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HPLC Method Development for Simultaneous Estimation of Telmisartan and Chlorthalidone in Tablet Dosage Form

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ABSTRACT

A simple, precise and rapid high performance liquid chromatography method is developed for the simultaneous quantitative determination of Telmisartan and Chlorthalidone from their combination drug product. It involves a Xterra 150 mm x 4.6 mm, 5µm, C-18 column. The separation is achieved on a simple isocratic method. The mobile phase contains a mixture of potassium dihydrogen phosphate buffer pH 2.5 (0.025M): acetonitrile in the ratio 60:40, *v/v*. The flow rate is 1.0 mL min^{-1} and the column is maintained at normal temperature. The detector wavelength is 235 nm. The retention times of Chlorthalidone and Telmisartan are 2.5 minutes and 4.4 minutes respectively. The total runtime for the separation of the two active compounds is 6.0 minutes. The described method is validated with respect to system suitability, specificity, linearity, precision and accuracy.

KEYWORDS

HPLC, Telmisartan, Chlorthalidone, HPLC, Validation.

INTRODUCTION

The combined dosage form of any pharmaceuticals is for the synergistic effect or to give longer time effect. Chlorthalidone and telmisartan[1] combination is used as antihypertensive to decrease blood pressure. Chlorthalidone known as (*RS*)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzene-1-sulfonamide is a is a diuretic drug used to treat hypertension. It is also described as a thiazide diuretic. Telmisartan known as 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-]methyl}phenyl)benzoic acid, is angiotensin receptor blocker and is used for the treatment of hypertension. It also is used for reducing the risk of stroke, heart attack, or death from cardiovascular disease events.

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Both plays significant role in reducing the risk of combined cardiovascular disease events, especially heart failure. Various methods[2-7] have already been reported for the estimation of the drugs studied either single or in combination with others. Proposed method is carried out for the simultaneous determination of chlorthalidone and telmisartan in tablet formulation by RP-HPLC method. The validation of methods was carried out as per ICH guidelines. [8-9]

MATERIALS AND METHODS

Chlorthalidone and telmisartan was obtained as a gift sample from Aurobindi Pharma Ltd, Hyderabad. HPLC grade Methanol, Acetonitrile (Merck) and AR grade potassium dihydrogen phosphate (Merck) was used. Milli-Q water was used in mobile phase preparation. Commercially available chlorthalidone and telmisartan dosage forms were purchased from local market.

Instrument

Chromatography system (HPLC, Waters, Milford, USA) with two Waters 515 pumps, a fixed wavelength programmable 2487 dual λ absorbance detector (Waters, Milford, USA).

Chromatographic Conditions

Chromatographic separations were achieved by using Xterra C-18 (150 x 4.6 mm, 5µ) analytical column. The mobile phase is consisting of a mixture of potassium dihydrogen phosphate buffer pH 2.5(pH adjusted by orthophosphoric acid) (0.025M): acetonitrile in the ratio 60:40, *v/v*. The flow rate was maintained at 1.0 ml/min with injection volume of 20µl and the absorbance was measured at 235 nm. The column and the HPLC system were kept in ambient temperature.

Preparation of Mobile Phase

Potassium Dihydrogen phosphate (0.025M) buffer was prepared by dissolving 3.5 gms of buffer in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthro phosphoric acid. For complete extraction of actives from formulations, trials were taken and mixture of methanol and buffer in 50:50 ratio were finalized as diluent.

Preparation of Standard Stock Solution

Accurately 25mg of chlorthalidone, 80 mg of Telmisartan standards were weighed and taken in 100 and 50 ml volumetric flask respectively. Dissolved by sonication in sufficient quantity of diluent and then diluted up to the mark.

Working Standard Solution

5 ml of the above standard stock solution from both were taken in 100 ml volumetric flask and made up to mark with diluent to get aconcentration of 12.5 and 80 µg/ml for chlorthalidone, and telmisartan respectively.

Preparation of Sample Solution

Ten tablets were accurately weighed and crushed into a fine powder. The powder equivalent to one tablet (12.5mg of chlorthalidone, and 80 mg of Telmisartan) was taken in 100 ml volumetric flask. About 50 ml diluent was added, shaken for 5min on rotary shaker and then sonicated for 20 mins with intermediate shaking. Then the volume was finally made up to the mark (100ml). Sample solution was centrifuged at 5000 rpm for 5 mins to get a clear solution. Then 10 ml of supernatant solution was further diluted to 100 ml to get final sample concentration of 12.5 µg/ml of chlorthalidone and 80 µg /ml of telmisartan.

Standard and sample solutions were injected five and two times respectively to get the chromatograms. Responses obtained were calculated with other important variables taken into consideration.

RESULTS AND DISCUSSION

After getting the desired assay values, the method was passed through the different parameters of validation.

Method Validation

The developed LC method extensively validated for assay of chlorthalidone and telmisartan using the following parameters.

