

---

## **SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND METHYLDOPA IN TABLETS USING FIRST DERIVATIVE SPECTROPHOTOMETRY AND PLS**

---

AHMED A. OMRAN

*Chemistry Department, Faculty of Science, Al-Azhar University, Assiut 71524, Egypt*

e-mail: [ahmed\\_omran@scia.azhar.edu.eg](mailto:ahmed_omran@scia.azhar.edu.eg)

---

### **Abstract**

Two new analytical methods are described as convenient and useful alternatives for simultaneous determination of hydrochlorothiazide (HCTZ) and methyldopa (MD) in their combinations. The first method depends on the first order derivative spectrophotometry by measuring the amplitudes at zero-crossing wavelengths of 286 and 270 nm for HCTZ and MD, respectively. In the second method, partial least squares (PLS) analysis of the ultraviolet absorption spectra of the samples in the 240-340 nm region was applied. The methods were calibrated between 3.5 and 35.7  $\mu\text{g mL}^{-1}$  for HCTZ and between 2.5 and 25.3  $\mu\text{g mL}^{-1}$  for MD. For PLS chemometric calibration a concentration set of random mixture consisting of the two drugs in ethanol was prepared. The absorbance data in UV spectra and their  $dA/d\lambda$  values were measured for the 24  $\lambda$  points considering  $\Delta\lambda = 4$  nm. The calibration of the PLS method involves both absorbance-concentration and  $dA/d\lambda$ -concentration data matrices. The numerical values were calculated by using Matlab version 6.5 and origin 7.0 software. The results of the methods were statistically compared with each other and a good harmony was found. The developed calibrations were successfully applied for assaying the pharmaceutical formulation of Aldoril<sup>®</sup> tablets.

**Keywords:** Hydrochlorothiazide . Methyldopa . Aldoril tablets . Derivative spectrophotometry . PLS

### **Introduction**

A combination of hydrochlorothiazide (HCTZ) and methyldopa (MD), in the form of tablets, is widely prescribed to patients for moderate to severe hypertension not controlled by initial therapy using a single antihypertensive agent. HCTZ, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1 dioxide is a diuretic and an antihypertensive agent of the class benzothiadiazines which decreases active sodium reabsorption and reduces peripheral vascular resistance. MD, levo-3-(3,4-dihydroxyphenyl)-2-methylalanine, is an old antihypertensive agent which is used in the treatment of mild to moderate hypertension. It is converted to  $\alpha$ -methylnorepinephrine in adrenergic nerve terminals and its

hypertensive action appears to be due to stimulation of central  $\alpha$ -adrenoreceptors by this agent [1].

Several analytical procedures have been described for individual determination of HCTZ, by using electrochemical [2,3], spectrophotometric [4-6], flow injection [7] and HPLC methods [8,9] and in combination with other pharmaceutical substances including spectrophotometric [10,11], polarographic [12], HPTLC-dansitometric [13], HPLC [14,15] and chemometric [16,17]. Numerous analytical techniques including spectrofluorimetric [18-20], chromatographic [21-27] and flow injection [28,29] are now available for the analysis of MD in biological fluids and pharmaceutical formulations. Also, many spectrophotometric methods have been proposed for the determination of catecholamines including MD [30-47].

So far, no method has been described for the simultaneous determination of HCTZ and MD in pharmaceutical formulations. Therefore, it is required simple, precise, accurate and reliable methods that can be applied in quality control laboratories for the determination of HCTZ and MD simultaneously. For this purpose UV-derivative spectrophotometric and PLS assay methods have been developed in this study. These methods were especially chosen because of their usefulness in simultaneous analysis of several mixture components without any prior chemical treatment and during a short time, as well as no expensive costs and complex instruments are required.

## **Experimental**

### *Chemicals*

HCTZ was purchased from Sigma (St. Louis, MO, USA) and MD was kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO.). Commercially available Aldoril<sup>®</sup> tablets are products of Merck & CO. INC (Whitehouse station, USA), each tablet was labeled to contain 25 mg of HCTZ and 250 mg of MD. Analytical grade ethanol and bidistilled water were used throughout the experiments.

### *Apparatus*

Spectro double 8 autocell UV-Vis spectrophotometer labomed, Inc., USA was used under the following operating conditions; scan speed 120 nm min<sup>-1</sup>, scan range 240-340 nm, slit width 2 nm and  $\Delta\lambda = 1$  nm. Zero- and first-order spectra were

automatically obtained using 1 cm quartz cell by using UVWin 5.0 analysis software.

