

Simultaneous Derivative Spectrophotometric Determination of Candesartan Cilexetil and Hydrochlorothiazide

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ABSTRACT

A new simple derivative spectrophotometric method has been developed for the simultaneous determination of Candesartan Cilexetil and Hydrochlorothiazide in tablet dosage forms. Candesartan antagonizes the effect of angiotensin II (vasoconstriction and aldosterone secretion) by blocking the angiotensin II receptor in vascular smooth muscle and the adrenal gland and decrease the blood pressure whereas Hydrochlorothiazide increases chloride, sodium and water excretion by interfering with transport of sodium ions across renal tubular epithelium. And there by show the diuretic action. The first derivative method is based on the measurement of absorbance of one drug at the zero crossing point of another drug. Candesartan Cilexetil and Hydrochlorothiazide were determined at two different wavelengths 222.69 (zero crossing point of Hydrochlorothiazide) and 254.63 nm (zero crossing point of Candesartan Cilexetil) from the derivative spectra respectively. The methods shows linearity over the concentration range 0.5-50 and 0.1-50 $\mu\text{g/ml}$ for Candesartan Cilexetil and Hydrochlorothiazide respectively in phosphate buffer. The proposed method was validated and

can be used for routine analysis of combined tablet dosage forms containing Candesartan Cilexetil and Hydrochlorothiazide.

Key words: Candesartan Cilexetil, Derivative spectroscopy, Hydrochlorothiazide, Simultaneous determination.

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DOI: 10.5530/phm.2015.6.23

INTRODUCTION

Candesartan Cilexetil¹ is used as an angiotensin II receptor antagonist. Hydrochlorothiazide (HTZ) is a diuretic used for the treatment of high blood pressure and swelling due to fluid build-up.² Candesartan is also available in combination with a low dose of thiazide diuretic, hydrochlorothiazide to achieve an additive antihypertensive effect. According to the previous literature LC-MS/MS,³ UPLC-MS/MS,⁴ HPTLC,⁵ HPLC⁶⁻¹⁶ and spectrophotometric¹⁷⁻⁹ methods were developed for the simultaneous estimation of Candesartan Cilexetil and Hydrochlorothiazide in human plasma as well as in tablet dosage forms. In the present the authors have proposed a derivative spectrophotometric method for the simultaneous determination of CAN and HTZ and the method was validated.

MATERIALS AND METHODS

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm path length matched quartz cells. For scanning, the wave length range selected was 400 nm to 200 nm with medium scanning speed. All weights were taken using electronic balance (Shimadzu, Japan). All experiments were performed at room temperature (25 ± 1)°C.

Chemicals and reagents

CAN and HTZ were obtained as gift samples from Dr. Reddy's Labs (India). Methanol (MERCK), Di-Hydrogen phosphate (Rankem) and Potassium Di-Hydrogen phosphate (KH_2PO_4) (Rankem) were purchased and used as received. All the chemicals are of analytical grade. Both CAN and HTZ are available as a combined dosage form in the local market as tablets with trade names CANDESAR-H (16 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) (Ranbaxy Laboratories Ltd.;

India) and CANDELONG-H (8 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) (Micro Labs Ltd.; India)

Preparation of stock solution

Stock solutions (1000 $\mu\text{g/ml}$) of Candesartan Cilexetil and Hydrochlorothiazide were prepared by dissolving about 25 mg of each of Candesartan Cilexetil and Hydrochlorothiazide in two separate 25 ml volumetric flasks in methanol. Working standard solutions were prepared from the stock solution with phosphate buffer as per the requirement.

Preparation of phosphate buffer solution (pH 7.5)

6.8 gm of potassium di-hydrogen orthophosphate and 1.56 gm of sodium hydroxide were weighed accurately in a 1000 ml volumetric flask. First 900 ml of double distilled water was added to mix them thoroughly by Sonication for 15 minutes, then adjust the pH 7.5 with sodium hydroxide solution and dilute with water to produce 1000ml.

