

## Application of first derivative UV-spectrophotometry and ratio derivative spectrophotometry for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide

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Two-component mixtures of candesartan cilexetil (CAN) and hydrochlorothiazide (HYD) were assayed by first derivative and ratio derivative spectrophotometry. The first method depends on zero-crossing and peak to base measurement. The first derivative amplitudes at 270.1 and 255.5 nm were selected for the assay of (CAN) and (HYD), respectively. The second method depends on first derivative of the ratio spectra by division of the absorption spectrum of the binary mixture by a normalized spectrum of one of the components and then calculating the first derivative of the ratio spectrum. The first derivative of the ratio amplitudes at 236, 250, 232, 267 and 280 nm were selected for the determination of (CAN) and (HYD), respectively. Calibration curves were established for  $6.0\text{--}38.0\text{ }\mu\text{g}\cdot\text{ml}^{-1}$  for (CAN) and  $4.0\text{--}28.0\text{ }\mu\text{g}\cdot\text{ml}^{-1}$  for (HYD) in binary mixtures. Good linearity, precision and selectivity were found, and the two methods were successfully applied to the pharmaceutical dosage form containing the above-mentioned drug combination without any interference by the excipients.

### 1. Introduction

Candesartan (CAN), ( $\pm$ -1-cyclohexyloxycarbonyloxy) ethyl-2-ethoxyl {[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-1H-benzimidazole-7-carboxylate is a potent, long-acting angiotensin II receptor antagonist, with high selectivity for the AT1 subtype [1]. Hydrochlorothiazide (HYD) (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide) is a diuretic of the class of benzothiadiazines.

Recently, CAN has been marketed in combination with HYD in tablets.

In the available literature, only three HPLC methods have been reported for the quantitative determination of CAN and its metabolites in biological fluids [2–4]. Several analytical methods have been reported for the determination of HYD, including voltammetry [5], capillary zone electrophoresis [6–8], spectrophotometry [9–19], and HPLC [20–29].

To my knowledge there is no report about the simultaneous determination of these drugs in pharmaceutical forms. Moreover, an official method for their determination has not yet been described in any national pharmacopoeia. It would therefore be beneficial to provide accurate, precise and reliable methods for the simultaneous determination of both drugs.

This work constitutes the first stage of a research schedule focused on the proposal of new spectrophotometric methods for the simultaneous determination of CAN and HYD. The proposed methods were applied to the determination of both analytes in synthetic mixtures and pharmaceutical preparations.

### 2. Investigations, results and discussion

#### 2.1. First derivative spectrophotometry

Fig. 1a shows the absorption (zero-order) spectra of (a) CAN ( $30.0\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ ) with a maximum at 255 nm and (b) HYD ( $24.0\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ ) with a maximum at 270 nm. The solutions showed overlapping UV spectra in 0.1 N HCl, making it difficult to resolve mixtures by classical spectrophotometry. However, derivative spectrophotometry can be used for resolving this problem satisfactorily. Fig. 1b shows the first derivative spectra ( $^1D$ ), they display features, which may permit the determination of both drugs in the presence of each other. The  $^1D$  amplitudes at 270 nm (zero-crossing of HYD) and at 255 nm (peak-to-baseline, nil contribution from CAN) were chosen for the simultaneous determination of both drugs in binary mixture. Linear relationships between the selected amplitudes from the  $^1D$  spectra and drug concentration were observed. Under the described experimental conditions, the graphs obtained by plotting the derivative values of each drug in this mixture versus concentration, in the range stated in Table 1, show a linear relationship. A critical evaluation of the proposed method was performed by the statistical analysis of the data. Slope intercepts and correlation coefficients are shown in Table 1. The relative standard deviation values of the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed method.

The stability of the working solutions was studied by recording the time-dependence of their absorption spectra; no changes in the spectra were observed for at least three days when the solutions are stored at room temperature in the dark.

