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Method development and validation for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine by RP-UPLC in pharmaceutical dosage form

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Article History:	ABSTRACT
Received on: 25.03.2018 Revised on: 19.05.2018 Accepted on: 23.05.2018	A novel, simple, accurate reverse phase ultra performance liquid chromatographic method for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine in pharmaceutical dosage form was developed and validated. This method was performed on Thermo scientific-Hypersil gold C18, 1.9 μ m (50mm X 2.1mm) column with Mobile Phase 60 volume of buffer and 40 volume of Acetonitrile. Buffer Solution was mixing 1ml of O-Phosphoric acid in 1000ml of water. The flow rate was 0.3 ml/min and detection were carried out using a PDA detector at 232 nm. The retention times were 2.6, 3.0, & 3.7 min for Chlorthalidone, Telmisartan and, Amlodipine respectively. The method was linear over the concentration range of 8-12 μ g/ml for Chlorthalidone and 24-36 μ g/ml for Telmisartan and 3-7 μ g/ml for Amlodipine with a correlation coefficient of 0.999, 0.998 and 0.997 respectively. The developed method was validated as per ICH guidelines for linearity, accuracy, precision, robustness, specificity, limit of detection, limit of quantitation.
Keywords:	
Telmisartan, Chlorthalidone, Amlodipine besylate, RP-UPLC, Simultaneous Estimation	



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INTRODUCTION

A newer separation technique, UPLC is a new invention in liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography, which brings dramatic improvements in sensitivity, resolution and speed of analysis. It has instrumentation that operates at high pressure than that used in HPLC and in this system, uses fine particles (less than 2.5 μ m) and mobile phases at high linear velocities decreases the length of the column, reduces solvent consumption and saves time. In the present work, this technology has been applied to the

method development, and validation, of simultaneous estimation of Chlorthalidone, Telmisartan, and Amlodipine in tablet dosage form. Chlorthalidone is a diuretic drug used to treat hypertension. Chemically it is 2-Chloro-5- (1-hydroxy-3-oxo-2, 3-dihydro-1Hisoindol-1-yl) benzene-1-sulfonamide. It is official in Indian Pharmacopoeia, British Pharmacopoeia, and United States Pharmacopoeia. Telmisartan is an angiotensin receptor blocker (ARB). Chemically it is 2- (4- {[4-methyl-6- (1-benzodiazol-1-yl) methyl} phenyl] benzoic acid. It is official in Indian Pharmacopoeia, British Pharmacopoeia.

Amlodipine (AML) is a Dihydropyridine calcium antagonist that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells. Chemically it is a (RS) -3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl) -6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate. It is official in Indian Pharmacopoeia, British Pharmacopoeia, United States Pharmacopoeia. The chemical structure of Chlorthalidone, Telmisartan, and Amlodipine are shown in figure 1, 2, 3.

