IJPSR (2019), Volume 10, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 May 2018; received in revised form, 19 June 2018; accepted, 13 July 2018; published 01 January 2019

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND LOSARTAN IN TABLET DOSAGE FORM BY RP-HPLC

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Keywords:

Hydrochlorothiazide, Losartan, Hypertension, RP- HPLC, Method validation

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ABSTRACT: Fixed-dose combinations have several advantages comparing with a single drug and separate agents. For simple dosing regimens and their synergistic antihypertensive action, a combination of hydrochlorothiazide and losartan potassium is widely prescribed. In this study, an HPLC-UV method was developed and validated for the simultaneous determination of hydrochlorothiazide and losartan in bulk and pharmaceutical formulation. The method was optimized selecting chromatographic conditions of 60: 40 acetonitrile: water, ACE3-C18 column (250 mm × 4.6 mm 5 µm), 20 µl injection volume, the flow rate of 1 ml/min at ambient temperature (25 °C), and 226 nm. The method was validated giving good precision (RSD% < 1), acceptable linearity (R2 ≥ 0.997), and low LOD and LOQ (0.5 and 1.5 µg/ml, respectively). Successful application on pharmaceutical dosage tablet form gave recovery percent within acceptance criteria (92% and above) indicating that the proposed method is simple and reliable for the determination of LOP and HCTZ and hence can be applied for routine analysis in quality control laboratories.

INTRODUCTION: Hypertension or high blood pressure is a chronic medical condition in which the blood pressure in the arteries is elevated. It is one of the most prevalent vascular diseases and considered as the main risk factor for cardiovascular, cerebrovascular, and peripheral vascular diseases that include coronary disease, stroke, peripheral artery disease, renal disease, and heart failure. Treatment with many medications known as anti-hypertensives is available to lower high blood pressure, and they include some different classes of drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(1).227-31

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(1).227-31

Fixed-dose combinations have many benefits comparing with a single drug and separate agents. Clinical practices proved that using combinations of antihypertensive drugs with complementary mechanisms of action is a marked increase regarding effects to reduce blood pressure levels more rapidly, convenience, improving treatment compliance, and low-cost treatment of hypertension [1]. Combination of hydrochlorothiazide and losartan potassium is widely prescribed by the physicians due to simple dosing regimens and their synergistic antihypertensive action, improved hypertension control and fewer dose-dependent side effects [2]. So it is essential to develop a simple method for simultaneous estimation of HCT and LOP in a combined formulation. Hydrochlorothiazide chemical name is 2H-1, 2, 4-Benzothiadiazine-7-sulfonamide, 6-chloro-3, 4-dihydro-, 1, 1-dioxide **Fig. 1**.

It belongs to a class of drugs called as thiazide diuretics antihypertensive. Hydrochlorothiazide binds to and inhibits the carbonic enzyme anhydrase. It is frequently used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hypoparathyroidism, edema, prevention of kidney stones and used in the treatment of osteoporosis [3]. There are many published methods for determination HCTZ in tablets and biological samples such as spectrophotometric [4], TLC [5], voltammetry [6], GC [7], flow injection [8], polarography [9], and HPLC [10-13].

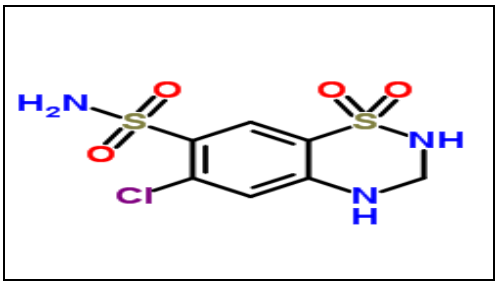

FIG. 1: CHEMICAL STRUCTURE OF HYDRO-CHLOROTHIAZIDE

Losartan potassium is (LOP) is chemically described as monopotassium salt of 4-butyl-4-chloro-1- [[2'-(1H-tetrazole-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-methanol **Fig. 2**. LOP is an angiotensin II blocker and used mainly to treat high blood pressure (hypertension). It is official in United States Pharmacopeia, British Pharmacopoeia, and Japanese Pharmacopoeia.

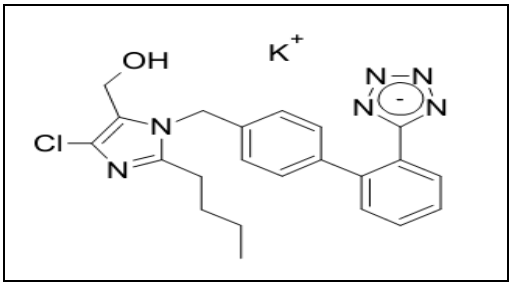

FIG. 2: CHEMICAL STRUCTURE OF LOSARTAN POTASSIUM

In reviewing the literature, various analytical methods were found for the determination of LOP whether alone or in its combination with other drugs in pharmaceutical preparations including spectrophotometry [14], potentiometry [15], voltammetry [16], capillary electrophoresis [17] and HPLC [18, 19, 20].

