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Stability indicating simultaneous validation of telmisartan, cilnidipine and chlorthalidone with forced degradation behavior study by RP-HPLC in tablet dosage form

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ABSTRACT

A simple, precise, and accurate RP-HPLC method has been developed and validated for the simultaneous assay of Telmisartan, Cilnidipine and Chlorthalidone in tablets. Isocratic RP-HPLC method was developed on BDS hypersil C18, 250mm × 4.6mm, 5µm column using mobile phase as Methanol: buffer (0.05M ammonium acetate) pH 5.0 with orthophosphoric acid (40 : 60) at a flow rate of 1.0 mL/min and the detection was carried out at 270nm using waters 486 tunable absorbance detector detector. Forced degradation study was carried out by acid degradation, base degradation, thermal degradation, oxidation of the drug. The method was validated for linearity, precision, accuracy and robustness. The method was found to be linear in the concentration range of 20–60 μg/mL with correlation coefficient of 0.9997 for Telmisartan, 5–15 μg/mL with correlation coefficient of 0.9989 for Cilnidipine and 6.25–18.75 μg/mL with correlation coefficient of 0.9989 for Chlorthalidone. Degradation products produced as a result of stress studies did not interfere with the detection of Telmisartan, Cilnidipine and Chlorthalidone; therefore, the assay can be considered to be stability indicating.

Keywords: HPLC, Telmisartan, Cilnidipine, Chlorthalidone, Validation, Forced degradation.

1. INTRODUCTION

Telmisartan is an antihypertensive drug (IUPAC name: 2-(4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl] methyl} phenyl) benzoic acid)(Figure 1). The molecular formula is $C_{33}H_{30}N_4O_2$ and molecular weight is 514.617 g/mol. Telmisartan is long acting and works as antagonist of Angiotensin II receptor (Angiotensin receptor blocker ARB).[1]

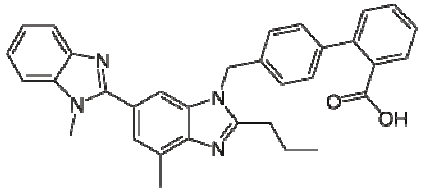

Figure - 1: Structure of Telmisartan.

Cilnidipine is an antihypertensive drug (IUPAC name: 3-(E)-3-Phenyl-2-propenyl 5-2-methoxyethyl 2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) (Figure 2). The molecular formula is $C_{27}H_{28}N_2O_7$ and molecular weight is 492.52 g/mol. It is a calcium channel blocker with L-type and N-type calcium channel blocking function.[2]

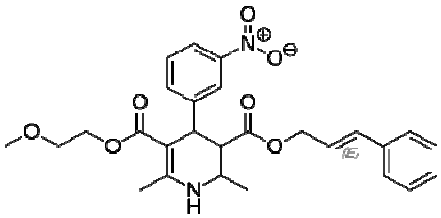

Figure - 2: Structure of Cilnidipine

Chlorthalidone is a diuretic drug and used in treatment of hypertension (IUPAC name: (RS)-2-Chloro-5- (1-hydroxy -3 –oxo -2,3 –dihydro -1H –isoindol -1 -yl) benzene -1 - sulfonamide) (Figure 3). The molecular formula is $C_{14}H_{11}ClN_2O_4S$ and molecular weight is 338.766 g/mol. It is a thiazide-like diuretic drug and prevents reabsorption of Chloride and Sodium.[3]

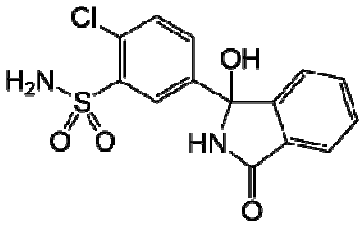

Figure - 3: Structure of Chlorthalidone

In literature survey some analytical methods have been reported for analysis of individual drugs (i.e. Telmisartan, Chlorthalidone and Cilnidipine) or in combination with other drugs in pharmaceutical dosage form. However, no stability indicating HPLC method for simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine have been reported.

The HPLC method has been developed and suitably validated in accordance with ICH guideline Q2(R1). The stability indicating method was developed by applying different stress conditions like acidic, alkali, H_2O_2, thermal, and photo-degradation.

