

Research Article**ISOCRATIC RP-HPLC-UV METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF RAMIPRIL AND TELMISARTAN IN TABLET DOSAGE FORM**

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ABSTRACT

An RP-HPLC method has been developed for the simultaneous estimation of ramipril and telmisartan in tablet dosage forms, using UV-detector. The developed method was validated as per ICH guidelines and specificity, linearity & range, accuracy, precision and robustness was performed. Specificity was determined by comparing the results obtained by running the placebo solution with that of standard and method was found to be specific due to no interference between placebo peaks and drugs peaks. Linearity range was found to be 4 to 16 µg/ml and 32 to 128 µg/ml of ramipril and telmisartan respectively. The method was found to be linear in the range of 4 to 16 µg/ml and 32 to 128 µg/ml for ramipril and telmisartan respectively. In the linearity study, regression equation and coefficient of correlation for ramipril and telmisartan were found to be ($y = 924480x - 151831$, $r = 0.9997$) and ($y = 2901878.3558x + 3803877$, $r = 0.9996$) respectively. This newly developed method was successfully utilized for the simultaneous estimation of ramipril and telmisartan in pharmaceutical tablet dosage forms.

KEYWORDS Ramipril, Telmisartan, RP-HPLC**INTRODUCTION**

Ramipril (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 infraction and prophylaxis of cardiovascular events in high risk patients. It is white crystalline powder that is sparingly soluble in water, freely soluble in Methanol and Acetonitrile.

Telmisartan (2-[4-[[4-methyl-6-(1-methylbenzoimidazol-2-yl)-2-propyl-benzoimidazol-1-yl]methyl]phenyl] benzoic acid) is an orally active nonpeptide angiotensin II antagonist that acts on the AT₁ receptor subtype. It is not official in any pharmacopoeia an effective agent for the treatment of hypertension and renal impairment. It is white to off-white crystalline powder that is insoluble in water, freely soluble in Methanol and Acetonitrile.

Literature review shows that there are developed methods including spectrophotometric, atomic absorption, HPLC and LC-MS method for the estimation of ramipril alone and in combination of other drugs losartan, hydrochlorthiazide, amlodipine, enalapril, benzanepiril, fosinopril, lisinopril and quinalopril. The developed methods for telmisartan includes spectrophotometric, electrophoresis, HPLC and HPLC with fluorimetric detection. But till this date no simultaneous method has been published anywhere for the simultaneous estimation of both drugs. So the aim of our study is to develop simple, fast, accurate and specific HPLC with UV detection method for simultaneous estimation of ramipril and telmisartan in bulk and combined dosage formulations.

MATERIALS AND METHOD

Shimadzu HPLC equipped with pump model LC-10Advp and SPD-10Avp UV detector. ODS column hypersil, 250×4.6 mm, 5 µm of thermo electron

corporation. Solvents HPLC grade and sodium dihydrogen phosphate purchased from Merck ltd. API were supplied from Lupin ltd and formulation Telma-R (Glenmark) and Telmista-RM (Lupin) were purchased from local market for estimation. Standards of both ramipril and telmisartan were provided by Oasis lab, Jaipur.

Chromatographic conditions were optimized after using mobile phase acetonitrile: buffer (Phosphate buffer: 3.9 gm NaH₂PO₄ in 1000 ml water) (65:35) at pH 4.5 ± 0.1, the flow rate was 1.0 ml/min at 210 nm. Run time, injection volume and flow rate were 12 min., 20 µl and 1 ml/min respectively. RT of ramipril and telmisartan were found to be 4.25 mins and 7.99 mins respectively.

Preparation of calibration graph

To prepare the calibration curve for ramipril and telmisartan, 4, 6, 8, 10, 12, 14 and 16 ml of the synthetic stock solution of ramipril (50.4 µg/ml) and telmisartan (404 µg/ml) were transferred to a series of seven, 50 ml volumetric flasks.

The volume in each flask was adjusted to 50 ml with mobile phase and mixed the contents to obtain a final concentration in the range of about 4 to 16 µg/ml and 32 to 128 µg/ml of ramipril and telmisartan respectively. Regression equation and coefficient of correlation for ramipril and telmisartan were found to be ($y = 924480x - 151831$, $r = 0.9997$) and ($y = 2901878.3558x + 3803877$, $r = 0.9996$) respectively.

