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VALIDATED SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND CANDESARTAN CILEXETIL IN TABLET DOSAGE FORM

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ABSTRACT
A method for the simultaneous determination of hydrochlorothiazide and candesartan cilexetil in bulk and tablet dosage form was developed. The method employs formation and solving of simultaneous equations using 225.8 and 255 nm as two analytical wavelengths. The absorbance maxima of hydrochlorothiazide and candesartan cilexetil were found to be 225.8 nm and 255 nm respectively in methanol. The linearity range lies between 1-7 µg/ml for hydrochlorothiazide and 2-10 µg/ml for candesartan cilexetil at their respective wavelength. Both the drugs obey Beer's law. The molar absorptivity and sandell's sensitivity were found to be 4.11 x 10^4 and 0.0073 respectively for Hydrochlorothiazide and for Candesartan cilexetil 6.21 x 10^4 and 0.0098 respectively. The recovery studies confirmed the accuracy of the developed method.

Keywords: *Simultaneous equation; simultaneous estimation; Hydrochlorothiazide; Candesartan cilexetil*

INTRODUCTION

Hydrochlorothiazide, 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a thiazide diuretic that inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. A survey of literature reveals that various HPLC [1-6] (in plasma) and spectrophotometric methods have been reported for the estimation of Hydrochlorothiazide.

Candesartan cilexetil, (±)-1-[[Cyclohexyloxy) carbonyl]oxy]ethyl ester of 2-ethoxy-1-[[22 -(1*H*-tetrazol-5-yl)[1,12 -biphenyl]-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid, is an angiotensin II receptor antagonist used for the management of hypertension, treatment of diabetic nephropathy, treatment of congestive heart failure. Methods such as HPTLC andHPLC[7-11] have been reported in the literature.

Even though various methods were reported in the literature for estimation of hydrochlorothiazide and candesartan cilexetil individually or in combination with other drugs no method had been reported for simultaneous estimation of these two drugs using simultaneous equations in bulk drug and dosage form. The present study was aimed at simultaneous estimation of hydrochlorothiazide and candesartan cilexetil by simultaneous equation method. This method was validated according to the ICH guidelines[12].

MATERIAL AND METHODS

Instrument

Shimadzu UV-1800; UV spectrophotometer with Spectral bandwidth of 1.8 nm, wavelength accuracy of 2 nm and Matched quartz cells of 10 mm optical path length.

Drug Sample

Hydrochlorothiazide and Candesartan cilexetil were obtained as gift sample from Zydus Cadila Healthcare Pvt. Ltd., Ahmedabad (Gujarat). The tablets were procured from the market.

Chemicals and Reagents

Methanol G.R grade was procured from Loba Chem. Ltd., Mumbai.

Procedure

Hydrochlorothiazide (25 mg) and candesartan cilexetil (25 mg) were dissolved separately in methanol (50 ml) and volume made up to 250 ml with methanol to get a stock solution of 100 µg/ml. From these stock solutions, working standard solutions were prepared. These were scanned in the entire UV range to determine the λ max. The λ max of hydrochlorothiazide and candesartan cilexetil were found to be 225.8 nm and 255 nm respectively. The overlain spectra of both the drugs are shown in Figure 1.

The regression analysis of the calibration curves suggests the level of precision of the method and the optical characteristics such as Beer's law limits, detection limit, molar absorptivities and Sandell's sensitivities as presented in Table 1.

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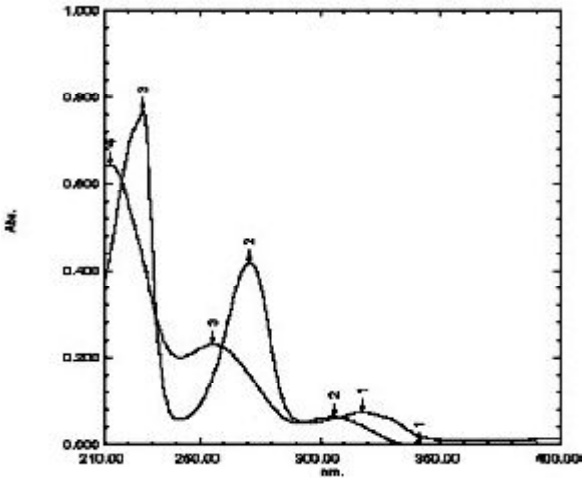

Fig. 1: *Overlain spectra of Hydrochlorothiazide and Candesartan Cilexetil*

Table 1: *Optical and Regression Characteristics of Hydrochlorothiazide and Candesartan Cilexetil*

