SimultaneousQuantificationof Metolazoneand Ramiprilin Their Combined Dosage FormbyFirst Order Derivative Spectroscopic Method.

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A simple, accurate and precise first order derivative spectrophotometric method was developed for simultaneous determination of Metolazone (MET) and Ramipril (RAM) in their combined pharmaceutical dosage form. MET and RAM were quantified using first derivative responses at 285nm and 212nm respectively. Methanol was taken as a common solvent. The calibration curve were linear in the concentration range of 5-25 µg/mL for MET and 8-24 µg/mL for RAM. The method was validated and found to be accurate and precise. Developed method was successfully applied for the estimation of MET and RAM in their combined dosage form

KEYWORDS: Metolazone (MET), Ramipril (RAM), first order derivative, Validation.

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INTRODUCTION

Metolazone is chemically7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2dihydroquinazoline-6-sulfonamide(Figure 1).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. 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.

Ramipril is chemically (2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[*b*] pyrrole-2-carboxylic acid (Figure 2).Ramipril is an angiotensin-converting enzyme (ACE) inhibitor, used to treathypertension and congestive heart failure. ACE inhibits lower the production of angiotensin II,therefore relaxing arterial muscles while at the same time enlarging the arteries, allowing theheart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passage ways. Ramipril is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes. Ramiprilat is mostly excreted by the kidneys.

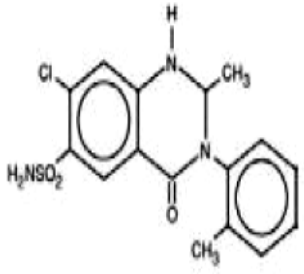

Figure 1. Structure of Metolazone

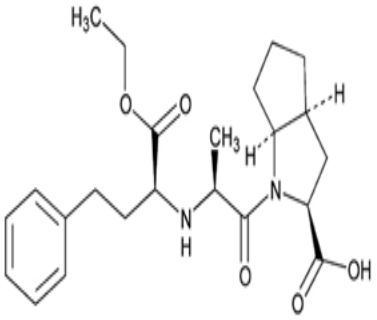

Figure 2. Structure of Ramipril

Methods for the estimation of MET and RAM alone and with other drugs have been reported (1-27).One RP-HPLC and HPTLC method has been reported for the estimation of MET and RAM in their combined dosage form, but no UV spectrophotometric method reported which is more economic. Present study involves development and validation of first order derivative spectrophotometric method for the estimation of MET and RAM in their combined dosage formas per ICH guide lines (28).

In comparison to LC, TLC and LC-MS/MS methods, Derivative spectrophotometricmethod is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis. This reduces the time and cost of analysis.

MATERIALS AND METHODS

Analytically pure MET and RAM was procured from RPG LIFE SCIENCES, ANKLESHWAR. Marketedformulation METOZ-R (mfg by: Centaur pharmaceuticals) procured from the local pharmacy store.All other reagent like methanol was used of analytical grade.

Instrument

A uv-visible spectrophotometer (double beam) shimadzu model 1800 was used for analysis. Uv-probe software of 2.34 version was used to convert zero order spectra into first order spectra.

Preparation of standard stock solution

10 mg of MET and RAM transferred in separate 10 mL volumetric flask and make up the volume up to the mark with methanol to obtain the stock solution of 1000 ppm for both the drugs, respectively.

Method

MET and RAM having good solubility in methanol.So,methanol was selected as a commonsolvent. Appropriate aliquots of MET and RAM from standard stock solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with methanol to obtain final concentrations of 5-25 µg/mL of MET and 8-24 µg/mL of RAM respectively. The solutions were scanned between 200 – 400 nm and by the use of UV probe software, zero order spectra has been converted in to first order derivative spectra (scaling 15 and Δλ-4). From the overlain of first order spectra ZCP of MET and RAM was found to be 212nm and 285nm, respectively.

RESULT AND DISCUSSION

Derivative method was selected for the estimation of MET and RAM in the presence of each other. Overlain of the first order derivative spectra (figure 4) (D1) of MET showed ZCP of MET at 212nm where RAM gives significant derivative response,

while the D1 spectrum of RAM(figure 4) showed ZCP of RAM at 285 nm, where MET gives significant derivative response. The ZCP of both the drugs remained constant and no shift was observed. Therefore, 285 nm was selected for the estimation of MET and 212nm was selected for the estimation of RAM. With increase in the concentration of MET, the derivative response at 285 nm increased. The responses for MET were found to be linear in the concentration range of 5-25 µg/mL, with r^2 of 0.9980. Similarly, the derivative responses for RAM at 212nm were linear in the concentration range of 8-24 µg/mL with r^2 of 0.9980. The regression analysis of the calibration curves is shown in Table-1. The % recoveries of MET and RAM were found to be in the range of 98.46-99.09 and 99.36-99.70, respectively (Table 2) Precision studies were carried out to study the intra-day and inter-day variability of the responses. The low % RSD value indicates that the method is precise. Excipients used in the specificity studies did not interfere with the derivative response of either of the drugs at their respective analytical wavelengths. In robustness study, no significant change in the derivative response of both the drugs was observed. The validation parameters are summarized in (Table 3). Assay of MET and RAM in marketing formulation has been studied (Table 4).