Validation of proposed method

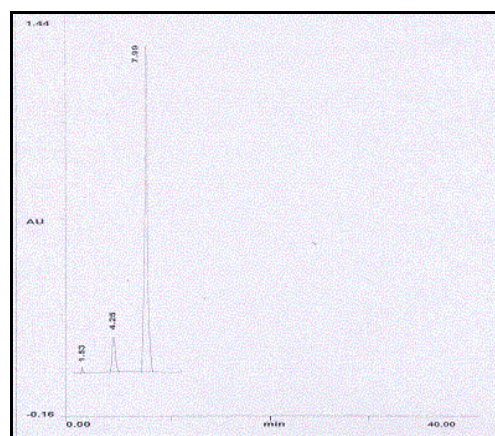


Fig. 1: Chromatogram of Ramipril and Telmisartan.

Table 1- Result of recovery study of Telma-R tablet

S.No.	Conc. before spiking (µg/ml)	Reference Std. added* (µg/ml)	Conc. After spiking* (µg/ml)	% Recovery
Ramipril				
1		4.13	9.10	99.26
2	5.03	5.05	10.18	100.99
3		6.17	11.13	99.83
Telmisartan				
1		31.79	71.58	99.15
2	40.33	40.15	81.15	100.83
3		48.04	88.70	100.44
Mean ± SD	Rap		99.63±0.500	
	Tel		100.14±0.880	

* Mean of three triplicate determinations

Table 2- Result of recovery study of Telmista-RM tablet

S.No.	Conc. before spiking (mg/ml)	Reference Std. added* (mg/ml)	Conc. After spiking* (mg/ml)	% Recovery
Ramipril				
1		4.12	9.08	99.34
2		5.07	10.18	100.89
3	5.02	6.12	11.13	99.91
Telmisartan				
1		31.79	71.58	99.541093
2		40.25	80.23	99.825806
3	40.12	48.04	88.7	100.61252
Mean ±	Rap		100.04±0.500	
SD	Tel		99.99±0.554	

* Mean of three triplicate determinations

Table 3- Result of recovery study of Ramipril and Telmisartan in synthetic mixture

S.No.	Conc. before spiking (µg/ml)	Reference Std. added* (µg/ml)	Conc. After spiking* (µg/ml)	% Recovery
Ramipril				
1		4.15	9.03	99.50
2	4.98	5.01	10.10	101.29
3		6.05	11.01	99.75
Telmisartan				
1		32.56	72.90	100.45
2	40.01	38.96	79.95	101.35
3		48.07	88.52	100.47
Mean ±	Rap		100.18±0.971	
SD	Tel		100.73±0.509	

* Mean of three triplicate determinations.

Specificity

Specificity of the method was determined by using commonly used excipients starch 60 mg, Talc 5 mg, Magnesium stearate 2 mg and titanium dioxide 2 mg transferred to 100 ml volumetric flask and 20 ml of ACN was added. The solution was sonicated for 20 minutes, filled up to mark and filtered. 10 ml of this solution was transferred to 50 ml volumetric flask, diluted, filtered with 0.22 micron filter and 20 µl volume was injected. No interference from placebo was observed at the retention time of the drugs.

Linearity and range

Linearity range: 4-16 µg/ml for RPL and 32-128 µg/ml for TLR.

Target concentration: 10 µg/ml for RPL and 80 µg/ml for TLR.

Target range: 80%, 100% and 120% of the target concentration equals to 8, 10 and 12 µg/ml for RPL and 64, 80 and 96 µg/ml for TLR.

Accuracy

The powdered sample of the tablet (Telma-R) equivalent to 5 mg of ramipril and 40 mg of telmisartan was transferred to a 100 ml volumetric flask and about 90 ml of HPLC grade acetonitrile was added and sonicated to dissolve the contents. The volume was made up to the mark with acetonitrile. This solution was filtered through whatman filter paper 41. The dilution contained about 50 µg/ml ramipril and 400 µg/ml of telmisartan.

