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Stability indicating RP-HPLC Method for Simultaneous Determination of Telmisartan and Chlorthalidone in Bulk and Pharmaceutical Dosage Form

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Abstract : A simple, precise and accurate stability indicating reverse-phase HPLC method has been developed for simultaneous estimation of Telmisartan and Chlorthalidone in bulk and tablet formulations. Separation was performed on a C -18 column (250 × 4.6mm ID,5 µm) with Acetonitrile : Methanol (85:15v/v) , flow rate of 1.0 ml/ min and UV detection at wavelength 242 nm. The retention time of Telmisartan and Chlorthalidone was found to be 3.96 and 2.63 minutes respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantitation and robustness as per the International Conference on Harmonisation (ICH) guidelines.. Linearity of Telmisartan and Chlorthalidone were in the range of 16-56 µg/mL and 5-17.5 µg/mL respectively. The percentage recoveries of both the drugs were 99.85 % and 99.06 % for Telmisartan and Chlorthalidone respectively. Degradation products produced as a result of stress studies did not interfere with the detection of Telmisartan and Chlorthalidone and the assay can thus be considered stability-indicating. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.
Key words: Telmisartan, Chlorthalidone, RP-HPLC, validation, assay.

Introduction

Telmisartan (TEL) is chemically 4′-[[4-methyl-6-(1-methyl-2-benzimidazolyl)-2-propyl-1-benzimidazolyl] methyl]-2-biphenylcarboxylic acid (Fig. 1) is a Antihypertensive drug[1-5].It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia(BP) and U.S. Pharmacopoeia(USP). It is estimated by Liquid Chromatography as per IP and Potentiometric titration as per BP and USP[3-5]. Literature review reveals that HPLC[7-9], UVspectrophotometric[10-13] and HPTLC[14-15] methods has been reported for estimation of TEL in pharmaceutical dosage forms. Chlorthalidone (CHL) is chemically (RS)-2-chloro-5-(1-hydroxy-3oxoisoindolin-l-yl)benzene sulphonamide (Fig.2) used as Diuretic[1-5]. It is official in IP, BP and USP and estimated by potentiometric titration as per IP and Liquid Chromatography as per BP and USP[3-5]. Literature review also reveals that HPLC [16-19], UVspectrophotometric [20] methods has been reported for the estimation of CHL in pharmaceutical dosage forms. Hence an attempt has been made to develop a simple, precise, reliable, and accurate stability indicating HPLC method for simultaneous estimation of TEL and CHL in bulk samples and in combined tablet dosage form. The present developed method is simple, precise and accurate for simultaneous determination of both drugs in their Pharmaceutical Dosage forms as per International Conference on Harmonization (ICH)

guidelines[6]. The method can be successfully employed for the simultaneous determination of Telmisartan and Chlorthalidone in pharmaceutical formulations.

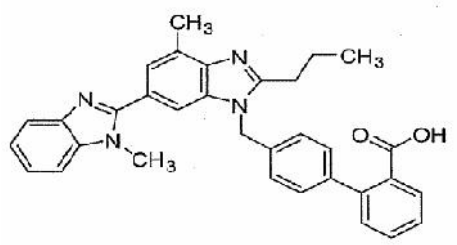

Fig. 1: Structure of Telmisartan (TEL)

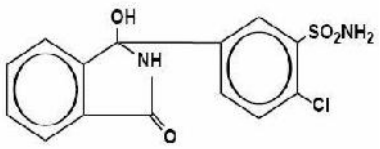

Fig. 2: Structure of Chlorthalidone (CHL)

Materials and Methods

Chemicals and reagents

Pure drug samples of Telmisartan & Chlorthalidone were provided as a gift sample by Alembic Limited, Vadodara, Gujarat, India. Commercial pharmaceutical tablets ERITEL-CH40 (Eris Lifesciences Pvt. Ltd, Ahmedabad, Gujarat, India) was procured from local pharmacy. All solvents used like Methanol, Acetonitrile which are of HPLC grade were purchased from E.Merck, Mumbai.

Instrumentation and analytical conditions

The analysis of the drug was carried out on Perkin Elmer model (series 200) containing Diode array detector (UV-visible) and Perkin elmer Rheodyne 7725 injector with 20µl fixed loop. Chromatographic analysis was performed using C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (BL- 220H) was used for weighing. Isocratic elution with Acetonitrile : Methanol :85:15(v/v) was selected with a flow rate of 1 ml/ min .The detection wavelength was set at 242 nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. 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.

