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Development and validation of RP-HPLC method for simultaneous estimation of telmisartan and clorthalidone in bulk and tablet dosage form

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ABSTRACT

A simple, fast, accurate and precise method has been developed for the simultaneous determination of Telmisartan and Clorthalidone from pharmaceutical formulation by reversed-phase high performance liquid chromatography. The separation was carried out on C_{18} column using mobile phase consisting of a mixture of acetonitrile: methanol and pH adjusted to 3.4 with orthophosphoric acid in the ratio (80:20 v/v). The flow rate was maintained at 1 ml/min. The UV detection was carried out at a wavelength of 225 nm. The retention time for Telmisartan and Clorthalidone was found to be 3.1 min and 4.6 min respectively. Linear response obtained for Telmisartan was in the concentration range 10-60 µg/ml (r^2 = 0.999) and Clorthalidone in the range 10-50 µg/ml (r^2 = 0.999). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, ruggedness and robustness. Thus, proposed method can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs without any interference of excipients.

Key words: Telmisartan (TEL); Clorthalidone (CLO); RP-HPLC; Validation.

INTRODUCTION

Chemically Telmisartan is 2-(4-{[4-methyl-6-(1-methyl-1*H*-1, 3-benzodiazol-2-yl)-2-propyl-1*H*-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid. Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin receptor II type 1(AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution. In addition to blocking the RAs, telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular diseases (CVD).

Chemically Clorthalidone is (*RS*)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzene-1-sulfonamide. Chlorthalidone increases the excretion of sodium, chloride, and water into the renal lumen by inhibiting sodium ion transport across the renal tubular epithelium. Its primary site of action is in the cortical diluting segment of the ascending limb of the loop of Henle. Thiazides and related compounds also decrease the glomerular filtration rate, which further reduces the drug's efficacy in patients with renal impairment (e.g. renal insufficiency). By increasing the delivery of sodium to the distal renal tubule, chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism (i.e. apical ROMK/Na channels coupled with basolateral NKATPases). This can result in hypokalemia and hypochloremia as well as a mild metabolic alkalosis; however, the diuretic efficacy of chlorthalidone is not affected by the acid-base balance of the patient being treated.

MATERIALS AND METHODS

Active pharmaceutical ingredient of TEL and CLO standards were received as gift samples from Eris Pharmaceuticals Ltd. Ahemdabad. Acetonitrile (HPLC grade), methanol (HPLC grade) and orthophosphoric acid (AR grade) were procured from Merck Chemicals, India. Tablet containing TEL and CLO (40:12.5 mg) were purchased from local pharmacy shop (Eritel cH-40, Pharmaceuticals Pvt. Ltd).

Equipment and chromatographic conditions

The HPLC system used was a Waters 510 HPLC system equipped with a Rheodyne injector (20 µl) and UV detector. Chromatographic separation was carried isocratically at room temperature with a Purosphere STAR RP-18 (250 mm × 4 mm i.d., 5 µm) column from Merck KGA 64271 Darmstadt, Germany. Data acquisition was made with Data Ace software. The mobile phase consisted of acetonitriile and methanol in the ratio 80:20 v/v (pH adjusted to 3.4 with orthophosphoric acid). The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed. The injection volume was 20 µl and eluted at a flow rate of 1 ml/min. The detection wavelength was 225 nm.

Preperation of standard solution

Standard stock solutions (100 µg/ml) of TEL and CLO were prepared by dissolving accurately weighed 10 mg of each drug separately in mobile phase in 100 ml volumetric flask and filtered through 0.45µ nylon filter. The working standard solutions of these drugs were further diluted with mobile phase to get required concentration of TEL (40 µg/ml) and CLO (12.5 µg/ml).

Preperation of standard stock solution

Twenty tablets were weighed and crushed to a fine powder. The quantity of the powder equivalent to 40 mg of TEL and 12.5 mg of CLO was weighed accurately and then transferred to 100 ml volumetric flask containing 60 ml of mobile phase. It was then sonicated for 15 minutes. The solution was filtered through a 0.45µ nylon filter and volume was made up to the mark with mobile phase. The final dilution made with mobile phase, contained about 40 µg/ml and 12.5 µg/ml of TEL and CLO respectively.

