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A new method development and validation for simultaneous estimation of ramipril and telmisartan by RP-HPLC method in combined dosage form

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Abstract

The present research work was development and validation of new RP-HPLC method for simultaneous estimation of Ramipril and Telmisartan in combined dosage form. In simultaneous RP-HPLC method development, Waters 2695 Separations Module with PDA Detector and column used is C8 SB ZORBAX (150 X 4.6mm) column with 3.5-micron particle size. Injection volume of 10 µL is injected and eluted with the mobile phase selected after optimization was Phosphate buffer and Acetonitrile in the ratio of 70:30 was found to be ideal. The optimized flow rate was at 1.0 mL/min. Detection was carried out at 230 nm. This system produced symmetric peak shape, good resolution and reasonable retention times of Ramipril and Telmisartan were found to be 2.275 and 4.261 minutes respectively. The Ramipril and Telmisartan showed linearity in the range of 20-60 µg/mL and 160-480 µg/mL respectively. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust and the %RSD value for percentage recovery of Ramipril and Telmisartan was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the Ramipril and Telmisartan.

Keywords: RP-HPLC; Ramipril; Telmisartan; UV Spectrum; HPLC water; Phosphate buffer

1. Introduction

Analytical chemistry has played a major role in the changes facing the pharmaceutical Industry today. Traditionally viewed as a service organization, the analytical department has become a significant parameter in the drug development process. Indeed, the demand for analytical data has become a critical path activity for selection of molecule for full development. The pharmaceutical analysis plays a major role in assuring, identity, safety, efficacy, purity and quality of drug product the need for pharmaceutical analysis is driven largely by regulatory requirements. In Reverse phase chromatography hydrophobic bonds were packed with an octadecyl or octyl functional group and a polar mobile phase, often a partially or fully aqueous mobile phase. Polar substances choose the mobile phase and elute first. As the hydrophobic nature of the solutes increases, retention also increases. Generally, the lower the polarity of the mobile phase, the higher is its eluting strength.

Ramipril is ACE inhibitor that inhibits the action of angiotensin converting enzyme (ACE), so it lowers the production of angiotensin II and decreasing the breakdown of bradykinin

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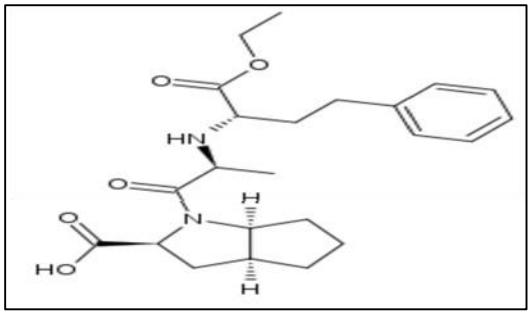

Figure 1 Structure of Ramipril

Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin I receptor type II (AT1), with a binding affinity 3000 times greater for AT1 than AT2.

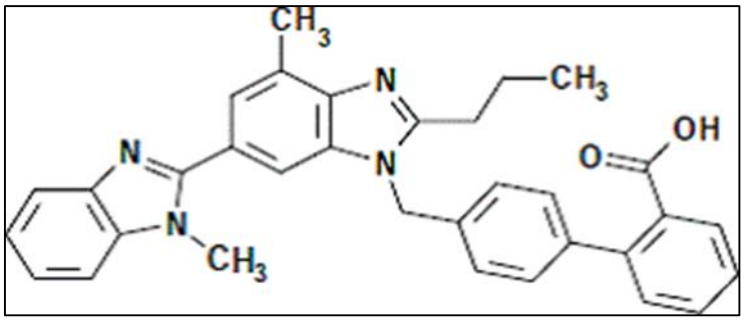

Figure 2 Structure of Telmisartan

2. Methodology

2.1. Selection of column

Different C8 and C18 columns were chosen for selected composition of mobile phase and quality of peaks were observed for the drugs. The column was finalized upon the satisfactory results of various system suitability parameters such as column efficiency, retention time, tailing factor, peak, and asymmetry of the peaks.

2.2. Selection of Wavelength

Wavelength for the analysis of ramipril and telmisartan was scanning in the range of 200-400nm on the UV spectrum. The wavelength of 230nm was selected for the RP-HPLC method as these drugs has shown good absorbances.

2.3. Preparation of Phosphate buffer

2.68 grams of KH_2PO_4 was weighed, placed into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Orthophosphoric acid was used to adjust the pH to 5.

2.4. Preparation of mobile phase

A mixture of Phosphate buffer 700 ml (70%) and Acetonitrile 300 ml (30%) was degassed in ultrasonic water bath for 5 minutes. Filtration was done by 0.45 µl under vacuum filtration.

2.5. Preparation of standard solution

Weighted accurately about 10 mg of Ramipril 80 mg of Telmisartan and transferred into a clean 100 ml volumetric flask, dissolved in few ml of methanol and made up to the volume with methanol and sonicated for 10 minutes and filtered through membrane filter, marked as standard stock solution.