A marketed tablet of brand name Angizaar-H which is an antihypertensive formulation contains 12.5 mg hydrochlorothiazide and 50 mg losartan. Meaning, the quantity of hydrochlorothiazide in this combination is four times smaller than losartan, which makes analysis more complicated and tedious. The present study aimed to develop a simple and fast HPLC method for routine analysis of both drugs without tedious extraction procedure. The analytical method was validated according to international conference on Harmonization (ICH) guidance for validation of analytical procedure by examining the precision, the linearity of the calibration curve and calculating the limit of detection (LOD) and the limit of quantification (LOQ) [21].

MATERIALS AND METHODS:

Instruments: HPLC system comprised an LC-20AT Shimadzu equipped with LC-20AT pump and on-line degassing system DGU- 20A5 coupled with Flom manual sample injector (20 µl loop) and SPD-20A UV/visible detector and LC solution software. The analytical column was ACE3-C18 (250 mm × 4.6 mm, ultra pure silica). Quigg digital ultrasonic cleaner was used for the mobile phase. The spectrophotometric measurement was made on a Cecil-7200 UV- visible double beam spectrophotometer with 1 cm matched Quartz cells.

Chemicals: Pharmaceutically pure samples of hydrochlorothiazide and losartan potassium drugs were obtained from Awamedica Company (Erbil City - Kurdistan region of Iraq). Commercial tablet of Angizaar-H (losartan potassium USP 50 mg, HCTZ USP 12.5 mg, Micro labs limited) was brought from the local drug market. Acetonitrile was purchased from Romil SpS LTD (99.9%) for HPLC application. Double deionizer water filtrated with 0.2 µl cellulose filter paper was used to prepare standards solutions and mobile phase in this study.

Preparation the Mobile Phase: The mobile phase of HPLC analysis was prepared from the organic solvent of acetonitrile and water (v:v %) at different concentration of 30, 40 and 50% ACN.

Stock and Working Solutions: Stock solutions of 1000 µg/ml of each of HCTZ and LOP were prepared by dissolving 0.05 g of sample in 50 ml of

acetonitrile. The mixed standard solution was prepared by a dilute specific volume of each stock solution in a proper volumetric flask and make up the volume with mobile phase.

Sample Solution Preparation: 20 tablets were crushed to fine powder. An accurately weighed portion of the powder (equivalent to 37.16 µg/ml of HCTZ and 148.64 µg/ml of LOP) was taken and dissolved in 100 ml solvent of acetonitrile.

RESULTS AND DISCUSSION:

Development and Optimization of the HPLC Method: An isocratic programming was employed by analyzing a solution of 20 µg/ml at a different concentration of mobile phase (v/v) of ACN and water. The HPLC chromatogram in **Fig. 3** shows the analysis of 20 µg/ml solution of a mixture of LOP and HCTZ under isocratic chromatographic conditions.

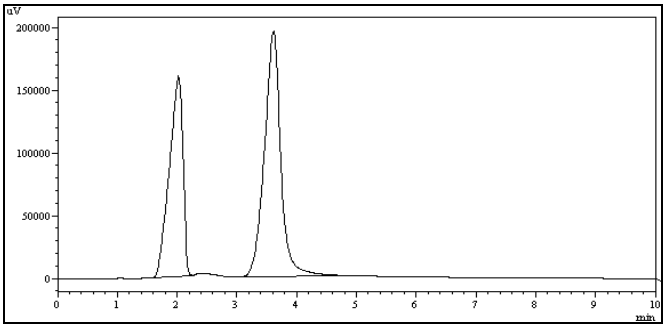

FIG. 3: CHROMATOGRAM OF MIXTURE OF 20 µg/ml LOP AND HCTZ

A good separation of two peaks was obtained at 40% strength of the mobile phase, although the chromatogram showed impurity peak that has little effect on the background of the baseline of the LOP peak, this impurity peak can be considered particularly at a very low concentration of LOP. The best chromatographic parameters for the proposed method were summarized in **Table 1**.

TABLE 1: CHROMATOGRAPHIC CONDITIONS OF PROPOSED METHOD

Parameter	HPLC System
Column	ACE3-C18 (250 mm × 4.6 mm, ultra pure slica)
Detector	SPD-20A UV/visible
Wavelength detection	226 nm
Mobile phase	40 % ACN
Flow rate	1 ml/min
Chromatographic run	10 min
LOP retention time	2.1 min
HCTZ retention time	3.6 min
Injection volume	20 µl
Column temperature	ambient temperature (25 °C)

Method Validation: Validation of the analytical method before determination of LOP and HCTZ in dosage sample was done by examining such parameters:

Precision: The precision of the method was estimated by repeated injections (n = 5) of 1 µg/ml of a mixture of LOP and HCTZ. The proposed method achieved good precision reporting RSD% values of the peak area of less than 1% as shown in **Table 2**.