Preparation of standard stock solution

A 100 mg of standard Telmisartan and Chlorthalidone was weighed accurately and transferred to a two separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flask was sonicated for 2 min. Volume was made up to the mark with methanol to give a solution containing 1000 µg/ml Telmisartan and Chlorthalidone.

Preparation of working standard solution

8 ml of standard stock solution of TEL (1000 µg/ml) and 2.5 ml of standard stock solution of CHL(1000 µg/ml) was pipette out in to 50 ml volumetric flask and volume was adjusted to the mark with mobile phase to get 160 µg/ml of TEL and 50 µg/ml of CHL.

Calibration curves for TEL and CHL

Appropriate volume of aliquots from standard TEL and CHL working standard solution were transferred to 10 ml flask. The volume was adjusted to mark with mobile phase to give solutions containing TEL (16, 24, 32, 40, 48 and 56 µg/ml) and CHL (5, 7.5, 10, 12.5, 15 and 17.5 µg/ml). The mixed standard solution was chromatographed for 10 minutes using mobile phase (Acetonitrile: Methanol: 85:15) at a flow rate of 1.0 ml/min. The graph was plotted for peak area vs. concentration for the drug. (Figure 3)

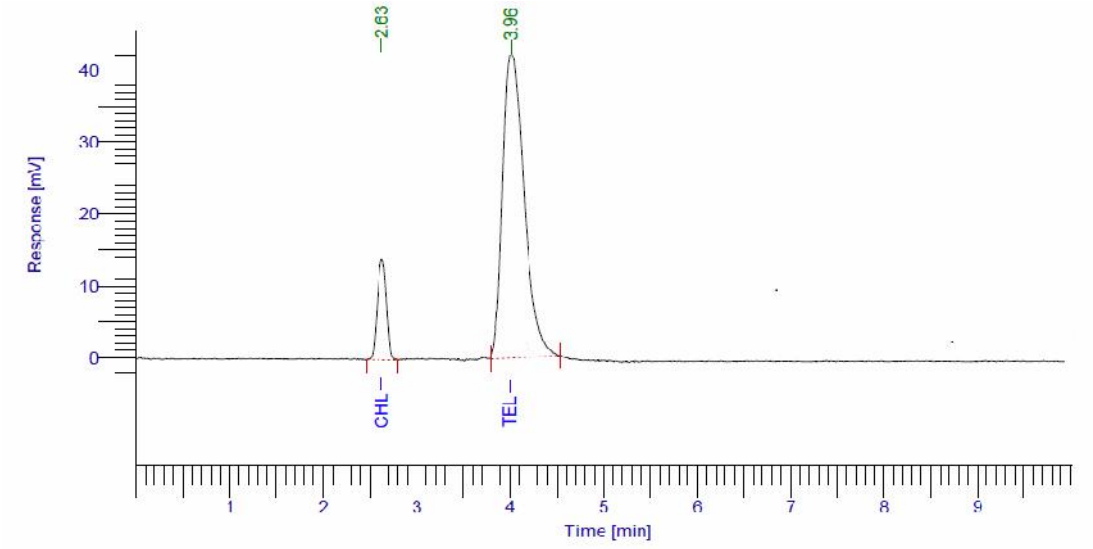

Figure 3: Chromatogram of mix standard solution of TEL (16 µg/ml) and CHL (5 µg/ml) using Acetonitrile: Methanol (85:15 v/v)

Analysis of TEL and CHL in marketed Tablet Formulation

To determine the content of TEL and CHL simultaneously in conventional tablets (Eritel- CH40 label claim 40 mg TEL and 12.5 mg CHL); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 40 mg TEL and 12.5 mg CHL was transferred into 50 ml volumetric flask containing 25 ml methanol, sonicated for 20 min and diluted to mark with mobile phase to obtain 800 µg/ml of TEL and 250 µg/ml of CHL. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). 1ml of the above filtrate was diluted to 10 ml with mobile phase to obtain 80 µg/ml of TEL and 25 µg/ml of CHL. 5 ml of above solution was further diluted to 10 ml with mobile phase to obtain 40 µg/ml of TEL and 12.5 µg/ml of CHL.

The prepared sample solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. From the peak area obtained in the chromatogram, the amount of Telmisartan and Chlorthalidone was calculated.(Table 6)

Method validation

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.