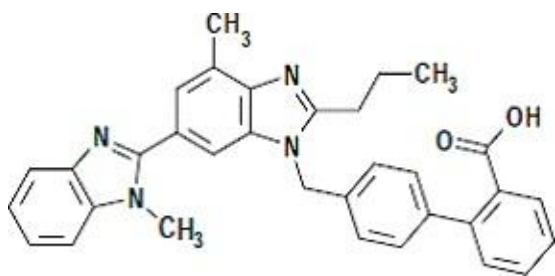


Figure 1: Structure of Telmisartan

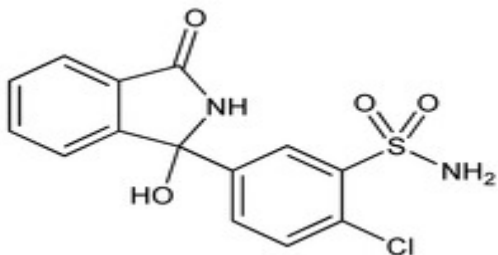


Figure 2: Structure of chlorthalidone

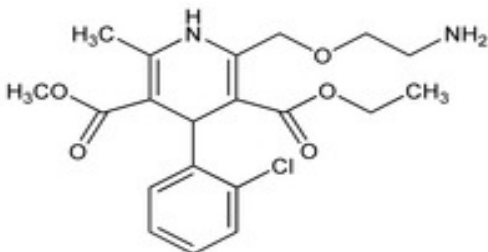


Figure 3: Structure of amlodipine

In literature survey reveals that several analytical methods such as UV (Abdullah *et al.*, 2014; Nanda Gopal *et al.*, 2015), HPLC (Brines Singh *et al.*, 2009; Lakshmi Suresh *et al.* 2012) have been reported for analysis of individual drugs of Chlorthalidone, Telmisartan and Amlodipine and in combination with them or other drugs UV (Padmane *et al.*, 2014), HPLC (Kreny E. Parmar *et al.*, 2013), UPLC (Santaji Nalwade *et al.*, 2016), capillary electrophoresis (Modroiu Adriana *et al.*, 2016) methods have been reported. However, no UPLC method for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine has been reported. Therefore, an attempt has been made to develop a new UPLC method for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine.

MATERIALS AND METHODS

Reagents and chemicals

The reference sample of Chlorthalidone, Telmisartan, and Amlodipine was supplied by Ideal laboratory Pondicherry. Tablet brand TELISTA TRIO (Lupin) was procured from the local market having the combination of Chlorthalidone-12.5mg, Telmisartan-40mg, and Amlodipine-5 mg respectively. HPLC grade Methanol and Acetonitrile were

purchased from S.D. Fine Chemicals (Mumbai, India). O-phosphoric acid AR Grade was purchased from Qualigens (Mumbai, India). Water was collected from Milli Q purification unit with 0.22 μ filters PVDF membrane filter (Millipore, India)

Instrumentation and chromatographic condition

Chromatography was performed with a THERMO-SCIENTIFIC UPLC system equipped with ACCELA-1250 Pump with Auto Sampler and a Photo Diode Array Detector. CHROM-QUEST software performed data acquisition, data handling and Instrumentation control. Column Thermo scientific - Hypersil gold C18, 1.9 μ m (50mm X 2.1mm) was used for chromatographic separation under a suitable condition. The mobile phase was a 60:40 (v/v) mixture of freshly prepared buffer (1 ml of O-phosphoric acid in 1000 ml of water) and acetonitrile. The mobile phase was sonicated and degassed before use. The diluent was methanol. It was pumped through the column at a Flow rate of 0.3 ml/min. The detection was carried out at 232 nm. The runtime was set at 4 minutes under these optimised chromatographic conditions. The retention time obtained for the drugs were 2.6 minutes for Chlorthalidone, 3.0 minutes for Telmisartan, 3.7 minutes for Amlodipine respectively. A typical chromatogram is shown in figure 4.

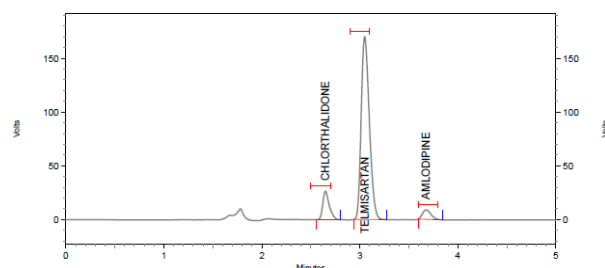


Figure 4: Optimized Chromatogram of Chlorthalidone, Telmisartan, and Amlodipine

Mixed Standard Preparation

Individual standard stock solution of Chlorthalidone (0.26mg/ml), Telmisartan (0.82mg/ml), Amlodipine (0.115mg/ml) was prepared with diluent methanol. 1 ml of each stock solution was pipetted out into 25ml volumetric flask and diluted to volume with mobile phase to achieve the final concentration of Chlorthalidone (10 μ g/ml), Telmisartan (33 μ g/ml), Amlodipine (6 μ g/ml). System suitability test was performed for five replicate standards.

