Research Article

Simultaneous Determination of Telmisartan Impurities and Chlorthalidone impurities by UPLC

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ABSTRACT

A rapid specific UPLC method has been developed for simultaneous determination of Telmisartan (TS) impurities and Chlorthalidone (CT) impurities in their formulations. Chromatographic separation of these two compounds impurities was carried out an Acquity BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm column is used for development of method pH 4.5 buffer is prepared using 0.025M Potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1 mL of triethyl amine in milli-Q water, pH 4.5 ± 0.05 adjusted with diluted ortho phosphoric acid. The mobile phase A consists pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v). Mobile phase B consists pH 4.5 buffers & acetonitrile in the ratio 20:80 (v/v). The flow rate of mobile phase is 0.3 mL/min with a gradient elution. The column temperature is 25°C and detection wavelength is 290 nm. The injection volume is 3 µL. The gradient program is as follows: time (min)/% mobile phase B: 0/20, 2/30, 5/45, 8/55, 10/80, 14/80, 14.1/20 and 18/20. The developed RP-UPLC method was validated with respect to specificity, linearity, accuracy, precision, robustness and high sensitivity with detection limits and quantification limits. To the best of our knowledge, a rapid LC method, which separates all the impurities of Telmisartan and Chlorthalidone, disclosed in this investigation was not published elsewhere.

Keywords: Pharmaceutical formulations, Telmisartan, Chlorthalidone, RP-UPLC, Validation.

INTRODUCTION

Telmisartan (TS),4'-[[4-Methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl] methyl] biphenyl-2-carboxylic acid is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as Telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure[1]. Chlorthalidone (CT), 2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzene sulfona-mide is thiazide-like diuretic, with molecular formula $C_{14}H_{11}ClN_2O_4S$. CT inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, CT indirectly increases potassium excretion via the sodium-potassium exchange mechanism. CT is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance [2].

TS and CT drug substances are official in USP/EP/BP/IP where as Telmisartan drug product is official in USP/IP. CT drug product is official in USP/BP/IP [3-6]. The combination product is not official in any of the pharmacopeias. So far to our current knowledge there is no method reported in any of the Pharmacopoeia or in the literature for the simultaneous determination of TS+CT in pharmaceutical formulation for assay and impurities. Literature survey reveals that methods have been reported for estimation of impurities in TS and CT drug substance and individual drug products but none of the reported articles described the single method for estimation of impurities in fixed dosage combination product of TS+CT. Instead of following two individual methods, author has seen an opportunity to develop a single analytical method for estimating impurities for this combination product. The chemical structures of Telmisartan and Chlorthalidone were shown in the figure 1.

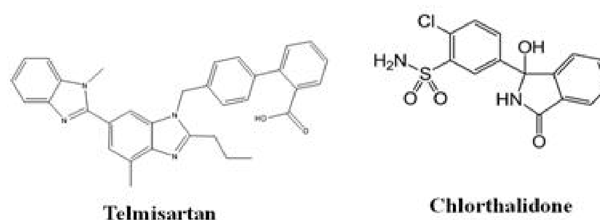

Figure 1

MATERIALS AND METHODS

Chemicals and reagents

Telmisartan API and its 6 impurities are procured from Shakhty chemicals Laboratories Ltd., Hyderabad, India. Chlorthalidone API, Chlorthalidone Impurity A and impurity B are procured from Bio - Leo chemicals Pvt Ltd., India. Analytical reagents of potassium dihydrogen orthophosphate and 1-hexane sulphonic acid sodium salt are purchased from Merck, Germany. HPLC grade acetonitrile, methanol, triethyl amine and ortho phosphoric acid are purchased from Merck, Germany and high pure water is prepared by using Millipore Milli Q plus purification system.

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Instrumentation and chromatographic conditions

The Waters UPLC system with a diode array detector is used for method development and method validation. The output signal is monitored and processed using M/s waters Empower software. Cinex degital water bath is used for hydrolysis studies. Mettler XS 205 Dual Range balance is used for weighing of samples and standard. Photo stability studies are carried out in a Sun Text XLS+ photo stability chamber. Thermal stability studies are performed in a Merck hot air oven. The pH of the buffer is measured using Thermo orin pH meter.

