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Development and Validation of Simultaneous HPLC method for Estimation of Telmisartan and Ramipril in Pharmaceutical Formulations

Sunil Jawla*, K Jeyalakshmi, T Krishnamurthy[1], Y. Kumar

***I. T. S. Paramedical College (Pharmacy), Delhi-Meerut Road, Murad Nagar, Ghaziabad, Utter Pradesh-201206. India.**

[1]Analytical Research and Development Department, Dr. Reddy's Laboratories, Hyderabad. India.

***Corres.author: suniljawla777@gmail.com**
Contact no. +919456039139

Abstract: A simple isocratic HPLC method has been developed and subsequently validated for simultaneous determination of Telmisartan and Ramipril in pharmaceutical dosage forms. The method employs an Inertsil ODS C18 column, 5 µ, 250mm x 4.60mm id with flow rate of 1.5 ml/min using PDA detection at 210nm. The separation was carried out using a mobile phase consisting of potassium di hydrogen phosphate buffer having pH 2.8 and Acetonitrile in the ratio of 60:40 respectively. The retention time for Telmisartan and Ramipril was found to be 5.7min and 10.8min respectively. A linear response was observed over the concentration range of 2.5-25.5ppm and 3.0-7.25ppm for the assay of Telmisartan and Ramipril respectively. The limit of detection and the limit of quantification for ramipril were found to be 0.75ppm and 2.5ppm respectively and for telmisartan were found to be 1.25ppm and 3.0ppm respectively. The results of analysis were validated statically and by recovery studies. Hence the proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulation.

Key Words: Telmisartan, Ramipril, HPLC, Acetonitrile, Chromatography.

Introduction

Telmisartan,2-[4-[[4-methyl-6-(1-methylbenzoimidazol-2-yl)-2-propyl benzoimidazol-1-yl]methyl] phenyl] benzoic acid, is a specific angiotensin II receptor (type AT1) antagonist, and Ramipril, (1S,5S,7S)-6-[(2S)-2-[[(1S)-1-ethoxycarbonyl-3-phenyl-propyl]amino] propanoyl]-6-azabicyclo[3.3.0]octane-7-carboxylicacid, is an angiotensin-converting-enzyme (ACE) inhibitor, is the new class of angiotensin receptor antagonist. It reduces effectively hypertension by suppressing the effects of angiotensin II at its receptor, thereby blocking the renin-angiotensin system. Telmisartan has been demonstrated to be superior to previous peptide receptor antagonists and angiotensin converting enzyme (ACE) inhibitors because of its enhanced specificity, selectivity, and tolerability. Currently, Telmisartan is marketed alone or combined with Ramipril or Hydrochlorothiazide

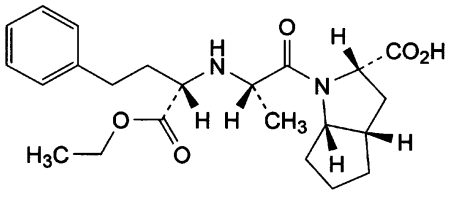

Fig.1 Chemical structure of Ramipril

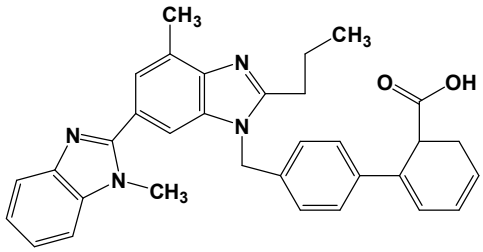

Fig 2. Chemical structure of Telmisartan

There are many analytical methods available for the quantization of Telmisartan and Ramipril individually. The purpose of this study was the development of a simple isocratic HPLC method with ultraviolet detection for Telmisartan and Ramipril assay in tablets and validates the same as per the ICH guidelines. [1-26]

Material and Methods

HPLC grade acetonitrile, methanol, reagent grade hydrochloric acid, Ortho -phosphoric acid, sodium per chlorate, potassium di hydrogen phosphate, triethyl amine, sodium hydroxide, 3% hydrogen peroxide, sodium meta bisulfate were purchased from Merck (Darmstadt, Germany). Distilled water (>18μΩ), purified using a Millipore MilliQ plus water system was used to prepare the mobile phase and standard solutions. The analytical samples Telmisartan (40 mg) and Ramipril (2.5 mg) tablets procured from market.