2. MATERIALS AND METHODS

2.1. Instruments

Waters HPLC M510 model containing Waters 510 HPLC pump and Rheodyne injector with 20µl fixed loop. Chromatographic separation was performed by using BDS hypersil C18, 250mm × 4.6mm, with 5µ particle size from Thermo scientific using Empower 3 chromatographic data software. AX00 electronic balance was used for weighing. Chemiline India pH meter was used.

2.2. Chemicals and reagents

Methanol and water were of HPLC grade and ortho phosphoric acid (OPA), ammonium acetate AR grade were obtained from Merck, Mumbai India. Telmisartan, Cilnidipine and Chlorthalidone standards were obtained as gift samples from IPCA, Mumbai. Tablet dosage form containing 40 mg of Telmisartan, 12.5 mg of Chlorthalidone and 10 mg of Cilnidipine (Cilacar TC) was procured from the local market. Analytical grade Hydrochloric acid, sodium hydroxide pellets, and hydrogen peroxide solution were obtained from Merck, India.

2.3. HPLC condition

The mobile phase consisted of pH 5 ammonium acetate buffer : methanol (60:40 v/v). The mobile phase was prepared freshly. BDS hypersil C18 column (250mm × 4.6mm, 5µm particle size). The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature. Flow rate at 1.0 ml/min. Detection at 270nm.Volume of injection 20µl.

2.4. Preparation of mobile phase

The mobile phase was prepared by mixing 0.05M ammonium acetate (pH 5) and methanol in the ratio of (60:40 %v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

2.5. Preparation of 0.05M ammonium acetate buffer

Dissolve 3.85 gm of ammonium acetate into 1000ml beaker. Add 800ml water and dissolve. Adjust the pH 5.0 of this solution with 1% orthophosphoric acid. Add sufficient water to produce 1000ml.

2.6. Preparation of stock solution

2.6.1. Standard stock solution of Telmisartan

Weigh accurate 40mg of Telmisartan and transferred to 100 ml volumetric flask and volume make up to 100ml with methanol.

2.6.2. Standard stock solution of Chlorthalidone

Weigh accurate 12.5mg of Chlorthalidone and transferred to 100 ml volumetric flask and volume make up to 100ml with methanol.

2.6.3. Standard stock solution of Telmisartan

Weigh accurate 10mg of Cilnidipine and transferred to 100 ml volumetric flask and volume make up to 100ml with methanol.

2.7. Determination of λ max

The UV spectra of standard stock solutions of Temisartan, Chlorthalidone and Cilnidipine taken between the wave length range of 200-400nm using methanol as blank (Figure 4). The λ max was found to be 228.77 nm, 255.74 nm and 255.14 nm for Temisartan, Chlorthalidone and Cilnidipine

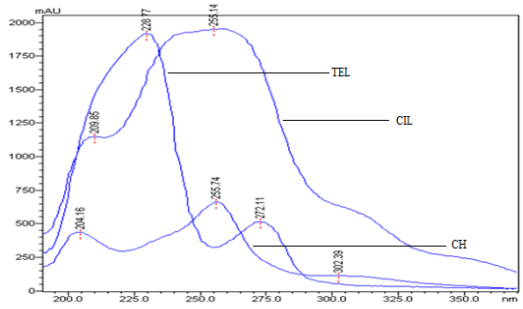

Figure - 4: Overlay absorption spectrum for Temisartan (TEL), Chlorthalidone (CH) and Cilnidipine (CIL).

respectively. Overlay of the three spectra taken and iso-absorptive point was selected and it was found that all three drugs show appreciable absorbance at 270 nm, so it is used for the further study.

2.8. Preparation of working standard solution

Add 1ml each of standard stock solution of Temisartan, Chlorthalidone and Cilnidipine in 10 ml volumetric flask and volume make up to 10ml with methanol.