2.6. Preparation of sample solution

Weighed and powdered 20 tablets accurately a quantity of powder equivalent to 10 mg of ramipril and 80 mg of telmisartan and transferred it into a clean 100 ml standard flask. Few ml of Methanol was added, dissolved, make up the volume with methanol and sonicated for 10 minutes and filtered through membrane filter and marked as sample stock solution.

Validation studies were performed as per the protocol.

- The standard solution was prepared using Ramipril and Telmisartan as working standard as per test method was injected six times into HPLC system.
- The system suitability parameters and % relative standard deviation (RSD) for peak areas for six replicate injections was found to be within limits.

2.7. Accuracy

The accuracy of an analytical method demonstrates the intimacy of agreement between the value accepted either as a conventional true value or an accepted reference value and the value established. Accuracy is generally noted by recovery studies in the analytes that spiked into a solution containing the matrix. To determine recovery studies of Ramipril and Telmisartan, combined a known quantity of standard drug with the preanalyzed sample and then contents were reanalysed by the method. Sample solutions were placed in triplicate for each spike level and assayed as per the test method.

2.8. Roubustness

The robustness of an analytical method is the determination of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. As part of the Robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to determine the impact on the method.

2.9. Limit of Detection (LOD)

Limit of Detection is the analyte smallest concentration that gives a measurable response (signal to noise ratio of 3). The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample that can be detected but not necessarily quantified as an exact value. Detection limit corresponds to the concentration that will give a signal-to-noise ratio of 3:1.

2.10. Limit of Quantitation (LOQ)

Limit of Quantitation is the smallest concentration of the analyte, which gives response that can be accurately quantified (Signal to noise ratio of 10). The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample that can be determined quantitatively with suitable precision and accuracy. The Quantification limit is the concentration of related substances in the sample that will give a signal to noise ratio of 10:1.

3. Results and discussion

Estimation of Ramipril and Telmisartan tablet dosage forms by RP- HPLC method was carried out using optimized chromatographic conditions. Selected drug combination Ramipril and Telmisartan is available in fixed dosage combination mainly in tablet dosage forms. In this present research work a simple, accurate, less expensive, and sensitive RP-HPLC method for the simultaneous estimation of ramipril and telmisartan was developed and validated. The separation was performed by using a mobile phase consisting of 20 mM phosphate buffer of pH 5.0: Acetonitrile in the ratio of 70:30%v/v. The column used was Zorbax SB C8, 150 X 4.6mm, particle size 3.5µ with flow rate of 1.0ml/min using PDA detection at 230 nm.

The chromatogram of Ramipril and Telmisartan reference standards are presented in figure 3 & 4 respectively. For quantitative estimation 230 nm was selected as suitable wavelength. The individual peaks of Ramipril and Telmisartan was identified by knowing the retention time 2.285 and 4.288 minutes respectively.