Parameters	Hydrochlorothiazide		Candesartan cilexetil	
	225.8 nm	255 nm	255 nm	225.8 nm
Beer's law limit (µg/ml)	1-7	1-7	2-10	2-10
Molar absorptivity (l mole^{-1} cm^{-1})	$4.1079 * 10^{3}$	$0.8050 * 10^{3}$	$6.2059 * 10^{3}$	$2.8617 * 10^{3}$
Sandell's sensitivity (mg/cm^{2}/0.001 absorbance unit)	$7.297 * 10^{-2}$	$3.69 * 10^{-2}$	$9.84 * 10^{-2}$	$2.134 * 10^{-2}$
Regression equation (y = a + bc) Slope (b) Intercept (a)	0.131 0.0426	0.0238 0.0168	0.0984 0.0159	0.0451 0.0105
Correlation coefficient	0.9996	0.9998	0.9999	0.9999

Preparation of API mixture of hydrochlorothiazide and candesartan cilexetil

The API mixture and synthetic mixture of hydrochlorothiazide and candesartan cilexetil were prepared in ratio of 4:5. For API mixture, accurately weighed 20 mg of hydrochlorothiazide and 25 mg of candesartan cilexetil were transferred to a 250 ml volumetric flask, dissolved and diluted upto the mark with methanol. The API mixture was based upon the dosage strength of combination, which is available in the market.

Preparation of calibration curve for hydrochlorothiazide and candesartan cilexetil

Standard solutions of hydrochlorothiazide (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml) and standard solutions of candesartan cilexetil (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml) were transferred to a series of 10 ml volumetric flasks. The volumes in each were adjusted with methanol. The absorbances of the solutions were measured at 225.8 nm and 255 nm against methanol as blank.

Estimation of hydrochlorothiazide and candesartan cilexetil in API mixture

The API mixtures solution were transferred and diluted to mark with methanol. The absorbances of these mixtures were measured at 225.8 nm and 255 nm.

Amounts of hydrochlorothiazide and candesartan cilexetil were determined by solving the simultaneous equations. Two simultaneous equations were formed using absorptivity coefficient values.

$$C_1 = \frac{A_1 \times 0.062817 - A_2 \times 0.041136}{0.005265} \quad \text{— (1)}$$

$$C_2 = \frac{A_1 \times 0.121136 - A_2 \times 0.056084}{0.005265} \quad \text{— (2)}$$

Where C_1 and C_2 are concentrations of hydrochlorothiazide and candesartan cilexetil respectively, in gm/liter in the sample solution. A_1 and A_2 are the absorbances of the mixture at 225.8 nm and 255 nm respectively.

Estimation of Hydrochlorothiazide and Candesartan cilexetil in tablet dosage form.

Twenty tablets (of same respective batch number) were accurately weighted and quantity of powder equivalent to 4 mg of Hydrochlorothiazide and 5 mg of Candesartan Cilexetil was separately transferred to 100 ml volumetric flask and then dissolved in 50ml of methanol. The solution was sonicated for 10 minutes thereafter volume was made up to 100 ml with methanol. The solution was filtered through whatman filter paper no. 40. From the filtrate 1ml was pipetted out in 10 ml volumetric flask and diluted to mark with methanol. The absorbance of this solution was measured at 225.8 and 255 nm against methanol as blank. Results are shown in Table 2.

Table 2: *Results of Determination of Hydrochlorothiazide and Candesartan Cilexetil in Marketed Tablet Dosage Form*

Brand	Drug Name	Label claim (mg/cap)g	Amount Found (mg/cap)	% Recovery	Mean ± S.D.	% C.V.
1	HCTZ	12.5	12.39	99.12	HCTZ	HCTZ
	CANDE	16	15.88	99.25	98.96 ± 0.1665	0.1682
2	HCTZ	12.5	12.35	98.8		
	CANDE	16	15.91	99.44		
3	HCTZ	12.5	12.38	99.04	CANDE	CANDE
	CANDE	16	15.87	99.18	99.29 ± 0.1305	0.1314

* *Average ± standard deviation of three determinations*

Recovery Studies and Validation of the Method according to ICH Q2A Guidelines

To study the validation parameters; accuracy, reproducibility, reliability, interference and recovery experiments were carried out by standard addition. The

recovery of added standard (80%, 100%, and 120%) was found at four same concentration levels for each drug. From the total amount of drug found, the percentage recovery was calculated. From the total amount of drug found, the percentage recovery was calculated.

RESULTS AND DISCUSSION

The molar absorptivity and Sandell's sensitivity values show the sensitivity of hydrochlorothiazide and candesartan cilexetil at respective wavelengths, while precision is confirmed by % RSD. The reproducibility, repeatability and accuracy of these methods were found to be good, evidenced by low standard deviation.

Thus, the proposed method for simultaneous estimation of hydrochlorothiazide and candesartan cilexetil in bulk and tablet dosage form was found to be simple, accurate, sensitive and economical. Therefore, the method can be useful in routine quality control analysis.

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