The development and validation of the assay was performed on a HPLC Quatenary system with VWD Model Agilent 1200 series, consisting of a Quatenary pump-G1311A, a Degasser- G1322A, a ALS- G1329A, a TCC-G1316A and Waters-2996 Photodiode Array Detector, a Waters HPLC with PDA Detectors, Waters 2695- Separation Module with EMPOWER software.

The analytical column used to achieve chromatographic separation was Inersil ODS column (250 x 4.6 mm), 5μm particle size. The peak purity was determined on a 2996 Photodiode Array Detector (PDA).

The mobile phase consists of sodium per chlorate, potassium di hydrogen phosphate, triethylamine solution (0.5%) pH 2.8 and Acetonitrile (60:40) v/v. The pH of the solution adjusted to 2.8 + 2 to 5 ml of ortho phosphoric acid prior to mixing with acetonitrile. Injections were carried out using a 100μl loop at 45°C and flow rate was 1.5 ml/min. Detection was performed at 210 nm and the identification of Telmisartan and Ramipril was made comparing its retention time and their UV spectra using the PDA detector.

Buffer solution was prepared by dissolving accurately weighed amount of about 2g of sodium per chlorate monohydrate and 3.5g of potassium di hydrogen phosphate in 100ml of Milli-Q water. Added 0.5ml of triethylamine, mixed well and filtered through 0.45 μm membrane filter. Adjusted the buffer to pH 2.5 with Orthophosphoric acid. Mobile phase was prepared by mixing the buffer solution and acetronitrile in the ratio of 60:40 v/v respectively.

Diluent for standard preparation of Ramipril was prepared by mixing buffer solution, acetonitrile, methanol and 0.1N sodium hydroxide in the ratio of 45:22.5:22.5:10 v/v respectively. Diluent for standard preparation of Telmisartan was prepared by mixing buffer solution, acetonitrile and 0.1N sodium hydroxide in the ratio of 54:36:10 v/v respectively.

Appropriate aliquots of standard stock solutions of Ramiril and Telmisartan were diluted with suitable diluents to obtain concentrations in the range of 3.0-7.25ppm of Ramipril and 20.5-51.5ppm of Telmisartan respectively.

The solutions were injected in triplicates for each concentration using a 20 μl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average content of the drug versus respective concentrations and regression equations were computed for both the drugs. The plots of average content Vs respective concentration of Ramipril and Telmisartan were found to be linear in the range of 3.0-7.25ppm and 20.5-51.5ppm with correlation coefficient 0.9985 and 0.9992 for Ramipril and Telmisartan, respectively.

Ten tablets were weighed and finely powdered. Tablet powder equivalent to 5 mg of Ramipril and 40 mg of Telmisartan was accurately weighed and transferred to a 100 ml volumetric flask. To this was added about 50 ml of diluent and flask was sonicated for 10 min. Centrifuged this solution in a centrifuge tube with cap at 4000 RPM for 10 minutes. The flask was shaken, and the volume was made up to the mark with suitable diluent. The above solution was then filtered through 0.45 μ Whatman filter paper. One ml of the above filtrate was further diluted up to 10 ml with suitable diluent to obtain final concentrations of 5ppm and 50 ppm of Ramipril and Telmisartan, respectively. Sample solutions were filtered and injected.

Analysis of formulations:

Commercial formulation of Ramipril and Telmisartan (Ramipril 2.5mg & Telmisartan 40mg) were selected for analysis. Sample preparations were done as per the procedure given for pure samples of Ramipril and Telmisartan and analyzed. Analysis was done with six injection and data were observed statistically. (Table 1)

Recovery studies:

To study the accuracy, reproducibility and precision of the above methods, were carred out by addition of standard drug solution to pre-analyzed sample at different levels. Results of recovery were found to be satisfactory and are reported in Table 1.

Validation

System suitability

Having optimized the efficacy of a chromatographic separation the quality of the chromatography was monitored by applying following system suitability tests: Capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all the analyte peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substances solution is presented in Fig 3.

