

Application of the Bivariate Calibration for Simultaneous Determinations of Hydrochlorothiazide/Enalapril Maleate and Hydrochlorothiazide/Bisoprolol Fumarate in Drug Tablets

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Abstract

The bivariate calibration method was used for quantification of hydrochlorothiazide/enalapril maleate and hydrochlorothiazide/bisoprolol fumarate in pharmaceutical tablets. The determination of hydrochlorothiazide/enalapril maleate is made using new absorption wavelengths at 211 and 272 nm, while hydrochlorothiazide/bisoprolol fumarate is made at new wavelengths 273.5 and 293 nm. The results are compared favorably to those obtained from HPLC. The method is simple, time saving, and costly effective; it could be used to determine the pharmaceutical compounds in commercial available products.

Keywords: hydrochlorothiazide, enalapril maleate, bisoprolol fumarate, bivariate calibration, drug tablets

1. Introduction

Angiozide[®] and Ziac[®] are both formulated to treat hypertension in the form of tablets. The two drugs contain hydrochlorothiazide (HCT) at various proportions. HCT is a very effective thiazide diuretic. HCT is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, Figure 1. In addition, Angiozide[®] contains enalapril (EN) maleate salt, EN is (S)-1-((S)-2-((S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino)propanoyl)pyrrolidine-2-carboxylic acid, Figure 1, which is known to treat hypertension and congestive heart failure.

Ziac[®] contains Bisoprolol (BIS) Fumarate salt, BIS is a well known beta blocker. It is used to treat cardiac arrhythmias and hypertension. BIS is 2-Propanol-1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino], Figure 1.

Simultaneous determination of EN and HCT has been carried by high performance liquid chromatography (HPLC) and by the first- and second-derivative ultraviolet spectrophotometry (Carlucci, Giuseppe, & Mazzeo, 1993; El Walily, Belal, Heaba, & El Kersh, 1995; Pawar, Nageswara-Rao, & Sankar, 2011; Shetkar & Shinde, 1997; Sowjanya, Gangadhar, Ramalingeswara-Rao, Subrahmanyam, & Suresh, 2012). While, simultaneous determination of BIS and HCT has been reported using only HPLC methods (Joshi, Karbhari, Bhoir, Bindu, & Das, 2010; Shaikh et al., 2008).

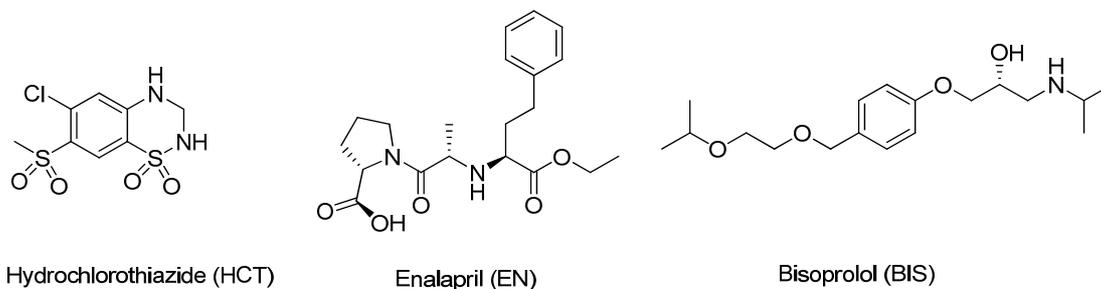


Figure 1. Schematic chemical structure of hydrochlorothiazide (HCT), enalapril (EN), and bisoprolol (BIS)

The bivariate calibration spectrophotometric method is simple and reliable method for estimation and resolution of binary mixtures, since it works to produce optimal wavelengths for severely overlapped absorption spectra of binary mixtures. This property is considered the main advantage of the bivariate calibration method over the derivative spectrophotometric and HPLC methods (Dinç & Üstündağ, 2005; Hoizey et al., 2002; Karpínska, Sokół, & Skoczylas, 2008; Laud, Pednekar, Vaidya, Joshi, & Parekh, 2009; López-Martínez, López-de-Albab, & Cerdá-Martín, 2001; Metwally, 2008; Salama, Nassar, Sharaf El-Din, Attia, & Yousri, 2011; Vujić, Crevar, Obradović, Kuntić, & Uskoković-Marković, 2009).

