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Research Article

NOVEL UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE BY SIMULTANEOUS EQUATION AND AREA UNDER CURVE METHOD

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ABSTRACT

A new, simple, rapid and novel spectrophotometric method has been developed for simultaneous estimation of Ramipril (RAM) and Hydrochlorothiazide (HCT). For this, simultaneous method (method A) is used. The method involved measurement of absorbance at two wavelengths, 210 nm and 270 nm, λmax of RAM and HCT respectively. The combination is also estimated by AUC method (method B). It involved measurement of area under curve in the wavelength range 202-237.5 and 247-289 for RAM and HCT respectively. Beer's law obeyed in concentration range of 0.1- 3.5 µg/ mL and 0.5- 17.5 µg/ mL for RAM and HCT respectively by both the methods.

The proposed methods are recommended for routine analysis since they are rapid, simple, accurate and also sensitive and specific. It involves no heating and no organic solvent extraction. These methods were validated for precision, reproducibility, linearity and accuracy as per ICH guidelines.

Keywords: Ramipril, Hydrochlorothiazide, simultaneous method, Area under curve.

INTRODUCTION

Ramipril, 2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-3-0]octane carboxylic acid (Fig 1), is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin-I to the highly potent vasoconstrictor, angiotensin-II, and also reduce the degradation of bradykinin[1].

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiadiazine- 7-Sulphonamide 1, 1-dioxide (Fig.2), is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water[1].

Literature survey reveals few analytical methods for the determination of ramipril in pharmaceutical preparations and biological fluids, viz.radioimmunoassay[2], spectrophotometry[3,4], potentiometry [5,6] GC, [7,8] and HPLC [9,10.] and LCMS[11].

UV Spectroscopy (12-15), Ratio Spectra Derivative Spectrophotometry (16), HPLC (16, 17, 18) and HPTLC (19) methods are reported for simultaneous estimation of Hydrochlothiazide in combined dosage form.

There are no reports yet for determination of this combination by proposed methods. Present work emphasizes on the quantitative estimation of Ramipril and Hydrochlorothiazide in their combined dosage form by UV Spectroscopic methods.

MATERIALS AND METHODS

Instrumentation

A Double beam UV-Visible spectrophotometer (Jasco V 530) with 10 mm matched quartz cells was used. All weighing were done on single pan balance (Shimadzu).

Reagents and chemicals

RAM and HCT reference standards were kindly provided by Emcure Pharmaceuticals Pvt. Ltd, Pune. Analytical grade methanol was purchased from Merck Specialities Private Ltd., Mumbai. All the reagents were of analytical grade. Glass double distilled water was used throughout the experiment.

Tablets were purchased from local market each containing 2.5 mg of RAM and 12.5 mg of HCT. RAM and HCT are available in the ratio of 1:5 respectively in the formulation and were used in same ratio for preparation of calibration curves.

Preparation of standard stock solutions and calibration curve

Standard stock solution of pure drug containing 50 µg mL^{-1} of RAM and 250 µg mL^{-1} of HCT were prepared in methanol distilled water system. The working standard solutions of these drugs were obtained by dilution of the stock solution in the distilled water. Series of solutions with conc. 0.1-4 µg mL^{-1} and 0.5 – 20 µg mL^{-1} of RAM and HCT respectively were used to prepare calibration curve. Solutions were scanned and proposed methods were applied. For determination of absorptivity values, calibration curves using standard serial dilutions of individual drugs were plotted.

Preparation of sample stock solution and formulation analysis

A quantity of powder from twenty tablets equivalent to 50mg of RAM (250 mg of HCT) was weighed and transferred to a flask containing 50 ml of methanol and ultrasonicated for 15 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Volume was made with distilled water. The solution was further diluted with distilled water to get 2.5 µg mL^{-1} and 12.5 µg mL^{-1} of RAM and HCT resp.