Table 1. Analysis of formulation and recovery studies

Drugs	Labeled Amount(mg)	Amount taken (ppm)	*Amount found(mg)	% Label claim	% Recovery*
Telmisartan	40	40	39.98±0.687	99.92	100.87± 1.136
Ramipril	2.5	2.5	2.49±0.356	99.87	99.66±0.872

*Each value is a mean of six observations

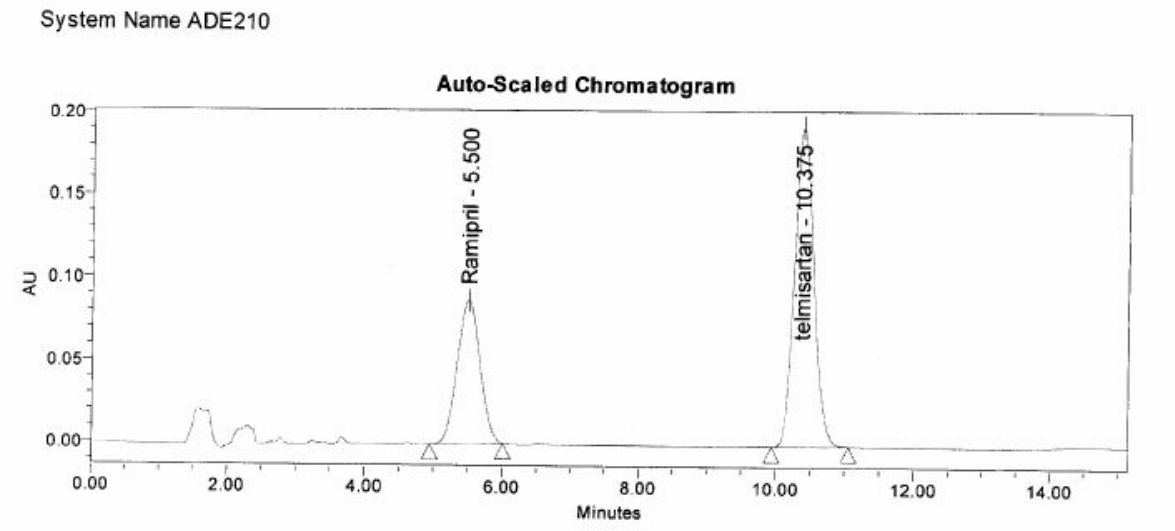

Figure 3. Chromatogram of Telmisartan and Ramipril reference substances.

Specificity

The excipients in the tablets used in this study contained the following inactive ingredients: Sodium hydroxide, Povidone, Megulumine, Sorbitol, Magnesium stereate, Microcrystaline cellulose, Lacose, Hydroxypropyl methylcellulose, Sodium starch glycolate, Sodium stearl fumarate as excipients. Injections of placebo and tablet solutions showed no interference with the main peak (Fig 4 & 5). The two point peak purity of the Telmisartan and Ramipril peaks obtained from reference substance of both Telmisartan and Ramipril and tablets were determined by PDA detector.

Range of linearity

Standard curves were constructed daily, for three consecutive days, using five standard concentrations in a range of 4.25 to 34mg/ml for Ramipril and 2.01 to 16.01 mg/ml for Telmisartan. This concentration range corresponds to the levels of 25-150% w/w of the nominal analytical concentration. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 99293x + 184818 and y = 363394x + 1E + 06.The R.S.D. values of the slope were 363394 and 99293 (n=5) and the R.S.D of y-intercept was (n=5).

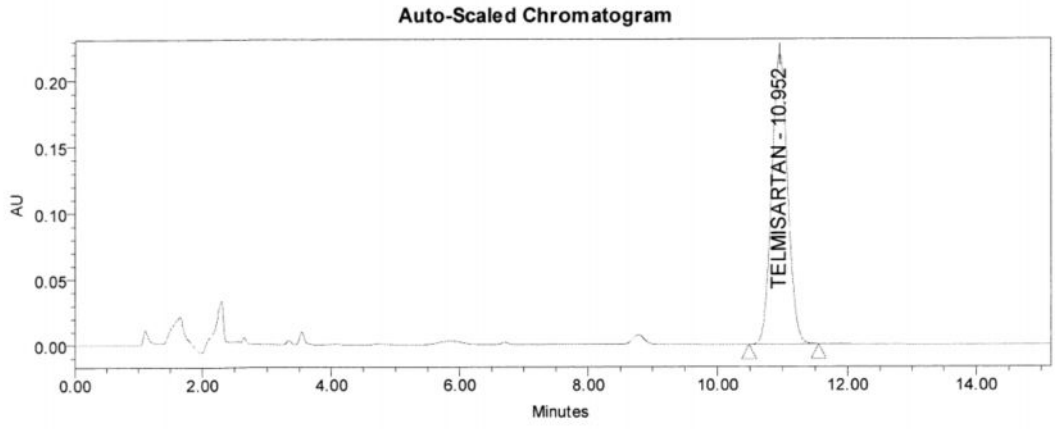

Figure 4. Chromatogram of Telmisartan in branded tablets.