The objective of this work is to investigate the feasibility of the bivariate calibration method to provide optimal wavelengths that could be used for the simultaneous determination of EN Maleate/HCT and BIS fumarate/HCT in pharmaceutical binary mixtures with good accuracy and precision of the results.

2. Experimental

2.1 Bivariate Method

2.1.1 Instrument

A Shimadzu (UV-1800) spectrophotometer (Japan) for UV-vis measurements using 1-cm quartz cuvette. The spectrophotometer is connected to an HP Compaq computer, the data analyses are carried out using UV-probe 2.33 software.

2.1.2 Materials, Chemicals and Reagents

HCT (purity, 99.2%) is kindly provided by Unichem Laboratories Limited (India), Batch No. ROHCT/g0414. BIS Fumarate (purity, 98.1%) is supplied by Unichem Laboratories Limited (India), Batch No. ROBFU/g0305. EN (purity, 98.5%) is supplied by Jordan-Sweedan Medical and Sterilization Co., Batch No. EM10305005. The commercial mixture of HCT and EN Maleate drug (Anjzide[®]) is available as tablets at 12.5 mg HCT and 20 mg EN Maleate. The Anjzide[®] tablet is manufactured by the Jordanian Pharmaceutical Manufacturing Co., Batch No. 110401. The commercial mixture of HCT and BIS Fumarate drug (Ziac[®]) is available as tablets at 6.25 mg HCT and 10 mg BIS Fumarate. Ziac[®] tablet is manufactured by Watson Pharmaceuticals, Inc., USA. All solutions were prepared using analytical-reagent grade chemicals. Doubly distilled water was used to prepare the stock and working solutions.

2.1.3 Preparation of Standards

500 mg/L stock solutions of HCT, EN, and BIS Fumarate were prepared in methanol/water (10:90, v/v) solutions. Working solutions in the range of 10 to 50 mg/L HCT, 10 to 50 mg/L EN, and 20 to 100 mg/L BIS Fumarate were prepared to construct the calibration curves. Several mixtures of HCT/EN and HCT/BIS Fumarate were prepared in the methanol/water solutions.

The stock of drug samples were prepared by crushing several tablets from each drug to powder, then accurately weighed powders were dissolved in 20 ml methanol/water (10:90, v/v) solution and sonicated for 10 minutes to dissolve the drug before the solutions were filtered using 0.45 micron Whatman filter paper to remove insoluble additives, and after that the mixtures dissolved drugs were diluted in 100 ml volumetric flasks. Several concentrations of each drug were prepared by the step dilution procedure.

2.2 Liquid Chromatography Method

2.2.1 Instrument

Integrated HPLC system from Shimadzu Corporation (Chromatographic and Spectrophotometric Division, Kyoto, Japan) was used. The system consisted of a LC 20AT pump, column, and a SPD-M20A detector. The chromatograms were recorded and integrated on PC installed with LC solution chromatographic software, version 1.22 SP1 (Shimadzu, Kyoto, Japan).

2.2.2 Reagents and Chromatographic Conditions

All solutions were prepared using HPLC grade solvents. Doubly distilled water was used to stock and working solutions. The chromatographic separations were carried out on a C-18 TRACER EXTRASIL ODS 15 μm 25 \times 0.46. Chromatographic conditions: (1) Analysis of the mixture of HCT/EN were set using the method by El Walily et al. (1995), in which the flow rate, 1.0 mL min⁻¹; wavelength at 215 for 3.5 min, and then changed to 275 nm; Mobile phase: acetonitrile-water (20:80, v/v) with pH = 2.8; (2) Analysis of the mixture of HCT/BIS Fumarate were set using the method that proposed by Joshi et al. (2010), in which the flow rate, 1.0 mL min⁻¹; wavelength at 228 nm; Mobile phase: 0.10 M potassium dihydrogen phosphate buffer and acetonitrile (70:30, v/v).

