ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF TELMISARTAN AND CILNIDIPINE BY RP-HPLC

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ABSTRACT: The study's primary goal was to create and validate an RP-HPLC method for determining the pharmaceutical dose form and bulk levels of Telmisartan (TEL) and Cilnidipine (CIL). The linearity data was obtained in the concentration range of 48 µg/mL to 112 µg/mL for Telmisartan and 12 µg/mL to 28 µg/mL for Cilnidipine. Trails were conducted to optimize various parameters such as wave length, column, mobile phase ratio etc. The optimized parameters were Zodiac ODS C18 (250×4.6× 5µ) column, buffer: Phosphate buffer (KH_2PO_4): Methanol: Tetra hydro furan (45:45:10v/v/v). Optimal detector response for the drugs was achieved at a detection wavelength of 232 nm, and the developed methods were verified for specificity, accuracy, precision, sensitivity, robustness, and ruggedness. All parameters met the specification limits as outlined in the ICH guidelines. From linearity response of Telmisartan and Cilnidipine R^2 was calculated as 0.9982 and 0.9987. Telmisartan and Cilnidipine had retention times (RT) of 2.78 min and 4.96 min respectively. The developed method can be employed for quality control checks for the pharmaceutical dosage forms.

KEYWORDS: Cilnidipine, telmisartan, dosage form, Rp-Hplc, quality control

INTRODUCTION: HPLC is one of important analytical techniques used for separation, purification and identification. Apart from the above the other fields that utilize hplc techniques are in pharmaceutical industry, forensic labs, environmental sciences, clinical and in food industries[1,2] Telmisartan and Cilnidipine are antihypertensive agents act by different mechanisms. Telmisartan [3] acts by inhibiting the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Cilnidipine [4] acts by blocking N-type calcium channel and dilates both arteriole & venules as a result the pressure in the capillary bed is reduced. The accumulated fluid in the tissues flows back to veins & thus Cilnidipine minimizes the incidence of pedal edema. There are various spectrophotometric[5-13], HPLC[14-18] and other hyphenated techniques[19-21] for estimation of Telmisartan and Cilnidipine either in single or in combination or in combination with other dosage forms. In this study, a straightforward and precise RP-HPLC technique was developed for the simultaneous determination of Telmisartan (Figure 1) in bulk and dose form and the method was validated in accordance with the given requirements.

MATERIALS AND METHODS:

Materials

Telmisartan and Cilnidipine bulk drugs, Telmisartan and Cilnidipine (Telmisartan 40 mg and 10 mg Cilnidipine), manufactured by JB Chemicals, all the reagents and solvents were of analytical grade and HPLC grade respectively obtained from standard reagent Pvt Ltd. In the current work UV-Visible Spectrophotometer (Nicolet evolution 100), HPLC Shimadzu (LC 20 AT VP), Agilent 1200 series, Ultra sonicator (Citizen, Digital Ultrasonic Cleaner), pH meter (Global digital), Electronic balance (Shimadzu, Syringe (Hamilton) and HPLC Column Zodiac ODS C18 (250×4.6× 5µ) were used.

Method development

Preparation of Standard stock solution

10 mg of Telmisartan and cilnidipine were weighed and transferred in to two 100 mL volumetric flasks and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /mL of solution by diluting 1mL to 10 mL with methanol.

Preparation of mobile phase

A mixture of 45 volumes of Phosphate buffer (KH_2PO_4) pH 3.5, 45 volumes of methanol and 10 volumes of Tetra hydro furan. The mobile phase was sonicated for 10min to remove gases.

Preparation of buffer solution

1.36 gm of potassium di hydrogen phosphate (KH_2PO_4) was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 3.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Sample stock solutions

Accurately weighed 20 tablets (each tablet contains 40 mg of Telmisartan and 10 mg of Cilnidipine) were taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Telmisartan and Cilnidipine (µg/mL) were prepared by dissolving weight equivalent to 80 mg of Telmisartan and 20 mg of Cilnidipine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100mL with mobile phase. Further dilutions are prepared in 5 replicates of 80 µg/mL of Telmisartan and 20 µg/mL of Cilnidipine was made by adding 1 mL of stock solution to 10 mL of mobile phase.

