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RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOLAZONE AND RAMIPRIL IN ORAL SOLID DOSAGE FORM .

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ABSTRACT

A Simple, efficient and reproducible reverse phase high performance liquid chromatographic method was developed and validated for the Simultaneous determination of metolazone (MET) and ramipril (RAM) in combined dosage form. The separation was effected on a Hypersil ODS C18 column (250 mm x 4.6 mm;5µ) using a mobile phase mixture of 30mM sodium dihydrogen phosphate buffer adjusted to pH 2.5.0 with orthophosphoric acid and acetonitrile in a ratio of 40:60 v/v at a flow rate of 1.0ml/min. The detection was made at 242 nm. The retention time of metolazone and ramipril was found to be 4.140± 0.007min and 7.007± 0.006min. Calibration curve was linear over the concentration range of 10-70 µg/ml for bothMetolazone and Ramipril .All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of Metolazone and Ramipril in tablet dosage forms.

KEY WORDS: Method development and validation, Metolazone ,Ramipril, Hypersil C18 column, RP-HPLC.

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INTRODUCTION

Ramipril (RAM) is chemically (2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-l] amino} propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.[1,2](Figure 1a). Ramipril is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. Metolazone (MET) is chemically 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2,3,4-tetrahydro- quinazoline-6-sulfonamide[3](Figure 1b). Metolazone is an oral diuretic drug, commonly classified with the thiazide diuretics. It is primarily used to treat congestive heart failure and high blood pressure[4]. Metolazone indirectly decreases the amount of water reabsorbed into the bloodstream by the kidney, so that blood volume decreases and urine volume increases. This lowers blood pressure and prevents excess fluid accumulation in heart failure.Hence, the combination of Ramipril and Metolazone (extended release), such as in the marketed product Metoz-R®, complements each other and provides an additive effect on blood pressure control, which is sustained for at least 24 hours.

Literature survey revealed spectrophotometric and chromatographic methods reported for estimation of MET [5-8] and RAM [9-12] individually or in combination with other drugs. However, there is no analytical method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. Present work describes simple, rapid, accurate and precise Isocractic RP-HPLC method for simultaneous determination of MET and RAM in oral solid dosage forms. The proposed method was validated as per ICH guidelines [13].

Figure.1
Structure of Metolazone and Ramipril

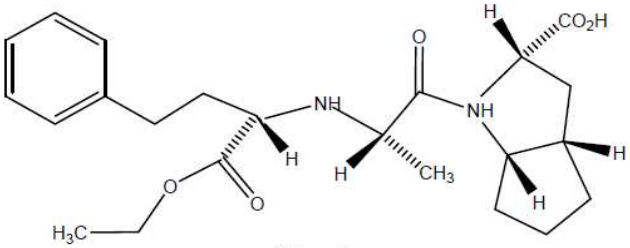


Figure 1a **Ramipril**

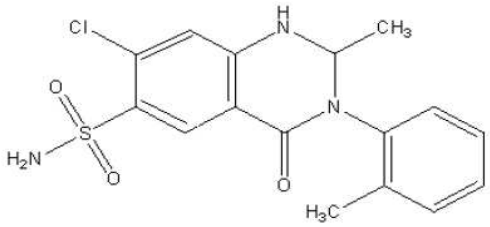


Figure 1b **Metolazone**

EXPERIMENTAL

Chemicals and Reagents

MET and RAM working standards were generous gift from Micro Labs, (Bangalore, India) and Sun PharmaceuticalsPvt.Ltd,(Mumbai,India). HPLC grade of acetonitrile and water were procured from E.Merck, Mumbai(India).Ortho phosphoric acid,sodium dihydrogen phosphate mono hydrate, sodium phosphate were of analytical grade delivered by S.D.Fine Chemicals ,India. Metoz-R® tablets manufactured by Centaur Pharmaceuticals Pvt .Ltd. were procured from local market (Chennai, India).The 0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt.Ltd, (Chandigarh,India).

Instrumentations

HPLC system (Waters Milford, USA) equipped with inbuilt auto sampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control, photodiode array (PDA) detector was employed throughout the analysis. Chromatographic data was acquired using empower software.

Chromatographic condition

Hypersil C18 column (250×4.6mm id., 5µm) column was used as a stationary phase maintained at 30ºC. The mobile phase used in a composition of 40:60 v/v solution containing 30mM sodium dihydrogen phosphate buffer adjusted to pH 2.5.0 with orthophosphoric acid and acetonitrile.The mobile phase was pumped through the column with flow rate of 1.0 mL/min .Injection volume 20µL was used in all experiments. The optimum wavelength selected was242nm, which represents the wavelength of maximum response for both MET and RAM. All samples were analyzed using a PDA detector covering the range of 200-400nm.Peak homogeneity was expressed in terms of peak purity values, and was obtained directly from the spectral analysis report obtained using the above mentioned software.

