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Research Article

A VALIDATED METHOD FOR THE SIMULTANEOUS ESTIMATION OF RAMIPRIL, TELMISARTAN AND HYDROCHLOROTHIAZIDE BY RP-HPLC IN BULK AND THE PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A RP-HPLC method was developed and validated for the simultaneous quantitative estimation of Ramipril, Telmisartan and Hydrochlorothiazide in bulk and pharmaceutical dosage form. Chromatography was carried on Hibar® Lichrospher® 100 RP-18 (5 µm, 250mm x 4.60mm id) Column with mobile phase comprising of acetonitrile and water (containing 0.1% orthophosphoric acid) in the ratio 65:35 v/v pH adjusted to 2.5 with Triethylamine (TEA). The flow rate was adjusted to 0.8 ml /min with UV detection at 225 nm. The retention times of Hydrochlorothiazide, Ramipril and Telmisartan were found to be 3.658min, 7.367min, and 10.28min, respectively. The different analytical parameters such as linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 5 to 30µg/ml, 40 to 320 µg/ml and 10 to 60 µg/ml for Ramipril, Telmisartan and Hydrochlorothiazide respectively. In the linearity study, the regression equation and coefficient of correlation for Ramipril, Telmisartan and Hydrochlorothiazide were found to be (y = 6106x + 66458, r = 0.9994), (y = 57157x-60937, r = 0.999) and (y = 10054x + 55335, r = 0.9992) respectively. The proposed method is highly sensitive, precise and accurate and hence was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations.

Keywords: Ramipril, Telmisartan, Hydrochlorothiazide, RP-HPLC and Simultaneous Estimation.

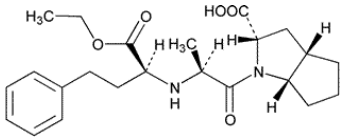
INTRODUCTION

The use of combination drug therapy for hypertension is common, using smaller amounts of one or more drugs in combination can minimize side effects while maximizing the antihypertensive effect.

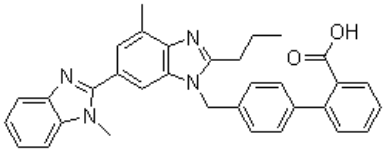
Ramipril(RP) chemically (4-[2-(1-ethoxycarbonyl-3-phenyl-propyl) aminopropanoyl]–4 azabicyclo[3.3.0]octane-3-carboxylic acid)is an angiotensin-converting enzyme (ACE) inhibitor, an effective agent for the treatment of hypertension, management of heart failure, treatment of myocardial infarction and prophylaxis of cardiovascular events in high risk patients. It is a white crystalline powder that is sparingly soluble in water, freely soluble in methanol and acetonitrile[1].

Telmisartan (TEL) is a new angiotensin II receptor antagonist for the treatment of essential hypertension usually given in combination with Ramipril and Hydrochlorothiazide. Telmisartan is chemically 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl) methyl)biphenyl-2- Carboxylic acid, blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland[2].

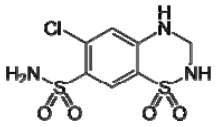
Hydrochlorothiazide (HCTZ) chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine- 7- Sulphonamide 1, 1-dioxide, is a diuretic which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co transporter, resulting in an increase in the excretion of sodium, chloride and water[2]. Indirectly it reduces plasma volume with consequent increases in aldosterone secretion and decreases in serum potassium.



RAMIPRIL



TELMISARTAN



HYDROCHLORTHIAZIDE

Literature survey revealed that there are many methods like HPLC, UV-Spectrophotometric and HPTLC for individual[1-8] and two combinations of RP, HCT and TEL[9-22] but there is no HPLC method reported for simultaneous estimation of RP, HCT and TEL in their combined dosage form. The purpose of this study was the development of a simple isocratic HPLC method with ultraviolet detection for Hydrochlorothiazide, Ramipril and Telmisartan, assay in tablets and validation as per the ICH guidelines.