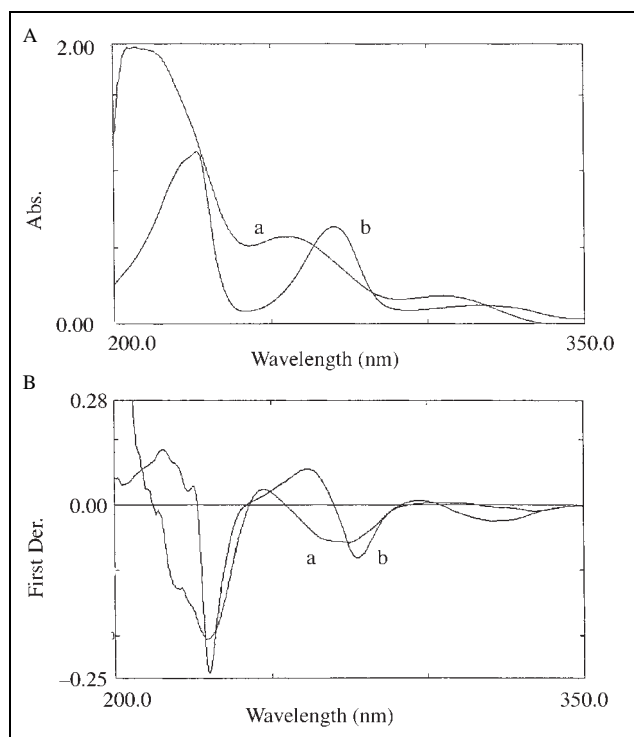


Fig. 1: A: Zero-order (a)  $30.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN, (b)  $24.0 \mu\text{g} \cdot \text{ml}^{-1}$  HYD in  $0.1 \text{ N HCl}$ ; B: First derivative spectra (a)  $30.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN, (b)  $24.0 \mu\text{g} \cdot \text{ml}^{-1}$  HYD in  $0.1 \text{ N HCl}$

**Table 1: Calibration equations for the determination of candesartan cilexetil and hydrochlorothiazide by zero-crossing first derivative spectrophotometry**

| Parameters                                              | Candesartan cilexetil | Hydrochlorothiazide   |
|---------------------------------------------------------|-----------------------|-----------------------|
| Wavelength (nm)                                         | 270                   | 255                   |
| Range ( $\mu\text{g} \cdot \text{ml}^{-1}$ )            | 6.0–30.0              | 4.0–28.0              |
| Detection limits ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | 0.73                  | 1.04                  |
| Regression equation ( $Y$ ) <sup>a</sup>                |                       |                       |
| Slope (b)                                               | $1.31 \times 10^{-3}$ | $3.91 \times 10^{-3}$ |
| Std. dev. on slope ( $S_b$ )                            | $1.87 \times 10^{-5}$ | $1.12 \times 10^{-4}$ |
| Intercept (a)                                           | $5.85 \times 10^{-5}$ | $2.45 \times 10^{-5}$ |
| Std. dev. on intercept ( $S_a$ )                        | $1.19 \times 10^{-5}$ | $4.19 \times 10^{-7}$ |
| Correlation coefficient (r)                             | 0.9998                | 0.9995                |
| Rel. std. dev. (%) <sup>b</sup>                         | 0.68                  | 0.58                  |
| % Range of error <sup>b</sup>                           | 0.38                  | 0.65                  |
| (% 95 confidence limit)                                 |                       |                       |

<sup>a</sup>  $Y = a + bC$  where  $C$  is concentration in  $\mu\text{g} \cdot \text{ml}^{-1}$  and  $Y$  in absorbance units