Method validation

The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, accuracy, limit of detection, limit of quantitation, intraday and interday precision, repeatability and robustness.

To establish the linearity a series of dilutions ranging from 10-60 µg/ml for TEL and 10-60 µg/ml for CLO were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

The ICH document defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. For this diluent was used as blank. Standard and sample were prepared as per test procedure. Check for the interference of blank with the analyte peak. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

The accuracy of the method was determined by calculating percent recovery of TEL and CLO by the standard addition method. The recovery experiments were carried out in triplicate (80, 100 and 120 %) by spiking previously analyzed samples of the tablets with three different concentrations of standards. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 40 µg/ml of TEL and 12.5 µg/ml of CLO for both the methods. The results are reported in term of percent recovery.

Precision of estimation of TEL and CLO by proposed method was ascertained by replicate analysis of homogenous samples of tablet powder at different time intervals on the same day (Intraday precision) and on second day (Interday precision). The relative standard deviation (% RSD) was determined to assess the precision of the assay and it was found to be not more than 2.0%.

Repeatability of the method was performed by injecting 100% concentration of TEL and CLO of the regular analytical working value consecutively for six times and the effects on the results were examined. Results were reported in terms relative standard deviation.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated for the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves. LOD and LOQ were

calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10(SD)/S. Where, SD = standard deviation of response (peak area) and S = slope of the calibration curve.

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate ±0.2, PH of mobile phase ±0.4 and percentage of acetonitrile in the mobile phase ±10.

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of TEL and CLO in bulk as well as in pharmaceutical preparation was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines.

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of acetonitrile: methanol (PH 3.4 adjusted with orthophosphoric acid) in the ratio of 80:20 v/v at 1 ml/min flow rate. The optimum wavelength for detection was set at 225 nm at which much better detector responses for both drugs were obtained. The retention time for TEL and CLO was found to be 3.1 min and 4.6 min respectively.

System suitability testing
To know reproducibility of the method, system suitability test was employed to establish the parameters such as retention time, tailing factor, etc. The results obtained for system suitability are summarized in Table 1.

Linearity
TEL and CLO showed a linearity of response between 10-60 µg/ml and 10-50 µg/ml with a correlation coefficient of 0.999 and 0.999 respectively. The results obtained for linearity of TEL and CLO are summarized in Table 1.

Precision
The precision of this method was determined by intraday and interday precision. The % R.S.D was found less than 2 this indicate that the method is precise. The results of precision studies are shown in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The sensitivity of method is described in terms of LOD and LOQ. LOD and LOQ values for TEL were found to be 2.54 µg/ml and 7.71 µg/ml and that for CLO were found to be 0.62 µg/ml and 0.62 µg/ml respectively. The results of LOD and LOQ studies are shown in Table 1.

Accuracy
The accuracy was evaluated by the recovery of TEL and CLO at three different levels (80, 100 and 120%). The percentage recovery was found to be 98% and 102% for TEL and CLO respectively, % RSD was found to be less than 2, ensuring that the method is accurate. The results of accuracy studies are shown in Table 2.

Repeatability
The experimental values obtained for the repeatability of TEL and CLO in samples is presented in Table 3. The result obtained shows % R.S.D. < 2, indicating good repeatability of method.

Robustness
Robustness of the method was carried out by deliberately made small change in the flow rate ±0.2, pH of mobile phase ±0.4 and mobile phase composition (acetonitrile: methanol) ± 10. The results of robustness studies are shown in Table 4.

Specificity
Specificity was observed that the excipients present in the formulation and diluents did not interfere with detection of TEL and CLO.

Label claim recoveries from tablets
The proposed method was evaluated in the assay of commercially available tablets containing TEL (40 mg) and CLO (12.5 mg). Six replicate determinations were carried out on an accurately weighted amount of the pulverized tablets equivalent to 40 mg of TEL and 12.5 mg of CLO. The results of label claim studies are shown in Table 5. Chromatogram of the sample is shown in Figure 3.