MATERIALS AND METHOD

Materials

Pure samples of Ramipril, Telmisartan and Hydrochlorothiazide were obtained from Aurobindo pharma limited, Hyderabad, the commercial pharmaceutical preparation Ramtel-H* 5 containing 5 mg, 40 mg and 12.5 mg respectively (Marketed by Piramal Healthcare Limited) were procured from local pharmacy. Methanol (HPLC grade), Acetonitrile (HPLC grade), Orthophosphoric Acid, Triethylamine (TEA) were a product of Sigma Aldrich limited. Purified water was prepared using a Millipore Milli-Q water purification system.

Instrument used

Chromatography was performed using a JASCO HPLC instrument (Japan) equipped with a PU-2080 pump and detection was achieved by UV-2075 detector (JASCO) using a column Hibar® Lichrospher® 100 RP-18 (5 µm, 250mm x 4.60mm id). Data acquisition and processing was performed using JASCO BORWIN software (Japan). Sample injection was performed with a Rheodyne 7725 injection valve via a 20 µl loop. Dissolution of the compound was enhanced by sonication on Bandelin sonorex sonicator. Degassing of the mobile phase and the other solvents was achieved through by helium purging before the use. The pH of the solution was adjusted by using a pH meter (Cyber scan pH 2100) made by EUTECH and analytical balance (Model DI 707 of Digisum Electronic).

Methodology

Chromatographic conditions

Chromatographic conditions were achieved by using Hibar® Lichrospher® 100 RP-18 (5 µm, 250mm x 4.60mm id) analytical column. The mobile phase used in this study was a mixture of acetonitrile and water (containing 0.1% OPA) in the ratio (65:35v/v), pH was adjusted to 2.5 with triethylamine (TEA). The mobile phase was filtered through a 0.45 µ membrane filter and degassed using helium before use. The mobile was pumped from the solvent reservoir to column at a flow rate of 0.8 ml/min with injection volume of 20 µl and the retention times obtained for Hydrochlorothiazide, Ramipril and Telmisartan were 3.658 min, 7.367 min and 10.283 min respectively at UV detection point 225nm. The identification of the separated Ramipril, Telmisartan and Hydrochlorothiazide was confirmed by running the chromatograms of the individual compounds under identical conditions.

Preparation of stock and working stock solutions

Primary stock solutions of Ramipril, Telmisartan and Hydrochlorothiazide of 1000 µg/ml were prepared in methanol. From these stock solutions six mixed standards were prepared with mobile phase having Ramipril, Hydrochlorothiazide and Telmisartan in the ratio of 1:2:8 respectively. The concentration ranges of three drugs in working standard solutions were RAM 5-30 µg/ml, TEL 40-320 µg/ml and HCTZ 10- µg/ml, Before injecting drug solutions, the column was equilibrated at least for 30-45 min with the mobile phase flowing through the system. Each of the samples (20 µl) prepared were injected three times into the column. The amount of drug is calculated by the peak area, standard graph was plotted by taking concentration of drug on X-axis and peak area of drug on Y-axis.

Assay determination of Ramipril, Telmisartan and Hydrochlorothiazide

Twenty tablets of Ramtel-H* 5 (each containing 5 mg, 12.5 mg and 40mg of Ramipril, Hydrochlorothiazide and Telmisartan respectively) were made into fine powder, an amount equivalent to 50 mg of tablet powder accurately weighed and then extracted with methanol in a 50 ml volumetric flask , filtered through 0.45 µ filter and sonicated for 20 min. The solution was centrifuged and the supernatant was taken into a thoroughly cleaned and dried volumetric flask. The sample solution (20 µL) was injected and the chromatogram was recorded. The peak area of each of the drugs was calculated and the amount of each drug present per tablet was estimated from the respective regression equations.

Method Validation

As per the International Conference on Harmonization (ICH) guidelines[23-25], the method validation parameters checked were specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity

Method Validation is done according to ICH Q2B guidelines for validation of analytical procedures, calibration curves were generated with appropriate volumes of working standard solutions for HPLC method. Linearity was determined by plotting the standard curve in the concentration range of 5-30 µg/ml, 10-60 µg/ml and 40-320 µg/ml for Ramipril, Hydrochlorothiazide and Telmisartan respectively. The linearity of these methods was evaluated by linear regression analysis, using least square method.