Acquity BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm column is used for development of method. pH 4.5 buffer is prepared using 0.025M Potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1 mL of triethyl amine in milli-Q water, pH 4.5 ± 0.05 adjusted with diluted ortho phosphoric acid. The mobile phase A consists pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v). Mobile phase B consists pH 4.5 buffers & acetonitrile in the ratio 20:80 (v/v). The flow rate of mobile phase is 0.3 mL/min with a gradient elution. The column temperature is 25°C and detection wavelength is 290 nm. The injection volume is 3 µL. The gradient program is as follows: time (min)/% mobile phase B: 0/20, 2/30, 5/45, 8/55, 10/80, 14/80, 14.1/20 and 18/20.

Preparation of standard solution

A stock solution of TS (1000 µg/mL) and CT (315 µg/mL) is prepared by dissolving an appropriate amount in methanol. Working solution is prepared from above stock solution for impurities determination (1.60 µg/mL of TS and 0.25 µg/mL of CT) in diluent. Impurity stock solutions are prepared in methanol. A mixture of all impurities (1.6 µg/mL of TC impurities and 0.25 µg/mL of CT impurity) along with TS and CT (1.60 µg/mL of TS and 0.25 µg/mL of CT) is prepared in diluent.

Preparation for Test solution

Twenty tablets of TS+CT are weighed and crushed to a powder in a mortar and pestle. Accurately tablet powder equivalent to 80 mg of TS and 12.5 mg of CT is transferred to 100 mL volumetric flask, 50 mL of methanol is added and sonicated for 15 minutes with intermediate shaking and made up to volume with diluent to give a solution containing 800 µg/mL of TS and 125 µg/mL of CT. Part portion of solution is centrifuged at 3000 rpm for 10 min. The supernatant test solution is used for analysis. Placebo sample is prepared in the same way by taking the placebo equivalent weight present in a test preparation.

Impurity stock preparations

Impurity stock solutions are prepared individually by weighing accurately about 10 mg each of TS and 10 mg of CT impurities into two separate 100 mL volumetric flasks. 25 mL of diluent is added and dissolved with aid of sonication and made up to volume with diluent to obtain stock solutions of 100 µg/mL each of TS impurities and 100 µg/mL of CT impurities.

Preparation of Test solution with spiking of Impurities

Twenty tablets of TS+CT are weighed and crushed to a powder in a mortar and pestle. Accurately tablet powder equivalent to 80 mg of TS and 12.5 mg of CT is transferred to 100 mL volumetric flask, 1.6 mL of TS and 0.25 mL of CT impurities stock solutions are added to obtain target level of 0.2% of impurity, 50 mL of methanol is added and sonicated for 15 minutes with intermediate shaking and made up to volume with diluent. Part portion of solution is centrifuged at 3000 rpm for 10 minutes. The supernatant test solution is used for analysis. The overlay chromatogram of test spiked with impurities are shown in figure 2.

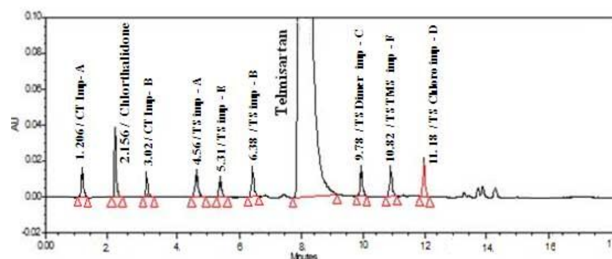

Figure 2: Chromatogram of test spiked with impurities

Diluent

Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio 60:20:20 (v/v/v) is used as diluent.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective of developing a RP-UPLC method is to get the separation of all impurities in TS+CT pharmaceutical dosage form in a single chromatographic condition with emphasis on the method being precise, accurate, linear, reproducible, robust, stability-indicating and free of interference from excipients which are used in formulation.