TABLE 2: RSD% VALUES OF FIVE REPLICATE INJECTIONS OF 1 µg/ml OF MIXTURE OF LOP AND HCTZ

Injection no.	LOP peak area	HCTZ peak area
1	136553	202849
2	138453	202215
3	136053	203700
4	136475	203856
5	134998	205141
Mean	136506	203552
STDEV	1252	1109
% RSD	0.92	0.54

Linearity: Calibration curve was constructed for HCTZ and LOP standard by plotting the concentration of each drug versus peak area response at two ranges of 2-10 and 5-50 µg/ml. Using Excel® software, the coefficient of determination (R^2) was obtained from the regression line to statistically assess the linearity of the method.

The results illustrate good linearity between the peak area and the concentrations of the standard solutions of two components giving the coefficient of determination (R^2) ≥ 0.997 over the selected ranges of concentrations.

Limit of Detection and Limit of Quantification: The limit of detection **(**LOD) and limit of quantification (LOQ) of HCTZ and LOP in the present method were calculated statistically based on the data from the calibration curve at a low level of 2-10 µg/ml using the following equations:

$$LOD = 3.3\ SD/S$$

$$LOQ = 10\ SD/S$$

Where, SD is the standard deviation of the response and S is the slope of the calibration curve. The results in **Table 3** show close values of LOD and LOQ of both components.

TABLE 3: LOD AND LOQ VALUES OBTAINED FROM THE CALIBRATION CURVE OF LOWER CONCENTRATIONS (2-10 µg/ml)

Parameter	LOP	HCTZ
Slope	124626.5	192826.6
Intercept	7738.6	15689.7
SD	18352.57314	27147.15385
LOD peak area	62796.31942	105275.3
LOQ peak area	191264.3	287161.2385
LOD	0.4 µg/ml	0.5 µg/ml
LOQ	1.5 µg/ml	1.4 µg/ml

System Suitability: System suitability was applying by testing such characteristics including capacity factor, tailing factor, theoretical plates and resolution. The results obtained are presented in **Fig. 3** and **Table 4** showing acceptance criteria set in method validation.

TABLE 4: SYSTEM SUITABILITY OF LOP AND HCTZ BY PROPOSED METHOD

Characteristic	LOP	HCTZ
Capacity factor	0.92	2.44
Tailing factor	0.74	1.03
Theoretical plates	1324	3844
Resolution		3.51

Recovery: The accuracy of the proposed method was determined by average recovery % of HCTZ and LOP in their pharmaceutical preparation as tablets (50 mg losartan potassium 12.5 mg and HCTZ) by triplicate injections under the same chromatographic conditions of the proposed method. As shown in **Table 5**, the results gave recovery percent within acceptance criteria (90% and above) indicating that the proposed method is suitable and reliable for the determination of LOP and HCTZ in pharmaceutical dosage forms.

TABLE 5: THE PERCENT RECOVERY (n = 3) OF DOSAGE SAMPLE

		Peak area of recovered amount	Peak area of injected amount	Mean of Recovery	RSD %
LOP	Injection 1	951619	1032785	92	0.2
	Injection 2	948405			
	Injection 3	950222			
HCTZ	Injection 1	336508	349640	96	1.0
	Injection 2	335909			
	Injection 3	330030			

CONCLUSION: An isocratic HPLC-UV method for the determination of LOP and HCTZ was developed and validated. Optimizing the method using an ACE3-C18 (250 mm × 4.6 mm, ultra pure silica) column showed reliable chromatography conditions of 40% ACN of mobile phase strength, 226 nm wavelength detection, 20 µl injection volume, 1 ml/min flow rate and retention time of 2.1 and 3.6 min of LOP and HCTZ respectively at a run time of 10 min. The proposed method was validated and offered acceptable precision (RSD % < 1), good linearity ($R^2 \geq 0.997$) and low values of LOD (~ 0.5 µg/ml) and LOQ (~ 1.5 µg/ml) of both components. The proposed method was applied to pharmaceutical formulation sample giving recovery of 92 and 96% of LOP and HCTZ respectively.

ACKNOWLEDGEMENT: This research was supported by Duhok environmental directorate, Duhok, Kurdistan, Iraq, particularly, Nidhal S. Mohammed would like to thank them for their help during this study.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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How to cite this article:
Mohammed NMS, Abdo HR and Hassan HM: Method development and validation of simultaneous determination of hydrochlorothiazide and losartan in tablet dosage form by RP-HPLC. Int J Pharm Sci & Res 2019; 10(1): 227-31. doi: 10.13040/IJPSR.0975-8232.10(1).227-31.