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Original Article

Method Development and Its Validation for Simultaneous Estimation of Ramipril & Clopidogrel by RP-HPLC in Combination Tablet Dosage Form

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ABSTRACT

A selective and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Ramipril & Clopidogrel. The λmax of the two ingredients i.e. Ramipril & Clopidogrel , were found to be 210 nm and 225 nm respectively in methanol as solvent system. Accurately weighed 100 mg of Ramipril and 100 mg of Clopidogrel were transferred to 100 ml volumetric flask. About 40 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 10 ml of the above solution was diluted to 100 ml with the solvent system. Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer + Dipotassium hydrogrn phosphate (0.01 M, pH 3.0): acetonitrile (30:70). Mobile phase was filtered through 0.45 m membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Clopidogrel and Ramipril for 15 min. The retention time were found to be 2.03 min and 9.93 min respectively

Keywords: Ramipril & Clopidogrel, RP-HPLC, Acetonitrile (30:70), Retention time.

1. INTRODUCTION

The oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been employed for the systemic delivery of drug via various pharmaceutical products of

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different dosage forms. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration and the belief that oral administration of the drug is well absorbed. Ramipril (Altace) is an ACE inhibitor. ACE stands for angiotensin converting enzyme. Ramipril is used to treat high blood pressure (hypertension) or congestive heart failure, and to improve survival after a heart attack.

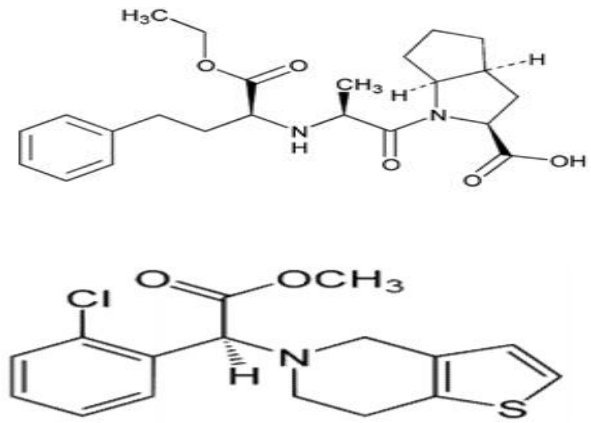

Fig 1a, b: Structure of Ramipril and Clopidogrel

Clopidogrel (INN) is an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, cerebrovascular disease, and to prevent myocardial infarction (heart attack). It is marketed by Bristol-Myers Squibb and Sanofi under the trade name **Plavix**. The drug works by irreversibly inhibiting a receptor called $P2Y_{12}$, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes.

2. EXPERIMENTAL WORK

2.1 Method Development

Standard & sample preparation for UV-spectrophotometer analysis:

Selection of wavelength

The λmax of the two ingredients i.e. Ramipril & Clopidogrel , were found to be 210 nm and 225 nm respectively in methanol as solvent system.the isobestic point for the drugs were found at 225 nm.

Preparation of standard solution of Ramipril 10 mg of Ramipril was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 µg/ml of Ramipril. Preparation of standard solution of Clopidogrel by 10 mg of Clopidogrel was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 µg/ml of Clopidogrel.Preparation of mix. standard solution of Ramipril & Clopidogrel . Accurately weighed 100 mg of Ramipril and 100 mg of Clopidogrel were transferred to 100 ml volumetric flask. About 40 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 10 ml of the above solution was diluted to 100 ml with the solvent system. The resultant solution was filtered through a 0.45 m membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique.