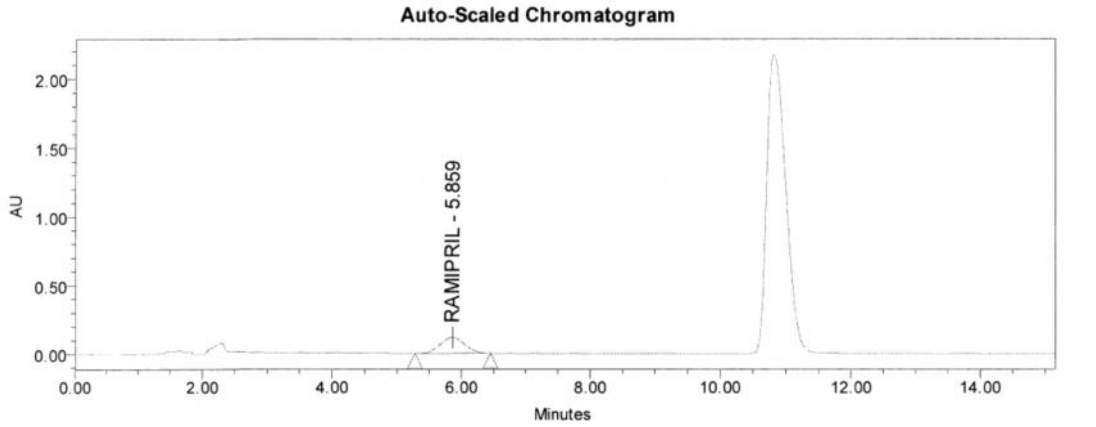

Figure 5. Chromatogram of Ramipril in branded tablets.

Precision

Method precision was demonstrated by the assay of a series of six samples, prepared as described above, on three consecutive days. The inter- and intra-day means and relative standard deviation (R.S.D) were calculated (Table 2). The assay method precision acceptance criteria set in the validation were a R.S.D ≤ 2.0% for each data set (1 day) and for all the data combined (3 days). The data of Table 3 meet these acceptance criteria.

Accuracy

The accuracy of the method was evaluated by determination of the recovery of Telmisartan and Ramipril on three days at three levels concentration. Tablets sample solutions were spiked with Telmisartan and Ramipril standard solution, corresponding to 50%

To 150% of the nominal analytical concentration (12 to 22ppm/ml).Their results showed good recoveries ranging from 98.3% to 105.1% for Ramipril and 100.1% to 104.0% for Telmisartan. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study. (Table 4)

Table: 2 Inter-day precision for Ramipril and Telmisartan

Day	Ramipril*		Telmisartan*	
	Mean	**%RSD**	**Mean**	**% RSD**
1	102.6	1.9	98.3	1.1
2	102.8	2.0	98.8	1.3
3	102.0	1.7	98.2	1.1

*(n=6) precision results for Ramipril and Telmisartan tablets assay on three consecutive days.

Table: 3 Intra-day precision for Ramipril and Telmisartan

Ramipril*		Telmisartan*	
Mean	%RSD	Mean	% RSD
104.2	0.5	99.6	0.5

(n=6) precision results for Ramipril and Telmisartan tablets.

