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Simultaneous Determination of Ramipril, Hydrochlorothiazide and Telmisartan in tablet dosage form using High-Performance liquid chromatography method

Susheel John Varghese* and Thengungal Kochupappy Ravi

Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, Tamil Nadu, India

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ABSTRACT

This paper describes validated reversed phase high performance liquid chromatography (HPLC) method for the simultaneous determination of ramipril(RPL), telmisartan(TLM), and Hydrochlorothiazide((HTZ) in combined tablet dosage forms. The isocratic reverse phase HPLC analysis was done on a Merck C_{18} 4.0 mm× 250 mm, column with mobile phase consisting of 0.1%phosphoric acid(pH adjusted to 2.5 with triethylamine) - acetonitrile(58:42,v/v) at a flow rate of 1 mL/min. Quantification was carried out using a photo-diode array UV detector at 210 nm. The developed analytical method was validated according to International Conference on Harmonization guidelines and the acceptance criteria for parameters like accuracy, precision, linearity, specificity and system suitability were met in all cases. The method is simple, precise, and sensitive, and hence applicable for simultaneous determination of RPL, TLM, and HTZ in pure powder and tablets.

Key words: Ramipril, Hydrochlorothiazide, Telmisartan, High-Performance liquid chromatography method.

INTRODUCTION

Tablet formulations containing angiotensin converting enzyme (ACE) inhibitor ramipril, angiotensin II receptor antagonist telmisartan, and the diuretic hydrochlorothiazide are used in the therapy to treat high blood pressure. Ramipril is chemically described as (2*S*,3*aS*,6*aS*)-1[(*S*)-N-[(*S*)-1-Carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta [*b*]pyrrole-2-carboxylic acid, 1-ethyl ester, telmisartan as 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-

[1,1'-biphenyl]-2-carboxylic acid, and hydrochlorothiazide as 6-chloro-3,4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1,1dioxide [1], Figure 1.

The increasing use of ramipril-hydrochlorothiazide-telmisartan combination as an effective treatment for hypertension demands the need of analytical methods to simultaneously quantify these drugs in tablets in order to evaluate its quality. Literature survey revealed no HPLC method for the simultaneous determination of RPL, TLM, and HTZ in combined dosage forms. Some papers have described different analytical methods like HPLC [2-4] for the determination of RPL in its single dosage form and also in combination with other drugs. Analytical methods that have been reported for the determination of TLM in pharmaceutical formulations and in bulk [5-8], as well as in biological fluids [9], include HPLC, HPLC/MS-MS, and HPTLC. For HTZ, the analytical methods reported in pharmaceutical formulations [10-14], include HPLC and HPTLC. An ultra -violet spectroscopy method has been reported for the simultaneous analysis of RPL, TLM, and HTZ in combined dosage forms [15].

Hence, the aim of this study was to develop a new validated HPLC method to simultaneously quantify RPL, TLM, and HTZ in fixed dose combination tablets.

Method validation is an important requirement in analytical method development. For this, International Conference on Harmonization (ICH) [16] provide guidelines for performing these validations. Taking into account these guidelines, the developed method has been validated.

MATERIALS AND METHODS

2.1. Chemicals, Reagents, and Standards

RPL and TLM reference standards were provided by Aristo Pharmaceuticals Pvt. Ltd., (Mumbai, India). HTZ was obtained from Medreich Pvt. Ltd.,(Bangalore, India).The pharmaceutical dosage form used in this study was Teram-H® tablets labeled to contain TLM 40 mg, RPL 5 mg, and HTZ 12.5 mg(Atoz Life Sciences, Pondicherry, India). Triethylamine (AR grade) was purchased from Qualigens Fine Chemicals Pvt. Ltd., (Mumbai, India). Formic acid (AR grade) was procured from Merck Specialities Pvt. Ltd., (Mumbai, India). Acetonitrile (HPLC grade) was obtained from Fischer Scientific Pvt. Ltd., (Mumbai, India). HPLC grade water was prepared by use of a Millipore Milli-Q Academic water purifier (Bangalore, India).