2.9. Analysis of tablet dosage forms

Twenty tablets containing 40 mg of Telmisartan, 12.5 mg of Chlorthalidone and 10 mg of Cilnidipine were weighed, and finely powdered. A quantity of powder sample equivalent to 40 mg of Telmisartan, 12.5 mg of Chlorthalidone and 10 mg of Cilnidipine transferred to 100ml volumetric flask. 60ml of methanol was added and shaked for 15 minutes and the volume was made up to 100ml with methanol. Filtered and 1ml of the aliquot was transferred to 10ml volumetric flask and the volume was made up to the mark with methanol. Twenty micro liters of the solution was injected into the chromatographic system and the peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of corresponding calibration curve.

Optimized chromatographic conditions

Stationary phase v : BDS hypersil C18, 250mm × 4.6mm, 5µm

Mobile phase : Ammonium acetate buffer (pH 5): methanol (60:40)

Flow rate : 1 ml/min

Run time (min) : 10min

Detection : At 270 nm

Injection (volume) : 20µl

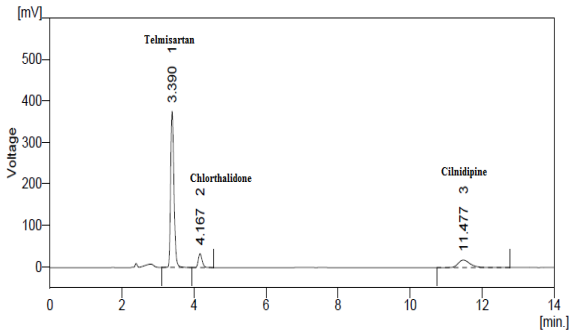

Figure - 5: Standard chromatograms of Telmisartan, Chlorthalidone and Cilnidipine

2.10. Method validation procedure

The developed method was validated for the parameters listed in ICH guidelines [4-7].

2.10.1. Linearity

The method was linear in the range of 20-60 µgm, 6.25-18-75 µgm and 5-15 µgm for Telmisartan, Cilnidipine and Chlorthalidone respectively. The linear correlation coefficient for Telmisartan, Cilnidipine and Chlorthalidone were found to be 0.9997, 0.9988 and 0.9989 respectively, and are recorded in table 2, 3 and 4. Calibration curve of Telmisartan, Cilnidipine and Chlorthalidone was obtained by plotting the peak area ratio versus the respective concentrations (Figure 6, 7 and 8).

Table - 1: System suitability of proposed method

Parameters	Telmisartan	Chlorthalidone	Cilnidipine
Theoritical plates	5962.499	7488.106	5956.693
Resolution	-	4.219	18.567
Asymmetry	1.435	1.333	1.532
Retention time	3.390 min	4.167 min	11.477 min

Table - 2: Linearity results of Telmisartan

Linearity results of Telmisartan		
Linearity Level	**Concentration**	**Area**
I	20 µg/ml	1238.63
II	30 µg/ml	1876.79
III	40 µg/ml	2457.18
IV	50 µg/ml	3022.71
V	60 µg/ml	3651.75
Correlation coefficient		0.9997

Table - 3: Linearity of Chlorthalidone

Linearity results of Chlorthalidone		
Linearity Level	**Concentration**	**Area**
I	6.250 µg/ml	123.275
II	9.375 µg/ml	187.157
III	12.50 µg/ml	243.520
IV	15.625µg/ml	302.451
V	18.750 µg/ml	350.179
Correlation coefficient		0.9988

Table - 4: Linearity of Cilnidipine		
Linearity results of Cilnidipine		
Linearity Level	**Concentration**	**Area**
I	5.0 µg/ml	208.575
II	7.5 µg/ml	319.724
III	10.0 µg/ml	419.61
IV	12.5 µg/ml	515.148
V	15.0 µg/ml	601.678
Correlation coefficient		0.9989

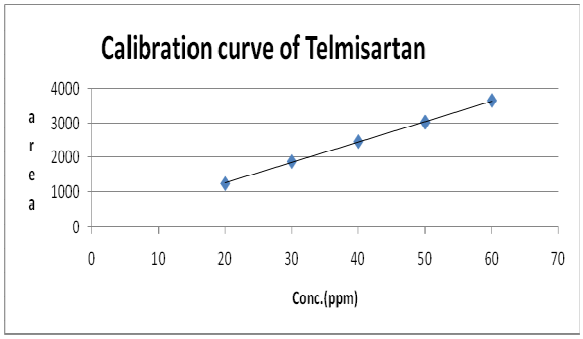

Figure - 6: Calibration curve of Telmisartan.