Table 4: Statistical Data for Accuracy

Statistical data	Ramipril					Telmisartan				
Spike Level	**50%**	**75%**	**100%**	**125%**	**150%**	**50%**	**75%**	**100%**	**125%**	**150%**
% Mean	102.8	99.3	100	97.7	99.6	99.3	99.2	102.3	101.4	100.7
%R.S.D.	1.7	1.4	1.2	1.8	1.4	1.6	0.8	1.7	0.0	1.4

Robustness

Typical variations in high performance liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

a) Variation in mobile phase

The effect of variations in percent Buffer and acetonitrile (ACN) from 60:40 to 50:40 and 60:50 ratio mobile phase was evaluated. While the tailing factor, number of theoretical plates and resolution showed a little change with acetonitrile ratio variations, the retention time and, consequently, the capacity factor were significantly altered with these changes (Table 5). Considering that the capacity factor obtained in this method is already close to the minimum value acceptable, that is 2.0, an increase in ACN concentration is not recommended, although the order parameters were still fine.

b) Variation in pH

The pH variations tested were higher values than the value set this method because extremes pH can cause damage to the chromatographic column. The chromatographic parameters of Telmisartan and Ramipril showed only major fluctuations with mobile phase pH changes.

c) Different column

A Inertsil ODS 3V- column (250x4.6 mm, 5µm particle size) was used with the original mobile phase and the chromatographic parameters of Telmisartan and Ramipril show only minor fluctuations with different column employed.

d) Variation in temperature

The temperature variations tested were higher and lower values than the value set this method because temperature can cause changes on the elution of components. The chromatographic parameters of Telmisartan and Ramipril showed only minor fluctuations with temperature changes.

e) Variation in flow rate

The effect temperature variation above and below the value of assay method showed a little change on the peak area. The higher flow rate reduces peak area both Ramipril and Telmisartan vice versa for lower flow rate.

Table 5. Chromatographic parameters of robustness evaluation.

Variation	Retention time		Peak area		Resolution	Tailing factor		Theoretical plates	
	R	T	R	T		R	T	R	T
ACN 40%	4.18	5.76	2026262	3743421	4.3	1.1	1.2	150377	3990
ACN 50%	4.1	5.53	184585	3780372	6.2	1.1	1.2	12084	4814
pH 2	6.8	8.0	2038968	3601058	2.5	1.1	1.2	3121	4439
pH 3	5.6	11.7	2020695	3781210	8.7	1.0	1.2	1066	4610
Temp(35^{o}C)	5.53	10.4	2042332	3780372	7.8	1.1	1.2	1016	6001
Temp(45^{o}C)	5.48	10.33	2078337	3764735	8.9	1.0	1.2	1534	6420
Flow(1.3ml)	6.32	11.9	2230098	4333456	8.9	1.1	1.2	1455	6813
Flow(1.7ml)	4.86	9.20	1804322	3061614	8.1	1.0	1.2	1122	6028
Different column	5.56	9.19	2127256	3786868	8.0	1.1	1.1	2120	8801
Without variations	5.50	10.37	2004411	3734162	8.6	1.0	1.2	1258	6488

R- Ramipril, T- Telmisartan

Result and Discussion

All the system suitability parameters were optimized to obtain best resolution between multi-component formulations. ODS Inertsil column (250mm X 4.60mm) having particle size 5 µ and bonded phase octadecylsilane (C-18) were optimized with following conditions.

Variable	Conditions
Column	ODS Inertsil
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C18)
Mobile Phase	
Sodium per chlorate & potassium di hydrogen phosphate buffer (pH 2.5)	60%
Acetonitrile	40%
Diluent	Buffer: ACN : NaOH (56:34:10)
Flow rate	1.5 ml/min
Temperature	45°C
Sample Size	100µl
Detection wavelength	210 nm
Retention time	Telmisartan 5.7miinutes Ramipril 10.8minutes

Under optimized conditions, Telmisartan and Ramipril showed good selectivity and resolution, which can be well understood by looking at the validation data for the developed method are given as follows.

Validation parameters	Telmisartan	Ramipril
Linearity (r2)	0.9991	0.9995
Repeatability (%RSD)	1.1	1.6
Inter day Variation (%RSD)	1.1	1.6
Accuracy by recovery study (%found)	99.87	99.45
Robustness (%RSD)	0.6	0.9
Tablets	99.6	100.2
Intra day Variation (%RSD)	0.5	0.4

Linearity was observed by linear regression equation method for Telmisartan and Ramipril in different concentration range. The correlation coefficient of these drugs was formed to be close to 1.00, indicating good linearity. The developed HPLC method was validated for simultaneous estimation of Telmisartan and Ramipril using linearity, range, accuracy and precision. The %RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

Analysis of the results shows that the results obtained by various methods do not differ significantly and are quite comparable to the label claim of the formulations.

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