisartan and Ramipril was optimized by a series of trials. At each trial the mobile phase composition was changed to improve the fineness of the chromatogram. Finally the method was optimized with the mobile phase buffer (pH3.0): ACN: Methanol (20:50:30 v/v/v %).The retention times of Telmisartan and Ramipril were 3.033 and 4.003 respectively. The chromatogram has fulfilled the system suitability parameters.

Specificity:

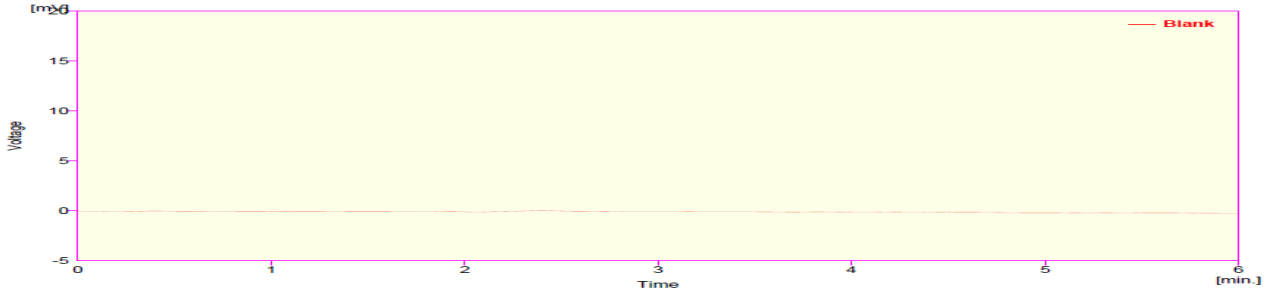

Fig-3 chromatogram of blank

Table -2 system suitability results of Ramipril

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.017	350897	3642	1.444	4.072
2	4.033	350553	3833	1.543	4.112
3	3.997	343520	3764	1.486	4.045
4	3.990	356161	3593	1.500	3.985
5	3.987	358580	4093	1.265	4.175
Mean		350255	-	-	-
SD		6.651	-	-	-
%RSD		**1.90**	-	-	-

Linearity:

The linearity of the method was determined by five replicate injections at 48-112µg/ml & 6 -14 µg/ml concentration levels of Telimasartan and Ramipril respectively. Linearity of detector response was established by plotting graph between concentrations versus average area counts of the analytes.The results are as follows:

Table -3 linearity data of Telmisartan and Ramipril

Injection no	Telmisartan		Ramipril	
	Concentration	Peak Areas	Concentration	Peak Areas
1	48	3363643	6	2110843
2	64	4114285	8	2650755
3	80	5011948	10	3147016
4	96	5770474	12	3690537
5	112	6630852	14	4202841
Correlation coeffecient		0.999		0.999