Matlab version 6.5 and origin 7.0 software were used for PLS and statistical treatment of data.

#### *Standard solutions*

Stock solutions of HCTZ and MD were prepared separately by dissolving 11.92 mg and 8.45 mg of each drug in 100 mL ethanol, respectively. The standard solutions were prepared by further dilution of the stock solutions with ethanol to reach a concentration range of 3.5-35.7  $\mu\text{g mL}^{-1}$  for HCTZ and 2.5-25.3  $\mu\text{g mL}^{-1}$  for MD.

#### *Procedures*

##### *First derivative spectrophotometric method*

The absorption spectra were recorded against ethanol to solutions in 10 mL calibrated flask containing 3.5-35.7  $\mu\text{g mL}^{-1}$  HCTZ and 2.5-25.3  $\mu\text{g mL}^{-1}$  MD and diluted the mark with ethanol. By measuring derivative values from the first derivative spectra at 286 and 270 nm, the concentrations of HCTZ and MD could be determined, respectively.

##### *PLS chemometric method and validation*

A training set of standard mixture solutions containing between 3.5-35.7  $\mu\text{g mL}^{-1}$  HCTZ and 2.5-25.3  $\mu\text{g mL}^{-1}$  MD was made daily from stock solutions. A validation set of seven synthetic mixtures containing various concentrations of both drugs was also prepared by using the same stock solutions.

##### *Sample preparation*

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 2.5 mg of HCTZ and 25 mg of MD was weighed accurately, dissolved and diluted to 250 mL with ethanol. The sample solution was filtered. Further dilution with ethanol was carried out to provide a solution of 10  $\mu\text{g mL}^{-1}$  of HCTZ and 100  $\mu\text{g mL}^{-1}$  of MD. The procedures for first derivative and PLS described above were followed and the concentrations of HCTZ and MD were calculated.

## Results and Discussion

### *Spectrophotometric measurements*

In Fig. 2 (a) the zero order derivative spectra of  $4.7 \mu\text{g mL}^{-1}$  of HCTZ (curve 1),  $21.1 \mu\text{g mL}^{-1}$  MD (curve 2) and their mixture (curve 3), in the wavelength range 240-340 nm, are shown. HCTZ exhibits two absorbance maxima at 270 and 317 nm. MD exhibits a defined absorbance maximum at 286 nm. As can be seen, that of HCTZ spectrum substantially overlaps the absorption spectrum of MD, which prevents the determination of MD in the presence of HCTZ by direct absorbance measurements. Derivative spectrophotometry is suitable technique for overcoming this problem, with the zero-crossing technique being the most common procedure for the preparation of analytical calibration graphs. In practice, the measurements selected are those which exhibit the best linear response, give a zero or near to zero intercept on the ordinate of the calibration graphs and which are least affected by the concentration of any other component. Thus, a zero-crossing measurement technique was utilized. In Fig. 2 (b), zero-crossing wavelengths of HCTZ and MD were 270 and 286 nm, respectively.

Due to the overlapping spectra, zero-crossing method is clearly the most appropriate approach for resolving mixtures of these components, as such, was used in this work without satisfactory results. Preliminary investigations showed that the derivative intensities of the signals at 270 nm (working zero-crossing wavelength of HCTZ) are proportional to the MD present in the binary mixture with HCTZ. Table 1 represents the statistical analysis of calibration graphs for the simultaneous determination of HCTZ and MD by using zero-crossing wavelength technique of the first derivative spectra of their mixtures.

### *Optimization of the instrumental conditions*

The main instrumental parameters that affect the shape of derivative spectra are the scan rate,  $\Delta\lambda$  (the wavelength increment used for derivatization) and smoothing. All of these parameters need to be optimized to give a well resolved large peak. Generally, the noise level decreases as  $\Delta\lambda$  increases, which leads to less pronounced fluctuations in the derivative spectrum. Since spectral resolution is very poor at excessively high  $\Delta\lambda$  values, the optimum value of  $\Delta\lambda$  should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Several values of  $\Delta\lambda$  were tested and 4.0 nm was selected as the optimum for a satisfactory signal-to-noise ratio.