Validation

Linearity

A series of solutions containing Candesartan Cilexetil (0.5-50 $\mu\text{g/ml}$) and Hydrochlorothiazide (0.1- 50 $\mu\text{g/ml}$) were prepared and scanned (200- 400 nm) against their reagent blank. The absorption spectra was transformed into first order derivative spectra (D_1) by the inbuilt software.

Precision and Accuracy

The intra-day precision studies were carried out at three different concentration levels (5, 10 and 20 $\mu\text{g/ml}$) on the same day and the % RSD was calculated. The inter-day precision study was also performed at three different concentration levels on three different days i.e. day 1, day 2 and day 3 and the % RSD was calculated. The accuracy of the assay method was performed by spiking the formulation solution with the pre analysed pure drug solutions at three levels (80, 100 and 120%) and the percentage recoveries were calculated.

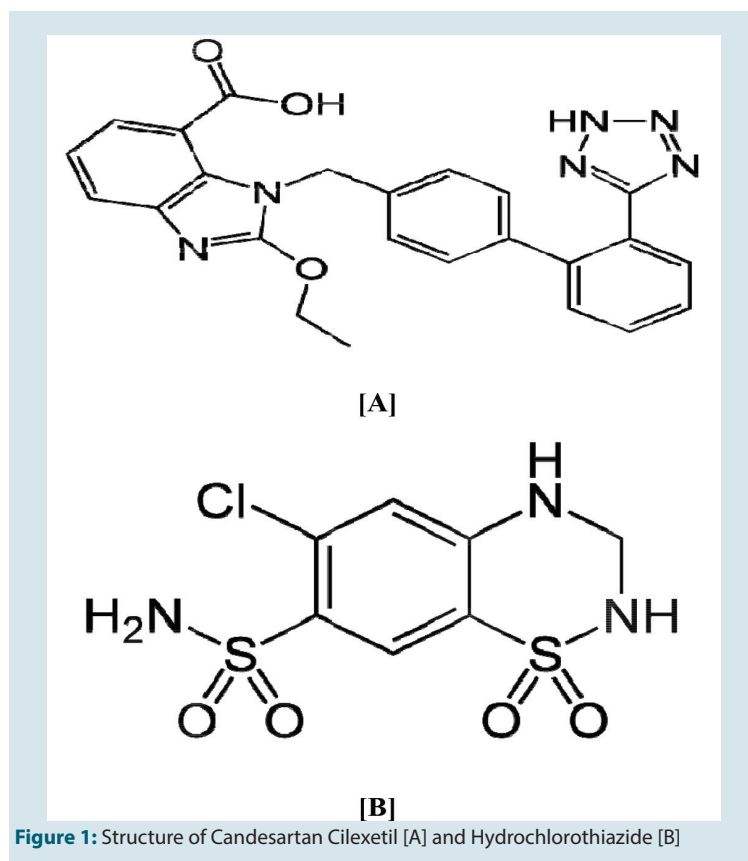
Table 1: Optical characteristics of Candesartan Cilexetil and Hydrochlorothiazide

Parameters	CAN	HTZ
Wavelength (nm)	222.53	255.69
Linearity range ($\mu\text{g/ml}$)	0.5-50	0.1-50
Regression equation	$y = 0.002x - 0.0003$	$y = 0.0024x + 0.0003$
Slope	0.002	0.0018
Intercept	0.0003	0.0003
Correlation coefficient	0.9996	0.9997
Precision (% RSD)		
Intra-day (n=3)	0.29-0.73	0.34-0.67
Inter-day (n=3)	0.49-0.94	0.57-0.63
Accuracy (% Recovery) (% RSD)	99.35-99.87 (0.28)	99.54-99.79 (0.49)