2.2. Instrumentation and chromatography

The HPLC analyses was carried out on a Shimadzu Prominence UFLC(Shimadzu corporation, Kyoto, Japan) equipped with LC-20 AD pump, SPD-M20A diode array detector, DGU-20A_3 degasser, SIL-20 AC auto sampler, CTO-10ASVP column oven and LC solutions software. Isocratic mobile phase consisted of 0.1% phosphoric acid (pH adjusted to 2.5 with triethylamine)- acetonitrile , (58:42, v/v), filtered through a nylon membrane filter (Rankem, New Delhi, India) of 0.45µm porosity. Lichrospher, 250 mm × 4 mm i.d., 5 µm particle size, RP-18, analytical column from Merck, Germany, was used as stationary phase. The flow rate was 1.0 mL/min and the detector was set at 210 nm. The injection volume was 20 µL. Chromatograms were recorded and integrated on PC installed with LC solutions

chromatographic software, version 3.41.324. All weighing was done on a Shimadzu electronic balance(Japan), Model BL-220H.

2.3. Preparation of standard solutions

2.3.1. Preparation of the standard stock solution

RPL, HTZ, and TLM standard stock solution were prepared by transferring accurately about 5mg of RPL, 12.5 mg of HTZ, and 40 mg of TLM reference standards to a 50 mL volumetric flask. Twenty milliliters of methanol was added initially to solubilize the drugs and the solution was diluted to volume with methanol and mixed well to get 100 µg/mL of RPL, 250 µg/mL of HTZ, and 800 µg/mL of TLM.

2.3.2. Preparation of working solution

An aliquot of 1 mL of above stock solution was transferred to a 10 mL volumetric flask and diluted with methanol, to obtain a solution of concentrations 10, 25 and 80 µg/mL of RPL, HTZ and TLM, respectively.

2.3.3. Preparation of calibration curves

From the working solution, standard solutions containing 0.5- 4.0, µg/mL of RPL, 1.25-10 µg/mL of HTZ, and 4-32 µg/mL were prepared in water-methanol (50:50,v/v) mixture and analyzed in triplicate. The peaks obtained were integrated, the peak areas were noted, and respective calibration curves were plotted as response factor against concentration of each drug.

2.3.4. Analysis of formulation

Twenty tablets, containing 5mg of RPL, 12.5 mg of HTZ, and 40 mg of TLM, were weighed and average weight was calculated. An amount of powder equivalent 5mg of RPL, 12.5 mg of HTZ, and 40 mg of TLM were transferred to a 50 mL volumetric flask, added 20 mL methanol and sonicated for a few minutes. A 30 mL portion of methanol was then added and sonicated for 15 min followed by shaking on a shaker for 10 min to ensure complete extraction. This solution was centrifuged at 4000 rpm for 10 min. A 1 mL portion of the supernatant solution was transferred to a 10 mL volumetric flask and diluted to volume with methanol. Aliquots of this solution was transferred to a 10 mL volumetric flask and diluted with water-methanol (50:50, v/v) mixture, to obtain concentrations in the linearity range. The solutions were filtered through a 0.45 µ membrane filter before injection into the column.

2.4. Method validation

The developed methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

2.4.1. Linearity

Linearity was studied for the standards using standard drug solutions prepared as described above. Peak areas of drugs were plotted versus concentration, and least-squares analysis was performed.

2.4.2. Precision

The precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) and was expressed as RSD of a series of measurements. Intraday precision was evaluated by six replicate readings at three concentration levels within the linearity range. Interday precision was studied by comparing the results on 3 different days.

2.4.3. Accuracy

To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample at three different levels: 80, 100, and 120%. The resulting solutions were then reanalyzed by the proposed method.

2.4.4. Specificity

Specificity of the method was evaluated by studying the peak purity index values. Spectral purities of RPL, HTZ, and TLM were evaluated using the UV spectra recorded by a diode array detector. In addition, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure and injected onto the chromatograph.

2.4.5. Robustness

Robustness of the HPLC method was evaluated by studying the influence of small deliberate variations of the analytical parameters on the retention time, peak area, or peak shape. The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use. Sample solutions were prepared and analyzed under the established conditions and by variation of the following analytical parameters: flow rate of the mobile phase (± 0.1mL/min), acetonitrile proportion in the mobile phase (±2 %), Column temperature (±2º C), buffer pH (±0.5 units) and buffer strength (±0.5%). The drug contents were determined for each condition and analyzed.