Determination of Working Wavelength (λ max)

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/mL solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption spectra showed characteristic absorption maxima at 298 nm for Telmisartan, 237 nm for Cilnidipine and 224 nm for the combination.

Method validation

System Suitability

In order to confirm that the analytical system is functioning correctly and capable of producing precise and accurate results, six injections of Cilnidipine and Telmisartan as per test method were made, and the corresponding chromatograms were recorded.

Specificity

The specificity in the method was established by recording blank, placebo and the analyte chromatograms.

Linearity

A series of sample concentrations ranging from 48 µg/mL to 112 µg/mL for Telmisartan and 12 µg/mL to 28 µg/mL for Cilnidipine were prepared and a linearity plot showing concentration values on X- axis and peak area values on Y- axis were plotted. From the above plot regression coefficient (r^2) was determined.

Accuracy

Recovery studies determined the method's accuracy. The drug reference standards were added to the formulation (pre-analysed sample) at 80%, 100%, and 120%. The percentage recovery and percentage mean recovery were computed for each drug after the recovery studies were conducted three times.

Precision

Prepared sample solutions of Cilnidipine and Telmisartan as per test method were injected six times each, separately prepared, the precision of the method was ascertained.

Sensitivity

Sensitivity of the method was determined by determining LOD and LOQ of the drugs.

Robustness

Chromatograms were recorded and various conditions, such as changes in wave length and flow rate, were used to assess how robust the method was.

Ruggedness

Degree of reproducibility of the results obtained under a variety of conditions is the ruggedness i.e., injection by different analysts and instruments. The ruggedness was estimated by different analyst.

Assay

Standard solution of 20 µL was infused into the chromatographic system five times; peak areas and chromatograms were noted. Sample solution of 20 µL was infused into the chromatographic system five times, chromatograms and peak areas were documented.

Results and discussion

Method optimization

Table 1. Trials

Trial	Column	Buffer: Mobile phase	Wave length (nm)	Run time (min)	Flow rate (mL/min)	Observation
1	Inertsil ODS (250×4.6× 5µ)	Ammonium acetate buffer: Methanol: Acetonitrile (30:40:30 v/v/v)	232 nm	4.417 (CIL) 8.217 (TEL)	1mL/min	Though the retention time is satisfactory for Cilnidipine and telmisartan. The Efficiency is good but peak Asymmetry factor is more than 2 for telmisartan.
2	Thermo Betasil ODS C18 (250×4.6 ×5µ)	Buffer (NaH_2PO_4): Acetonitrile (60:40 v/v)	: 232 nm	2.380 (CIL) 2.380 (TEL)	1mL/min	There is no separation of two drugs because they have the same retention time in this mobile phase.
3	Waters ODS, C_{18} (250×4.6× 5µ)	Phosphate buffer: methanol (55:45 v/v)	232 nm	3.020 (CIL) 3.230 (TEL)	1 mL/ min	There is Asymmetry factor was less than 1 for Cilnidipine and more than 2 for Telmisartan, the theoretical plates were satisfactory for but there is less resolution and response also less.
4	Kromosil ODS (150×4.6× 5µ)	Phosphate buffer : Acetonitrile : Methanol (30:40:30 v/v/v)	232 nm	5.127 (CIL) 3.307 (TEL)	1 mL/ min	Though the retention time was satisfactory for both Telmisartan and Cilnidipine and the peak Asymmetry factor was more than 2 for Telmisartan and the efficiency was not good for Cilnidipine and Telmisartan.
5	Zodiac ODS C18 (250×4.6× 5µ)	Phosphate buffer(KH_2PO_4) : Methanol : Tetra hydro furan (45:45:10v/v/v)	232 nm	4.960 (CIL) 2.780 (TEL)	1 mL/ min	The peak asymmetry factor was less than 2 for both Telmisartan and Cilnidipine and the efficiency also good, and the retention time was also satisfactory for both Telmisartan and Cilnidipine.