Sample Preparation

Twenty tablets were weighed and finely powdered. 299.6 mg of tablet powder weighed, transferred into 50 ml volumetric flask, diluted with methanol, Sonicated for 30 minutes. The solution was filtered through a 0.22 μ m filter and then 1ml

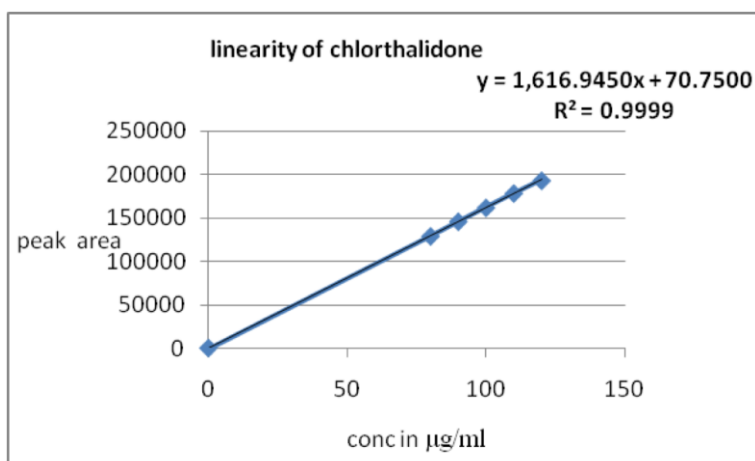


Figure 5: Linearity for Chlorthalidone

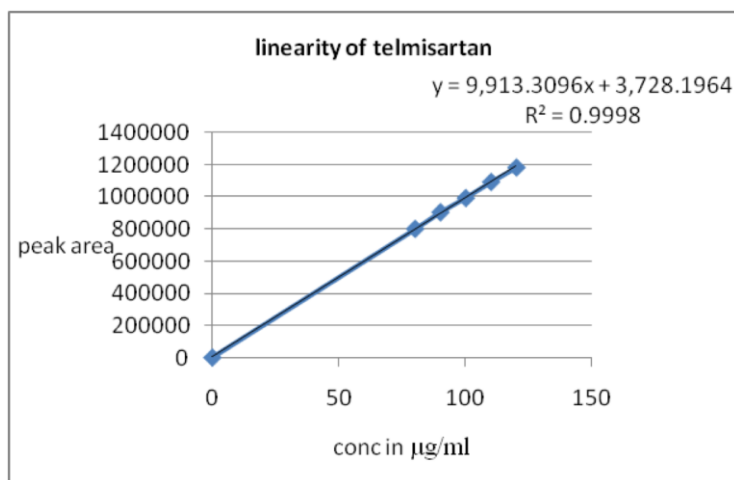


Figure 6: Linearity for Telmisartan

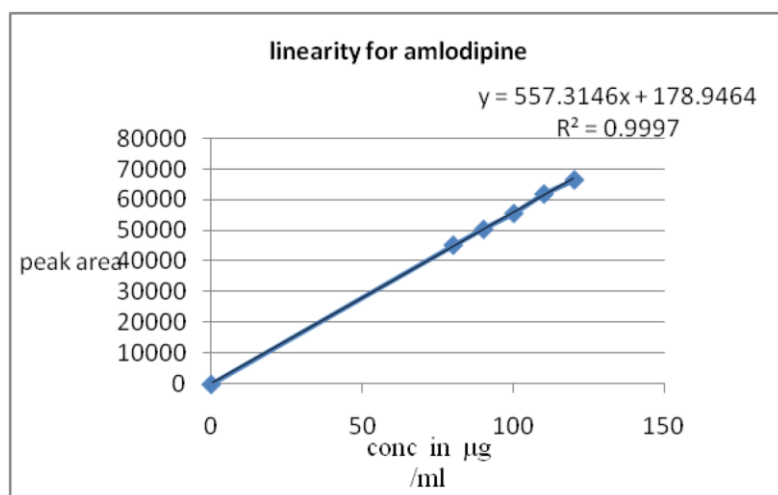


Figure 7: Linearity for Amlodipine

of the stock solution was transferred into 25 ml volumetric flask and diluted to volume with mobile phase