Fig. 3 (a) shows a set of the first derivative spectra of mixtures containing 21.1  $\mu\text{g mL}^{-1}$  of MD plus increasing amounts of HCTZ (3.5-35.7  $\mu\text{g mL}^{-1}$ ). A further set was performed by keeping the HCTZ concentration constant at 4.7  $\mu\text{g mL}^{-1}$ , while MD concentration was varied from 2.5-25.3  $\mu\text{g mL}^{-1}$  (Fig. 3 (b)). The results in Fig. 3 ((a) and (b)) indicate that when the concentration of MD (HCTZ) was kept constant and the HCTZ (MD) was varied, the peak amplitudes at 270 nm (286 nm) were unaltered. The amplitudes at 286 nm ( $h_1$ ) and 270 nm ( $h_2$ ) were proportional to the HCTZ and MD concentration, respectively.

***Determination of HCTZ in the presence of MD by using direct absorbance measurements at 317 nm***

Fig 2 (a) shows the zero-order spectra of 4.7  $\mu\text{g mL}^{-1}$  of HCTZ (curve 1) and 21.1  $\mu\text{g mL}^{-1}$  of MD (curve 2) in the wavelength range 240-340 nm. As can be seen at 317 nm (maximum absorption of HCTZ), MD does not absorb even at high ratio assayed. The measurements of HCTZ absorbance were used for determining HCTZ in the presence of MD. Binary mixtures of HCTZ in the presence of MD in ethanol were prepared by keeping the MD concentration constant at 21.1  $\mu\text{g mL}^{-1}$  while the HCTZ concentration was varied from 0.0 to 35.7  $\mu\text{g mL}^{-1}$ . The calibration graph was linear between 3.5-35.7  $\mu\text{g mL}^{-1}$  of HCTZ. The regression equation found was  $A = 2.4 \times 10^{-3} (\pm 0.9 \times 10^{-3}) + 1 \times 10^{-2} (\pm 2.8 \times 10^{-5})C$ , where C is HCTZ concentration in  $\mu\text{g mL}^{-1}$  with a correlation coefficient of 0.9997. The detection and quantitation limits were 0.31 and 0.79  $\mu\text{g mL}^{-1}$  of HCTZ, respectively. The between-run precision of the method was evaluated with a series of sample containing the concentrations of the above drugs being analyzed during five consecutive days. The obtained variation coefficient was 2.1 %.

***PLS for the zero and the first-order derivative spectra***

Based on the diagram of binary matrix design [48], a training set of 20 representative mixtures at different ratios of two drugs was prepared as described in Table 2. The absorption spectra of the training set were recorded in computer and their first derivative spectra were calculated with  $\Delta\lambda = 4.0$  nm. The values of the absorbance were measured at 24 points with  $\Delta\lambda$  interval of 4.0 nm in the selected range of 220-340 nm. The obtained zero-order and first derivative absorbance data were used to compose the proposed PLS calibration.

Utilizing a cross-validation, 15 calibration spectra were used for the selection of the optimum number of factors in PLS. The predicted concentrations of each sample

in calibration step were compared with the actual concentrations. The prediction residual error sum of squares (PRESS) was computed by using the following formula:

$$PRESS = \sum_{i=1}^n (C_i^{predicted} - C_i^{actual})^2 \quad \text{----- (1)}$$

Moreover, two statistical criterions were considered for calibration steps of PLS. The first statistical parameter is the root mean square difference (RMSD) which represent the average error in the analysis for each component in the training samples. The RMSD was calculated using the following equation:

$$RMSD = \sqrt{\frac{1}{n} \sum_{i=1}^n (C_i^{predicted} - C_i^{actual})^2} \quad \text{----- (2)}$$

Where  $C_i^{predicted}$  and  $C_i^{actual}$  are the predicted and actual concentration in calibration samples, respectively.  $n$  is the total number of calibration samples.

The relative error of the prediction (REP) as a second statistical parameter was also calculated to control the predictive ability of the estimated calibration model for training set as follow:

$$REP = \frac{100}{\bar{C}} \sqrt{\frac{1}{n} \sum_{i=1}^n (C_i^{predicted} - C_i^{actual})^2} \quad \text{----- (3)}$$

Where  $\bar{C}$  represents the mean of the predicted concentration in the calibration step for all samples.