Aliquots of working standard solutions of TEL and CHL were taken in 10 ml volumetric flasks and diluted with mobile phase to get final concentrations in range of 16-56µg/ml for TEL and of 5-17.5 µg/ml of CHL. This calibration range was prepared six times and chromatographs were recorded. Co-relation coefficient was calculated. (Table 1,2 and Figure 4,5)

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. (Table 3)

Table 1: Result of Calibration readings for TEL

Concentration (µg/ml)	Peak Area Mean (n=6) ± SD	%RSD
16	963852.047±3711.524	0.385
24	1393478.46±2216.357	0.159
32	1866827.84±2564.166	0.137
40	2167181.78±2424.892	0.111
48	2637053.43±2866.871	0.108
56	3065908.02±2788.241	0.090

Table 2: Result of Calibration readings for CHL

Concentration (µg/ml)	Peak Area Mean (n=6) ± SD	%RSD
5	154238.188±377.5638	0.244
7.5	201252.258±384.6525	0.191
10	257046.5±460.3449	0.179
12.5	296259.345±414.4803	0.139
15	341901.765±418.4197	0.122
17.5	386149.61±413.1933	0.107

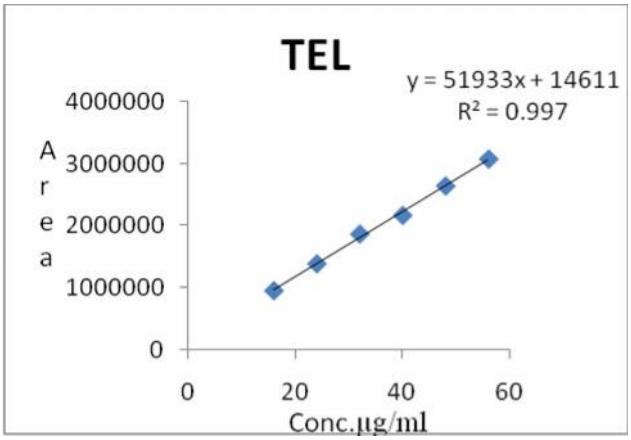

Figure 4 : Calibration curve of TEL

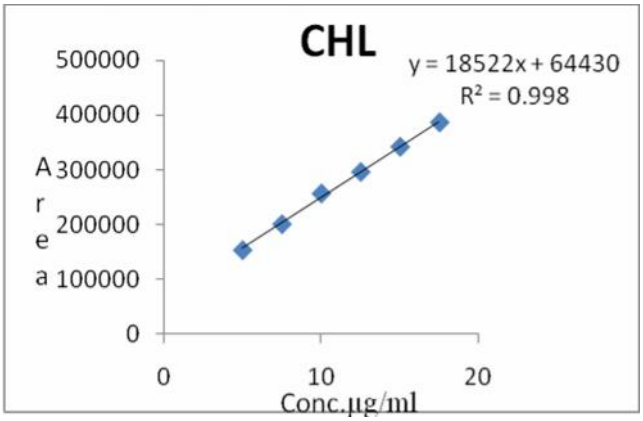

Figure 5: Calibration curve of CHL

Table 3: Determination of Accuracy

Concentration of Sample Taken (µg/ml)		Concentration of Pure API spiked (µg/ml)	Total amount recovered (µg/ml)	Mean Total Concentration Found (n=3) (µg/ml)	%Recovery Mean (n=3)	%RSD
TEL	24	19.2	43.2	43.19	99.94	0.376
	24	24	48	47.93	99.72	0.112
	24	28.8	52.8	52.76	99.87	0.312
Average					99.85	
CHL	7.5	6	13.5	13.46	99.45	0.541
	7.5	7.5	15	14.92	98.98	0.361
	7.5	9	16.5	16.38	98.74	0.516
Average					99.06	

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It was expressed as percentage Relative Standard Deviation (%RSD).