The sample spiked with impurities at 0.2% concentration is used for method development. Initially, the separation of all impurities from TS, CT and placebo peaks is verified using literature method conditions. These conditions resulted in separation of the TS peak with the placebo peaks and impurities peaks, but CT, CT impurity A and impurity B are co-eluting with TS impurities. Different gradient program are tried by changing polarities of mobile phase to separate CT , impurity A and impurity B, but adequate separation is not achieved. Different C18 and C8 stationary phases are screened like BEH phenyl, BEH Shield, BEH C8 and BEH C18 etc., Finally better separation is seen on BEH shield –RP18 column compared against C8 stationary phase columns. Hence Acquity BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm column is chosen to work further.

Based on above experiments, changes are made in mobile phase by altering buffer from ammonium acetate to potassium dihydrogen phosphate by incorporating ion pair reagent (1-hexane sulphonic acid sodium salt) for

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better separation. Different gradient programs are verified at different pH of buffer and finally the chromatographic separation is achieved with following conditions. A reversed phase Acquity BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm column operated at 25°C and flow rate of mobile phase is 0.3 mL/min with gradient elution. The detection wavelength is 290 nm. The mobile phase A consists pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v). Mobile phase B consists pH 4.5 buffers & acetonitrile in the ratio 20:80 (v/v). pH 4.5 buffer prepared using 0.025M potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1 mL of triethyl amine in milli-Q water, pH 4.5 ± 0.05 adjusted with diluted ortho phosphoric acid. The injection volume is 3 µL. The gradient program is as follows: time (min)/% mobile phase B: 0/20, 2/30, 5/45, 8/55, 10/80, 14/80, 14.1/20 and 18/20. Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio 60:20:20 (v/v/v) is used as diluent for sample preparation. No chromatographic interference due to blank (diluent) and other excipients (placebo).

Method validation

Precision

The % RSD of replicate test preparations spiked with impurities (Intra-day and inter-day precision study) is found to be less than 4.0, conforming good precision of the method. All values are well within the acceptance criteria i.e. % RSD not more than 15.0 %. The data is shown in Table 1.

Accuracy

The percentage recoveries of all impurities in TS+CT samples are found to be between 85.0 to 115.0 for TS and CT impurities. The % recovery values are presented in Table 2.

LOQ and LOD

The determined limit of detection (LOD), limit of quantification (LOQ) and precision at LOQ values for TS and CT impurities are reported in Table 3.

Table 1: Results of precision for TS impurities

Preparation	Imp- B	Imp - A	Imp - F	Dimer Imp -C	TMS1 Imp -D	Chloro Imp -E	CT imp-A	CT imp-B
Prep-1	0.202	0.194	0.200	0.205	0.197	0.201	0.196	0.196
Prep-2	0.200	0.197	0.200	0.205	0.199	0.200	0.195	0.195
Prep-3	0.200	0.194	0.200	0.206	0.202	0.212	0.198	0.198
Prep-4	0.202	0.197	0.199	0.205	0.202	0.197	0.197	0.197
Prep-5	0.202	0.192	0.202	0.208	0.206	0.216	0.197	0.197
Prep-6	0.202	0.196	0.201	0.204	0.204	0.197	0.196	0.196
Avg	0.202	0.195	0.200	0.206	0.202	0.204	0.197	0.197
%RSD	0.5	1.0	0.5	0.6	1.6	4.0	0.5	0.5

Table 2: Recovery results of TS impurities in TS+CT tablet

Amount spiked	% Recovery							
	TS- Impurity B	TS-Impurity A	TS-Impurity F	TS-Dimer Impurity C	TS-TMS1 Impurity D	TS-Chloro analogue E	CT-Impurity A	CT-Impurity B
LOQ	95.8±3.7	96.7±3.1	98.5±1.9	97.4±2.8	95.4±2.3	98.5±2.8	96.9±4.1	98.6±2.7
50%	101.6±0.7	99.9±2.9	98.3±0.8	102.4±2.4	96.0±3.4	98.5±3.1	102.6±3.3	104.5±2z3
100%	95.5±2.3	91.9±2.2	94.8±4.3	99.5±0.9	93.5±3.1	99.6±1.7	95.4±2.9	98.5±0.9
150%	96.9±3.8	94.8±3.5	99.5±2.7	98.5±4.3	93.8±1.2	100.1±1.9	103.3±0.9	95.2±2.1
200%	94.4±4.1	95.4±4.4	98.8±3.6	97.9±1.7	93.8±2.9	98.5±2.9	99.0±2.3	95.5±1.8
300%	93.6±3.6	93.1±3.8	100.5±3.1	95.8±2.6	94.8±3.7	99.5±3.5	94.5±2.4.	95.4±3.2