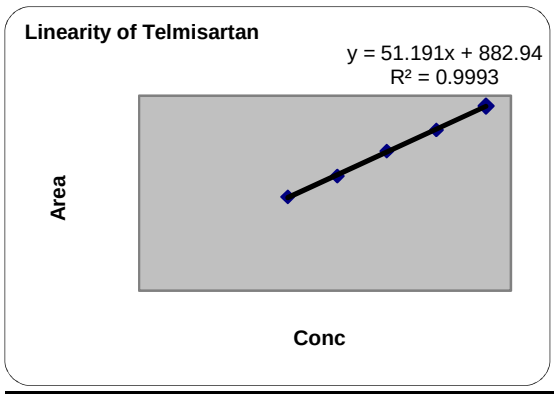

Fig no-5 linearity of Telmisartan

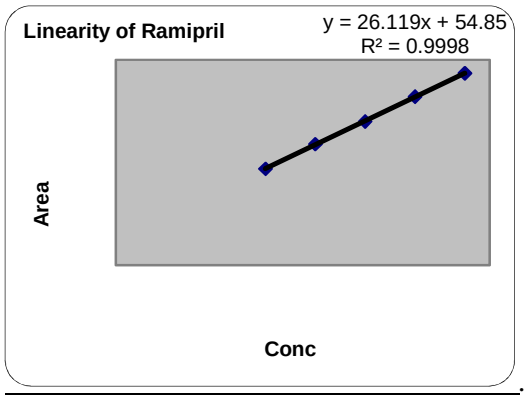

Fig no-6linearity of Ramipril

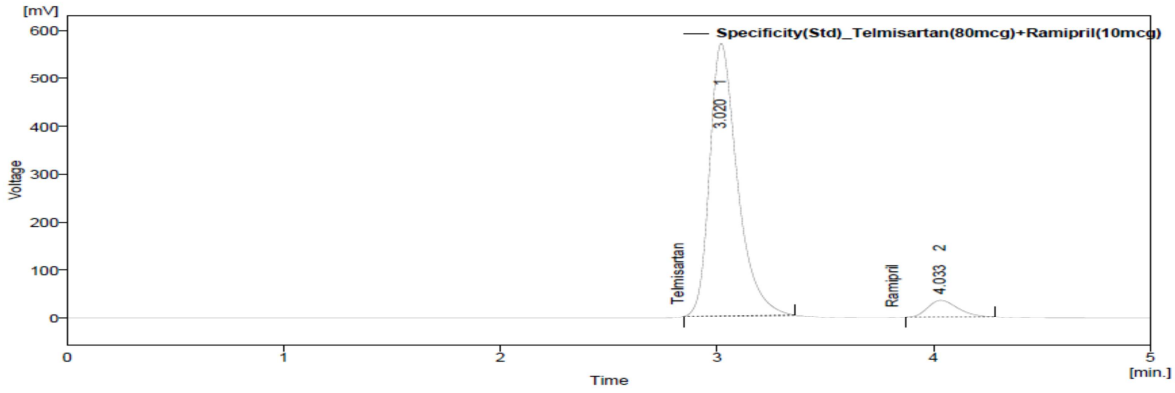

Fig-4 chromatogram of Telmisartan and Ramipril

Precision:
Six replicate injections of sample solutions were injected into the HPLC system and the areas were measured. The %RSD of the peak areas were within the limits.

No peaks were observed near the retention times of Telmisartan and Ramipril in the chromatogram of blank indicating no interference from mobile phase. Therefore the method is specific

System suitability:
The % RSD of retention time and peak areas of both the drugs was less than 2 and the other system suitability parameters were within the acceptable limits.

Table -1 system suitability results of Telmisartan

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.013	5143100	2830	1.645
2	3.013	5069727	2705	1.645
3	3.000	5097817	2669	1.677
4	2.997	5116744	2664	1.677
5	2.993	5120530	2792	1.645
Mean		5101080	-	-
SD		32.221	-	-
%RSD		**0.63**	-	-

Table-4 precision results of Telmisartan and Ramipril

Injection No.	RT	Area	RT	Area
1	3.013	5143100	4.017	350897
2	3.020	5069727	4.033	350553
3	3.000	5097817	3.997	343520
4	2.997	5116744	3.990	356161
5	2.993	5120530	3.987	358580
6	3.027	5058561	4.020	341821
AVG	3.0083	5101080	350255	350255
SD	0.0137	32.221	0.019	6.651
%RSD	0.46	0.63	0.46	1.90

Accuracy:
Accuracy was evaluated in triplicates by recovery studies at various concentrations of Telmisartan and Ramipril equivalent to 50,100& 150% and percentage recovery values were calculated and reported.

Table no-5 accuracy results of Telmisartan

Sample no.	Spiked Amount (mcg)	Recovered Amount (mcg)	%Recovered	Average mean recovery
1	80	79.91	99.88	98.81%
2	96	94.52	98.46	
3	112	109.87	98.10	

Table no-6 accuracy results of Ramipril

Sample no.	Spiked Amount (mcg)	Recovered Amount (mcg)	%Recovered	%Average recovery
1	10	10.12	101.23	100.50%
2	12	12.06	100.47	
3	14	13.97	99.81	

The percentage recovery for each level should be between 98.0 -102.0%.

The percentage recovery of Telmisartan and Ramipril are within the limits i.e.98.81 & 100.50%respectively.

LOD & LOQ:
Limit of Detection and Limit of Quantification were calculated

Robustness:

Table-8 robustness results –variation of flow rate & wave length.

Parameter	Telimasartan			Ramipril		
Flowrate (ml/min)	**RT**	**Tailing factor**	**Efficiency**	**RT**	**Tailing factor**	**Efficiency**
0.8	3.743	1.861	2790	4.980	1.550	3643
1.0	3.013	1.645	2805	4.017	1.444	3653
1.2	2.543	1.483	2569	3.367	1.375	3855
Wave length (nm)	**RT**	**Tailing factor**	**Efficiency**	**RT**	**Tailing factor**	**Efficiency**
218	2.997	1.700	2644	3.998	1.598	3586
220	3.013	1.645	2872	3.984	1.265	4067
222	2.990	1.667	2896	3.886	1.45	3675

The method is robust since the effeciency and tailing factors were within the limits even after little deliberate variations in flow rate (±0.2ml/m) and detection wavelength (±2).

CONCLUSION

A rational and valid attempt has been made for the development of a new liquid chromatographic method for the routine analysis of Telimasartan and Ramipril in tablet dosage form. The accountability of the proposed method has been established by evaluating validation parameters as per ICH guidelines. The results were in good agreement with acceptable limits. Therefore the method has been proven to be linear, precise, accurate, specific and robust. Therefore the present method can be adopted for the estimation of Telmisartan and Ramipril in bulk and also in combined dosage forms as a part of regular quality control analysis.

Acknowledgement
The authors are thankful for the management, St.Pauls College of Pharmacy, Hyderabad for providing necessary facilities.

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