Precision

The assay of the precision was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %RSD. The intra and inter-day variation in the peak area of drug solution containing (25 µg/ml, 50 µg/ml and 200 µg/ml) of Ramipril, Hydrochlorothiazide and Telmisartan respectively were calculated in terms of standard deviation and %RSD.

Accuracy

Accuracy is the percentage of analyte recovered by assay from a known added amount. Accuracy of HPLC method was done by adding known amount of standard solution of Hydrochlorothiazide (40, 50 and 60 µg/ml), Ramipril (20, 25 and 30 µg/ml) and Telmisartan (160, 200 and 240 µg/ml) to a pre-quantified sample solution of HCTZ 50 µg/ml, RAM 25 µg/ml and TEL 200 µg/ml. All the solutions were prepared and analyzed in triplicates.

Robustness and ruggedness

Robustness was performed by small changes in the chromatographic conditions such as % acetonitrile in the mobile phase, flow rate, buffer and pH was varied and effects of parameters were observed and calculated for mean standard deviation and relative standard deviation. Results remained unaffected by small variations in the parameters. Ruggedness was determined by varying the analyst, instrument and different column of different grades. The relative standard deviation of the results obtained from different analysts and instruments was <1.0%.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio. LOD is lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

LOD = 3.3 × SD/SLOPE

LOQ = 10 × SD/SLOPE

RESULTS AND DISCUSSIONS

Method of development

The chromatographic conditions were optimized in order to provide good performance in assay, various ratios and combinations of mobile phase were tried. Finally the mobile phase of 65:35 v/v mixture of acetonitrile and water (containing 0.1% OPA) , pH was adjusted to 2.5 with triethylamine (TEA) at a flow rate 0.8 ml/min and UV detection at 225 nm, at these conditions peaks obtained are with good shape and resolution.

Validation of the method

Linearity

These results indicate that the response is linear over the range of 5-30 µg/ml, 10-60 µg/ml and 40-320 µg/ml for Ramipril, Hydrochlorothiazide and Telmisartan respectively. The results were shown in below.

Precision

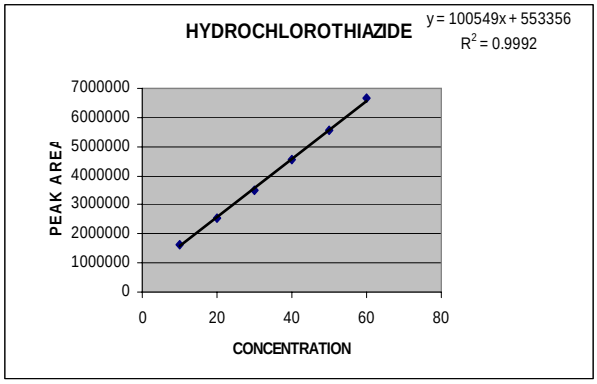
These results indicate that the proposed method is precise. The results were shown in Table: 2.

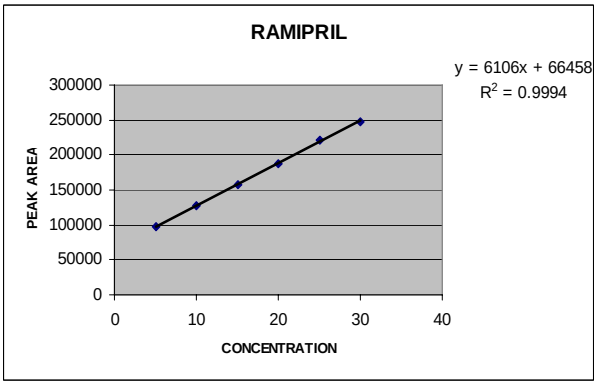
Accuracy

These results indicate that the proposed method is precise. The results were shown in Table: 3.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The S/N Ratio values of LOD and LOQ concentrations of HCTZ, RP and TEL were found to be 8.8ng/mL, 31ng/mL and 4.17ng/mL and 26ng/mL, 95ng/mL and 12ng/mL respectively.