Specificity

Blank and Placebo interference

To establish the interference of placebo, study was conducted. Assay was performed on placebo in duplicate equivalent to concentration of test preparation as per test method. Blank and Placebo chromatograms solutions showed no peaks at the retention time of chlorthalidone and telmisartan

peak. This indicates that the excipients used in the formulation do not interfere in estimation of chlorthalidone and telmisartan in chlorthalidone and telmisartan tablets. The chromatogram of blank, placebo, and standard using the proposed method is shown in Fig 1, 2 and 3.

Linearity

Linearity was studied by plotting a graph of concentration versus response and determining the correlation coefficient. A series of solutions of chlorthalidone and telmisartan standard solution were prepared in the concentration

range of about 6.4µg/mL to 18.9 µg/ mL for chlorthalidone and in the concentration range of about 39.8 µg/mL to 119.4 µg/ mL for telmisartan. Linearity results obtained are presented in Table 1 and 2.

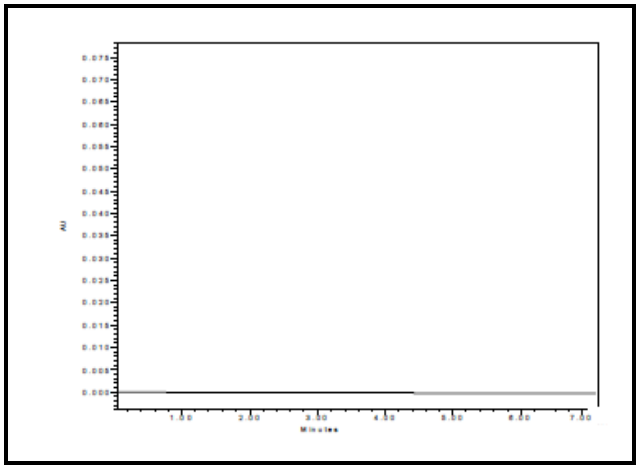

Figure 1: Blank chromatogram

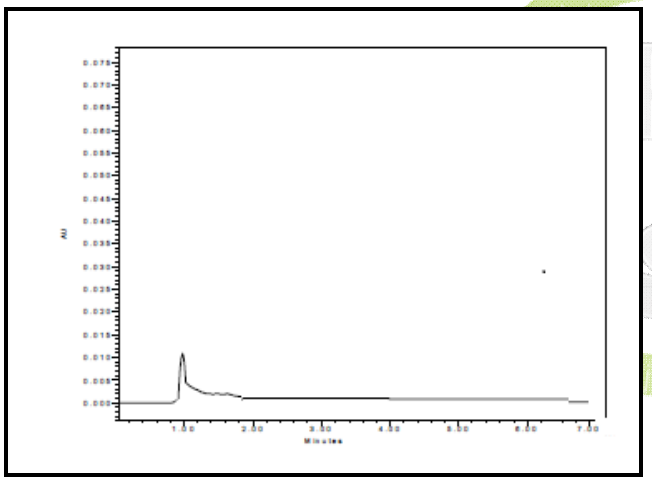

Figure 2: Placebo Chromatogram

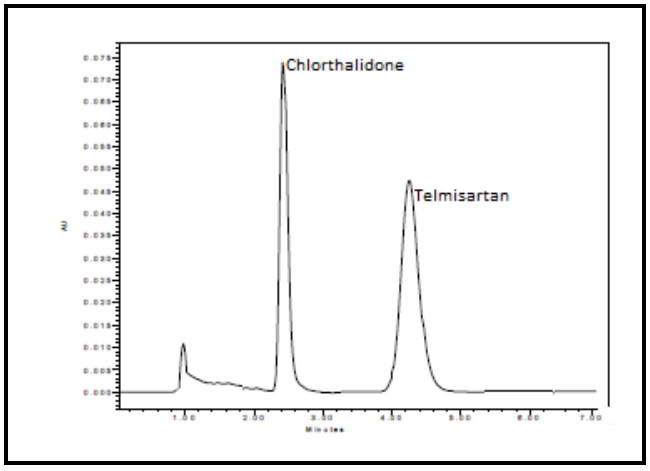

Figure 3: Standard Chromatogram

Table 1: Linearity Results Showing Correlation Coefficient for Chlorthalidone

Chlorthalidone	
Concentration (µg/ mL)	Response
6.4	2390
9.5	3701
12.5	4808
15.6	6098
18.9	7284
Slope	391.70
Corre. Coeff.	0.999
Intercept	-71.4

Table 2: Linearity Results Showing Correlation Coefficient for Telmisartan

Telmisartan	
Concentration (µg/ mL)	Response
39.8	27510
60.5	40684
80.5	53998
100.0	67514
119.4	81025
Slope	673.50
Corre. Coeff.	0.999
Intercept	238.9

The linearity was found to be linear with a correlation coefficient of 0.999 for both the actives. Linearity graph of chlorthalidone and telmisartan is shown in Fig 4 and fig 5.