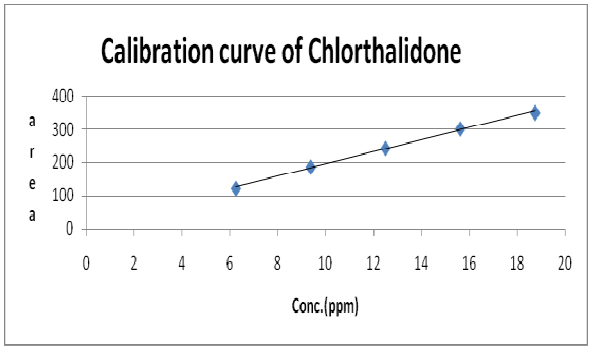

Figure - 7: Calibration curve of Chlorthalidone.

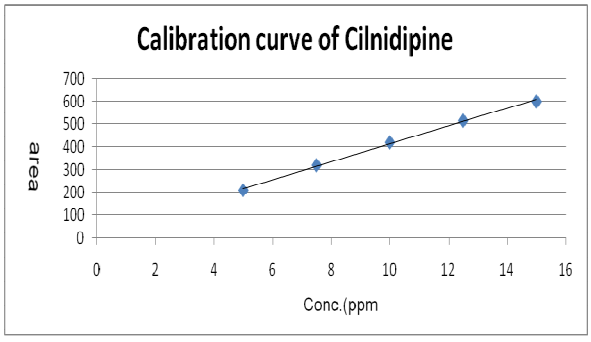

Figure - 8: Calibration curve of Cilnidipine.

2.10.2. Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For all the three drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100%, 120% level and the percentage recovery was calculated. Percent recovery was within the range of 99.48 to 100.89 for Telmisartan, 99.82 to 100.47 for Chlorthalidone and 100.23 to 100.71 for Cilnidipine which indicates that the method was accurate.

2.10.3. Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated. The %RSD for Telmisartan, Chlorthalidone and Cilnidipine were found to be 0.84, 0.88 and 0.50% respectively. From the data obtained the developed RP-HPLC method was found to be precise.

2.10.4. Limit of detection and limit of quantification

The limit of detection and quantification were calculated using standard deviation of response and slope of the calibration curve. The LOD for Telmisartan, Chlorthalidone and Cilnidipine was found to be 1.263 µg/ml, 0.429 µg/ml and 0.0.699 µg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ for Telmisartan, Chlorthalidone and Cilnidipine was 3.827 µg/ml, 1.300 µg/ml and 2.118 µg/ml.

2.10.5. Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like flow rate, mobile phase ratio and pH of buffer. It was observed that there were no marked changes in chromatograms and % relative standard deviation was found below 2%, which demonstrated that the developed RP-HPLC method is robust.

2.11. Degradation Study

The degradation samples were prepared by transferring powdered tablets, which had been equivalent to 40.0mg Telmisartan, 12.5 mg Chlorthalidone and 10.0mg Cilnidipine, into a 100mL volumetric flask. Then drug content was employed for acid media, alkali media, oxidative media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature. Specific degradation conditions were described as follows.

Table 5: Results of Accuracy

Sample	Accuracy (%)	Standard Drug	Formulation	% of recovery	Standard deviation	% Relative standard deviation
Telmisartan	80	16	20	100.90	0.818	0.81
	100	20	20	99.93	1.313	1.31
	120	24	20	99.48	1.180	1.19
Chlorthalidone	80	5	6.25	99.87	1.345	1.35
	100	6.25	6.25	100.47	0.945	0.94
	120	7.5	6.25	99.82	1.481	1.48
Cilnidipine	80	4	5	100.71	1.022	1.01
	100	5	5	100.45	0.641	0.64
	120	6	5	100.24	1.067	1.06