a. All impurities of TS individually spiked on test preparation at 0.2 % level (0.8 mg/mL of TS and 0.125mg/mL of CT), b. Mean ± % RSD for three determinations; c. CT- Impurity A spiked on test preparation at 0.2 % level (0.8 mg/mL of TS and 0.125 mg/mL of CT); d. Mean ± % RSD for three determinations

Table 3: LOD, LOQ and precision at LOQ for TS and CT impurities

Name of the Impurity		LOD		LOQ		
		Con. in %	S/N ratio	Con. in '%'	S/N ratio	% RSD LOQ
CT- Impurity A		0.043	3.23	0.014	10.05	3.5
TS impurities	TS – Impurity A	0.030	3.15	0.010	9.84	3.4
	TS – Impurity B	0.038	2.76	0.012	9.83	1.9
	TS – Impurity E	0.038	3.17	0.013	10.23	2.5
	TS – Impurity F	0.027	3,29	0.009	10.12	4.4
	TS – Dimer Impurity	0.037	2.85	0.012	10.42	3.7
	TS – TMS1 Impurity	0.039	2.97	0.013	9.82	2.4
	TS – Chloro analogue	0.029	3.43	0.010	9.93	1.7

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Table 4: Results of Robustness study

Impurity Name	RRT's of the impurities										
	*	Flow rate		Column temperature		pH of the buffer		Acetonitrile composition			
								Mobile Phase A		Mobile Phase B	
		0.25 mL/min	0.35 mL/min	20°C	30°C	4.3	4.7	90%	110%	90%	110%
CT- Impurity A	0.80	0.83	0.82	0.79	0.81	0.82	0.80	0.80	0.82	0.81	0.79
TS impurity B	1.13	1.16	1.11	1.12	1.12	1.15	1.17	1.11	1.13	1.12	1.14
TS impurity B	2.38	2.43	2.39	1.54	2.39	2.42	2.37	2.42	2.39	2.35	2.37
TS impurity A	1.55	1.59	1.52	1.56	1.56	1.60	1.57	1.57	1.55	1.59	1.54
TS impurity F	2.30	2.32	2.28	2.33	2.33	2.35	2.26	2.32	2.34	2.28	2.27
TS-Dimer impurity	3.10	3.15	3.09	3.15	3.09	3.12	3.07	3.15	3.12	3.10	3.11
TS-TMS1 impurity	3.45	3.44	3.43	3.47	3.41	3.44	3.42	3.46	3.45	3.47	3.41
TS-Chloro analogue	3.56	3.58	3.57	3.57	3.53	3.59	3.52	3.52	3.57	3.55	3.58

* As per the method conditions

Linearity

A linear calibration plot for TS and CT impurities is obtained over the calibration range LOQ (~0.05) to 6.81 µg/mL and the correlation co-efficient is found to be about 0.999. The result an excellent correlation exists between the peak area and concentration of the analyte for all the impurities.

Robustness

To determine the robustness of the developed method, experimental conditions are deliberately altered and the elution pattern, separation between TS and its impurities, CT and its impurities and tailing factor for TS and CT and its impurities are recorded. In all the deliberate varied chromatographic conditions (flow rate, column temperature, pH of buffer in mobile phase and composition of organic solvent), all analytes are adequately resolved and elution orders remained unchanged. RRT of all the known impurities for all deliberately varied conditions along with original conditions are summarized in Table 4.

CONCLUSION

A sensitive, specific, accurate, robust and validated stability indicating UPLC method is described for the determination of degradation products and process-related impurities in TS+CT tablets. The behavior of TS and CT under various stress conditions is studied. All degradation products and process impurities are well separated from each other and from TS and CT which indicates the stability-indicating power of the method. The information presented in this study is very useful for quality monitoring of TS+CT tablets and can be used to check drug product quality during stability studies.

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