Preparation of standard stock solution and linearity solutions for assay method.

The standard stock solutions of MET and RAM (1mg/mL) were prepared separately by dissolving 100 mg of each drug in 100 ml of methanol. The stock solutions were protected from light and stored at 4ºC to avoid degradation. The mixed standard calibration solutions of MET and RAM were prepared by diluting stock solution with the same solvent to obtain a range of 10- 70 µg/mL of both MET and RAM. All these solutions were prepared in triplicate. Before being subjected to analysis all the working standard solutions were filtered through a 25mm nylon membrane syringe filter pore size 0.45µm).The linearity was evaluated by the least square regression method with triplicate determinations at each concentration level.

Application to pharmaceutical formulation.

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed to a homogeneous powder and the quantity equivalent to one tablet (10 mg of both MET and RAM) was weighed and transferred in to a 200 mL volumetric flask. Fifty milliliters of methanol was then added and the mixture was sonicated for 30 minutes in an ultrasonic bath. The volume was diluted up to the mark with methanol to get the concentration of 50 µg/mL of both MET and RAM. The resultant solution was filtered through 0.45µm nylon syringe filter. The filtrate collected after discarding first few milliliters was injected on the above chromatographic system. All the solutions were protected from light.

System suitability studies

System suitabilty testing is used to verify that the proposed method was able to produce good resolution between the peak of interest with high reproucibilty.The system suitability was determined by making six replicate injections from freshly prepared standard solutions of assay and and analyzing each solute for their retention time, theoretical

plates,resolution,capacity factor and tailing factor.

LOQ and LOD

The LOQ and LOD were determined on the basis of the standard deviation of the response and the slope of calibration plot, as defined by ICH guideline.

Accuracy

Accuracy of the assay method as well as dissolution method was calculated by recovery studies at three concentrations of 50%, 100% and 150% levels by standard addition method. The peak areas were calculated and obtained values were fitted to the straight-line equation of the calibration curves.
The recovery percentages were calculated.

Precision

The precision of the proposed assay and dissolution method were assessed as repeatability (Intra-day precision) and Intermediate precision (inter-day precision) by performing five replicate injections of three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzing three times in the same day and performing the same procedure on the different day by another person under same experimental conditions.

Robustness

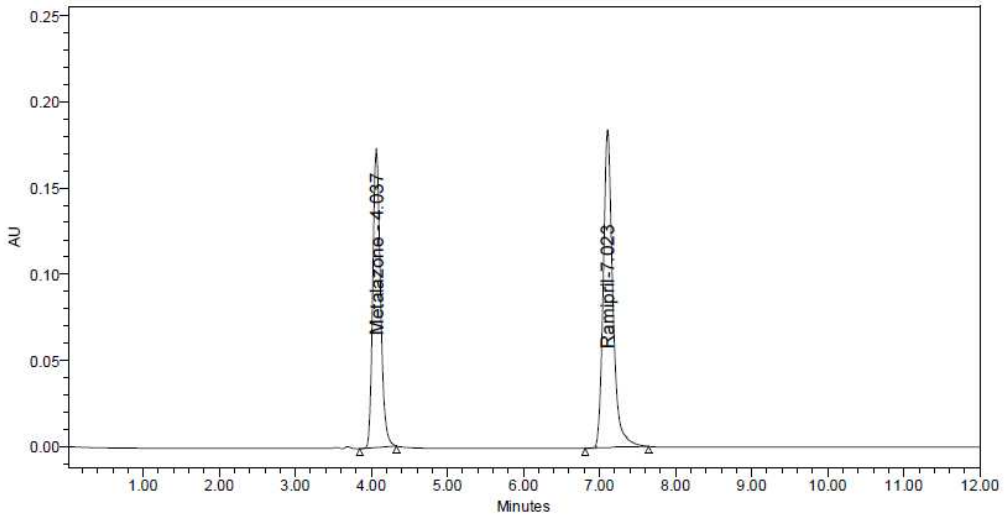
Robustness of a method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Minor changes in pH of the mobile phase, flow rate, column temperature and detector wavelength were studied to evaluate the robustness of the developed assay method.

RESULTS AND DISCUSSION

Optimization of the HPLC method

Proper selection of the methods depends upon the nature of the sample (acidic or basic or neutral molecule) its molecular weight and solubility. During the optimization of the method, different column such as RP-C_8, RP-C_{18}, RP-NH_2 and RP-phenyl columns were used for the selection of appropriate column. Amino and Phenyl columns were not eluted both drugs and RP-C_8 Column has shown less sensitivity compared with RP-$C_{18.}$ Mobile phase composition was also optimized by the use of several trails. Acetonitrile was chosen as the organic modifier, because it resolved the drugs well and produced sharp peaks with acidic mobile phase pH3-4. 30mM sodium di hydrogenphosphate buffer was employed to supply the ionic strength and pH was reduced to 2.5 to improve the peak shape, separation and reduce tailing factor. Finally, mixture containing 30mM sodium dihydrogen phosphate buffer adjusted to pH 2.5.0 with orthophosphoric acid and acetonitrile in a ratio of 40:60 v/v was selected as a mobile phase. The optimum wavelength for detection was 242nm at which better responses for both drugs were obtained and chromatogram was shown in Figure.2.