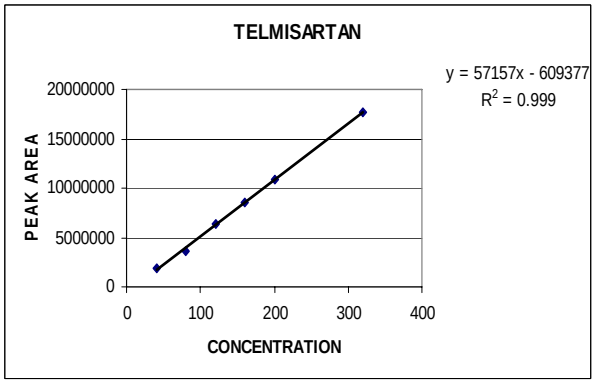


Table 2: Precision of the proposed RP-HPLC method

Drugs	Conc. (µg/mL)	Measured concentration (µg/mL) ± SD (n = 3)		% CV	
		Intra-day	Inter-day	Intra-day	Inter-day
Hydrochlorothiazide	50	49.87	49.57	0.007	0.0524
Ramipril	25	25.047	24.897	0.086	0.1325
Telmisartan	200	198.69	198.16	0.071	0.055

Table 3: Accuracy and recovery results of the proposed RP-HPLC method

Drug	Conc. (µg/mL)	Measured concentration (µg/mL) ± SD (n = 3)	% Recovery Spike level		
			80%	100%	120%
Hydrochlorothiazide	40	39.847± 0.003	99.61	99.73	100.9
	50	49.872± 0.0057			
	60	60.543,±0.0032			
Ramipril	20	19.913 ±0.0115	99.57	101	99.14
	25	25.39 ± 0.017			
	30	29.74 ± 0.011			
Telmisartan	160	161.11 ± 0.003	100.69	100.18	99.29
	200	200.3 ± 0.0057			
	240	238.30 ±0.0057			

Assay determination of Ramipril, Telmisartan and Hydrochlorothiazide

These results indicate the suitability of this method for routine analysis of Hydrochlorothiazide, Ramipril and Telmisartan in pharmaceutical dosage form. The results were shown in Table: 4.

Table 4: Assay Results

Drug	Label claim (mg)	Mean ± SD (amount recovered) (n=3)	Estimated % of label claim ± SD (n = 3)
Hydrochlorothiazide	12.5	12.49±0.01	99.92±0.02
Ramipril	5	4.95±0.011	99.0±0.01
Telmisartan	40	39.96±0.02	99.90±0.01

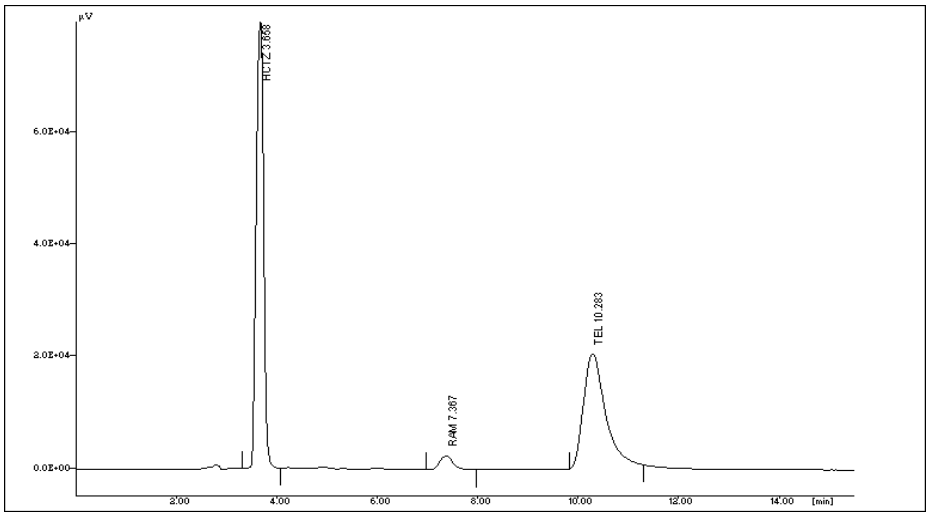


Fig. 1: Standard Chromatogram of HCTZ (3.658 min), RAM (7.367 min) and TEL (10.283 min)