The statistical results (PRESS, RMSD, REP,  $r^2$ , intercept and slope for the proposed calibrations) are summarized in Table 3. The statistical parameters in calibration step for the measurements zero- and first-order derivative spectra were compared and found to be acceptable for the proposed PLS calibration model.

#### ***Validation of PLS calibration for synthetic binary mixtures***

In order to test the proposed PLS calibration an independent set of the validation samples containing HCTZ and MD in the different compositions given in Table 4, was prepared and analyzed. The means, standard deviations (S.D.) and relative standard deviation (R.S.D.) are shown in Table 4.

For further demonstration of the validity, the predictive applicability of PLS was expressed by the standard error of the prediction (SEP) and standard error of calibration (SEC) which is given in the following formula:

$$SEP(SEC) = \frac{\sqrt{\sum_{i=1}^n (C_i^{predicted} - C_i^{actual})^2}}{n-1} \text{----- (4)}$$

Where,  $C_i^{actual}$  is the actual concentration of analyte,  $C_i^{predicted}$  is the predicted concentration of analyte and n is the total number of synthetic mixtures and calibration set.

The SEP and SEC results and other statistical evaluations obtained by applying PLS to synthetic mixtures and validation set were summarized in Table 5. Moreover, to check the precision of PLS method, the detection limit DL and quantification limit QL were calculated and presented in Table 4. As can be seen, all the statistical parameters indicate that PLS is applicable for the determination of two drugs in synthetic mixtures.

#### *Tablet analysis*

Recovery studies of the standard additions to commercial tablet preparation were realized. The recovery results for the validation of the two proposed methods were obtained in the average of six replicated for each drug in commercial tablet formulation. The results were found to be very close to each other and the drug recovery % was shown in Table 6.

#### **Conclusion**

Although the absorption spectra of both HCTZ and MD were superposed, the proposed derivative method as well as the PLS calibration using the absorption and first derivative spectrophotometric data were successfully applied to their simultaneous determination in synthetic mixtures and tablets. Thus, the proposed methods could be given as alternative determination techniques of two drugs in the same pharmaceutical preparation without any need for either prior separation procedures or more complex instrumentation being more expensive, tedious and time consuming. Consequently, the proposed methods are strongly believed to be successfully employed for the quality control of HCTZ and MD in their binary mixtures and pharmaceutical preparations.

**Figure captions**

**Fig. 1.** Chemical structures of HCTZ and MD.

**Fig. 2.** (a) Ultraviolet absorption and (b) first-derivative spectra of (1) 4.7  $\mu\text{g mL}^{-1}$  HCTZ, (2) 21.1  $\mu\text{g mL}^{-1}$  MD and (3) mixture of HCTZ (4.7  $\mu\text{g mL}^{-1}$ ) and MD (21.1  $\mu\text{g mL}^{-1}$ ) in 240-340 nm region.

**Fig. 3.** First-derivative spectra of:

- (a) 21.1  $\mu\text{g mL}^{-1}$  of MD in the presence of (1) 3.53; (2) 4.7; (3) 7.14; (4) 9.53; (5) 11.91; (6) 14.29; (7) 16.67; (8) 19.05; (9) 21.44; (10) 23.82; (11) 29.77; (12) 35.73  $\mu\text{g mL}^{-1}$  HCTZ and,  
 (b) 4.7  $\mu\text{g mL}^{-1}$  HCTZ in the presence of (1) 2.53; (2) 3.37; (3) 5.07; (4) 6.76; (5) 8.45; (6) 10.14; (7) 11.83; (8) 13.52; (9) 15.21; (10) 16.90; (11) 21.12; (12) 25.35  $\mu\text{g mL}^{-1}$  MD.

**Table 1: Statistical analysis of calibration graphs in the determination of HCTZ and MD using zero-crossing wavelength technique of the first derivative spectra**

Parameter	HCTZ	MD
$\lambda$ (nm)	286	270
Linearity range ( $\mu\text{g mL}^{-1}$ )	3.5-35.7	2.5-25.3
Detection Limit, DL	0.54	0.51
<u>Regression Equation</u> $Y^*$ :		
Slope (b)	$-2.1 \times 10^{-3}$	$0.6 \times 10^{-3}$
SD of slope ( $S_b$ )	$-1.3 \times 10^{-3}$	$4.0 \times 10^{-3}$
Intercept (a)	$0.7 \times 10^{-5}$	$-0.6 \times 10^{-3}$
SD of the intercept ( $S_a$ )	$0.5 \times 10^{-5}$	$3.3 \times 10^{-3}$
Correlation coefficient	0.9997	0.9996

\*  $Y = a + bC$ , where C is the concentration of the drug in  $\mu\text{g mL}^{-1}$  and Y is the first derivative value;  $D_1$  at the specified wavelength.