Figure.2
Chromatogram of Standard.



Method validation

The developed method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability. System suitability test The system suitability test performed according to ICH guidelines. The observed RSD values at 1% level of analyte concentration were well within the usually accepted values (≤ 2%). Theoretical plates, tailing factor, resolution between MET and RAM were determined for both assay and dissolution. The results are all within acceptable limits summarized in Table.1.

Table.1
System Suitability parameters

Parameters	MET	RAM
Theoretical plates	7245	5568
USP tailing	1.24	1.35
Resolution		6.24
Capacity factor	3.15	2.14
Retention time	4.12	7.04

Specificity

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 50 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both MET and RAM from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Linearity, Limit of detection, Limit of quantification

The linearity was evaluated by linear regression analysis. The calibration graph was constructed for the proposed method from the data points over the concentration range cited

in Table 2 .The linearity of the calibration graph and conformity of the HPLC method proved by the high values of the correlation coefficients (r^2) of the regression equation. According to ICH recommendations the approach based on the SD of the response and the slope was used for determining the detection and quantitation limits. The detection limit and quantitation limit of MET were found to be 2µg/ml and 4.0 µg/ml and RAM3.0 µg/ml and 5.0 µg/ml respectively.

Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of a method is evaluated using three separate determinations for repeatability, intermediate precision, and reproducibility. The results of intra- and inter-day variations are shown in Table 2. The results obtained from intermediate precision also indicated a good method precision. All the data were within the acceptance criteria.

Table 2

Summary of validation parameters of the proposed method

Parameters	Metolazone	Ramipril
Linearity	10-70µg/ml	10-70µg/ml
Intercept	20213	24480
Slope	9771.6	999.3
Correlation coefficient	0.9996	0.9996
LOD	2µg/ml	3µg/ml
LOQ	4µg/ml	5µg/ml
Precision(RSD)		
Intraday,(n=3),%	0.1156	0.2655
Interday,(n=3),%	0.6598	0.2446
Repeatability of injection,(n=10),%	0.8814	0.5478
Ruggedness(RSD)		
Analyst 1,(n=3),%	0.1153	0.3699
Analyst 2,(n=3),%	0.3698	0.1544
Instrument 1,(n=3),%,	0.3486	0.4278
Instrument 2,(n=3),%	0.6243	0.5172

Accuracy

The HPLC area responses for accuracy determination are depicted in Table .3.The results show that best recoveries (99.67% and 101.41%) of the drug were obtained at each added concentration, indicating that the method was accurate.

Table 3

Evaluation data for accuracy study

Level of Recovery	Amount of pure drug added		Percentage Recovery*	
	MET	RAM	MET	RAM
50%	25	25	99.89	99.84
100%	50	50	101.02	101.35
150%	75	75	100.56	99.86
	Mean Recovery		100.15	99.98
	S.D		±0.2354	±0.4125
	R.S.D		0.3214	0.5478

Robustness and Ruggedness

Robustness studies were carried out after deliberate alterations of flow rate and mobile phase compositions and pH. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results has proven that the method is robust and the data are summarized in Table.4. The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Shimadzu HPLC and by two different operators using different columns of similar type like Phenomenex C_{18},HypersilC_{18} and the results were shown in Table 2,the low RSD values confirms the ruggedness of the method.

Table 4
Robustness testing of the method

Parameter	Modification	Metolazone % Recovery	Ramipril% Recovery
pH	3.0	101.1	99.4
	2.5	99.5	99.9
	3.4	99.9	101.1
Buffer Composistion(M)	38	99.8	100.2
	40	99.6	99.2
	42	100.0	99.7
Flowrate (mL/min)	0.9	101.4	99.6
	1.0	99.7	101.3
	1.1	101.4	101.7

Tablet studies

The proposed method was successfully applied to the analysis of marketed products (. Metoz-R ®) and the results obtained are given in Table 5.

Table 5
Analysis of formulation

Drug	Labeled amount(mg)	Amount of mg/tab found*	%Label claim	%RSD
Metolazone	50	49.9	99.93	0.484
Ramipril	50	50.16	100.92	0.235

*Averageof sixdeterminations

CONCLUSION

The results showed that the method provided adequate accuracy, precision, sensitivity, reproducibility with better resolution for the analysis of MET and RAM in formulations either simultaneously or individually. The advantages of a proposed method are its short analysis time and a simple procedure for sample preparation.The RP-HPLC method developed for simultaneous analysis of MET and RAM can be used for routine quality control of their bulk drug mixture and their combined dosage form.

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