- **Intra and inter day precision**

  Variations of results within the same day (intra-day), variation of results between days (inter- day) were analyzed. Intraday precision was determined by analyzing TEL and CHL for three times in the same day.Inter day precision was determined by analyzing the drugs daily for three days. (Table 4)

Table 4: Intra-Day and Inter-Day study of TEL

DRUG	Concentration (µg/ml)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
TEL	24	1393355.933±453.31	0.032	1393323.953±427.6	0.03
	32	1865380.22±435.527	0.023	1865402.703±383.01	0.02
	40	2166131.62±382.013	0.017	2166294.863±439.58	0.02
CHL	7.5	201144.94±83.28	0.04	201222.38±149.94	0.074
	10	257264.19±59.09	0.02	257374.05±254.73	0.098
	12.5	296455.07±90.84	0.03	296523.6±284.91	0.096

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The LOD and LOQ of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 X σ/S,

LOQ = 10 X σ/S

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters.

a) Flow rate : ±0.2 ml/min

b) mobile phase ratio: ± 2 ml

System Suitability

System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter. The values of system suitability results obtained were recorded in Table 5.

Forced Degradation study:

- **Acid and Base Hydrolysis:** Forced degradation study was conducted on 50 mg drug powder of each drug substances by exposing with 5 ml of 5N hydrochloric acid/ 5 ml of 1N sodium hydroxide for 3 hr at at 60 ºC in water bath. Then neutralized with acid or base (when necessary) and dilute up to 50 ml with diluent. 1 ml of this solution in to 10 ml volumetric flask and diluted up to 10 with diluent. From this, appropriate volume was transferred to 10 ml volumetric flask and mobile phase was added upto the mark to get 56 µg/ml of TEL and 17.5 µg/ml of CHL.

- **Oxidation:** Forced degradation study was conducted on 50 mg drug substances by exposing with 5 ml of 6% H_2O_2 for 1.5 hrs at 60 ºC in water bath. and dilute up to 50 ml with diluent.1 ml of this solution in to 10 ml volumetric flask and diluted up to 10 with diluent. From this, appropriate volume was transferred to 10 ml volumetric flask and mobile phase was added upto the mark to get 56 µg/ml of TEL and 17.5 µg/ml of CHL.

- **Thermal degradation:** Solid drug powder was kept in dry oven at 100°C for 24 hours.

- **Photolysis:** Standard and sample solid drug was spread in 1 mm thickness uniform layer on a Petridish and exposed in UV chamber for 24 hrs.

The chromatograms were extracted for Peak purity and demonstrated as in (Table 7).

Table 5 : System suitability Test parameter

System suitability parameter	RESULTS	
	TEL	**CHL**
Retention Time(R_f)	3.94±0.02	2.65±0.02
Theoretical plates(N)	3743.25	4584.5
Asymmetry(A_s)	1.43	1.21
Resolution	2.983	-

Table 6: Assay Result of Marketed Formulation

Parameters	Eritel -CH40 TAB	
	TEL	**CHL**
Actual Concentration (µg/ml)	40	12.5
Concentration Obtained (µg/ml)	39.71	12.46
%Purity	99.27	99.7
%RSD	0.335	0.334
Limit	90-110%	

Table 7: Forced Degradation data of TEL and CHL

Condition	%Degradation			
	API		TAB	
	TEL	CHL	TEL	CHL
Acid(5N HCl for 3 hrs)	6.16	1.99	5.98	1.75
Alkali(1 N NaOH for 1.5 hrs)	10.12	8.67	9.81	8.13
Peroxide(6% H_2O_2 for 2 hrs)	12.12	1.91	11.56	0.92
UV Light (24 hrs)	-	-	-	-
Thermal (100˚c 24 hrs)	-	-	-	-

Results and Discussion

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase.The drugs Telmisartan and Chlorthalidone preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile : Methanol 85:15(v/v). The retention time of Telmisartan and Chlorthalidone was found to be 3.96 and 2.63 min, respectively. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. The average recovery was found to be 99.85% for Telmisartan and 99.06% for Chlorthalidone indicating that the proposed method is highly accurate. The LOD and LOQ were found to be 0.029 µg/ ml and 0.09 for TEL and 0.047 µg/ ml and 0.143 µg/ ml for CHL respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. TEL and CHL were found to be relatively stable following photolysis and Thermal degradation. Considerable degradation was observed for both in oxidation, acid and base hydrolysis. The validated method was applied to the determination of TEL and CHL in commercially available ERITEL CH-40 tablets. The results of the assay indicate that the method is selective for the analysis of both TEL and CHL without interference from the excipients used to formulate and produce these tablets.

Conclusion

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of TEL and CHL in API and tablet dosage forms. The results of the stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate these drugs from their degradation products; excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability studies.

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