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Short Communication

Optimized and Validated RP-UPLC Method for the Determination of Losartan Potassium and Chlorthalidone in Pharmaceutical Formulations

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Abstract

Purpose: A validated ultra performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous determination of losartan potassium and chlorthalidone in pharmaceutical preparations.

Methods: Waters-Acquity UPLC system equipped with Auto Sampler, PDA detector and operated with Empower-2 software was used for the present study. Detection was done at wavelength of 230 nm, HSS C18, 100 mm x 2.1x 1.8 µm column with a reverse phase elution and mobile phase composed of A and B mixed in the ratio 56:44 v/v (Where mobile phase A consists of potassium dihydrogen phosphate buffer of pH 3.0 and Mobile phase B consists of acetonitrile and methanol mixed in the ratio of 90:10 v/v) used at a flow rate of 0.4ml per minute.

Results: The retention times for losartan potassium and chlorthalidone were observed at 0.72 and 1.89 minutes. The developed method was validated as per ICH guidelines. Linearity ranges were found to be 12.5-125 µg/ml and 3.125-31.25 µg/ml for losartan potassium and chlorthalidone, respectively.

Conclusion: This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

Introduction

Losartan potassium (LSP), an angiotensin II receptor antagonist used in the treatment of hypertension. Losartan potassium is given to delay progression of diabetic nephropathy and also to reduce renal disease progression in patients with type 2 diabetes. Chlorthalidone (CLD), a thiazide diuretic used in the treatment of hypertension. Chlortalidone increases the excretion of sodium, chloride, and water into the renal lumen by inhibiting sodium ion transport across the renal tubular epithelium. Losartan and Chlorthalidone combination therapy is prescribed because they have complementary mechanism of action which allows for synergistic lowering of blood pressure and more over this combination therapy allows lower dosage requirements of each individual agent which leads to decreased side effects and improved compliance. The chemical structure of LSP and CLD were presented in Figure 1.

A number of analytical and bioanaytical methods have been reported for the estimation of LSP by using UV,[1,2] HPLC,[3-7] LC/MS/MS[8-11] and voltammetry.[12] A few of analytical methods have been reported for the estimation of chlorthalidone by using Potentiometry,[13] UV,[14] HPLC,[15] Capillary electrophorosis[16,17] and also by LC/MS/MS.[18]

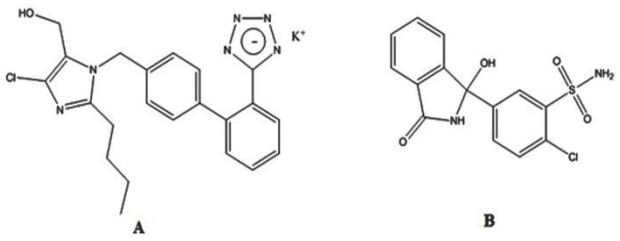

Figure 1. Chemical Structures of Losartan Potassium (A) and Chlorthalidone (B)

From the above literature, it was found that, there are no chromatographic methods available for the simultaneous estimation of losartan potassium and chlorthalidone in their combined dosage form, this work holds a challenge for developing a new method in ultra performance liquid chromatography. Moreover, among the existing liquid chromatographic methods, there exists no method in which LSP is eluted below 4 min. Hence UPLC was selected in order to reduce the elution time of both the drugs which in turn reduce the consumption of mobile phase and time of analysis.

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Materials and Methods

Instrumentation

Waters-Acquity UPLC system equipped with auto sampler, binary gradient pump, and PDA detector was used for the separation. An analytical column; HSS C18, (100 mm x 2.1x 1.8 µm) was used in the analysis. For data collection and processing Chromatographic software Empower -2 was used.

Chemicals and Reagents

Losartan potassium pure drug was obtained from Chemit laboratories, Hyderabad and chlorthalidone pure drug was obtained from Hetero Drugs, Hyderabad. The commercially available formulations of losartan potassium and chlorthalidone(ctd-L 25/6.25mg) were purchased from the local market. The HPLC grade water was obtained from Millipore. Acetonitrile and methanol of HPLC grade were obtained from E. Merck. (India) Ltd., Mumbai. Potassium dihydrogen phosphate and ortho phosphoric acid of analytical grade were purchased from Chempure pvt Ltd., India.

Preparation of standard solution

Stock solution (50µg/mL, 12.5 µg/mL) of losartan potassium and chlorthalidone was prepared by dissolving accurately weighed 25 mg of losartan potassium standard and 6.25mg of chlorthalidone standard into a 25ml volumetric flask, dissolved and made up to the volume. A series dilute solutions ranging from 12.5 to 125 µg/mL of losartan potassium and 3.125 to 31.25 µg/mL of chlorthalidone were prepared by taking different aliquots (0.125 to 1.25 mL) of the stock solution and diluted to 10ml with diluent in similar manner.

Preparation and sample solution

About 20 tablets of ctd-L were weighed and powdered and from that powder the amount of powder equivalent to 25mgof losartan potassium and 6.25mg of chlorthalidone was dissolved in 25 mL of diluent in a volumetric flask, sonicated and made up to the mark. Further working standard (50µg/mL ,12.5 µg/mL) of losartan potassium and chlorthalidone was prepared by transferring 0.5 mL of the stock solution into 10 mL volumetric flask and diluted up to the mark with diluent, sonicated and filter through 0.45 mm filter.