RESULTS AND DISCUSSION

Chromatographic conditions were optimized to achieve the best resolution and peak shapes. Mobile phase selection was based on peak parameters (symmetry, theoretical plates, and capacity factor), run time, ease of preparation, and cost. Preliminary trials using different compositions of mobile phases consisting of acetonitrile and orthophosphoric acid solutions gave poor peak shape and high tailing factors. Symmetrical peaks with good separation (retention time, HTZ=3.0 min, RPL=9.1 min, and TLM=10.5 min,) were obtained on a 250 mm × 4 mm, RP-18column with mobile phase consisting of 0.1% phosphoric acid(pH adjusted to 2.5 with triethylamine) and acetonitrile(58:42,v/v) at a flow rate of 1 mL/min. A typical chromatogram obtained from the analysis of the drugs using the proposed method is shown in Figure 2. The optimum wavelength for detection and quantification was 210 nm, which gave good detector response for both drugs.

3.1. Method validation

3.1.1. *Linearity*

The relationship between the concentration of RPL, HTZ, and TLM and peak areas was investigated. Good linearity was observed for in the concentration range of 0.5-4.0, 1.25-10, and

4.0-32.0 µg/mL for RPL, HTZ, and TLM, respectively. The regression equations for the drugs were found by plotting peak areas in AU (*y*) versus the concentration (*x*) in µg/mL. Table 1 summarizes the linearity ranges and linear regression equations for the drugs.

3.1.2. Precision

The precision of the method was determined by repeatability (intraday) and intermediate (interday) precision and was expressed as the RSD of the results. The values obtained for precision studies are presented in Table 1 and indicate good repeatability and low interday variability.

3.1.3. Accuracy

The recovery study results ranged from 98 to 102% for RPL, HTZ, and TLM, showing the accuracy of the method (Table 2). Low RSD values indicate that the method is precise.

3.1.4. Specificity

The specificity of the developed method was evaluated by studying the peak purity index values. The chromatographic peak of each drug was not attributable to more than one component (diode array detector peak purity index values for RPL, HTZ, and TLM were found to be 1).

3.1.5. Robustness

The robustness of the proposed method was evaluated by modifications in the organic composition and pH value of the aqueous phase of the mobile phase, and in the flow rate. During these investigations, it was found that there was not much change in the retention times, area, or symmetry of the peaks.

3.1.6. Stability studies

The stability of solutions was evaluated by storing them at ambient temperature and 2–5°C, and testing at regular intervals. The responses for the aged solutions were evaluated using a freshly prepared standard solution. The solutions stored at ambient temperature and 2–5°C was found to be stable for around 2 and 5 days, respectively.

Table 1. Summary of validation parameters for the proposed method

Parameters	RPL	HTZ	TLM
Linearity, µg/mL	0.5 to 4.0	1.25 to 10.0	4.0 to 32
Linear regression equation[a]			
Intercept	-5566.9	-30058.7	-197344.8
Slope	43521.9	81193.0	153453.3
Correlation coefficient	0.996	0.990	0.991
Precision			
Intraday(n=6), %	0.7623	0.5091	0.4817
Interday (n=6), %	1.0837	1.016	1.1195
Repeatability of injection (n=10), %	0.7640	0.6948	0.7207

[a] $y=mx+c$

3.1.7. System Suitability studies

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were made, and peak asymmetry, theoretical plate number, and RSD of the peak areas were determined. For all system suitability injections, asymmetry was <1.5, theoretical plate number was >5000, and RSD of the peak areas was less than 1.0%.

3.1.8. Analysis of fixed dose combination tablets

The proposed method was used for the assay of commercially available tablets containing RPL, HTZ, and TLM. Six replicate determinations were performed on accurately weighed tablets. Experimental values obtained for determination of RPL, HTZ, and TLM in samples is presented in Table 3.