<sup>b</sup> Five replicate samples

## 2.2. Ratio first derivative spectrophotometry

Fig. 2 shows the ratio spectra of different amounts of CAN standards (spectra divided by the standard spectrum of a  $12.5 \mu\text{g} \cdot \text{ml}^{-1}$  solution of HYD) and their first derivatives. As can be seen, the height of the maximum and minimum at  $236 \text{ nm}$  ( $^1\text{DD}_{236}$ ) and  $250 \text{ nm}$  ( $^1\text{DD}_{250}$ ) in the derivative spectra corresponds to the CAN present in the solution, so it can be used for quantitative determination. Likewise, Fig. 3 shows the ratio spectra of different HYD standards (spectra divided by the spectrum of a  $20.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN solution) as well as the corresponding first derivative spectra, on the basis of which the drug can be quantified by measuring at  $232 \text{ nm}$  ( $^1\text{DD}_{232}$ ),  $267 \text{ nm}$  ( $^1\text{DD}_{267}$ ) and  $280 \text{ nm}$  ( $^1\text{DD}_{280}$ ), corresponding to minimum and maximum wavelengths. Under the experimental conditions described, standard calibration curves

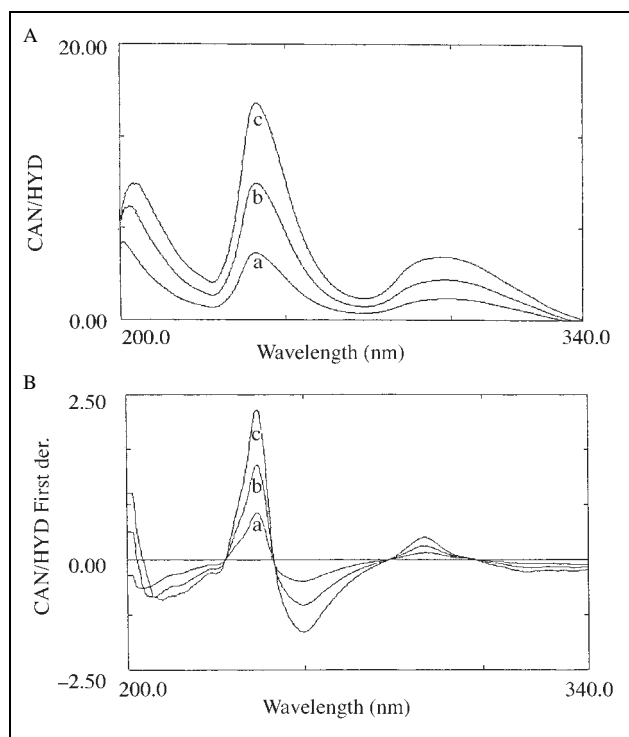


Fig. 2: A: Ratio spectra of the CAN (a)  $6.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; (b)  $22.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; and (c)  $38.0 \mu\text{g} \cdot \text{ml}^{-1}$ , where  $12.5 \mu\text{g} \cdot \text{ml}^{-1}$  HYD used as divisor in  $0.1 \text{ N HCl}$  ( $\Delta\lambda$ :  $4 \text{ nm}$ ); B: First ratio derivative spectra of the CAN (a)  $6.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; (b)  $22.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; and (c)  $38.0 \mu\text{g} \cdot \text{ml}^{-1}$ , where  $12.5 \mu\text{g} \cdot \text{ml}^{-1}$  HYD used as divisor in  $0.1 \text{ N HCl}$  ( $\Delta\lambda$ :  $4 \text{ nm}$ )