Results and Discussion

Optimisation of chromatographic method

The chromatographic separation was carried out under the isocratic conditions. The mobile phase was allowed to flow through the column at a flow rate of 0.4 mL/min for 3 min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 0.5µl of standard into HSS C18, (100 mm x 2.1x 1.8 µm) column .The mobile phase of composition 560 mL of solution A (1.36g of potassium dihydrogen phosphate buffer of pH 3.0) and 440ml mL of solution B (acetonitrile and methanol in 9:1 ratio) was allowed to flow through the column at a flow rate of 0.4 ml per minute for a period of 3.0 min. Detection of the component was carried out at a wavelength of 230 nm. The retention time of the components were found to be 0.72 and 1.89min for losartan potassium and chlorthalidone respectively (Figure 2). The system suitability parameters such as tailing factor and theoretical plate count were found to 1.36, 1.25 and 11040, 8325, respectively.

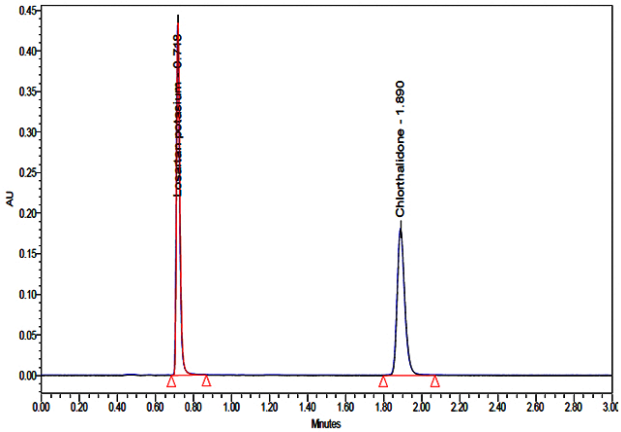

Figure 2. UPLC overlay chromatogram of Losartan Potassium and Chlorthalidone

Validation results of the method

Validation of the proposed method was done according to ICH[19] guidelines. In the repeatability study, the % relative standard deviation (%RSD) was 0.51 & 0.37 for the retention times of losartan potassium and chlorthalidone and 0.13 & 1.31 for the peak areas of both the drugs. In the intermediate precision which is the study of reproducibility of results in different days, the % relative standard deviation for the retention times and peak areas were 0.831, 0.523 and 0.522, 0.849 for both the drugs.

The limit of detection (LOD) and LOQ concentrations were estimated by using signal to noise ratio method.[20] At 3:10 S/N ratio the LOD and LOQ were found to be 497ng/mL and 1508 ng/mL for losartan potassium and 71 ng/ mL and 217 ng/ mL for chlorthalidone.

Good linearity was observed over the concentration range of 12.5 to 125 µg/ mL for losartan and 3.125 to 31.25 µg/ mL for chlorthalidone with correlation coefficient > 0.999 for both the drugs and data was presented in Table 1.

Table 1. Validation parameters of developed UPLC method for analysis of LSP and CLD

Parameters		LSP	CLD
Regression equation (y=mx+c)	Slope (m)	5919.3	2044.7
	Intercept (c)	8407.3	216.57
Correlation coefficient (r^2)		0.9994	0.9997
LOD (ng/mL)		497	71
LOQ(ng/mL)		1508	217
Precision (%RSD) (n=5)	Intra day	0.132	1.31
	Inter day	0.522	0.849

The standard addition and recovery experiments were conducted for both the drugs in triplicate at 50,100 and 150% of analyte concentration. The recovery was calculated from the slope and y-intercept of the calibration curve and the % recovery was ranged from 99.56% to 100.03% for losartan potassium and 98.73% to 100.34% for chlorthalidone (Table 2).

Table 2. Accuracy data

Drug name	%Concentration	Area	Amount added(µg/mL)	Amount found(µg/mL)	Mean %Recovery	%RSD
LSP	50%	452060	25	24.95	99.81	1.44
	100%	600403	50	50.01	100.03	1.10
	150%	746352	75	74.67	99.56	1.25
CLD	50%	38595	6.25	6.27	100.34	1.21
	100%	51002	12.5	12.34	98.73	0.41
	150%	89762	31.25	31.30	100.17	1.59

The chromatographic resolution of losartan potassium and chlorthalidone peaks was used to evaluate the method robustness under the modified conditions like change in flow rate, pH of the buffer and composition of organic phase. The resolution between both the peaks was greater than 2 under all the tested conditions; also both the drug peaks passed the system suitability parameters.

No significant change in the losartan potassium and chlorthalidone drug content was observed during solution stability and mobile phase stability experiments. Hence, standard solutions and mobile phase were stable for upto 48hr during assay determination.

Conclusion

The proposed RP-UPLC method was found to be simple, fast, precise, accurate and rugged. Both the drugs were eluted below 2min hence reduces the run time and the mobile phase composition, made the method economical. The drugs were found to be stable throughout the assay period. Therefore the developed method can be used as an alternative method for routine analysis in quality control.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

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