2.2.3 Sample Preparation and Procedures

Samples were dissolved using the mobile phases; filtered through an HPLC filter. Peak areas were used for quantification based on caffeine as an internal standard. The Caffeine is used in this study since it has similar chemical properties to the analyzed components of drugs and its chemistry is well established in the literature (Walily et al., 1995). The whole analysis was done using two working standard preparation; first standard was injected 6 times while the other injected twice; the standard deviation for six injection were less than 1% that indicates the stability of the HPLC over the analysis time, while the difference between the two standards was less than 2% that ruling out the possibility of personal errors. Reported numbers are average of four replicates and each replicate was injected twice.

3. Results and Discussion

3.1 The Bivariate Method

The analysis of a two-component mixture (AB) can be performed using the absorption method (Deming, Michotte, Massart, Kaufman, & Vandeginste, 1988; López-Martínez et al., 2001; Metwally, 2008). The absorption method is based on the additive absorbance of the two components in the mixture at a certain wavelength, which can be described by the following expression:

$$A_{AB} = \varepsilon_A b C_A + \varepsilon_B b C_B$$

Where A_{AB} is the absorbance of the two components A and B in the mixture; b is the optical path length (1 cm); ε_A , ε_B are the molar absorptivity coefficients of the two components in the mixture at a certain wavelength; C_A , C_B are the concentrations of A and B in the mixture. The concentrations of the two components in mixture can be determined through two sets of absorbance measurements at two different wavelengths, The two optimal wavelengths can be chosen by applying the bivariate calibration procedure which was proposed by Deming et al. (1988), the procedure consists of resolving the determinant of selectivity ($m = \varepsilon b$) matrix (\mathbf{K}):

$$\mathbf{K} = \begin{pmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{pmatrix}$$

where m_{A1} , m_{A2} represents the selectivity parameters of component A at the two selected wavelength (λ_1 and λ_2) and m_{B1} , m_{B2} correspond to the selectivity parameters of component B. In this case the selectivity parameters are considered as the calibration curve slopes for each component at the two selected wavelengths. The absorption values of mixtures at the two selected wavelengths were used for simultaneous determinations of individual components in the mixtures Deming et al. (1988).

3.1.1 Hydrochlorothiazide/Enalapril Maleate Mixture

The bivariate calibration method is applied to the mixture of HCT/EN. Figure 1 shows the absorption spectra of HCT and EN at 10 ppm each in methanol/water (10:90, v/v).

HCT shows three maximum absorbances at wavelengths of 226, 272, and 320 nm, while Enalapril shows a maximum absorbance at a wavelength of 207 nm. The absorption spectrum of the mixture shows an spectral overlapping in the region between 200–240 nm. The absorption in this region is resulted from $\pi \rightarrow \pi^*$ transitions in both compounds.

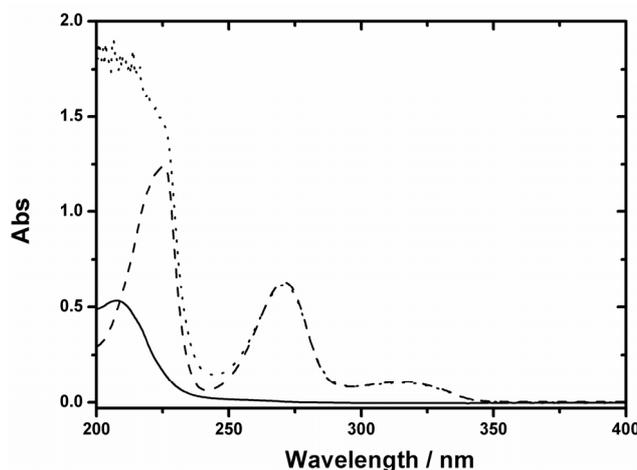


Figure 2. UV-vis spectra of EN (—), HCT (-----), and a mixture of EN/HCT at a concentration of 10 ppm