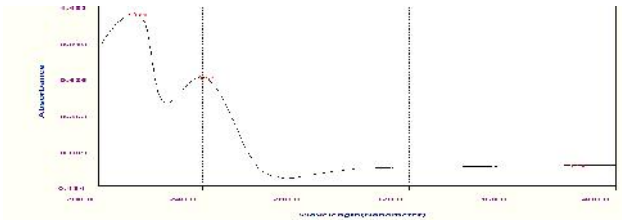

Fig 2: The λ_{max} of Ramipril

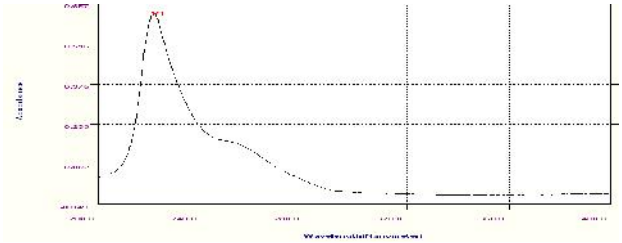

Fig 3: The λ_{max} of Clopidogrel

2.2 Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

2.3 Different chromatographic conditions used and their Optimizations

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs.

Table 1: Chromatographic condition

Mobile phase	**Potassium dihydrogen phosphate +Dipotassium hydrogrn phosphate buffer (0.01 M, pH 3.0): acetonitrile (30:70)**
Wavelength	225 nm
Flow rate	1.0 ml/ min.
Run time	15 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

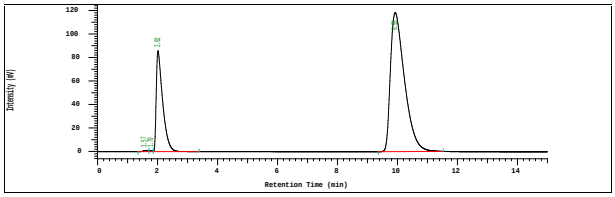

Fig 2: The chromatogram obtained after condition , typical chromatogram of Ramipril (rt=9.93 min) and Clopidogrel (rt= 2.02 min).

Here resolution was good, theoretical plate count and symmetry was appropriate. Also no unwanted little peaks were seen between two peaks. Hence it was acceptable. The selected and optimized mobile phase was Potassium dihydrogen phosphate buffer+Dipotassium hydrogrn phosphate (0.01 M, pH 3.0): acetonitrile (30:70) Run time was 15 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

2.4 Preparation of mobile phase

Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer+Dipotassium hydrogrn phosphate (0.01 M, pH 3.0): acetonitrile (30:70). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

2.5 Running the standard solution of Clopidogrel

2 ml of stock solution was pipetted out into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 27**.**

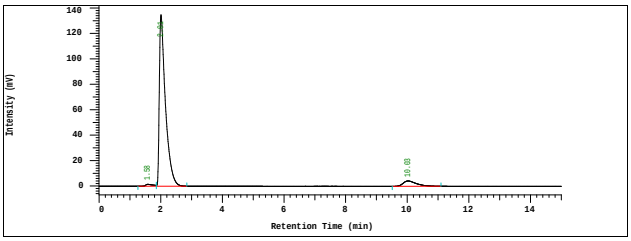

Fig 3: Chromatogram of Clopidogrel

Retention time was found to be 2.01 min.

2.6 Running the standard solution of Ramipril

2 ml of stock solution was pipetted into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure .

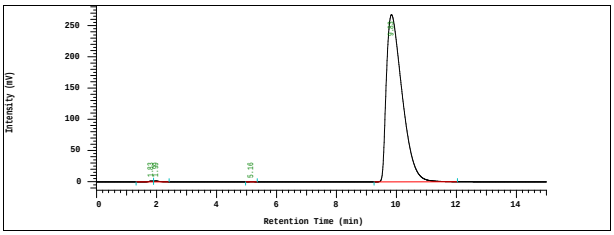

Fig 4: Chromatogram of Ramipril

Retention time was found to be 9.83 min.

3. RESULT & DISCUSSION

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Clopidogrel and Ramipril for 15 min. The retention time were found to be 2.03 min and 9.93 min respectively.

3.1 Method Validation

Preparation and running of synthetic mixture of *Clopidogrel and Ramipril*

For the specificity of the method the marketed formulations has been taken & the solution was injected into the HPLC system. The chromatograms obtained are shown in figure 5.