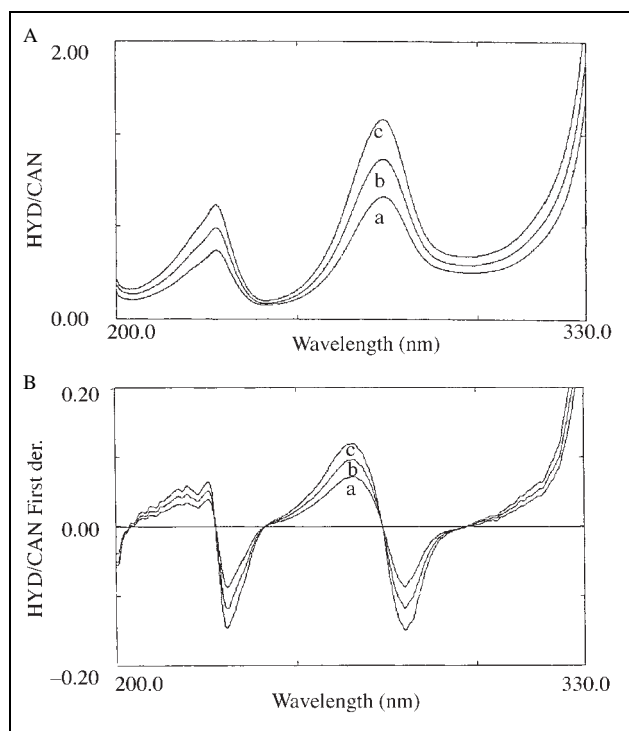


Fig. 3: A: Ratio spectra of the HYD (a)  $4.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; (b)  $16.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; and (c)  $28.0 \mu\text{g} \cdot \text{ml}^{-1}$ , where  $20.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN used as divisor in  $0.1 \text{ N HCl}$  ( $\Delta\lambda$ :  $4 \text{ nm}$ ); B: First ratio derivative spectra of the HYD (a)  $4.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; (b)  $16.0 \mu\text{g} \cdot \text{ml}^{-1}$ ; and (c)  $28.0 \mu\text{g} \cdot \text{ml}^{-1}$ , where  $20.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN used as divisor in  $0.1 \text{ N HCl}$  ( $\Delta\lambda$ :  $4 \text{ nm}$ )

**Table 2: Calibration equations of the determination of candesartan cilexetil and hydrochlorothiazide by the ratio first derivative spectrophotometry**

| Parameters                                              | Candesartan cilexetil |                       | Hydrochlorothiazide   |                       |                       |
|---------------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Wavelength (nm)                                         | 236                   | 250                   | 232                   | 267                   | 280                   |
| Range ( $\mu\text{g} \cdot \text{ml}^{-1}$ )            | 6.0–38.0              | 6.0–38.0              | 4.0–28.0              | 4.0–28.0              | 4.0–28.0              |
| Detection limits ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | 0.88                  |                       |                       |                       | 1.08                  |
| Regression equation (Y) <sup>a</sup>                    |                       |                       |                       |                       |                       |
| Slope (b)                                               | $1.64 \times 10^{-4}$ | $5.81 \times 10^{-4}$ | $6.63 \times 10^{-4}$ | $1.81 \times 10^{-3}$ | $7.01 \times 10^{-4}$ |
| Std. dev. on slope (S <sub>b</sub> )                    | $2.64 \times 10^{-7}$ | $6.11 \times 10^{-5}$ | $8.13 \times 10^{-6}$ | $2.17 \times 10^{-7}$ | $6.65 \times 10^{-7}$ |
| Intercept (a)                                           | $4.86 \times 10^{-3}$ | $8.84 \times 10^{-3}$ | $1.03 \times 10^{-4}$ | $2.63 \times 10^{-3}$ | $1.54 \times 10^{-3}$ |
| Std. dev. on intercept (S <sub>a</sub> )                | $6.64 \times 10^{-6}$ | $3.52 \times 10^{-5}$ | $6.19 \times 10^{-6}$ | $4.36 \times 10^{-6}$ | $3.28 \times 10^{-6}$ |
| Correlation coefficient (r)                             | 0.9999                | 0.9980                | 0.9985                | 0.9999                | 0.9989                |
| Rel. std. dev. (%) <sup>b</sup>                         | 0.84                  | 0.99                  | 1.28                  | 0.56                  | 1.02                  |
| % Range of error <sup>b</sup>                           | 0.14                  | 0.68                  | 0.91                  | 0.71                  | 1.04                  |
| (% 95 confidence limit)                                 |                       |                       |                       |                       |                       |

<sup>a</sup> Y = a + bC where C is concentration in  $\mu\text{g} \cdot \text{ml}^{-1}$  and Y in absorbance units

<sup>b</sup> Five replicate samples

for both drugs were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 2. Regression analysis with the method of least-squares was made for the slope, intercept and correlation coefficient values (Table 2). Wavelengths of 236 nm for CAN and 280 nm for HYD were selected for the determination in synthetic mixtures and pharmaceutical dosage forms.