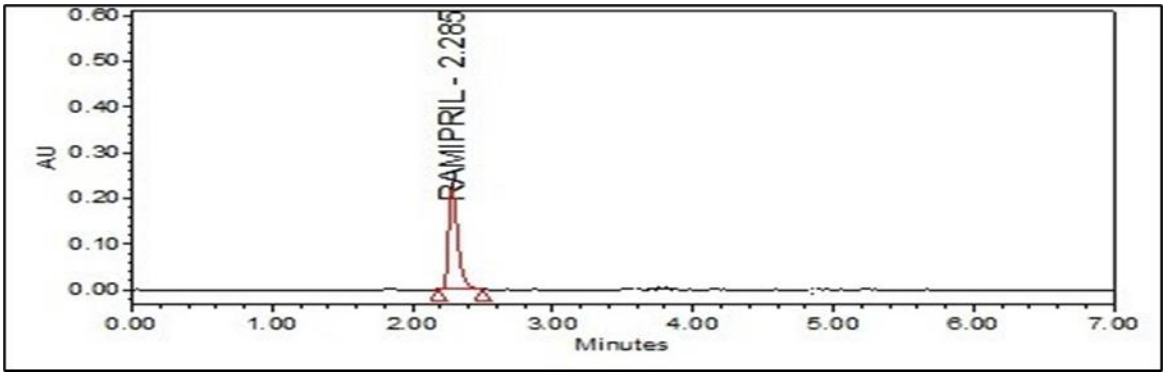

Figure 3 Chromatogram of Ramipril standard

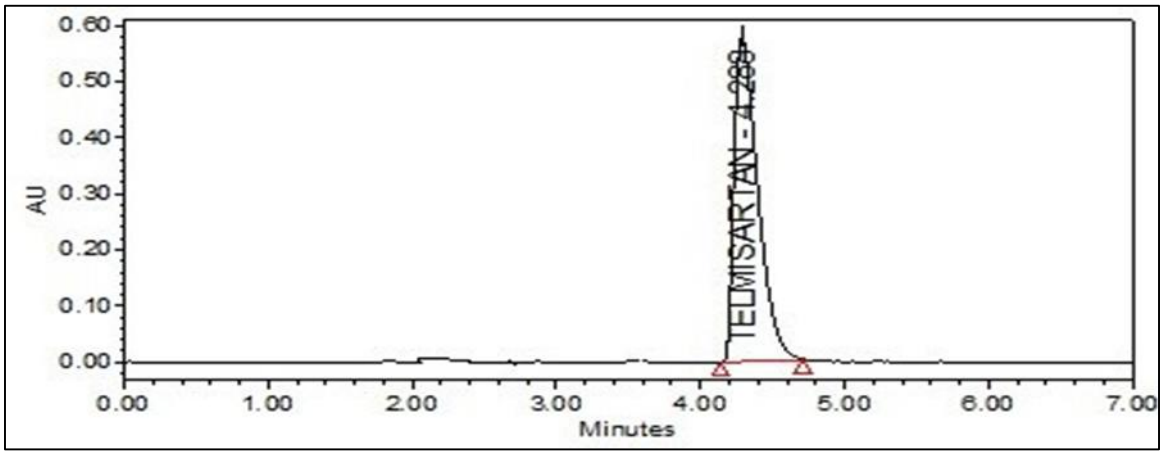

Figure 4 Chromatogram of Telmisartan standard

Linearity was evaluated by visual inspection of the plot of peak area as a function of analyte concentration for Ramipril and Telmisartan. The linearity of the method was determined at concentration levels ranging from 20-60 µg/ml for Ramipril and 160-480 µg/ml for Telmisartan. The correlation co-efficient of Ramipril was found to be 0.9999 and the correlation co-efficient of Telmisartan was found to be 1.0000, these are within limit.

System suitability parameters such as resolution, tailing factor and number of theoretical plates are presented in table 1. System precision was carried out, the % RSD for peak area of Ramipril and Telmisartan for six replicated injections was not more than 2.0%.

Table 1 System Suitability

System suitability parameters	Ramipril	Telmisartan
Tailing factor	1.427	1.831
Resolution	9.248	
No. of theoretical plates	5882	3045

The quantitative evaluation was carried out on Ramipril and Telmisartan by taking the same concentration as for standard solution. The data regarding quantitative evaluation was shown in table, percentage purity values 100.15 for Ramipril and 99.96 for Telmisartan respectively.

The acceptance criteria for method precision were found to be % RSD NMT 2.0% and the method shows precision of 0.2038 for Ramipril and 0.2575 for Telmisartan. The accuracy of the method was estimated by recovery experiments.

The recovery study was evaluated and the percentage recovery range found to be within the limit, 99.8-100.20 percentage for Ramipril and 100.10-100.11 percentages for Telmisartan. Table.2

Table 2 % Recovery of Ramipril and Telmisartan

Drug	Amount added in (µg / ml)	Amount Found in (µg / ml)	Percentage recovery	SD	% RSD
Ramipril	5	4.99	99.80	0.2314	0.2306
	10	10.02	100.20	0.0545	0.0545
	15	15.02	100.10	0.4894	0.4223
Telmisartan	40	40.04	100.10	0.5627	0.5349
	80	80.09	100.11	0.4164	0.5382
	120	120.13	100.10	0.4089	0.5347

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were evaluated by injecting low concentrations of the standard solutions using the developed RP-HPLC method. The LOD was 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 2.727 µg/ml for Ramipril and 2.990 µg/ml for Telmisartan. The LOQ was the smallest concentration of the analyte that gives response accurately quantified (signal to noise ratio of 10). The Limit of Quantification (LOQ) was found to be 9.091 µg/ml for Ramipril and 9.968 µg/ml for telmisartan. The present method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, precision, repeatability of measurement of peak area, repeatability of sample application.

4. Conclusion

The present research work was development and validation of new RP-HPLC method for simultaneous estimation of Ramipril and Telmisartan in combined dosage form. Injection volume of 10 µL is injected and eluted with the mobile phase selected after optimization was Phosphate buffer and Acetonitrile in the ratio of 70:30. The optimized flow rate was at 1.0 mL/min. Detection was carried out at 230 nm. The Ramipril and Telmisartan showed linearity in the range of 20-60 µg/mL and 160-480 µg/mL respectively. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust and the %RSD value for percentage recovery of Ramipril and Telmisartan was found to be within the acceptance criteria. The present chromatographic method development for the Ramipril and Telmisartan are rapid, simple, specific, sensitive, precise, accurate and reliable can be effectively applied for analysis in research work, testing laboratories, quality control departments, bio-pharmaceuticals and bio-equivalence and clinical pharmacokinetic studies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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