Simultaneous determination

For simultaneous estimation of RAM and HCT, a series of standard solutions were scanned in the range of 200 to 400 nm against Distilled water as blank for obtaining the overlain spectra. The overlain UV spectra are shown in Figure 3. Absorbance and absorptivities of series of standard solutions were recorded at selected wavelengths λ_1 (210 for RAM) and λ_2 (270 for HCT). Concentration of HCT was determined at 270 nm where RAM does not exhibit absorbance. Concentration of RAM was calculated using following equation.

$A\lambda_1 = a_1C_1 + a_2C_2$ --------1

Where, C1 and C2 are the concentrations of RAM and HCT in gms/lit respectively in sample solution. $A\lambda_1$ is the absorbance of the sample solution measured at 210 nm. a_1 (114.1) and a_2 (71.89) are absorptivities of RAM and HCT at 210 nm.

Area under curve

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions

(1000 µg/mL) of ASP and TIC were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm. For Area Under Curve method, calibration curve was plotted and the sampling wavelength ranges selected for estimation of RAM and HCT are 202- 237.5 (λ_1-λ_2) and 247 -289(λ_3-λ_4) and area were integrated between these selected wavelength ranges for both drugs (Fig 4), which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations.

Concentraction of HCT was determined at 247-289(λ_3-λ_4) where RAM does not exhibit absorbance. Concentration of RAM was calculated using following equation.

$A\lambda_1 - \lambda_2 = a_1C_1 + a_2C_2$ --------1

Where, C_1 and C_2 are the concentrations of RAM and HCT in gms/lit respectively in sample solution. $A\lambda_1$ is the AUC of the sample solution measured at 202- 237.5 (λ_1-λ_2). a_1 (1529) and a_2 (1875) are absorptivities of RAM and HCT at 202- 237.5 (λ_1-λ_2).

Results of both methods are displayed in Table 1 and 2.

Accuracy as recovery studies

Recovery studies were done at three different levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The pre-analyzed sample was spiked with known concentration of the pure samples, and the mixtures were reanalyzed by the proposed method. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 1 µg mL-1 of RAM and 2.5 µg mL-1 of HCT for both methods. Percentage recovery was calculated from the amount of drug found in the solution. Values are reported in Table 3.

Precision of the method

Precision of the methods was determined by repeating assay six times. To study intraday precision, method was repeated 6 times in a day and the average % RSD was calculated. Similarly the method was repeated on 3different days and average % RSD was calculated Values are reported in Table 4.

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of RAM and HCT in combined dosage form were found to be accurate, simple and rapid. Hence it can be used for routine analysis of two drugs in combined dosage forms.

There was no interference from tablet excipients was observed in these methods. The values of % RSD and correlation of coefficient for simultaneous determination (Tablet) were found to be (% RSD 0.49- 1.08) and correlation coefficient was 0.999 for RAM and HCT. The result of recovery studies for tablet was found to be in the range of 98.40 -100.96% for method A, 99.02-100.89 for method B. Values are reported in Table 3. It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. Both methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

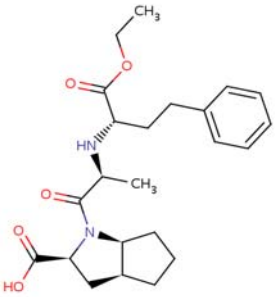

Fig.1. Ramipril

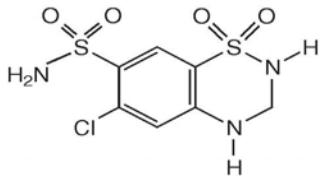

Fig. 2: Hydrochlorothiazide

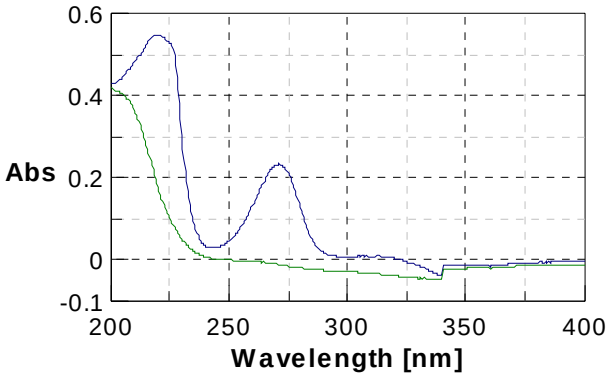

Fig. 3: Overlay spectra of RAM and HCT (210 nm and 270 nm, λmax of RAM and HCT)