In order to determine the concentration of each component in the mixture; the signals of the two components were measured at selected seven wavelengths: 211, 234, 242, 252, 272, 292, and 315 nm. The values of slopes obtained for each component represent the sensitivity values. Those sensitivity values for each wavelength pair were defined by resolving the determinants of the selectivity (K), Table 1. The optimal wavelength pairs with the highest absolute sensitivity values for HCT/EN were 211 and 272 nm (Table 1). The linearity was obtained for hydrochlorothiazide in the concentration range 10 to 50 mg/L and enalapril maleate in the concentration range 10 to 50 mg/L. The linear calibration equations used for the mixture are presented in Table 2; the slopes of these equations were used for direct determinations of the drug tablet.

Table 1. The values of the absolute selectivity of Kaiser's Determinant ($K \times 10^5$) for HCT/EN mixture

λ_1/λ_2 (nm)	211	234	242	254	272	292	315
211	—	176.67	10.45	77.85	369.86	49.54	64.53
234	—	—	10.97	3.623	54.12	6.033	9.606
242	—	—	—	5.095	26.09	3.432	4.574
254	—	—	—	—	16.33	1.649	2.918
272	—	—	—	—	—	2.584	0.2826
292	—	—	—	—	—	—	0.4915
315	—	—	—	—	—	—	—

Table 2. Linear calibration equations for HCT/EN mixture used for the bivariate method

Mixture	Components	Calibration Equations	
		$\lambda_1 = 211$ nm	$\lambda_2 = 272$ nm
HCT/EN	HCT/EN	$Y = 0.0661X - 0.2591, r^2 = 0.9999$ $Y = 0.0588X - 0.0604, r^2 = 0.9999$	$Y = 0.0643X - 0.1142, r^2 = 0.9985$ $Y = 0.0014X - 0.0087, r^2 = 0.9982$

Y is the absorbance values at selected wavelength; X is the concentrations in ppm. r^2 is the coefficient of determination.

The applicability of the proposed wavelength pairs to a routine analysis was applied by analyzing laboratory prepared mixtures, in which the concentration ratios of HCT to EN were in the range 1:5 to 5:1 by adjusting their concentrations in the mixtures. A selected set of prepared mixtures and the percentage recoveries are presented in

Table 3. The results of the analysis in Table 3 appear to be quite independent of the concentration ratios of the components in the mixtures. The analysis of a commercially available pharmaceutical drug (Angiozid®) and the percentage recoveries of the drug were calculated and given in Table 4.

Table 3. Determination of HCT/EN in selected sets of laboratory prepared mixtures by the proposed methods

Mixture	HCT			EN		
	Concentration (mg/L)	Found (mg/L)	% Recovery \pm S.D.	Concentration (mg/L)	Found (mg/L)	% Recovery \pm S.D.
1 (1:1)	50	49.6	99.4	50	50.2	100.6
2 (1:5)	10	9.9	99.0	50	48.3	96.6
3 (5:1)	50	50.1	100.2	10	9.4	94.0
Mean \pm S.D. ^a			99.5 \pm 0.61			97.1 \pm 3.32

^a Standard Deviation.

Table 4. Analysis of HCT/EN in a commercially available pharmaceutical drug

Drug	Label Claim (mg/L)		% Recoveries \pm S.D.			
			Bivariate Method ^a		HPLC ^b	
	HCT	EN Maleate	HCT	EN Maleate	HCT	EN Maleate
Angiozid®	12.5	20	97.5 \pm 2.18	98.7 \pm 1.59	96.9 \pm 2.31	99.6 \pm 0.84

^a average of five determinations.

^b average of four determinations.

3.1.2 Hydrochlorothiazide/Bisoprolol Fumarate Mixtures

The absorption spectra of hydrochlorothiazide and bisoprolol fumarate are shown in Figure 3. Bisoprolol shows two maximum absorbances at wavelengths of 226 and 272 nm, which are in complete overlapping with the first two bands of HCT as shown the absorption spectrum of the mixture. The bivariate method was applied on this mixture, the signal at seven wavelengths were measured at 226, 242, 253.5, 264, 270.5, 273.5, and 293. The analysis of (K) determinant showed that the wavelength pairs with highest absolute sensitivity were 273.5 and 293 nm (Table 5).