To optimize the simultaneous determination of CAN and HYD using the ratio derivative spectrophotometric method, it is necessary to test the influence of the variables: divisor standard concentration,  $\Delta\lambda$  and smoothing function. All these variables were studied. The influence of the  $\Delta\lambda$  for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval;  $\Delta\lambda = 4$  nm was selected as suitable. For selecting the standard solution as divisor, its concentration was modified  $20.0 \mu\text{g} \cdot \text{ml}^{-1}$  CAN and  $12.5 \mu\text{g} \cdot \text{ml}^{-1}$  HYD were selected as appropriate values of the divisor concentrations. Due to the extent of the noise levels on the ratio spectra a smoothing function was used and 16 experimental points were considered as suitable.

The detection limits of the proposed methods were found to be  $0.73$  and  $0.88 \mu\text{g} \cdot \text{ml}^{-1}$  for CAN and  $1.04$  and  $1.08 \mu\text{g} \cdot \text{ml}^{-1}$  for HYD, detected by first derivative spectrophotometric method and ratio derivative spectrophotometric methods, respectively. While the quantitation limits of the proposed methods were found to be  $1.64$  and  $1.53 \mu\text{g} \cdot \text{ml}^{-1}$  for CAN and  $1.74$  and  $1.96 \mu\text{g} \cdot \text{ml}^{-1}$  for HYD, determined by first derivative spectrophotometric method and ratio derivative spectrophotometric methods, respectively. The determinations of different concentration levels were carried out for each drug to test sensitivity, quantitation and reproducibility and of the first derivative and ratio derivative values [30, 31] (Table 1 and 2).

Comparison of the original, first derivative and ratio derivative spectrum in standard and drug formulations shows that the wavelength of maximum and minimum absorbance did not change.

Accuracy of the first derivative spectrophotometric and ratio derivative spectrophotometric methods for simultaneous determination were checked by analyzing synthetic mixtures at various concentrations within the linearity range. The mean recovery data for each level (at 95% confidence limits) and its percentage recoveries are presented

**Table 3: Recovery experiments obtained for different binary mixtures of candesartan cilexetil and hydrochlorothiazide by zero-crossing first derivative spectrophotometry**

| Sample        | Recovery (mean $\pm$ sd) % <sup>a</sup>             |                                                     |              |                                                     |                                                     |              |
|---------------|-----------------------------------------------------|-----------------------------------------------------|--------------|-----------------------------------------------------|-----------------------------------------------------|--------------|
|               | Candesartan cilexetil                               |                                                     |              | Hydrochlorothiazide                                 |                                                     |              |
|               | Amount added ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Amount found ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Recovery (%) | Amount added ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Amount found ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Recovery (%) |
|               | 15.00                                               |                                                     |              | 12.5                                                | 12.4                                                | 99.2         |
|               | 17.50                                               |                                                     |              | 12.5                                                | 12.7                                                | 101.6        |
|               | 20.00                                               |                                                     |              | 12.5                                                | 12.3                                                | 98.4         |
|               | 22.50                                               |                                                     |              | 12.5                                                | 12.4                                                | 99.2         |
|               | 25.00                                               |                                                     |              | 12.5                                                | 12.2                                                | 97.6         |
|               | 20.00                                               | 20.18                                               | 100.9        | 7.5                                                 |                                                     |              |
|               | 20.00                                               | 19.87                                               | 99.3         | 10.0                                                |                                                     |              |
|               | 20.00                                               | 19.81                                               | 99.1         | 15.0                                                |                                                     |              |
|               | 20.00                                               | 20.10                                               | 100.5        | 17.5                                                |                                                     |              |
|               | 20.00                                               | 19.98                                               | 99.9         | 20.0                                                |                                                     |              |
| $\bar{X}$ (%) |                                                     |                                                     | R.S.D (%)    |                                                     |                                                     |              |
| CAN           | 99.9                                                |                                                     | 0.68         |                                                     |                                                     |              |
| HYD           | 99.2                                                |                                                     | 1.34         |                                                     |                                                     |              |