Table - 6: Results of Precision

Injection	Area of Telmisartan	Area of Chlorthalidone	Area of Cilnidipine
Injection 1	2424.566	242.185	414.657
Injection 2	2451.261	244.842	419.226
Injection 3	2453.665	245.095	419.638
Injection 4	2488.101	248.535	420.466
Injection 5	2443.545	244.563	420.007
Injection 6	2448.789	243.374	418.944
Average	2451.655	244.766	418.823
Standard Deviation	20.69	2.14	2.11
% Relative standard deviation	0.84	0.88	0.50

Table - 7: Results of LOD and LOQ

Parameter	Telmisartan (µg/ml)	Chlorthalidone (µg/ml)	Cilnidipine (µg/ml)
LOD	1.263	0.429	0.699
LOQ	3.827	1.300	2.118

Table - 8: Results of Robustness

Condition	Variation	Average Area			% Relative standard deviation		
		Telmisartan	Chlorthalidone	Cilnidipine	Telmisartan	Chlorthalidone	Cilnidipine
Flow rate	0.8 min	2203.238	219.922	377.058	0.39	0.26	0.41
	1.2 min	2671.412	266.207	454.868	0.69	1.02	1.17
Mobile phase	Buffer: Methanol 58:42	2267.086	225.855	387.943	1.02	0.65	1.04
	Buffer: Methanol 62:38	2647.629	264.573	448.099	0.56	0.58	0.97
pH	4.8	2601.511	260.045	443.325	0.11	0.11	0.51
	5.2	2295.286	229.025	388.909	0.63	0.56	1.44

2.11.1. Acidic Degradation Condition

Acidic degradation study was performed by treating sample with 2 ml of 0.1N HCl for 4 hours and then neutralizing with 2ml 0.1N NaOH to stop further degradation.

2.11.2. Alkali Degradation Condition

Alkaline degradation study was performed by treating sample with 2 ml of 0.1N NaOH for 2.5 hours and then neutralizing with 2ml 0.1N HCl to stop further degradation.

2.11.3. Oxidative Degradation Condition

Oxidation degradation study was performed by treating sample with 3% v/v H_2O_2 for 4 hours.

2.11.4. Thermal Degradation Condition

Thermal degradation was performed by exposing solid drug to dry heat of 105°C in a conventional oven for 3 hours.

2.11.5. Photolytic Degradation Condition

Photolytic degradation study was performed by exposing the sample to sunlight for 2.5 hours.

3. RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with BDS hypersil C18, 250mm × 4.6mm, 5µm column or equivalent column and mobile phase comprising of Methanol: buffer (0.05M ammonium acetate) pH 5.0 with orthophosphoric acid (40 : 60) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 270nm based on peak area. The retention time for Telmisartan, Cilnidipine and Chlorthalidone were found to be 3.390 min, 4.167 min and 11.477 min, respectively.

The optimized method was validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported. The method was found to be linear in the concentration range of 20–60 μg/mL with correlation coefficient of 0.9997 for Telmisartan, 5–15 μg/mL with correlation coefficient of 0.9989 for Cilnidipine and 6.25–18.75 μg/mL with correlation coefficient of 0.9989 for Chlorthalidone. The results of recovery study (99.93% for Telmisartan, 100.47% for Chlorthalidone and 100.44% for Cilnidipine) suggest that the method has good recovery. The precision of the proposed method was carried in terms of the repeatability. The low% RSD (<2) values of 0.84%, 0.88% and 0.50% variation for Telmisartan, Chlorthalidone and Cilnidipine, respectively, reveals that the proposed method is precise. The LOD and LOQ values for Telmisartan were found to be 1.263µg/ml and 3.827µg/ml, for Chlorthalidone were 0.429µg/ml and 1.300µg/ml and for Cilnidipine were 0.699µg/ml and 2.118µg/ml. The results of robustness in the present method showed no significant changes. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method. Therefore, the proposed method can be used for routine analysis of three drugs in their combined pharmaceutical dosage form.

4. CONCLUSION

A simple, precise, accurate and rapid method was developed for simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims. Hence, this method can be easily and conveniently adopted for routine analysis of Telmisartan, Chlorthalidone and Cilnidipine in pure form and its dosage form.

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