5 ml of the above solution was diluted to 50 ml with mobile phase. The solution was filtered through a membrane filter (0.22 μm) and sonicated to degas. The dilution contained about 5 $\mu\text{g/ml}$ ramipril and 40 $\mu\text{g/ml}$ of telmisartan and estimation of the

drugs were done by proposed method. 5 ml of tablet solution (50 $\mu\text{g/ml}$ ramipril and 400 $\mu\text{g/ml}$ of telmisartan) and 4, 5 and 6 ml of synthetic stock solution (section 4.2.3.3) were mixed in three different 50 ml volumetric flasks separately and

Table 4: Calculation of overall RSD ramipril for robustness

S. No.	Control	Assay (mg/Tablet)					
		Flow rate (ml/min)		Column Oven Temp.		PH	
		0.9	1.1	25 $^{\circ}\text{c}$	35 $^{\circ}\text{c}$	4.3	4.7
1	5.03	5.01	4.97	4.95	5.03	5.02	5.08
2	4.98	4.94	5.05	4.98	4.94	4.97	5.06
3	5.05	4.92	4.96	5.02	5.04	4.99	5
Mean	5.02	4.95	4.993	4.983	5.00	4.99	5.05
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
SD	0.03	0.05	0.05	0.04	0.06	0.03	0.04
RSD	0.718	0.953	0.988	0.705	1.101	0.504	0.825

the RSD of each parameter was under acceptable limit, which is 2%.

Table 5: Calculation of overall RSD telmisartan for robustness

S. No.	Control	Assay (mg/Tablet)					
		Flow rate (ml/min)		Column Oven Temp.		pH	
		0.9	1.1	25 $^{\circ}\text{c}$	35 $^{\circ}\text{c}$	4.3	4.7
1	39.85	39.43	39.67	40.11	40.01	40.1	39.65
2	39.64	39.81	40.25	39.78	39.85	40.31	40.11
3	40.11	39.64	39.82	40.18	40.2	39.72	39.96
Mean	39.86	39.62	39.91	40.02	40.02	40.04	39.91
\pm SD	\pm	\pm	\pm	\pm	\pm	\pm	\pm
	0.23	0.19	0.30	0.21	0.17	0.30	0.23
RSD	0.591	0.480	0.754	0.534	0.438	0.747	0.588

Table 6: Results of system suitability parameter

S.No.	Parameter	Limit	Result
1	Resolution	$R_s > 2$	4.2
2	Injection precision	$RSD < 1\%$ for $n \geq 5$	Ramipril: $RSD=0.237\%$, ($n=6$) Telmisartan: $RSD=0.282\%$, ($n=6$)
3	Tailing factor	$T \leq 2$	Ramipril: 1.12 Telmisartan: 1.20
4	Theoretical plate	$N > 2000$	Ramipril: 4782 Telmisartan: 3890
5	Retention time		Ramipril: 4.25 min Telmisartan: 7.99 min

volume was made upto 50 ml with the mobile phase. This solution was filtered through the membrane filter (0.22 μ m) and sonicated to degas. The estimation of drugs was done by proposed method.

The result of recovery of Telma-R tablet is shown in Table 1.5 ml of synthetic stock solution (section 4.2.3.3) and 4, 5 and 6 ml of similar synthetic stock solution (section 4.2.3.3) were mixed in three different 50 ml volumetric flasks separately and volume was made upto 50 ml with mobile phase. This solution was filtered through membrane filter (0.22 μ m) and sonicated

to degas. The estimation of drugs was done by proposed method. The result of recovery of synthetic mixture is shown in Table 3.

Results

The Mean % recovery was found to be 99.63% and 100.18% for ramipril and 100.14% and 100.73% for telmisartan for Telma-R tablet and synthetic mixture respectively. The limit for mean recovery is 98-102%. Thus the method was found to be accurate.

