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Stability indicating RP-HPLC method for estimation of Hydrochlorothiazide and Clonidine hydrochloride in bulk and formulation

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ABSTRACT

An isocratic, reverse phase liquid chromatographic method was developed for the quantitative determination of Hydrochlorothiazide and Clonidine HCl in combined dosage form. A thermo Agilent zorebax sb (250 X 4.6 mm; 5 µ) C_{18} column with mobile phase containing methanol: ortho phosphate buffer in the ratio of (40:60 %) was used. The flow rate was 1.0 ml/min, column temperature was 30 °C and effluents were monitored at 217 nm. The retention times of Hydrochlorothiazide and Clonidine HCl were 2.753 min and 1.968 min, respectively. Correlation co-efficient for Hydrochlorothiazide and Clonidine HCl was found to be 0.9999 and 0.9999, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Mean recovery of Hydrochlorothiazide and Clonidine HCl in formulations was found to be in the range of 99.9-100.22 % and 100.01-100.10 %, respectively confirms the non-interferences of the excipients in the formulation. The method was successfully applied to the simultaneous estimation of Hydrochlorothiazide and Clonidine HCl in combined dosage form due to its simplicity, rapidness and high precision.

Keywords: RP-HPLC, Hydrochlorothiazide, Clonidine HCl and Simultaneous estimation

INTRODUCTION

Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Fig. 1). It is a diuretic drug of the thiazide class that acts by inhibiting the kidneys ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. It is frequently used in the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones. In addition, it is one of the most important medications needed in a basic health system listed by World Health Organization (Beermann et al., 1976; Duarte et al., 2010).

Clonidine HCl is chemically 2-[(2,6-dichlorophenyl)amino-2-imidazoline hydrochloride (Fig. 2). Clonidine (trade names Catapres, Kapvay, Nexiclon, Clophelin, and others) is a sympatholytic medication used to treat high blood pressure, attention-deficit/hyperactivity disorder, anxiety disorders, withdrawal (from alcohol, opioids, or smoking), migraine, menopausal flushing, diarrhoea, and certain pain conditions. It is classified as a centrally acting α_2 adrenergic agonist and imidazolin receptor agonist that has been in clinical use for over 40 years (Lowenthal et al., 1988; Neil, 2011).

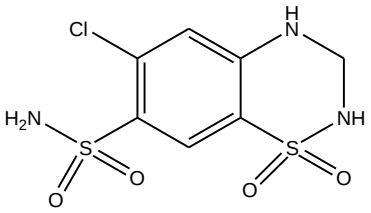

Figure 1: Structure of Hydrochlorothiazide

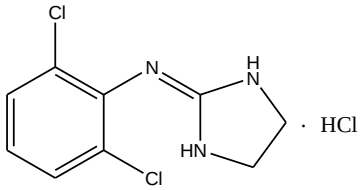

Figure 2: Structure of Clonidine HCl

The drug analysis plays an important role in the development of drugs, manufacturing and therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specification. The literature review indicates there are several analytical methods have been reported for estimation of these drugs as individual or in combination with other drugs, and also several analytical methods for the determination of simultaneous estimation of Hydrochlorothiazide and Clonidine HCl by HPTLC, UPLC and UV in dosage formulation and its bio-analytical applications (Walters and Stonys, 1983; Leena et al., 2007; Wankhede et al., 2007; Rim Said Haggag, 2011; Kamepalli Sujana, 2012; Anandkumar et al., 2013; Khushboom Gondaliya et al., 2014). Hence the main objective of this study is to develop a stability indicating RP-HPLC method for simultaneous estimation of Hydrochlorothiazide and Clonidine HCl & validate the developed method according to ICH guidelines by using various parameters.

MATERIALS AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and thermo Ajilent zorebax sb (250 X 4.6 mm, 5 µ) C_{18} column.

Chemicals and reagents: Hydrochlorothiazide and Clonidine HCl were obtained as a gift sample from Lara drugs, Hyderabad. Ortho phosphate AR grade were purchased from E. Merck (India) Ltd., Mumbai and milli Q water.

HPLC conditions: The mobile phase consisting methanol & ortho phosphate buffer (pH 2.4) and were filtered through 0.45 µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 40:60 % into the column at a flow rate of 1.0 ml/min. The column temperature was 30 °C. The detection was monitored at 217 nm and the run time was 5 min. The volume of injection loop was 20 µl; prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of standard solution: An accurately weighed quantity of 80 mg of Hydrochlorothiazide and 0.4 mg of Clonidine HCl were transferred into a 50 ml volumetric flask. Dissolved in distilled water and sonicated for 30 min. Finally the volume was made using distilled water. (1600 µg/ml of Hydrochlorothiazide and 8 µg/ml of and Clonidine HCl). From the standard stock solution 5 ml is pipetted out into 25 ml volumetric flask and made up the volume with distilled water (320 µg/ml of Hydrochlorothiazide and 1.6 µg/ml of and Clonidine HCl).