**Table 2: Composition of a training set HCTZ and MD**

Standard number	HCTZ	MD
	$\mu\text{g mL}^{-1}$	$\mu\text{g mL}^{-1}$
1	3.0	3.0
2	3.0	5.0
3	3.0	7.0
4	3.0	9.0
5	7.0	3.0
6	7.0	5.0
7	7.0	7.0
8	7.0	9.0
9	12.0	3.0
10	12.0	5.0
11	12.0	7.0
12	12.0	9.0
13	18.0	3.0
14	18.0	5.0
15	18.0	7.0
16	18.0	9.0
17	25.0	3.0
18	25.0	5.0
19	25.0	7.0
20	25.0	9.0

**Table 3: Statistical results for the PLS calibration model.**

Parameter	Zero-order spectra		First-order derivative spectra	
	HCTZ	MD	HCTZ	MD
PRESS	0.6165	0.3541	0.6502	0.5512
RMSD	0.1527	0.1108	0.1688	1.480
REB	1.5668	0.6371	1.1395	1.08033
$r^2$	0.9991	0.9989	0.9993	0.9997
Intercept	0.0152	0.0384	-0.0281	0.0292
Slope	1.0079	0.9953	1.0320	0.9992

**Table 4: Recovery results obtained for the simultaneous determination of HCTZ and MD in synthetic mixtures by PLS method.**

Amount added		% recovery			
$\mu\text{g mL}^{-1}$		zero-order derivative spectra		First-order derivative spectra	
HCTZ	MD	HCTZ	MD	HCTZ	MD
6.0	5.0	99.9	99.5	99.6	99.8
10.0	5.0	98.8	100.6	99.1	100.6
12.0	4.0	100.2	100.8	98.9	101.1
14.0	18.0	101.0	99.7	100.7	100.3
18.0	12.0	99.3	99.4	99.8	99.1
20.0	10.0	100.4	100.6	101.0	100.5
25.0	8.0	98.7	98.9	98.4	98.9
Mean $\pm$ R.S.D.*		99.76 0.86	99.92 0.73	99.64 0.95	100.03 0.84

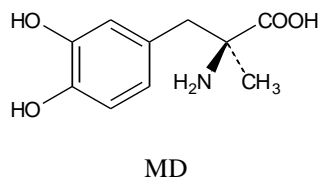
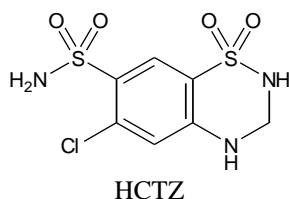
\* R.S.D., relative standard deviation.

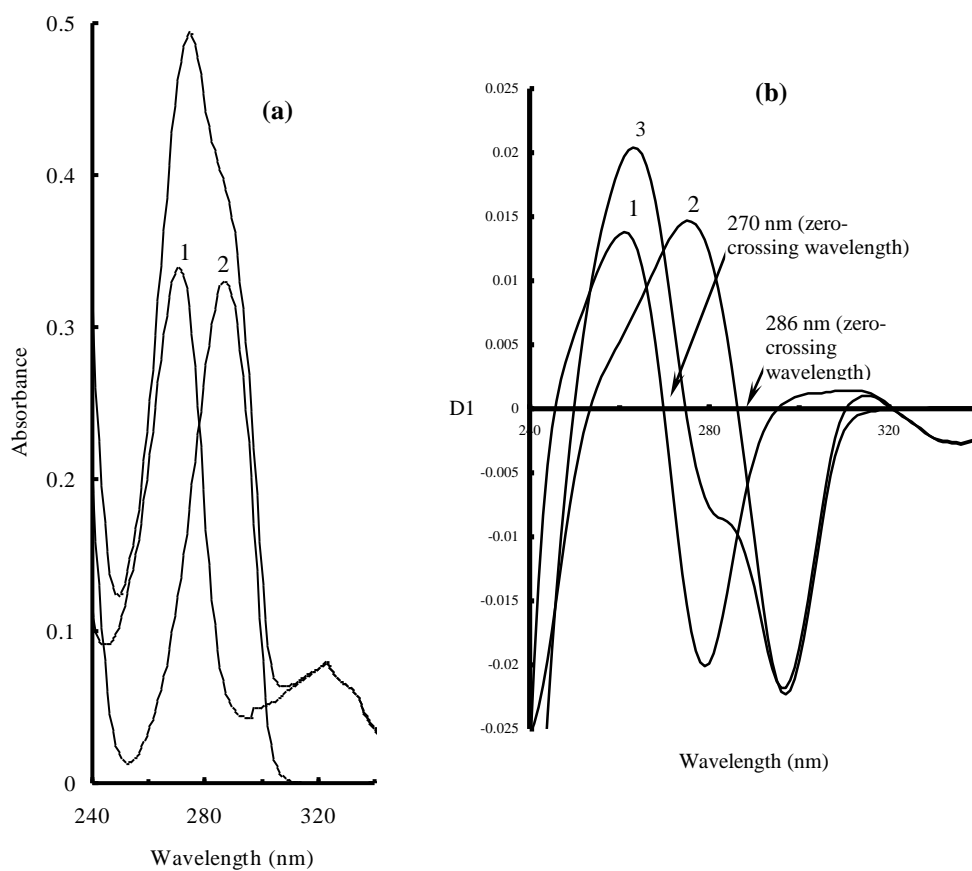
**Table 5: Statistical parameters by applying PLS technique to the synthetic mixtures**