Table 2. Accuracy data (analyte recovery)

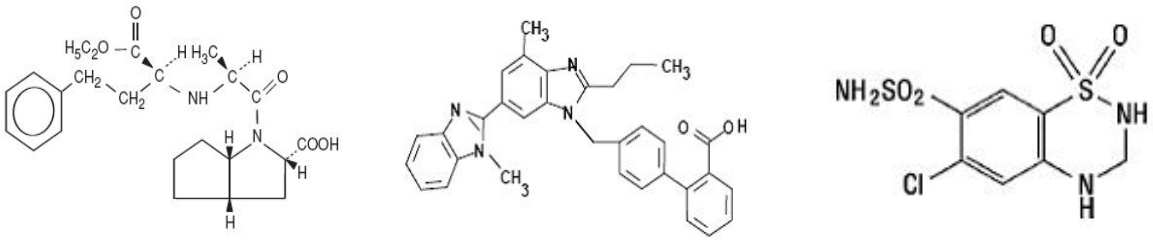
Drug	Level,%	Recovery, (%)[a]	R.S.D., (%)[a]
RPL	80	99.3	0.7490
	100	101.8	0.8631
	120	102.3	0.7537
TLM	80	98.5	0.9021
	100	101.2	0.7839
	120	98.9	0.8921
HTZ	80	102.4	0.8951
	100	101.6	0.7849
	120	102.9	0.9626

[a] *Average of six determinations.*

Table 3. Analysis of the formulation

Drug, labeled amount, mg/tablet	Amount found, mg/tablet	Amount found, %[a]	R.S.D., %[a]
RPL, 5	4.92	98.4	0.7630
TLM, 12.5	12.75	102	0.6962
HTZ, 40	40.75	101.9	0.9012

[a] *Average of six determinations.*



Ramipril **Telmisartan** **Hydrochlorothiazide**

Figure 1. Chemical structures of ramipril, telmisartan, and hydrochlorothiazide

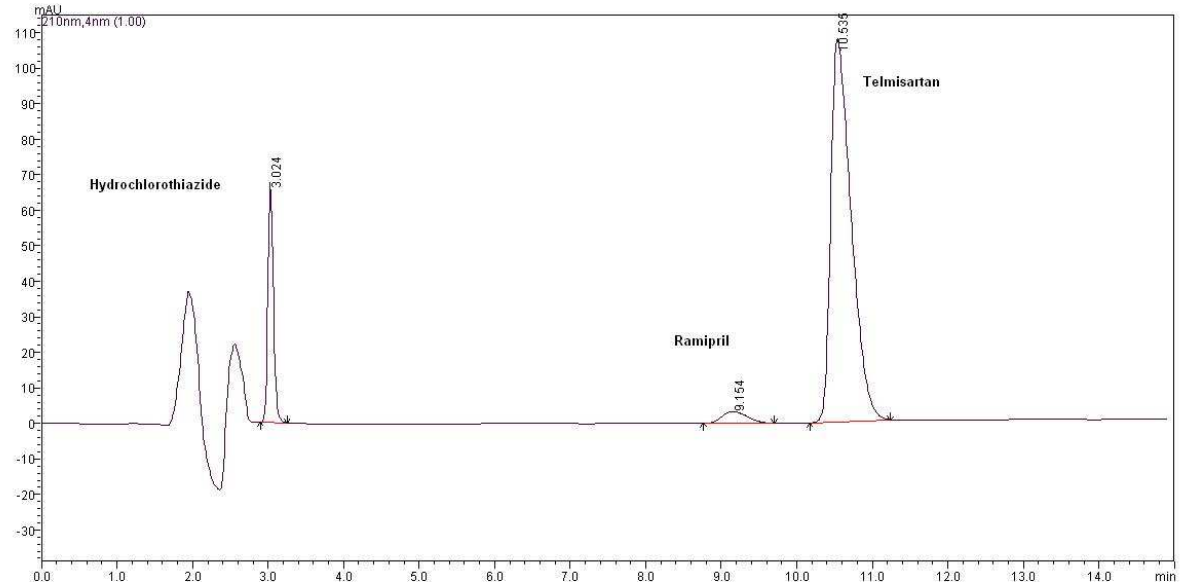


Figure 2. HPLC chromatogram of RPL (2 µg/mL), HTZ (5 µg/mL), and TLM (16 µg/mL)

CONCLUSION

The proposed HPTLC method is simple, precise, sensitive and accurate. It uses simple reagents with minimal sample preparation procedures. Hence, the developed method is suitable for routine analysis of RPL, HTZ, and TLM in combined tablet dosage forms.

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