Table 1. Method validation and system suitability parameters

Parameter		TEL	CLO
Linearity range		10-60 µg/ml	10-50 µg/ml
regression coefficients (r)		0.9995	0.9998
Limit of detection		2.54 µg/ml	0.20 µg/ml
Limit of quantitation		7.71 µg/ml	0.62 µg/ml
Precision	Intra-day (%RSD)	1.04	0.82
	Inter-day (%RSD)	1.04	0.82
Retention time (min)		3.1	4.6
Tailing factor		1.008	0.8

Table 2. Result of recovery study of TEL and CLO

Formulation	Label Claim mg/tablet	Amount added (%)	Total amount added (mg)	% Recovery* ± SD	% RSD
TEL	400	80	32	99.74 ± 0.0070	0.007
		100	40	100.05 ±0.0151	0.013
		120	48	99.68 ± 1.4424	1.440
CLO	50	80	10	98.57 ± 0.0070	0.0071
		100	12.5	100.83 ±1.0535	1.048
		120	15	101.09± 1.5877	1.601

**Average value ± SD of 6 determinations, SD is standard deviation and %RSD is relative standard deviation*

Table 3. Result of repeatability study of TEL and CLO

Parameter	TEL	CLO
% Mean*	24189.786	7759.987
SD	253.87	63.70
% RSD	1.04	0.82

**Average of six determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation.*

Table 4. Result of Robustness study

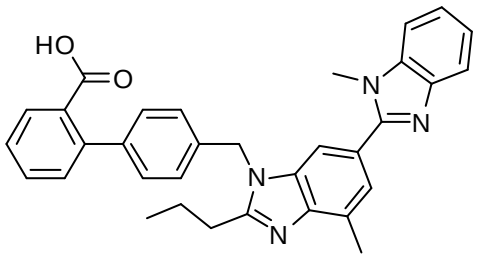
Parameter	Variation	SD		% RSD	
		TEL	CLO	TEL	CLO
Mobile phase composition (ACN : Methanol) ± 10*	70:30	405.24	65.29	1.64	0.84
	90:10	9.89	10.77	0.037	0.13
pH ± 0.4*	3.0	100.18	136.37	0.5	1.87
	3.8	51.54	2.69	0.208	0.05
Flow rate (ml/min) ± 0.2*	0.8	191.8	55.44	0.822	0.77
	1.2	79.84	20.57	0.88	0.29

***** *Average of three determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation.*

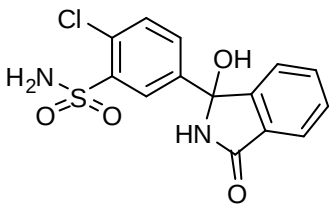
Table 5. Result of assay of Tablet formulation

Formulation	Label claim mg/tablet	Amount found in mg	% Label claim *mean ± SD
TEL	40	40.9	101.25 ± 0.6708
CLO	12.5	12.5	99.26 ± 0.5187