Preparation of sample solution: Twenty tablets were accurately weighed and ground to a fine powder. An 700.16 mg of powder equivalent to 0.4 mg of Clonidine HCl and 80 mg of Hydrochlorothiazide were weighed accurately and transferred into a 50 ml volumetric flask Dissolved in distilled water, sonicated for 30 min and madeup with distilled water and then the solution was filtered through 0.45 µm membrane filter. 5 ml of filtrate was taken into 25 ml volumetric flask and made up to the volume with mobile phase.

Procedure: 20 µl of the filtered portion of sample and standard preparations were injected into the chromatograph. The responses for the major peaks were recorded and the content of Hydrochlorothiazide and Clonidine HCl were calculated.

Validation parameters: For the above method all of the analytical validation parameters were determined according to ICH guidelines (USFDA, 1995; USFDA, 1996; ICH, 1996; USFDA, 2000; ICH, 2001). Obtained validation parameters are presented in Table 2.

System suitability: Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated.

Accuracy: Accuracy was determined in terms of % recovery. Sample solutions were prepared at three different concentration levels 50 %, 100 % and 150 %. Predetermined amount of standard was added to these solutions by spiking standard drug solution to the sample. % recovery was calculated by assaying these solutions.

System precision, method precision and intermediate precision: The system, method and intermediate precision of the proposed method are ascertained by injecting 6 replicates of test and standard sample, % RSD were calculated.

Specificity: Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

Linearity: A linear relationship was evaluated across the range of the analytical procedure. A series of standard dilutions were prepared from the working standard solution in the concentration range of 160-480 µg/ml for the Hydrochlorothiazide and 0.8-2.4 µg/ml for Clonidine HCl, respectively. 20 µl of each solution was injected into HPLC system. Linearity is evaluated by plotting the peak area as a function of analyte concentrations.

Robustness: Robustness was carried out by changing small variations in method parameters like flow rate (± 0.2 ml), wavelength (± 2 nm), mobile phase (± 5 % v/v) and temperature (± 5° C). Ruggedness wad done by studying changes with variation of analyst**.**

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were determined for Hydrochlorothiazide and Clonidine HCl.

Procedure for forced degradation studies: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. Further forced degradation studies were conducted for indicating the stability of the method developed. The results of the degradation studies are presented in Table 2.

Acid degradation: 700.16 mg of sample equivalent to 80 mg Hydrochlorothaizide & 0.4 mg of Clonidine HCl was taken into 50 ml volumetric flask & added 10 ml of 0.1N HCl. Then sonicated for 30 min & added 10 ml of 0.1N NaOH for neutralization & diluted volume with distilled water. Transferred 5 ml in 25 ml volumetric flask & made up volume with distilled water to get 100 % solution.

Base degradation: 700.16 mg of sample equivalent to 80 mg Hydrochlorothaizide & 0.4 mg of Clonidine HCl was taken into 50 ml volumetric flask & added 10 ml of 0.1N NaOH. Then sonicated for 30 min & added 10 ml of 0.1N HCl for neutralization & diluted volume with distilled water. Transferred 5 ml in 25 ml volumetric flask & made up volume with distilled water to get 100 % solution.

Peroxide degradation: 700.16 mg of sample equivalent to 80 mg Hydrochlorothaizide & 0.4 mg of Clonidine HCl was taken into 50 ml volumetric flask & added 10 ml of 1 % peroxide. Then sonicated for 30 min & diluted volume with distilled water. Transferred 5 ml in 25 ml volumetric flask & made up volume with distilled water to get 100 % solution.

Thermal degradation: Sample was put in oven for 1 h at 60 ºC. Above sample 700.16 mg equivalent to 80 mg Hydrochlorothaizide & 0.4 mg of Clonidine HCl was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with distilled water. Transferred 5 ml in 25 ml volumetric flask & made up volume with distilled water to get 100 % solution.

UV degradation: Sample was put in sun light for 1 day. Above sample 700.16 mg equivalent to 80 mg Hydrochlorothaizide & 0.4 mg of Clonidine HCl was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with distilled water. Transferred 5 ml in 25 ml volumetric flask & made up volume with distilled water to get 100 % solution.

RESULTS AND DISCUSSION

The preliminary studies indicated that the desired system suitability parameters were obtained with the mobile phase containing methanol: ortho phosphate buffer (pH 2.4) in the ratio of (40: 60 %). The mobile phase eluted the drug at lower retention times (2.753 and 1.968 min for Hydrochlorothaizide and Clonidine HCl, respectively). The corresponding chromatogram was shown in the Fig. 3 & 4 and the datas are presented in Table 1.