RESULTS AND DISCUSSION

Method Development

The RP-UPLC method aimed to estimate the Chlorthalidone, Telmisartan, and Amlodipine in the pharmaceutical dosage form and validate the

method as per ICH guidelines. Preliminary tests were performed to select optimum UPLC condition. Parameters such as choice of column mobile phase composition, detection wavelength and other factors were studied. After several trials the mobile phase selected as 60:40 (v/v) mixture of freshly prepared buffer (1 ml of O-phosphoric acid in 1000 ml of water) and acetonitrile. Column Thermo scientific - Hypersil gold C18, 1.9 μm (50mm X 2.1mm) was found to be suitable for

Table 1: Method Validation Results

Parameters	Chlorthalidone	Telmisartan	Amlodipine
System Suitability Test			
Theoretical plates	7282	6070	7275
Asymmetry	1.49	1.27	1.23
Resolution	0.00	3.09	3.80
Validation			
Linearity($\mu\text{g/ml}$)	8-12	24-36	3-7
Regression equation	$Y=1,616.9450x+70.7500$	$Y=9,913.3096x+3,728.1964$	$Y=557.3146x+178.9464$
The correlation coefficient (R^2 value)	0.999	0.998	0.997
Accuracy (%)			
100%	100.55	100.3	100.9
110 %	101.09	100.15	100.1
120%	100.63	100.07	101.8
130%	101.3	100.20	100.3
system precision(% RSD)	0.85	0.91	0.55
Method precision(%RSD)	0.25	0.27	0.28
Limit of detection ($\mu\text{g/ml}$)	0.015	0.020	0.012
Limit of quantitation ($\mu\text{g/ml}$)	0.040	0.050	0.030
Assay (%)	99.99	99.62	99.99
Retention time	2.640	3.073	3.703

chromatographic separation. The wavelength was Selected at 232 nm based on the UV –overlain spectra of three drugs using optimised mobile phase. The flow rate was 0.3 ml and retention time obtained for the drugs were 2.6 minutes for Chlorthalidone, 3.0 minutes for Telmisartan, 3.7 minutes for Amlodipine respectively.

Method Validation

The developed UPLC method was validated to confirm that it was suitable for its intended purposes as described in an international conference on harmonisation (Q2B) guidelines. The validation parameters include system suitability, linearity, accuracy, precision (system precision, method precision), limit of detection (LOD), limit of quantitation (LOQ), sensitivity, robustness (flow rate, wavelength).

Linearity

The linearity of the method was evaluated by analysing the different concentration of the drugs. According to ICH recommendations, at least five concentrations must be used. The method was linear in the range of concentration of Chlorthalidone was 8-12 $\mu\text{g/ml}$, 24-36 $\mu\text{g/ml}$ for Telmisartan and 3-7 $\mu\text{g/ml}$ for Amlodipine and calibration curve was constructed by plotting the concentration on X-axis and peak area on the Y-axis. The linearity graph for the individual drug is shown in figure 4, 5, 6.

Accuracy

Accuracy was determined by spiking the standard solutions in the sample solution at three different levels 110%, 120%, and 130% of the target concentration and calculating the percentage recovery. The percentage recovery was given in table 1.

Precision

The precision of the method was assessed by studying system precision, and method precision System precision was calculated on five to replicate injections of standard solutions method precision was calculated on six replicate injections of sample solutions. The percentage of RSD was calculated. The value of precision was given in table 1.

Robustness

Robustness is a measure of the capacity of the analytical method to remain unaffected by small but deliberate variations of the operating condition. This was tested by studying the effect of changing the flow rate and wavelength.

Limit of detection and Limit of quantitation

According to ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantitation limits. LOD and LOQ values were estimated as [standard devi-

ation of repeatability/slope of the regression equation] by multiplying with 3.3 and 10 respectively. The values are given in table 1.

CONCLUSION

The simple RP-UPLC method was developed and validated for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine in a pharmaceutical formulation. The method was precise, accurate, linear, robust and ultra-fast. The shorter run time of within 4 minutes enables rapid determination of the drugs individually and in combination. The method was found to be specific. This method exhibited an excellent performance in terms of speed. The method was more economical and suitable for laboratory use as solvent consumption was very less.

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