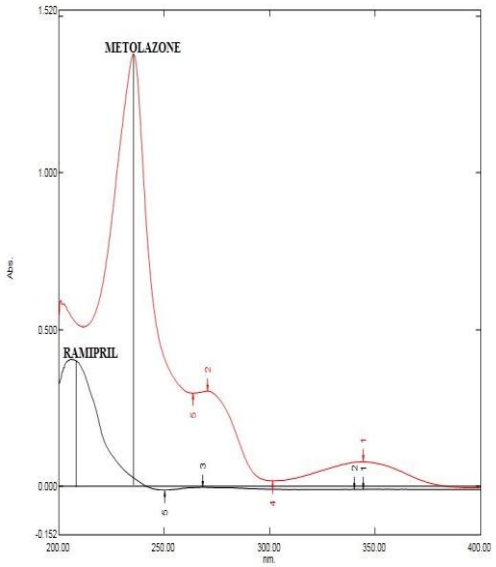

Figure 3. Overlain zero Order Spectra of 10ppm solution of MET and RAM

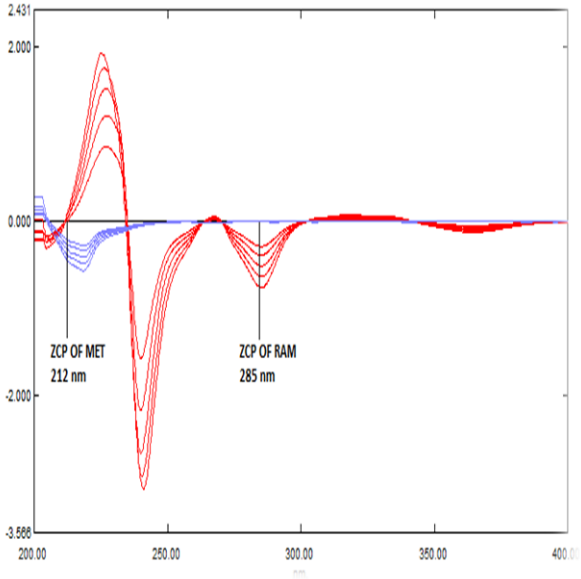

Figure 4. Overlain First Order Derivative Spectra of MET and RAM. Using Methanol as solvent at **Δ**λ-4 and scaling 15.

Validation

Method validation parameters like linearity, intraday and interday precision, and limit of detection, limit of quantification, accuracy, specificity, and robustness were performed as per ICH guidelines.

Linearity

Linearity was observed over a concentration range 5-25 µg/mL [measured at 285nm (ZCP of RAM)] and 8-24 µg/mL [measured at 212nm (ZCP of MET)] for MET and RAM, respectively.Statistical data of calibration curve are shown in table 1.

Accuracy

The accuracy of the method was determined by calculating recoveries of MET and RAM by method of standard

additions. Known amount of MET (0, 4, 5, 6 µg/mL) and RAM (0, 8, 10, 12 µg/mL) were added to a pre quantified sample solutions prepared from marketing formulation. The amount of MET and RAM were estimated by measuring derivative response at the appropriate wavelengths.

Precision

Intraday and interday precision study of MET and RAM was carried out by estimating different concentrations of MET (5, 15, 25 µg/mL) and RAM (8, 16, 24 µg/mL). The results are reported in terms of% RSD

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived from the calibration curves by using the following equations as per International Conference on Harmonization (ICH) guidelines:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ is the standard deviation of the intercept, and S is mean of Slope.

Specificity

For specificity study commonly used excipients present in selected tablet formulation were spiked into a pre weighed quantity of drugs. The absorbance was measured and the quantities of drugs were determined.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Table 1. Statistical data of calibration curve

Parameters	MET at 285 nm	RAM at 212nm
Linear range	5 – 25 µg/mL	8 – 24 µg/mL
Slope	0.022	0.027
Intercept	0.054	0.012
Standard deviation of slope	0.00054	0.00051
Standard deviation of intercept	0.0058	0.0041
Regression Coefficient (R^2)	0.9980	0.9980

Table 2. Accuracy study of MET and RAM

DRUG	Conc. of sample taken (µg/mL)	Conc. of pure API spiked (µg/mL)	Total Conc. (µg/mL)	Conc. of added Found (µg/mL)	%RECOERYV ±S.D	%RSD
MET	5	0	5	4.92	98.46±0.30	0.31
		4	9	3.89	98.90±0.57	0.58

		5	10	4.76	97.73±0.45	0.46
		6	11	5.90	99.09±0.45	0.48
RAM	10	0	10	9.94	99.36±0.15	0.15
		8	18	7.94	99.45±0.37	0.37
		10	20	9.92	99.61±0.20	0.20
		12	22	11.93	99.70±0.26	0.26

Table 3. Summary of validation parameters

Parameters	MET	RAM
Limit of Detection (PPM)	0.67	0.71
Limit of Quantitation (PPM)	2.05	2.17
Accuracy (%)(n=3)	98.46–99.09 %	99.36–99.70%
Precision (% RSD)		
Intraday (n = 3)	0.40-0.91 %	0.57 –1.40 %
Interday (n = 3)	0.67–1.32 %	0.80–1.67 %
Repeatability (n = 6)	0.833	0.773
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 48 hrs.	Suitable for 48 hrs.

Table 4. Assay of MET and RAM inmarketing formulation

PARAMETERS	METOZ-R Tablet (n = 3) (2.5 mg MET +5 mg RAM)	
	MET	RAM
Actual Conc. (µg/mL)	8	16
Conc. Obtained (µg/mL)	7.96	15.08
% Assay*	99.50	99.31
%RSD	0.27	0.51
Limit	97-102%	90-110%

CONCLUSION

The first order derivative spectrophotometric method has been developed for the estimation of MET and RAM in their combined dosage form. The method was validated and found to be simple, sensitive, accurate and precise. The First order derivative spectrophotometric method having advantage that it is simple, requires less analysis time and economic compare to chromatographic method. The proposed method was successfully applied in the estimation of MET and RAM in their combined dosage form.

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