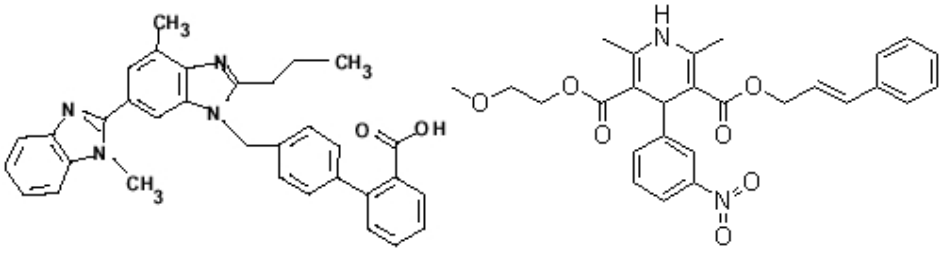

Figure 1. Structures of Telmisartan and Cilnidipine

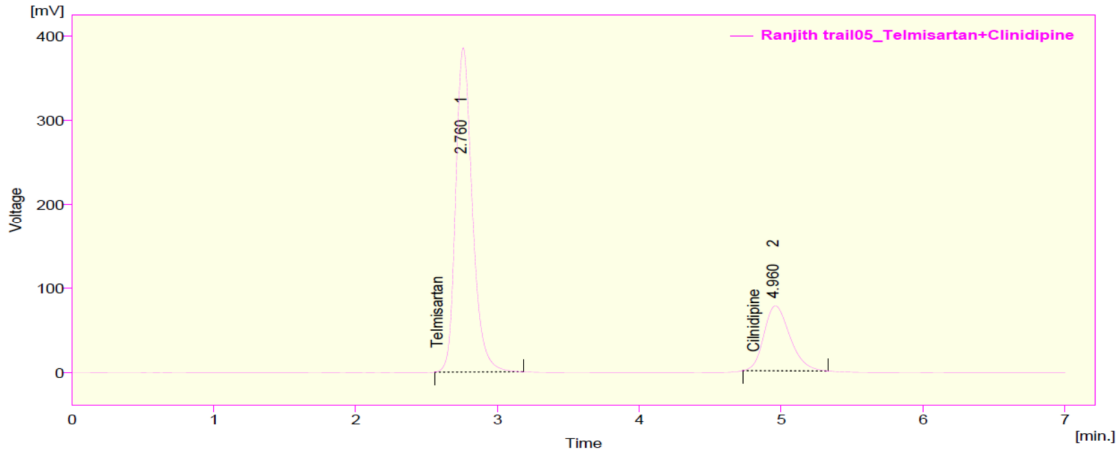

Figure 2. Chromatogram of trail 5

Table 2. Optimized reaction conditions.

Mobile phase	Phosphate buffer(KH2PO4): Methanol: Tetra hydro furan(45:45:10 v/v/v)
Column	Zodiac ODS, RP-18,250×4.6mm ID, 5µm Particle size
flow rate	1.0 mL/min
Column temperature	Room temperature(20-25ºC)
sample temperature	Room temperature(20-25ºC)
Wavelength	232 nm
Injection volume	20 µL
Run time	6 min
Retention time	About 2.780 min for Telmisartan and 4.960 min for Cilnidipine

Method validation

System suitability

System suitability parameters such as retention time (RT) and peak areas were within the range and the obtained values are shown in the Table 3.

Table 3. System suitability parameters.

S. No	Telmisartan		Cilnidipine	
Inj	**Retention time**	**Peak area**	**Retention time**	**Peak area**
1	2.76	3119.74	4.96	970.584
2	2.74	3160.88	4.943	952.008
3	2.743	3178.18	4.94	971.462
4	2.737	3171.44	4.953	991.337
5	2.747	3251.47	4.97	943.815
6	2.73	3212.05	4.957	970.902
Mean	2.743	3182.29	4.954	966.685
SD	0.01	45.116	0.011	16.745
% RSD	**0.371**	**1.418**	**0.225**	**1.732**

Specificity

Telmisartan and Cilnidipine had retention times (RT) of 2.78 min and 4.96 min respectively. At retention times of these drugs no interfering peaks in blank and placebo were observed. Hence this method was said to be specific.