**Table 4: Recovery experiments obtained for different binary mixtures of candesartan cilexetil and hydrochlorothiazide by the ratio first derivative spectrophotometry**

| Sample        | Recovery (mean $\pm$ sd) % <sup>a</sup>             |                                                     |              |                                                     |                                                     |              |
|---------------|-----------------------------------------------------|-----------------------------------------------------|--------------|-----------------------------------------------------|-----------------------------------------------------|--------------|
|               | Candesartan cilexetil                               |                                                     |              | Hydrochlorothiazide                                 |                                                     |              |
|               | Amount added ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Amount found ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Recovery (%) | Amount added ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Amount found ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) | Recovery (%) |
|               | 15.00                                               |                                                     |              | 12.5                                                | 12.4                                                | 99.2         |
|               | 17.50                                               |                                                     |              | 12.5                                                | 12.5                                                | 100.0        |
|               | 20.00                                               |                                                     |              | 12.5                                                | 12.3                                                | 98.4         |
|               | 22.50                                               |                                                     |              | 12.5                                                | 12.3                                                | 98.4         |
|               | 25.00                                               |                                                     |              | 12.5                                                | 12.7                                                | 101.6        |
|               | 20.00                                               | 20.04                                               | 100.2        | 7.5                                                 |                                                     |              |
|               | 20.00                                               | 20.07                                               | 100.3        | 10.0                                                |                                                     |              |
|               | 20.00                                               | 19.95                                               | 99.8         | 15.0                                                |                                                     |              |
|               | 20.00                                               | 19.89                                               | 99.4         | 17.5                                                |                                                     |              |
|               | 20.00                                               | 19.99                                               | 99.9         | 20.0                                                |                                                     |              |
| $\bar{X}$ (%) |                                                     | R.S.D (%)                                           |              |                                                     |                                                     |              |
| CAN           | 99.9                                                | 0.32                                                |              |                                                     |                                                     |              |
| HYD           | 99.5                                                | 1.20                                                |              |                                                     |                                                     |              |

**Table 5: Comparative studies for commercial preparations**

|         | Mean (mg) $\pm$ SD <sup>a</sup>    |                     |                                                  |                     |
|---------|------------------------------------|---------------------|--------------------------------------------------|---------------------|
|         | First derivative spectrophotometry |                     | Ratio first derivative spectra spectrophotometry |                     |
|         | Candesartan cilexetil              | Hydrochlorothiazide | Candesartan cilexetil                            | Hydrochlorothiazide |
| Batch 1 | 20.5 $\pm$ 1.13                    | 12.5 $\pm$ 0.63     | 20.1 $\pm$ 0.46                                  | 12.1 $\pm$ 0.86     |
| Batch 2 | 19.7 $\pm$ 0.96                    | 12.2 $\pm$ 0.55     | 20.1 $\pm$ 0.34                                  | 12.3 $\pm$ 0.81     |
| Batch 3 | 19.9 $\pm$ 0.92                    | 12.7 $\pm$ 0.52     | 20.4 $\pm$ 0.50                                  | 12.0 $\pm$ 0.93     |

<sup>a</sup> Each value is the mean of ten experiments; SD: standard deviation

in Tables 3 and 4. Satisfactory recoveries with small standard deviations were obtained, which indicated the high repeatability and accuracy of the two methods.

The developed method was applied to three batches of commercial formulations. The results presented in Table 5 are in good agreement with the labelled content. All data represent the average of ten determinations.