Parameter	zero-order spectra		first-order spectra	
	HCTZ	MD	HCTZ	MD
SEP	0.378	0.101	0.285	0.079
SEC	0.443	0.097	0.325	0.069
$r^2$	0.9991	0.9994	0.9988	0.9993
Intercept	0.014	0.009	-0.0002	-0.001
Slope	0.071	0.014	0.004	0.005
DL	0.592	0.425	0.561	0.492
QL	1.957	1.748	1.975	1.777

**Table 6: Recovery % of HCTZ and MD of Aldoril® tablets by using the proposed methods**

Drug	Recovery %*		
	First derivative method	PLS method	
		zero	first
HCTZ	100.1 $\pm$ 2.3	100.3 $\pm$ 0.7	99.8 $\pm$ 1.3
MD	99.8 $\pm$ 1.3	98.2 $\pm$ 2.5	97.4 $\pm$ 0.9

\* Mean  $\pm$  standard deviation for six determinations.**Fig. 1.** Chemical structures of (a) HCTZ and, (b) MD



**Fig. 2.** (a) Ultraviolet absorption and (b) first-derivative spectra of (1)  $4.7 \mu\text{g mL}^{-1}$  HCTZ, (2)  $21.1 \mu\text{g mL}^{-1}$  MD and (3) mixture of HCTZ ( $4.7 \mu\text{g mL}^{-1}$ ) and MD ( $21.1 \mu\text{g mL}^{-1}$ ) in 240-340 nm region.