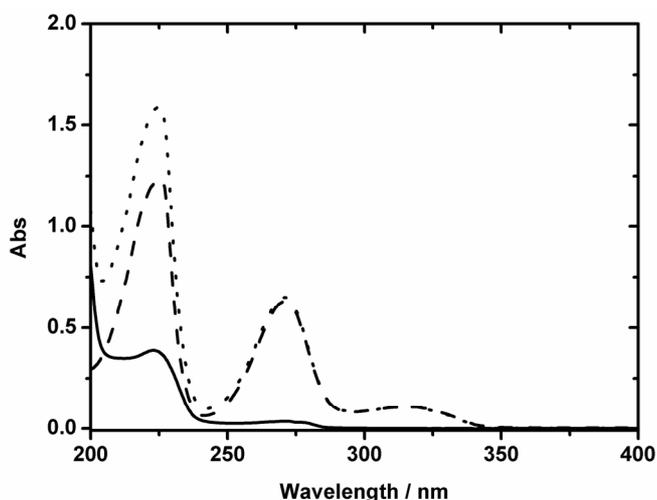


Figure 3. UV-vis spectra of BIS fumarate (—) at a concentration of 30 ppm, HCT (-----) at a concentration of 10 ppm, and a mixture of HCT/ BIS fumarate

Table 5. The values of the absolute selectivity of Kaiser's Determinant ($K \times 10^5$) for HCT/BIS fumarate mixture

λ_1/λ_2 (nm)	226	242	253.5	264	270.3	273.5	293
226	–	87.044	16.006	10.965	34.811	31.143	9.651
242	–	–	108.385	67.265	117.509	118.921	62.977
253.5	–	–	–	1.284	21.370	16.912	38.918
264	–	–	–	–	11.185	9.085	24.899
270.5	–	–	–	–	–	41.158	55.329
273.5	–	–	–	–	–	–	152.762
293	–	–	–	–	–	–	–

Table 6 shows the calibration equations of the mixture at the proposed wavelengths. These wavelengths were applied in the analysis of HCT and BIS Fumarate in different concentration ratios of laboratory prepared mixtures. Results of the percentage recoveries are shown in Table 7, which suggested that method was accurate for the simultaneous estimation of HCT and BIS Fumarate from their combination mixtures. The method was applied for the analysis of one marketed drug (Ziac[®]) containing BIS Fumarate 10 mg and HCT 6.25 mg per tablet. The results of the analysis are given in Table 8.

Table 6. Linear calibration equations of HCT/BIS fumarate used for the bivariate method

Mixture	Components	Calibration Equations	
		$\lambda_1 = 273.5$ nm	$\lambda_2 = 293$ nm
HCT/BIS	HCT/BIS	$Y=0.0832X-0.0149r^2=0.9987$	$Y=0.0337X-0.2254r^2=0.9886$
Fumarate	Fumarate	$Y=0.0172X-0.1019r^2=0.9997$	$Y=0.0007X-0.0261r^2=0.9821$

Y is the absorbance values at selected wavelength; X is the concentrations in ppm. r^2 is the coefficient of determination.

Table 7. Determination of HCT/BIS Fumarate in selected sets of laboratory prepared mixtures by the proposed methods

Mixture	HCT			BIS Fumarate		
	Concentration (mg/L)	Found (mg/L)	%Recovery \pm S.D.	Concentration (mg/L)	Found (mg/L)	% Recovery \pm S.D.
1 (1:1)	50	50.2	100.9	50	47.2	94.4
2 (1:5)	10	9.8	98.0	50	48.5	97.0
3 (5:1)	50	49.9	99.8	10	9.7	97.0
Mean \pm S.D. ^a			99.6 \pm 1.46			96.1 \pm 1.50

Table 8. Analysis of HCT/BIS Fumarate in a commercially available pharmaceutical drug

Drug	Label Claim (mg/L)		% Recoveries \pm S.D.			
	HCT	BIS fumarate	Bivariate Method ^a		HPLC ^b	
	HCT	BIS fumarate	HCT	BIS fumarate	HCT	BIS fumarate
Ziac [®]	6.25	10	98.2 \pm 1.41	98.1 \pm 0.99	97.8 \pm 1.81	98.8 \pm 1.67

^a average of five determinations.