Linearity

A series of sample concentrations ranging from 48 µg/mL to 112 µg/mL for Telmisartan and 12 µg/mL to 28 µg/mL for Cilnidipine a linearity plot showing concentration values on X- axis and peak area values on Y- axis were plotted. From the above plot regression coefficient (r^2) was determined.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table 4.

Precision

Prepared sample solutions of Cilnidipine and Telmisartan as per test method were injected six times each, separately prepared, the precision of the method was ascertained and the results are shown in the Table 5.

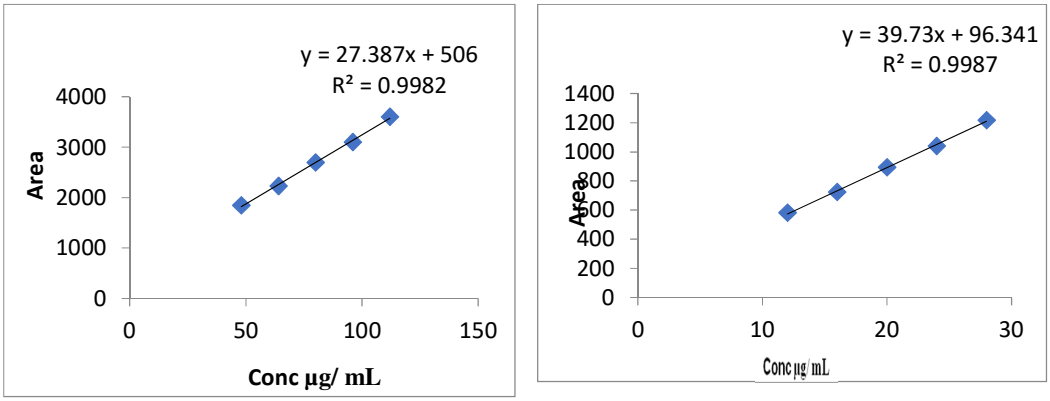

Figure 3. Linearity plot for Telmisartan and Cilnidipine

Table 4. Percentage recovery studies for Telmisartan and Cilnidipine

Telmisartan							Cilnidipine					
Recovery level	**Amount taken (mcg/mL)**	**Area**	**Average area**	**Amount recovered (mcg/mL)**	**% Recovery**	**Average % Recovery**	**Amount taken (mcg/mL)**	**Area**	**Average area**	**Amount recovered (mcg/mL)**	**% Recovery**	**Average % Recovery**
80%	80	2775.309	2743.306	78.67	98.34	98.05 %	20	907.530	899.272	19.89	99.47	99.85 %
	80	2751.487					20	908.264				
	80	2703.123					20	882.023				
100%	96	3285.082	3231.705	95.83	99.82		24	1081.858	1071.346	23.97	99.86	
	96	3269.902					24	1079.376				
	96	3140.131					24	1052.805				
120%	112	3595.532	3584.419	110.88	99.00		28	1190.648	1215.591	28.06	100.22	
	112	3564.311					28	1248.340				
	112	3593.415					28	1207.786				

Table 5. Precision data for Telmisartan and Cilnidipine

S. No	Telmisartan		Cilnidipine	
	Retention time	**Peak Area**	**Retention time**	**Peak Area**
1	2.760	3119.741	4.960	970.584
2	2.740	3160.876	4.943	952.008
3	2.743	3178.176	4.940	971.462
4	2.737	3171.436	4.953	991.337
5	2.747	3251.471	4.970	943.815
6	2.730	3212.049	4.957	970.902
Mean	2.743	3182.292	4.954	966.685
SD	0.010	45.116	0.011	16.745
%RSD	**0.371**	**1.418**	**0.225**	**1.732**

Sensitivity

The LOD for Telmisartan and Cilnidipine were 3.05µg/ mL and 0.35µg/ mL respectively. The LOQ for Telmisartan and Cilnidipine were 9.24 µg/ mL and 1.59 µg/ mL respectively.