Table 2: Assay of commercial formulation

Brand	Labeled amount (mg)		Amount obtained (mg)*		% Recovery*		% RSD*	
	CAN	HTZ	CAN	HTZ	CAN	HTZ	CAN	HTZ
I	16	12.5	15.94	12.41	99.65	99.32	0.72	0.47
II	8	12.5	7.91	12.44	98.96	99.58	0.59	0.62

*Mean of three replicates



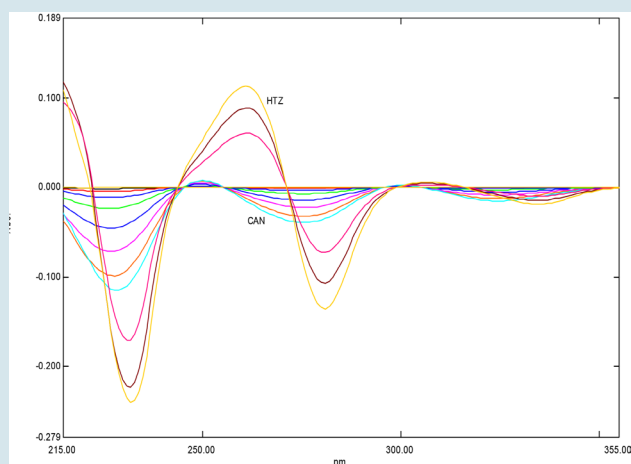


Figure 2: Overlay first derivative spectra of Candesartan Cilexetil and Hydrochlorothiazide

Assay of commercial formulations (Tablets)

The combined dosage forms of Candesartan and Hydrochlorothiazide (Tablets) are available with brand names CANDESAR-H (16 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) and CANDELONG-H (8 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) and were procured from the local pharmacy store. 20 tablets of each brand were weighed and powdered and powder equivalent w.r.t. 12.5 mg of Hydrochlorothiazide was taken and dissolved in a 100 ml volumetric

flasks containing methanol and sonicated for 30 minutes. The volume was made up to the mark with methanol and filtered. These solutions were further diluted with phosphate buffer as per the requirement for the two methods and the percentage purity was determined.

RESULTS AND DISCUSSION

The first order derivative spectrum of CAN shows zero crossing points at 208.36, 244.48, 254.63, 294.63 and 345.37 nm and that of HTZ at 222.69, 243.88, 271.34, 299.4, 316.42, and 350.75 nm. The overlay first order derivative spectrums of the CAN and HTZ was shown in Figure 2. CAN was determined at 222.69 nm which is one of the zero crossing points of HTZ in which the minima values were taken for the construction of calibration curve. Similarly HTZ was determined at 254.63 nm which is one of the zero crossing points of CAN in which the maxima values were taken for the construction of calibration curve.

A graph was drawn by taking the concentration on the x-axis and the corresponding derivative absorbance on the y-axis for Candesartan Cilexetil and Hydrochlorothiazide. Beer-Lambert's law was obeyed over the concentration range 0.5-50 µg/ml and 0.1-50 µg/ml for Candesartan Cilexetil and Hydrochlorothiazide respectively (Figure 3A and 3B) with linear regression equations $y = 0.002x - 0.0003$ ($R^2 = 0.9996$) and $y = 0.0024x + 0.0003$ ($R^2 = 0.9997$) for Candesartan Cilexetil and Hydrochlorothiazide respectively.

In the precision studies the % RSD was found to be 0.29-0.73 (Intra-day) and 0.49-0.94 (Inter-day) for CAN and for that of HTZ the % RSD was found to be 0.34-0.67 (Intra-day) and 0.57-0.63 (Inter-day) which is less than 2% indicating that the method is precise. In the accuracy studies the % recovery was found to be 99.35-99.87 (% RSD 0.28) and 99.54-99.79 (% RSD 0.49) for CAN and HTZ respectively indicating that the method is accurate. The Optical characteristics of Candesartan Cilexetil and Hydrochlorothiazide are shown in Table 1.