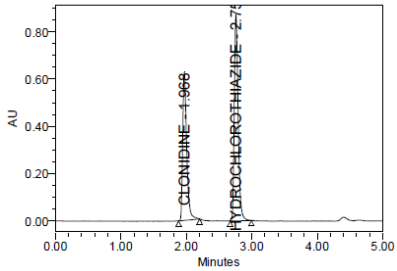

Figure 3: Standard chromatogram for Hydrochlorothiazide and Clonidine HCl

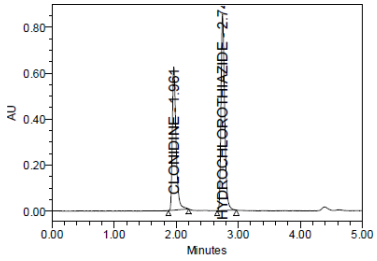

Figure 4: Formulation chromatogram for Hydrochlorothiazide and Clonidine HCl

Table 1: System suitability parameters

System suitability parameters	Hydrochlorothiazide	Clonidine HCl
Resolution	7.45	
Tailing factor	1.31	1.46
Number of theoretical Plates	11791	5526
Retention time	2.75	1.96

The % RSD in precision, accuracy and robustness studies were found to be less than 2.0 %, indicating that the method is precise, accurate and robust. The data are shown in Table 2.

The Rt of Hydrochlorothaizide and Clonidine HCl of standard solution and sample solution are identical. Moreover, the blank solution and placebo solution doesn't produce any peak. Hence the proposed analytical method is specific for the simultaneous estimation of Hydrochlorothaizide and Clonidine HCl.

The linearity for HPLC method was determined at five concentration levels ranging from 160-480 µg/ml for the Hydrochlorothiazide and 0.8-2.4 µg/ml for Clonidine HCl, respectively**.** The calibration curve was constructed by plotting response factor against respective concentration of Hydrochlorothaizide and Clonidine HCl. The plots of peak area Vs respective concentration of Hydrochlorothaizide and Clonidine HCl were found to be linear in the range of 160-480 µg/ml and 0.8-2.4 µg/ml with coefficient of correlation (r^2) 0.9999. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Hydrochlorothaizide and Clonidine HCl were given in Figure 5 and Figure 6.

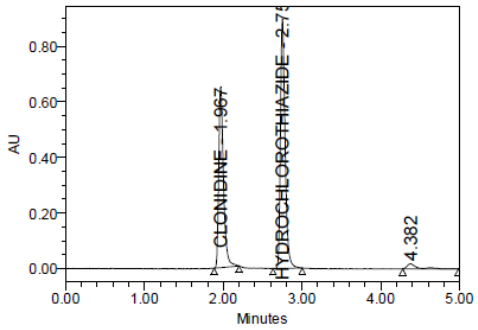

Figure 7a: Chromatogram of acid degradation

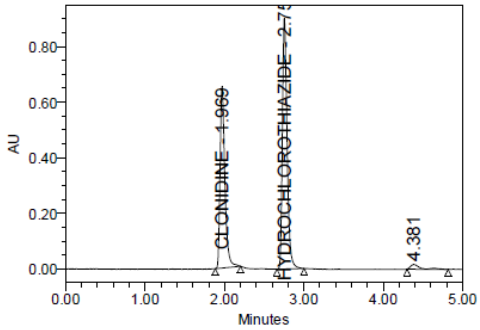

Figure 7b: Chromatogram of base degradation

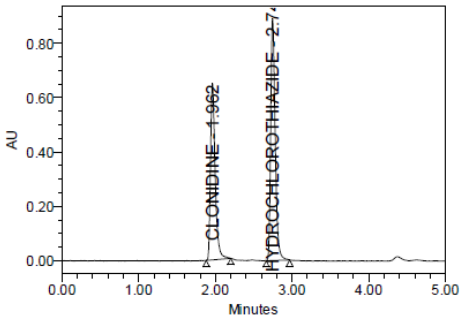

Figure 7c: Chromatogram of peroxide degradation

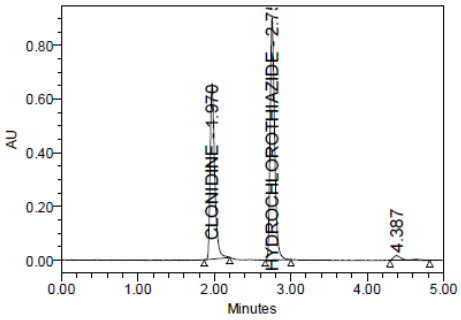

Figure 7d: Chromatogram of thermal degradation

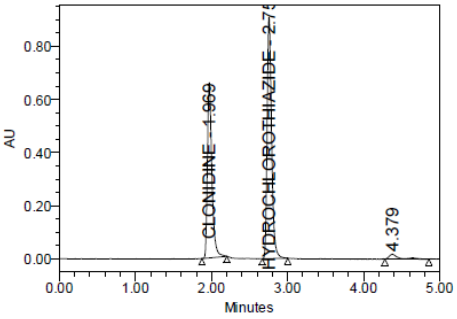

Figure 7e: Chromatogram of UV degradation

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate, sensitive and stability indicating for the simultaneous estimation of Hydrochlorothiazide and Clonidine HCl in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Hydochlorthazide and Clonidine HCl in pure and its pharmaceutical dosage forms.

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