Robustness

The results suggested that the minor variations in the method settings did not affect the system performance specifications. The values are represented in the Table 6.

Table 6. Robustness data for Telmisartan and Cilnidipine

Chromatographic changes		Retention time (min)		Tailing factor	
		Telmisartan	**Cilnidipine**	**Telmisartan**	**Cilnidipine**
Flow rate (mL/min)	0.8 mL/min	2.317	6.249	1.588	1.367
	1.0 mL/min	2.243	6.347	1.533	1.325
	1.2 mL/min	2.3	6.257	1.542	1.328
Mean		2.28	6.298	1.5605	1.346
S. D		0.03876	0.054418	0.029501	0.023431
% RSD		1.699998	0.864054	1.89051	1.740769
Wavelength	230nm	2.757	5.047	1.5	1.518
	232nm	2.743	4.94	1.533	1.57
	234nm	2.747	5.04	1.483	1.521
Mean		2.75	4.9935	1.5165	1.544
S. D		0.007	0.060	0.025	0.029
%RSD		0.26	1.20	1.68	1.89

Ruggedness

The ruggedness (Table 7) of the method was examined by analyst 1 and analyst 2 for both standard and sample.

Table 7. Ruggedness for both standard and sample by analyst1 and analyst 2 for Telmisartan and Cilnidipine

	Telmisartan				**Cilnidipine**				
	Area	**RT**	**Theoretical plates**	**Asymmetry**	**Area**	**RT**	**Theoretical plates**	**Asymmetry**	**Resolution**
Analyst 1 (Standard)	2749.07	2.723	3754	1.615	901.827	5.057	4715	1.6	9.483
Analyst 2 (Standard)	2739.907	2.675	3638	1.607	909.957	4.977	4835	1.548	9.366
Analyst 1 (Sample)	2741.911	2.742	3754	1.556	887.033	5.057	4902	1.575	9.595
Analyst 2 (Sample)	2739.907	2.649	3638	1.607	909.957	4.977	4735	1.548	9.466
Mean	2743.629	2.713333	3715.333	1.592667	899.6057	5.030333	4817.333	1.574333	9.481333
S.D	1.15701	0.047983	66.97263	0.029445	13.23518	0.046188	84.04166	0.015588	0.114806
% RSD	0.042171	1.768402	1.802601	1.848778	1.47122	0.91819	1.744568	0.990162	1.21086

Assay

The % purity of Telmisartan and Cilnidipine present in the taken drug form was found to be 101.37 % and 101.83 % respectively (Table 8).

Table 8. Results of assay of Telmisartan and Cilnidipine

Telmisartan			Cilnidipine	
Injections	**Standard Area**	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2708.76	2723.7	899.144	881.526
Injection-2	2735.59	3187.94	902.235	987.427
Injection-3	2683.74	2771.62	876.761	971.64
Injection-4	2719.84	2720.23	866.756	894.646
Injection-5	2707.98	2696.12	881.057	897.661
Average Area	2709.36	2819.92	885.191	926.58
Tablet average weight	250.2 mg		250.2 mg	
Standard weight	80.1 mg		20 mg	
Sample weight	504.2 mg		504.2 mg	
Label amount	40 mg		10 mg	
Std. purity	98.01		98.023	
Amount found in mg	40.55 mg		10.18 mg	
Assay (% purity)	101.37%		101.83%	

Conclusion

The suggested RP-HPLC method works well for figuring out Telmisartan and Cilnidipine concentrations. The drug parameters were all in compliance with the ICH guidelines for method validation. The method was found to be straightforward, exact, accurate, and fast with eluent that is affordable and easy to prepare. The sample recoveries indicated that the formulation excipients did not interfere with the estimation, and they were in good agreement with the claims made on their respective labels. As a result, this approach is simple to use and convenient for routine analysis of dosage forms and pure forms of Telmisartan and Cilnidipine. It can also be applied for dissolution, QC analysis, clinical pharmacokinetics, and other purposes.

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Availability of data and material

All data and materials are available on request.

Declarations

Ethics approval & consent to participate

Not applicable.

Consent of publication

Not applicable as our study does not include patients.

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