Assay of commercial formulations (Tablets)

The method was applied for the available marketed formulations with different brand names in which the % recovery was found to be 98.96-99.65 and 99.32-99.58 for CAN and HTZ respectively (Table 2).

CONCLUSION

The proposed validated derivative spectrophotometric method is simple, precise, accurate and can be applied for the simultaneous determination of Candesartan Cilexetil and Hydrochlorothiazide in pharmaceutical formulations successfully.

ACKNOWLEDGMENTS

The authors are grateful to M/s GITAM University, Visakhapatnam for providing the research facilities and (Ranbaxy Laboratories Ltd.; India) for providing the gift samples of Candesartan Cilexetil and Hydrochlorothiazide.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATION USED

HTZ: Hydrochlorothiazide; **LC-MS:** Liquid Chromatography Mass Spectroscopy; **UPLC:** Ultra Pressure Liquid Chromatography; **HPLC:** High Performance; **HPTLC:** High Pressure Thin Layer Chromatography; **CAN:** Candesartan Cilexetil; **UV:** Ultraviolet; **RSD:** Relative Standard Deviation; **ICH:** International Conference on Harmonization.

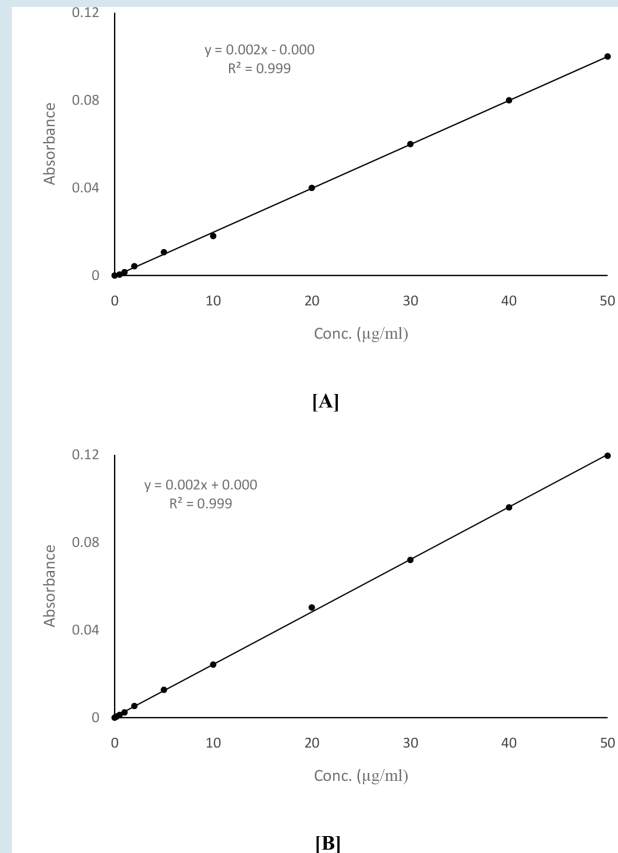
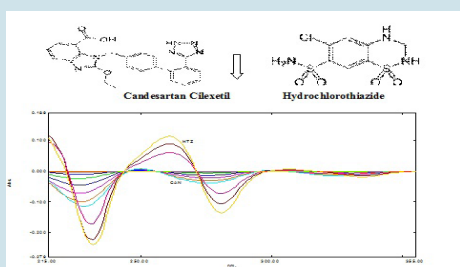


Figure 3: Calibration curves of Candesartan Cilexetil and Hydrochlorothiazide

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PICTORIAL ABSTRACT



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SUMMARY

- A simple first order derivative spectrophotometric method was developed for the simultaneous determination of Candesartan Cilexetil and Hydrochlorothiazide in tablets and validated.
- In this method each drug is determined at the zero crossing point of the other drug from the overlay derivative spectrum of Candesartan Cilexetil and Hydrochlorothiazide.

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