Table 7: Results of assay (Telma-R)

S. No.	Peak area (μ V*sec)	Labeled quantity in tablet (mg/tab)	% of Drug	Quantity found in (mg/tab)
Ramipril				
1	9024567	5.0	99.40	4.97
2	8914274		98.19	4.91
3	9010162		99.25	4.96
Mean			98.95	4.95
Telmisartan				
1	236457839	40.0	98.98	39.59
2	237852374		99.56	39.82
3	236178307		98.86	39.54
Mean			99.14	39.66

Table 8: Results of assay (Telmistan-RM)

S. No.	Peak area (μ V*sec)	Labeled quantity in tablet (mg/tab)	% of Drug	Quantity found in (mg/tab)
Ramipril				
1	8925647	5.0	98.30	4.91
2	9014274		99.19	4.96
3	9026784		99.25	4.96
Mean			98.91	4.94
Telmisartan				
1	237638672	40.0	99.98	39.99
2	236789036		99.32	39.72
3	236568903		98.63	39.45
Mean			99.31	39.72

Precision

Injection repeatability was assessed using six determinations at 100% of the test concentration (10 µg/ml and 80 µg/ml of ramipril and telmisartan respectively). For intra-day studies three concentrations were injected in triplicate in a day and for inter-day studies three concentrations were injected in triplicate for three days.

% RSD of repeatability, interday and intraday precision found to be 0.237, 0.569 and 0.311 for RPL and for TLR 0.282, 0.339 and 0.261 respectively proves that method is precise in nature.

Robustness

The Robustness was determined by injecting triplicate injections of standard and three sample solutions in single at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions; flow rate (± 0.1 ml/min), temperature ($\pm 5^\circ\text{C}$) and change in

pH of buffer (± 0.2). The result obtained for the robustness study by small and deliberate changes in different chromatographic parameters indicated that

System suitability parameter**Estimation of Ramipril and Telmisartan in tablet dosage forms**

Twenty tablets were taken and weighed. The tablets were crushed to the fine powder. The powdered sample equivalent to 5 mg of ramipril and 40 mg of telmisartan was transferred to a 100 ml volumetric flask and about 90 ml of HPLC grade acetonitrile was added and sonicated to dissolve the contents. The volume was made up to the mark with acetonitrile. This solution was filtered through whatman filter paper 41. 10 ml of this solution was diluted upto 50 ml with mobile phase.

Table 9: Summary of validation parameters

PARAMETER	OBSERVATION	
	Ramipril	Telmisartan
Specificity	No interference was found w.r.t. excipients	
Linearity (Correlation coefficient r)	R = 0.9997	R = 0.9996
Range	70 to 130%	70 to 130%
Accuracy* (% Recovery)	99.92%	100.28%
Precision RSD**		
Repeatability (n= 6)	0.237%	0.282%
Intra-day (n=3)	0.311%	0.331%
Inter-day (days=3)	0.569%	0.261%
Robustness Overall RSD***		
Change in pH of mobile phase		
pH 4.3	0.504%	0.747%
pH 4.7	0.825%	0.588%
Change in temperature		
Temp 25 ⁰ C	0.705%	0.534%
Temp 35 ⁰ C	1.101%	0.438%
Change in flow rate		
0.9 ml/min	0.953%	0.480%
1.1 ml/min	0.988%	0.754%

* Acceptance Criteria 98-102 %, ** Acceptance Criteria: RSD \leq 2 %,

*** Acceptance Criteria: RSD \leq 2 %

CONCLUSION

An RP-HPLC method has been developed for the simultaneous estimation of ramipril and telmisartan in tablet dosage forms, using UV-detector. The developed method was validated as per ICH guidelines and specificity, linearity & range, accuracy, precision and robustness was performed. Specificity was determined by comparing the results obtained by running the placebo solution with that of standard and method was found to be specific due to no interference between placebo peaks and drugs peaks. Estimation of the drugs was performed by developed method in market formulations, telma-R and telmista-RM. The assay was found to be 98.95% & 98.91% for ramipril and 99.14% & 99.31% for telmisartan in telma-R and telmista-RM tablets respectively.

It is evident from the study that the developed method is simple, specific, precise and accurate. This newly developed method can be used for routine analysis as method for the simultaneous estimation of ramipril and telmisartan in pharmaceutical tablet dosage forms.

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