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## DETERMINATION OF HYDROCHLOROTHIAZIDE AND CANDESARTAN CILEXETIL IN TABLET DOSAGE FORMS BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

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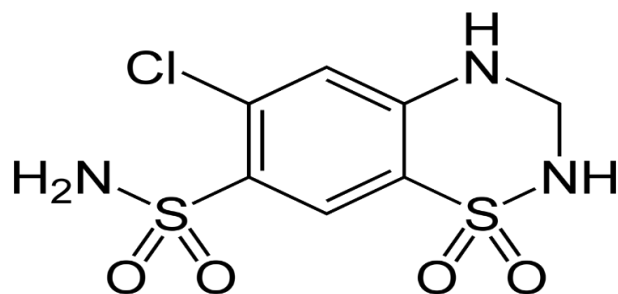
### Abstract

In this study the authors have developed a first order derivative spectrophotometric method for the determination of Hydrochlorothiazide and Candesartan Cilexetil in pharmaceutical dosage forms and validated. Candesartan cilexetil is an angiotensin II type 1 receptor antagonist and hydrochlorothiazide is a diuretic that belongs to thiazide class. The combination of these two drugs has been used in the treatment of hypertension. Candesartan Cilexetil was determined at the zero crossing point of Hydrochlorothiazide (272.69 nm) and Hydrochlorothiazide was determined at the zero crossing point of Candesartan Cilexetil (255.10 nm) from the first order derivative spectrum respectively. Linearity was observed over the concentration range 1-80 and 1-60 µg/ml for Candesartan Cilexetil and Hydrochlorothiazide respectively in borate buffer. The proposed method can be used for the analysis of dosage forms.

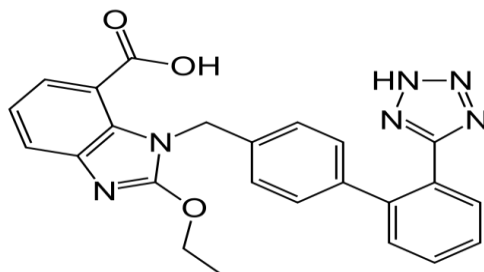
**Key Words:** Derivative spectroscopy, Hydrochlorothiazide, Candesartan Cilexetil, Borate buffer, Simultaneous determination.

### Introduction

Hydrochlorothiazide (HTZ) is a thiazide diuretic<sup>1</sup> and Candesartan Cilexetil (CAN) is an angiotensin II receptor antagonist<sup>2</sup>. The combination of these two drugs is used for the treatment of high blood pressure. Few analytical methods such as HPTLC<sup>3</sup>, LC-MS/MS<sup>4</sup>, UPLC-MS/MS<sup>5</sup>, HPLC<sup>6-16</sup> and spectrophotometric<sup>17-20</sup> were developed for the simultaneous determination of Candesartan Cilexetil and Hydrochlorothiazide in tablet dosage forms as well as in human plasma. In the present study the authors have developed a derivative spectrophotometric method for the simultaneous determination of CAN and HTZ and the method were validated.



[a]



[b]

**Figure 1: Structures of (a) Hydrochlorothiazide [HTZ] (b) Candesartan Cilexetil [CAN]**

## Materials and Methods

**Instrumentation:** Shimadzu double beam UV-VIS spectrophotometer (Model UV-1800) with spectral bandwidth of 1nm and wavelength accuracy of  $\pm 0.3$ nm was used throughout the spectral work. For scanning, the wavelength range selected was 400nm to 200nm with medium scanning speed. All weights were taken using electronic balance (Shimadzu, Japan) and all experiments were performed at room temperature ( $25 \pm 1$ )°C.

**Chemicals and reagents:** CAN and HTZ were obtained as gift samples from Dr. Reddy's Labs (India). Methanol (MERCK), Boric acid (Rankem) and sodium hydroxide (Merck) were purchased and used as received and all the chemicals are of AR grade. The combination of CAN and HTZ are available as tablet dosage forms with trade names CANDELONG-H (8 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) (Micro Labs Ltd.; India) and CANDESAR-H (16 mg Candesartan Cilexetil and 12.5 mg Hydrochlorothiazide) (Ranbaxy Laboratories Ltd.; India).

**Preparation of stock solution:** Stock solutions 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 and further diluted solutions were made with borate buffer solution as per the requirement.

### Preparation of borate buffer solution (pH 9.0)

6.20 g of boric acid was dissolved in 500 mL volumetric flask with water. This solution was transferred into a 1000ml volumetric flask along with 41.5 mL of 1M sodium hydroxide and diluted with water to 1000 mL to adjust pH 9.0.

## Method Validation<sup>21</sup>

### Linearity:

A series of solutions containing Candesartan Cilexetil (1-80 µg/ml) and Hydrochlorothiazide (1- 60 µg/ml) were prepared and scanned (200- 400 nm) against their reagent blank. The absorption spectra so obtained was transformed into first order derivative spectra (D<sub>1</sub>) by the inbuilt software and all analytical determinations were done from the derivative spectrum obtained.

### Precision and Accuracy

The intra-day precision studies were carried out at three different concentration levels (5, 10 and 20 µg/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 preanalysed pure drug solutions at three levels (80, 100 and 120%) and the percentage recoveries were calculated.