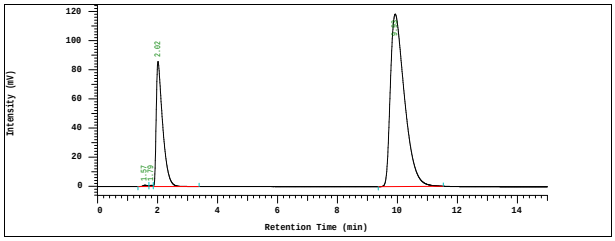

Fig 5: Chromatogram of synthetic mixture sample

No peaks were found at the retention of Clopidogrel and Ramipril. Specificity studies indicating that the excipients did not interfere with the analysis.

3.2 Linearity and Range

*Method***:** for linearity various concentrations like 10, 20, 30, 40, 50 of clopidogrel & ramipril were prepared in amixture & then injected into HPLC.

Linearity range was found to be 0-50 µg/ml for Clopidogrel and 0-50 µg/ml for Ramipril. The correlation coefficients were found to be 0.999 & 0.997, the slopes were found to be 44623 & 13801 and intercept were found to be 10569 & 10378 for Clopidogrel and Ramipril respectively.

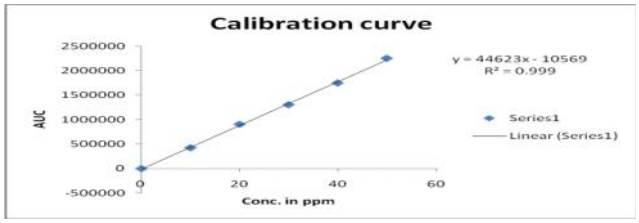

Fig 6: Standard curve for Clopidogrel

Table 2: Standard curve for Clopidogrel

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
10	424838
20	904737
30	1302869
40	1746831
50	2250813

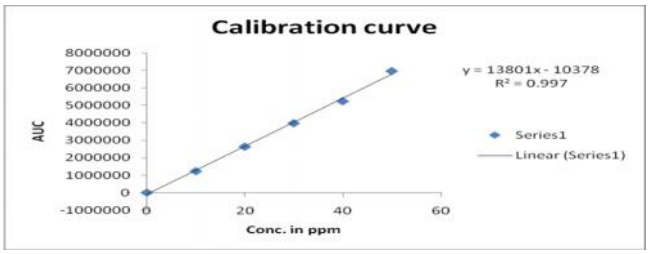

Fig 6: Standard curve for Ramipril

Table 3: Standard curve for Ramipril

CONC.	AUC
0	0
10	1228747
20	2638031
30	3983572
40	5249436
50	6979310

*3.3 Accuracy***:** Clopidogrel

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Clopidogrel were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-8.

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Ramipril were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table 4: Percentage Recovery

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	16	20	99.63	Mean= 99.67667%
S_2 : 80 %	16	20	99.92	S.D. = 0.223681
S_3 : 80 %	16	20	99.48	% R.S.D.= 0.224407
S_4 : 100 %	20	20	99.19	Mean= 99.19%
S_5 : 100 %	20	20	99.25	S.D. = 0.06
S_6 : 100 %	20	20	99.13	% R.S.D.= 0.06049
S_7 : 120 %	24	20	99.25	Mean= 99.49%
S_8 : 120 %	24	20	99.54	S.D. = 0.219317
S_9 : 120 %	24	20	99.68	% R.S.D. = 0.220441

The mean recoveries were found to be 99.67, 99.19, 99.49 % for Clopidogrel and 99.92, 100.72, 100.40% for Ramipril. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

3.4 Precision*: *Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug. Ramipril & Clopidogrel.

Table 5: The percent relative standard deviations

HPLC Injection Replicates of Clopidogrel	Retention Time	Area
Replicate – 1	2.02	1302869
Replicate – 2	2.02	1302586
Replicate – 3	2.02	1318521
Replicate – 4	2.01	1302569
Replicate – 5	2.02	1302896
Average	2.018	1305888
Standard Deviation	0.004472	7063.605
% RSD	0.221612	0.540904

4. CONCLUSION

The LOD was found to be 0.02 µg/ml and 0.06 µg/ml and LOQ was found to be 0.04 µg/ml and 1.2 µg/ml for Clopidogrel and Ramipril respectively which represents that sensitivity of the method is high.

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