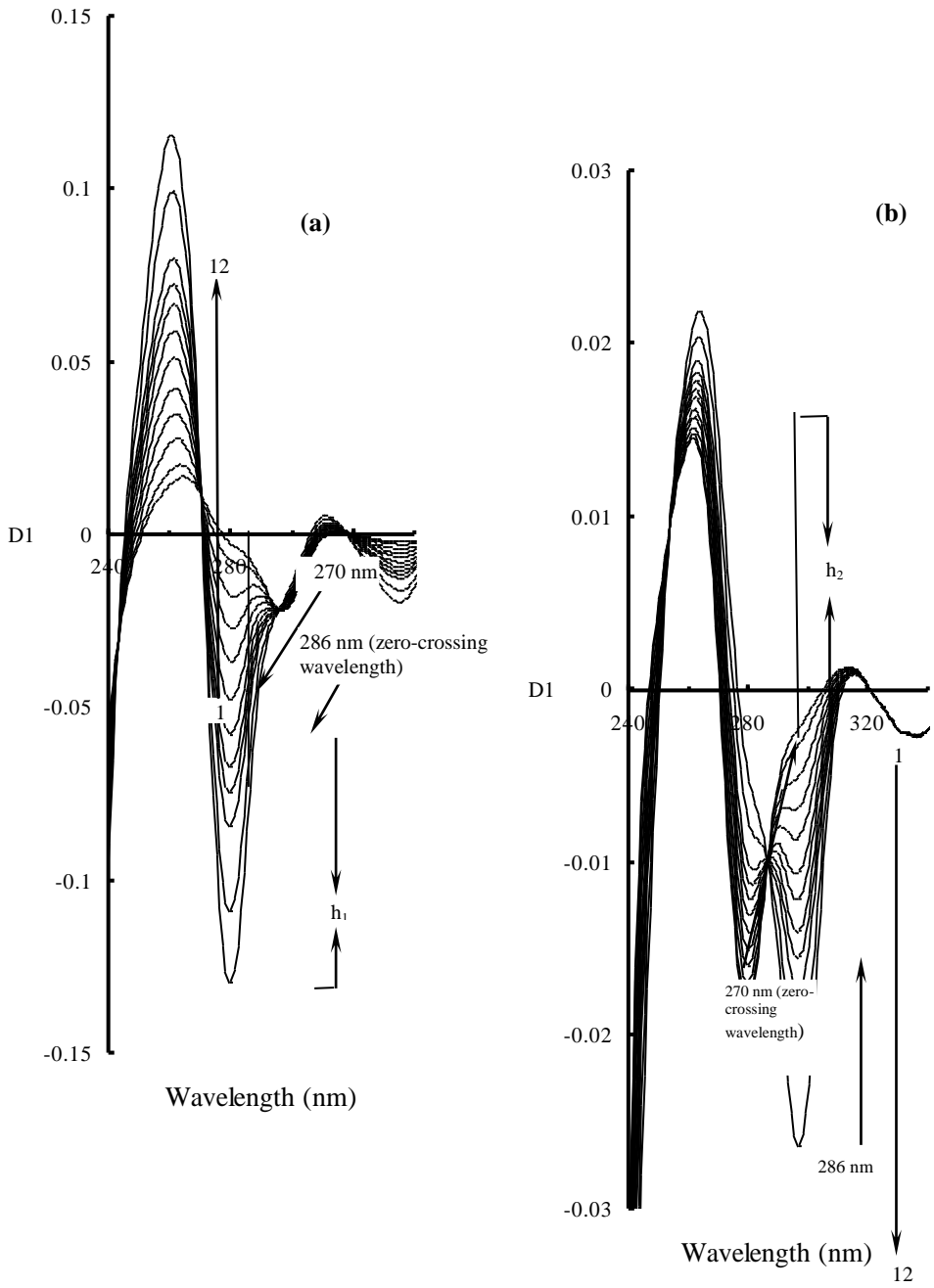


Fig. 3. First-derivative spectra of:

- (a) 21.1  $\mu\text{g mL}^{-1}$  of MD in the presence of (1) 3.53; (2) 4.7; (3) 7.14; (4) 9.53; (5) 11.91; (6) 14.29; (7) 16.67; (8) 19.05; (9) 21.44; (10) 23.82; (11) 29.77; (12) 35.73  $\mu\text{g mL}^{-1}$  HCTZ and,  
(b) 4.7  $\mu\text{g mL}^{-1}$  HCTZ in the presence of (1) 2.53; (2) 3.37; (3) 5.07; (4) 6.76; (5) 8.45; (6) 10.14; (7) 11.83; (8) 13.52; (9) 15.21; (10) 16.90; (11) 21.12; (12) 25.35  $\mu\text{g mL}^{-1}$  MD.