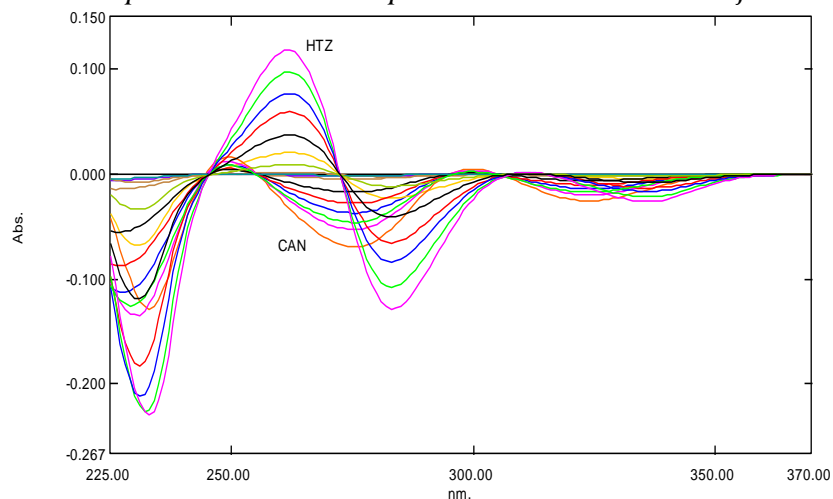
### Assay of marketed formulations (Tablets)

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 borate buffer solution as per the requirement for the two methods and the percentage purity was determined.

### Results and Discussion

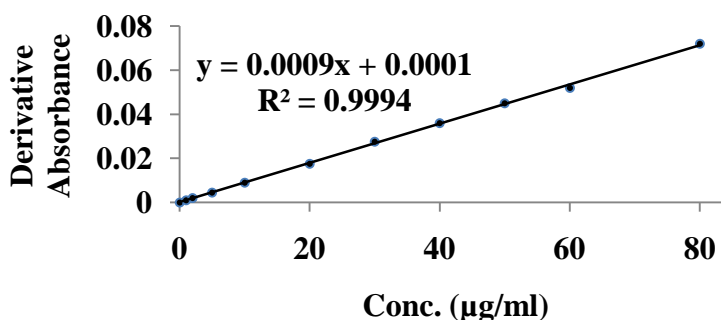
The first order derivative spectrum of Hydrochlorothiazide has shown zero crossing points at 221.14, 245.56, 272.69, 307.05 and 314.73 nm where as Candesartan Cilexetil has shown zero crossing points at 245.23, 255.10, 294.95 and 305.50 nm.

The overlay first order derivative spectra of CAN and HTZ in borate buffer was shown in Figure 2. CAN was determined at 272.69 nm which is one of the zero crossing points of HCZ in which the minima values were taken for the construction of calibration curve. Similarly, HCZ was determined at 255.10 nm which is one of the zero crossing points of CAN in which the maxima values were taken for the construction of calibration curve.

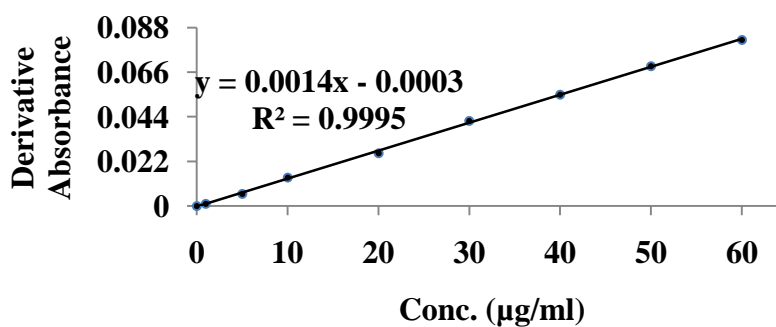


**Figure 2: Overlay first derivative spectra of Candesartan Cilexetil and Hydrochlorothiazide.**

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 1-80 µg/ml and 1-60 µg/ml for Candesartan Cilexetil and Hydrochlorothiazide respectively (Figure 3A and 3B) with linear regression equations  $y = 0.0009x + 0.0001$  ( $R^2 = 0.9994$ ) and  $y = 0.0014x - 0.0003$  ( $R^2 = 0.9995$ ) for Candesartan Cilexetil and Hydrochlorothiazide respectively.



[A]



[B]

**Figure 3: Calibration curves of Candesartan Cilexetil and Hydrochlorothiazide.**

In the precision studies the % RSD was found to be 0.38-0.64 (Intra-day) and 0.68-0.98 (Inter-day) for CAN and for that of HTZ the %RSD was found to be 0.21-0.45 (Intra-day) and 0.65-0.83 (Inter-day) which is less than 2%

indicating that the method is precise. In the accuracy studies the % recovery was found to be 99.18-99.64 (% RSD

0.35) and 99.45-99.98 (%RSD 0.32) for CAN and HTZ respectively indicating that the method is accurate. The

Optical characteristics of Candesartan Cilexetil and Hydrochlorothiazide are shown in Table 1.

**Table1: Optical characteristics of Candesartan Cilexetil and Hydrochlorothiazide.**

Parameters	CAN	HTZ
Wavelength (nm)	272.69	255.10
Linearity range (µg/ml)	1-80	1-60
Regression equation	$y = 0.0009x + 0.0001$	$y = 0.0014x - 0.0003$
Slope	0.0009	0.0014
Intercept	0.0001	0.0003
Correlation coefficient	0.9994	0.9995
Precision(% RSD)		
Intra-day(n=3)	0.38-0.64	0.21-0.45
Inter-day(n=3)	0.68-0.98	0.65-0.83
Accuracy(%Recovery) (% RSD)	99.18-99.64 (0.35)	99.45-99.98 (0.32)

#### Assay of marketed formulations (Tablets)

The method was applied for the available marketed formulations with different brand names in which the % recovery was found to be 99.25-99.50 and 99.04-99.52 for CAN and HTZ respectively (Table-2).

**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	15.88	12.44	99.25	99.52	0.34	0.54
II	8	12.5	7.96	12.38	99.50	99.04	0.44	0.48

\*Mean of three replicates

#### Conclusion

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

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The authors have no conflict of interest.

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