^b average of four determinations.

3.2 The HPLC Methods

The reversed-phase HPLC methods were used to confirm the results of analysis of bivariate method. Angiozide® which contains HCT/EN maleate was analyzed using the method El Walily et al. (1995), which involves use a C-18 column and mobile phase consisting of acetonitrile/water (20:80, v/v) (pH = 3.8). The wavelengths of detection were changed during the run to achieve maximum responses for the HCT and EN maleate in the drug sample. The chromatographic analysis of Angiozide® sample showed a good resolution of HCT and EN maleate. The order of elution of the Angiozide® sample was EN maleate ($t_r = 2.91$ min), Caffeine ($t_r = 5.70$ min) (the internal standard), and HCT ($t_r = 7.56$ min) at a flow rate of 1.0 mL/min. The chromatogram of the sample also showed the appearance of a few unidentified peaks which may be contributed to the presence of soluble additives in the sample. The results of this analysis are given in Table 4. Similarly, Ziac® which contains HCT/BIS fumarate was analyzed using method by Joshi et al. (2010), The retention times were 7.56 min BIS fumarate, 5.74 min for Caffeine, and 6.81 min for HCT. The results of Ziac® analysis are given in Table 8.

3.3 Statistical Analysis of the Results

The results obtained for the HCT/EN Maleate and HCT/BIS Fumarate in the pharmaceutical tablets in Tables 4 and 8 were statistically compared using Student's t -test and the variance ratio F -test, the results are given in Table 9 and Table 10.

Table 9. Statistical analysis of the results obtained by Bivariate method and the HPLC for Angiozide®

Parameters	Bivariate Method		HPLC	
	HCT	EN Maleate	HCT	EN Maleate
Mean \pm S.D.	97.5 \pm 2.18	98.7 \pm 1.59	96.9 \pm 2.31	99.6 \pm 0.84
N	5	5	4	4
Variance	4.752	2.528	5.336	0.706
t (2.37)*	0.402	1.015	–	–
F (5.19)*	1.123	3.581	–	–

* The values in parentheses are corresponding to the theoretical values of t and F at ($p = 0.05$).

Table 10. Statistical analysis of the results obtained by Bivariate method and the HPLC for Ziac®

Parameters	Bivariate Method		HPLC	
	HCT	BIS fumarate	HCT	BIS fumarate
Mean \pm S.D.	98.2 \pm 1.41	98.1 \pm 0.99	97.8 \pm 1.81	98.8 \pm 1.67
N	5	5	4	4
Variance	1.988	0.980	3.276	2.789
t (2.37)*	0.374	0.788	–	–
F (5.19)*	1.648	2.846	–	–

* The values in parentheses are corresponding to the theoretical values of t and F at ($p = 0.05$).

The Statistical analysis showed that there is no significant difference between the proposed bivariate methods and the HPLC methods, as well as there is no significant difference between the precision of proposed method and the HPLC methods.

The closeness of the results obtained in pharmaceutical tablets to the label claim supports the accuracy of the used method. It was noticed that the accuracy of the pharmaceutical mixtures was different from the claim labels, this may be attributed to the matrix effect since it is accustomed to use insoluble additives in pharmaceutical tablets which is used to hold the structure of tablets.

4. Conclusion

The bivariate method was applied for determining the hydrochlorothiazide/enalapril maleate and hydrochlorothiazide/bisoprolol fumarate in pharmaceutical tablets. The proposed method is simple, time saving,

and does not need any sophisticated apparatus. The samples were also analyzed by standard HPLC methods, the results are in excellent agreement with those obtained by the bivariate method.

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