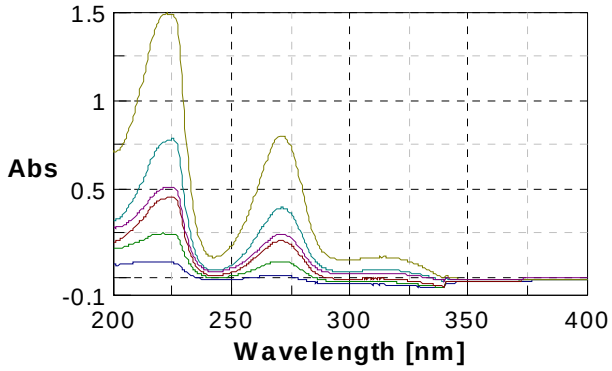

Fig. 4: Overlay spectrum of RAM and HCT in Methanol:Water. RAM (0.1-3.5µg mL-1) and TIC (0.5-17.5 µg mL-1) for AUC

Table 1: Optical characteristic of the methods and results of method validation of proposed methods

Parameters	Ramipril		Hydrochlorthiazide	
	Method 1	Method 2	Method 1	Method 2
λ (nm)	210	202-237.5	270	247-289
Beers law limit(µg/ml)	0.1-3.5	0.1-3.5	0.5-17.5	0.5-17.5
Regression Equation (y=mx)	y=735x	Y=10032x	Y=76.87x	Y=1363x
Correlation Coefficient (R^2)	0.998	0.998	0.997	0.999

Table 2: Assay results for the determination of RAM and HCT in its tablets by the proposed method

Lable Claim	Amount of drug found		% of Drug Found	
	Method A	Method B	Method A	Method B
2.5 mg RAM	2.472	2.5061	98.88	100.245
12.5 mg HCT	12.415	12.565	99.32	100.52

Table 3: Result of recovery studies by the proposed methods

Amount added (µg/ml)	Amount recovered (Method A)	% Recovery (Method A) ±S.D.	Amount recovered (Method B)	% Recovery (Method B) ±S.D.
RAM				
80% (2.0 µg/ml)	1.97	98.5 ±0.64	1.982	99.1 ± 0.42
100% (2.5 µg/ml)	2.474	98.96±0.87	2.482	99.3 ± 0.55
120 % (3.0 µg/ml)	2.9598	98.66±0.37	2.976	99.2 ± 0.49
HCT				
80 % (10.0 µg/ml)	10.118	101.18±0.51	9.90	99.0 ± 0.41
100 % (12.5 µg/ml)	12.535	100.28±0.44	12.40	99.2 ± 0.74
120% (15.0 µg/ml)	15.297	101.98±0.39	14.895	99.3 ± 0.60

Table 4: Intra and interday Precision (n=6)

Conc. (µg/ml)	Intraday Precision		Interday Precision		Intraday Precision		Interday Precision	
	Method A		Method A		Method B		Method B	
	S.D.	%R.S.D.	S.D.	%R.S.D.	S.D.	%R.S.D.	S.D.	%R.S.D.
RAM	0.39	0.40	0.78	0.79	1.149	1.1591	1.178	1.181
HCT	0.94	0.96	0.42	0.42	1.72	1.75	1.14	1.15

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. The developed UV spectroscopic methods were found suitable for determination of RAM and HCT as bulk drug and in marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of RAM and HCT. It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

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