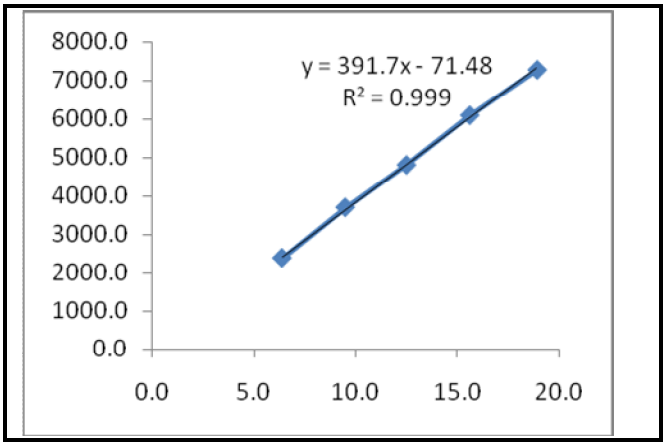

Figure 4: Linearity Graph of Chlorthalidone

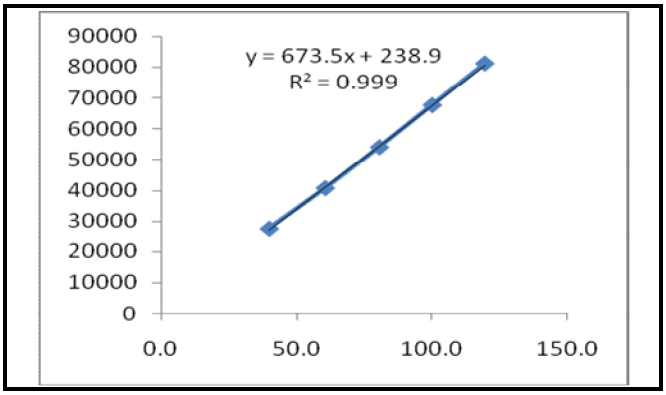

Figure 5: Linearity Graph of Telmisartan

Precision and Ruggedness

The precision of test method was established by conducting assay in six samples of chlorthalidone and telmisartan tablets. The average % assay of chlorthalidone and telmisartan in marketed tablets were found to be 99.8 and 100.1 respectively. The %RSD found to be 0.4 and 0.5 respectively. Ruggedness was studied by repeating the above steps by second analyst on another day. The results were given in Table 3 and table 4.

Table 3: Precision And Ruggedness Values Obtained for Chlorthalidone

Chlorthalidone							
Analyst 1/Day 1				Analyst 2/Day2			
	System Precision		Method Precision		System Precision		Method Precision
1	4840		100.2		4796		101.1
2	4847		100.1		4802		99.7
3	4798		99.8		4867		100.0
4	4901		99.0		4785		99.8
5	4835		99.8		4865		99.4
6	4789		99.9		4907		100.1
Mean	4835.0	Mean	99.8	Mean	4837	Mean	100.0
Std.Dev	40.0	Std.Dev	0.4	Std.Dev	49.4	Std.Dev	0.6
% RSD	0.8	% RSD	0.4	% RSD	1.0	% RSD	0.6

Table 4: Precision and Ruggedness Results Obtained for Chlorthalidone

Telmisartan							
Analyst 1/Day 1				Analyst 2/Day2			
	System Precision		Method Precision		System Precision		Method Precision
1	53978		99.8		53847		99.9
2	53615		99.6		53761		100.2
3	53897		100.0		53648		100.4
4	53712		100.2		53748		99.7
5	53899		100.0		54027		99.8
6	53717		101.1		53901		100.1
Mean	53803.0	Mean	100.1	Mean	53822	Mean	100.0
Std.Dev	141.2	Std.Dev	0.5	Std.Dev	132.9	Std.Dev	0.3
% RSD	0.3	% RSD	0.5	% RSD	0.2	% RSD	0.3

Accuracy

The recovery of chlorthalidone and telmisartan from spiked placebo was conducted at five different spike levels i.e.50, 75, 100, 125 and 150 %. Samples were prepared by mixing placebo with chlorthalidone and telmisartan raw material equivalent to test concentration. Sample solutions were prepared in triplicate for each spike level and assay was determined as per proposed method. The % recovery obtained was well within the ICH guidelines.

Robustness

A study was conducted to know the effect of deliberate variation in mobile phase composition, flow rate and pH of Buffer in mobile phase. As per proposed method, standard solutions prepared were injected into HPLC system. The system suitability parameters were evaluated. In all the cases, the retention time was observed and the %RSD obtained was less than 1. From the above study the proposed method was found to be robust.

CONCLUSION

In this study a simple, fast and reliable HPLC method was developed and validated for the simultaneous determination of chlorthalidone and telmisartan in tablet dosage forms. The developed method was successfully applied for the analysis of chlorthalidone and telmisartan. The method shows a good performance with respect to linearity, sensitivity, accuracy, precision, selectivity. So the proposed method can be used in routine quality control laboratories.

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