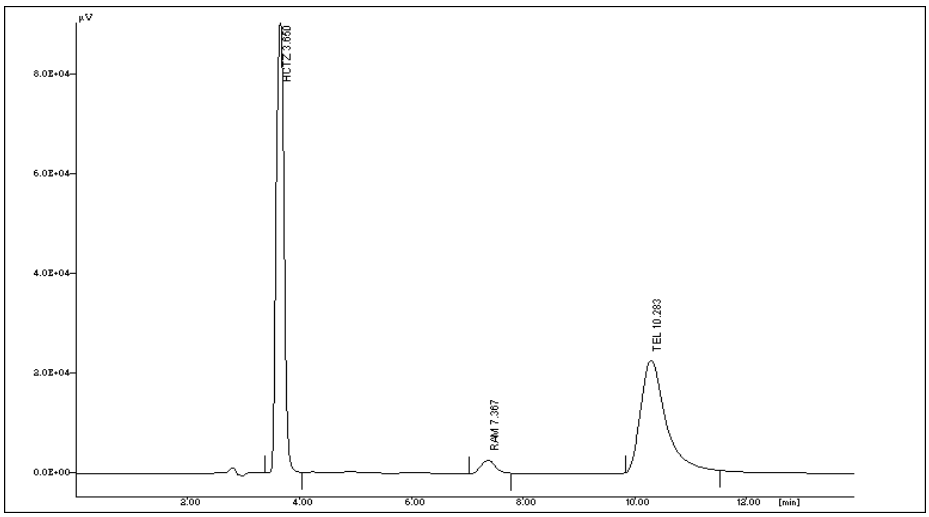


Fig. 2: Assay chromatogram of Ramtel-H* 5

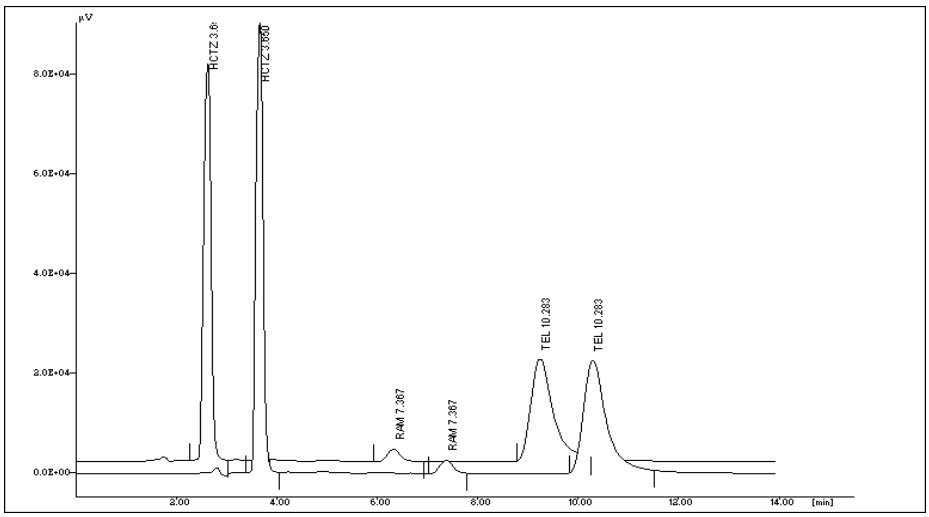


Fig. 3: Overlaid chromatogram of standard and marketed formulation.

CONCLUSION

The proposed RP-HPLC method is simple, reliable and selective providing satisfactory accuracy and precision with lower limit of detection and quantification. Moreover the shorter duration of analysis for Hydrochlorothiazide, Ramipril and Telmisartan made these reported method suitable for routine quantitative analysis in pharmaceutical dosage forms. Hence it can be easily and conveniently adopted for routine quality control analysis.

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