***** *Average of six determinations, S.D. is Standard deviation*



Telmisartan



Clorthalidone

Figure 1. The chemical Structure of Telmisartan and Clorthalidone

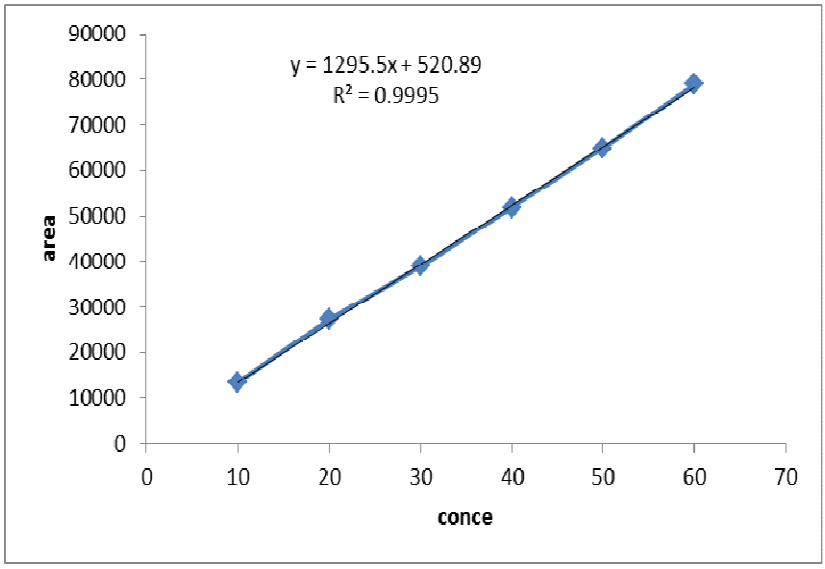


Figure 2 (a). Calibration curve for Telmisartan

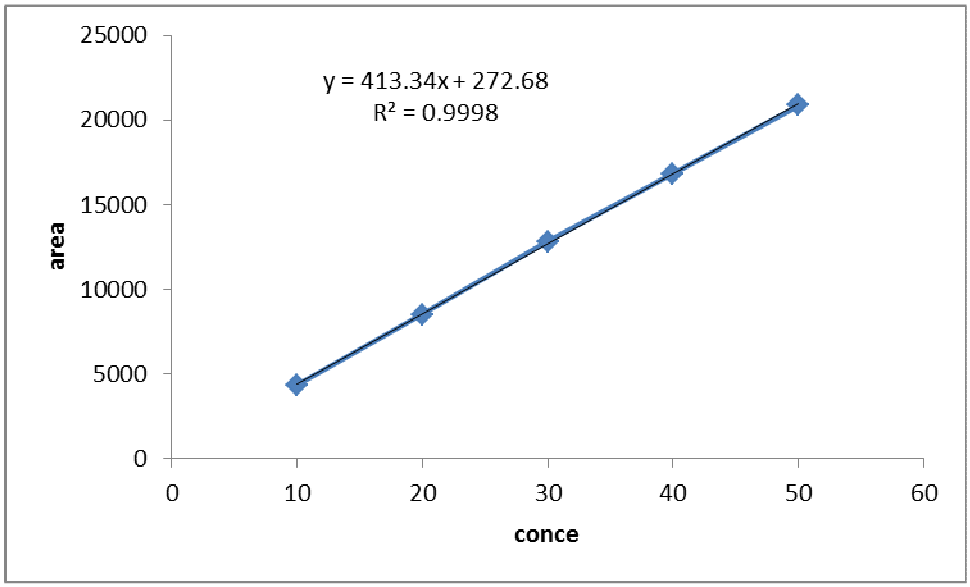


Figure 2 (b). Calibration curve for Clorthalidone

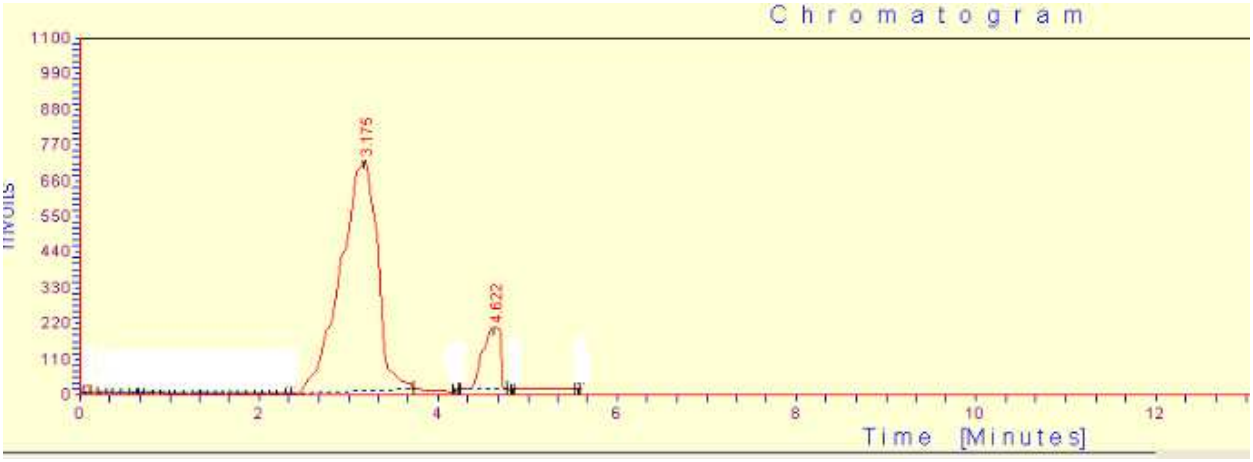


Figure 3. A RP-HPLC chromatogram of Telmisartan (RT 3.1) and Clorthalidone (RT 4.6) of marketed formulation.

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of TEL and CLO in pharmaceutical formulations without interference and with good sensitivity; hence it can be used for the routine analysis in quality control department.

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