## References

1. Benowitz NL (2004) in: Katzung BG Ed Basic and Clinical Pharmacology, 9<sup>th</sup> ed The McGraw-Hill Companies, New York, NY, p 166
2. Woodson AL, Smith DE (1970) *Anal Chem* 42:242-248
3. Stewart JT, Clark SS (1986) *J Pharm Sci* 75:413-415
4. Bigley FP, Grob RL, Brenner GS (1986) *Anal Chim Acta* 181:241-244
5. Magri AL, Balestrieri F, Magri AD, Marini D (1995) *Talanta* 42:1719-1723
6. The United States Pharmacopeia XX (1980), The USP Convention, Inc, MD, USA, pp. 378-379
7. Ouyang J, Baeyens WRG, Delanghe J, Van der weken G, Calokerinos AC (1998) *Talanta* 46:961-968
8. Farthing D, Fakhry I, Ripley BDE, Sica D (1998) *J Pharm Biomed Anal* 17:1455-1559
9. Richter K, Oertel R, Kirch W (1996) *J Chromatogr A* 729:293-296
10. Panderi IE (1999) *J Pharm Biomed Anal* 21:257-265
11. Saglik S, Sagirli O, Atmaca S, Ersoy L (2001) *Anal Chim Acta* 427:253-257
12. Martin ME, Hernandez OM, Jimenez AI, Arias JJ, Jimenez F (1999) *Anal Chim Acta* 381:247-256
13. El Gindy A, Ashour A, Abdel-Fattah L, Shabana MM (2001) *J Pharm Biomed Anal* 25:171-179
14. Carlucci G, Palumbo G, Mazzeo P, Quaglia MG (2000) *J Pharm Biomed Anal* 23:185-189
15. Ertürk S, Çetin SM, Atmaca S (2003) *J Pharm Biomed Anal* 33:505-511
16. Dinç E, Baleanu D (2002) *J Pharm Biomed Anal* 30:715-723
17. Vignaduzzo SE, Maggio RM, Castellano PM, Kaufman TS (2006) *Anal Bioanal Chem* 386:2239-2244
18. Myhre E (1972) *Acta Med Scand* 191:343-347
19. Kim BK, Koda RT (1977) *J Pharm Sci* 66:1632-1634
20. Rona K, Gachalyi B, Vereczkey L, Nadas B, Kaldor A (1987) *Int J Clin Pharm Ther Toxicol* 25:515-518
21. Oliveira CH, Barrientos-Astigarraga RE, Sucupira M, Graudenz GS, Muscará MN, De Nucci G (2002) *J Chromatogr B* 768:341-348

22. Róna K, Ary K, Gachályi B, Klebovich I (1996) *J Chromatogr A* 730:125-131
23. Lucarelli C, Betto P, Ricciarello G (1991) *J Chromatogr* 541:285-296
24. Zürcher G, Da Prada M (1990) *J Chromatogr* 530:253-262
25. Dilger C, Salama Z, Jaeger H, *Arzneim-Forsch* (1987) *Drug Res* 37:1399-1407
26. Mell LD, Gustafson AB (1978) *Clin Chem* 24:23-26
27. Kochak GM, Mason WD (1980) *J Pharm Sci* 69:897-900
28. Nevado JJB, Gallego JML, Laguna PB (1995) *Fresenius J Anal Chem* 353:221-223
29. Ribeiro PRS, Gomes Neto JA, Pezza L, Pezza HR (2005) *Talanta* 67:240-244
30. Walsh MI, Abou Ouf A, Salem FB (1982) *J Assoc Off Anal Chem* 65:1445-1551
31. Mohamed WI, Salem FB (1984) *Anal Lett* 17:191-203
32. Salem FB (1987) *Talanta* 34:810-812
33. Salem FB (1993) *Anal Lett* 26:1959-1966
34. Davidson AG (1984) *J Pharm Sci* 73:1582-1584
35. Salem FB (1985) *Anal Lett* 18:1063-1075
36. El-Rabbat NA, Omar NM (1978) *J Pharm Sci* 67:779-781
37. Zivanovic L, Vasiljevic S, Radulovic D (1991) *Boll Chim Farm* 130:162-165
38. Nagaraja P, Vasantha RA, Murthy KCS, Rangappa KS (2001) *Chem Anal (Warsaw, Pol)* 46:569-576
39. Issopoulos PB (1990) *Fresenius J Anal Chem* 336:124-128
40. Aman T, Khan IU, Aslam N, Ahmed I (1998) *Anal Lett* 31:1007-1020
41. Nagaraja P, Murthy KCS, Rangappa KS, Gowda NMM (1998) *Talanta* 46:39-44
42. Vieira IC, Fatibello-Filho O (1998) *Talanta* 46:559-564
43. Issopoulos PB, Economou PT (1993) *Farmaco* 48:127-135
44. Mohamed WI, Abou Ouf A, Salem FB (1985) *J Assoc Off Anal Chem* 68:91-95
45. Nagaraja P, Vasantha RA, Sunitha KR (2001) *Talanta* 55:1039-1046
46. Issopoulos PB (1989) *Pharm Acta Helv* 64:82-85
47. Oliveira CC, Zagatto EAG, Araújo AN, Lima JLF (1998) *Anal Chim Acta* 371:57-62
48. Haaland DM, Thomas EV (1988) *Anal Chem* 60:1193-1202