To further check the validity of the proposed methods, the standard addition method was applied by adding different amounts of CAN with concentrations of 6.0–30.0  $\mu\text{g} \cdot \text{ml}^{-1}$  (first derivative spectrophotometry) and 6.0–38.0  $\mu\text{g} \cdot \text{ml}^{-1}$  (ratio first derivative spectrophotometry); and HYD with concentration range 4.0–28.0  $\mu\text{g} \cdot \text{ml}^{-1}$  (first derivative spectrophotometry) and 4.0–28.0  $\mu\text{g} \cdot \text{ml}^{-1}$  (ratio first derivative spectrophotometry) to the previously analyzed tablets. The mean recoveries of the added drugs were found to be 99.7  $\pm$  0.78 (for first derivative spectrophotometry), 100.5  $\pm$  0.29 (for ratio first derivative spectrophotometry) and 99.1  $\pm$  0.52 (for first derivative spectrophotometry), 99.5  $\pm$  0.64 (for ratio first derivative spectrophotometry) for CAN and HYD, respectively. The results of analysis of the commercial tablets and recovery study (standard addition method) suggested that there is no interference from any excipients present in the tablets. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F-test at 95% confidence level. There is no significant difference between the first derivative spectrophotometric and ratio first derivative spectrophotometric methods.

To determine the precision of the method, the solutions at a concentration of 20.0  $\mu\text{g} \cdot \text{ml}^{-1}$  for CAN and

12.5  $\mu\text{g} \cdot \text{ml}^{-1}$  for HYD in 0.1 N HCl were analyzed 10 times and the mean CAN and HYD values were 19.8  $\pm$  0.05 and 12.3  $\pm$  0.03 for first derivative spectrophotometry and 20.1  $\pm$  0.02 and 12.5  $\pm$  0.06 for ratio first derivative spectrophotometric methods, respectively. The SD were found as 0.12 and 0.24 for first derivative spectrophotometry and 0.10 and 0.08 for ratio first derivative spectrophotometric methods, respectively, and the proposed methods have a good precision.

The proposed first derivative spectrophotometry and ratio first derivative spectrophotometry can be used for the simultaneous determination of CAN and HYD either in their pure powder form or in tablet preparations. The main advantage of the first derivative of the ratio spectra method may be the chance of doing measurements in correspondence of peaks, hence a potential greater sensitivity and accuracy. The main disadvantages of the zero-crossing method in derivative spectrophotometry for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the derivative spectrum.

The two methods are precise, accurate and simple. Also, no separation step is required. They are rapid and do not require any expensive or sophisticated apparatus compared with chromatographic methods. In the absence of an official monograph these methods can be used for the simultaneous determination of the cited drugs in pharmaceutical preparations but they cannot be considered as stability indicating assays.

### 3. Experimental

#### 3.1. Materials and reagents

Pharmaceutical grade of CAN and HYD were kindly supplied by AstraZeneca Pharm. Ind. and certified to contain 99.7%. The commercial ATACAND<sup>®</sup> plus tablet used was manufactured by AstraZeneca Pharm. Ind. Each tablet contains 20.0 mg of CAN and 12.5 mg of HYD. All other chemicals were of analytical-reagent grade.

#### 3.2. Apparatus

A double-beam Shimadzu UV-visible spectrophotometer, model UV-1601 PC connected to an IBM compatible computer loaded with Shimadzu UVPC software, and a HP 1100 laserjet model printer was used.

#### 3.3. Standard solutions and calibration

Stock standard solutions were prepared separately by dissolving 100 mg of each drug in 100 ml 0.1 N HCl. The standard solutions were prepared by dilution of the stock standard solutions with the same solvent to reach a concentration range of 6.0–30.0  $\mu\text{g} \cdot \text{ml}^{-1}$  of CAN and 4.0–28.0  $\mu\text{g} \cdot \text{ml}^{-1}$  of HYD for first derivative spectrophotometry and 6.0–38.0  $\mu\text{g} \cdot \text{ml}^{-1}$  and 4.0–28.0  $\mu\text{g} \cdot \text{ml}^{-1}$  for ratio first derivative spectrophotometry.

#### 3.4. Assay procedure for tablets

An accurately weighed amount of powdered tablets equivalent to about one tablet was transferred into a 100.0 ml conical flask in 0.1 N HCl. After 30 min of mechanical shaking, the solution was filtered in to a 100 ml calibrated flask through Whatman No 42 filter paper, and diluted to 100.0 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol.

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