ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 299–307

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Spectrophotometric determination of benazepril hydrochloride and hydrochlorothiazide in binary mixture using second derivative, second derivative of the ratio spectra and chemometric methods

Alaa El-Gindy [a,*], Ahmed Ashour [b], Laila Abdel-Fattah [c], Marwan M. Shabana [d]

[a] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt*
[b] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo 11562, Egypt*
[c] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Ainy st., Cairo 11562, Egypt*
[d] *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Ainy st., Cairo 11562, Egypt*

Received 31 July 2000; received in revised form 29 September 2000; accepted 14 October 2000

Abstract

Different spectrophotometric methods are presented for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in pharmaceutical tablets. The first method depends on second derivative (^{2}D) ultraviolet spectrophotometry, with zero crossing and peak to base measurement. The second derivative amplitudes at 214.8 and 227.4 nm were selected for the assay of benazepril hydrochloride and hydrochlorothiazide, respectively. The second method depends on second derivative of the ratio spectra by measurement of the amplitudes at 241.2 and 273.2 nm for benazepril hydrochloride and hydrochlorothiazide, respectively. Chemometric methods, classical least squares and principal component regression, were applied to analyze the mixture. Both the chemometric methods were applied to the zero and first order spectra of the mixture. The proposed methods were successfully applied for the determination of the two drugs in laboratory prepared mixtures and in commerical tablets.

Keywords: Benazepril hydrochloride; Chemometrics; Hydrochlorothiazide; Second derivative spectrophotometry; Second derivative of the ratio spectra

1. Introduction

Benazepril hydrochloride, {(3S)-3-[(1S)-1-ethoxycarbonyl-3-phenylpropylamino] − 2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-1-yl}acetic acid hydrochloride (**I**) is a potent angiotensin

* Corresponding author. Tel.: + 20-64-561877.
E-mail address: ghada74@ismailia.ie-eg.com (A. El-Gindy).

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00502-1

converting enzyme inhibitor that is used in the treatment of essential hypertension. Hydrochlorothiazide ,6-chhloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamidel,1dioxide (**II**) is one of the oldest and widely used thiazide diuretics. The binary mixture of the two drugs is used in the treatment of hypertension. Various spectrophotometric methods have been reported for the simultaneous determination of both drugs in pharmaceutical tablets using absorbancy ratio measured at 236.4, 258.3 and 264 nm with linearity range 2.5–6.5 and 3–24.8 μg ml^{-1} for (**I**) and (**II**), respectively [1]. Vierordt's method measured at 238.1 and 271.7 nm with linearity range 8–36 and 2–28 μg ml^{-1} for (**I**) and (**II**), respectively [2], first derivative measured at 260.7 and 239.8 nm with linearity range 10–50 and 25–125.1 μg ml^{-1} for (**I**) and (**II**), respectively [1], second derivative measured at 253.6 and 282.6 nm with linearity range 14.8–33.8 and 18.5–42.2 μg ml^{-1} for (**I**) and (**II**), respectively [3], and ratio spectra first derivative measured at 238.8 and 269.5 nm with linearity range 8–36 and 2–28 μg ml^{-1} for (**I**) and (**II**), respectively [2]. HPLC method has been described for the simultaneous determination of the two drugs using a C_{18} microbore column with 0.025 M sodium dihydrogen phosphate (pH 4.8) acetonitrile (55:45 v/v) as a mobile phase [4]. No chemometric method has been reported for the determination of this mixture.

Derivative spectrophotometry offers a convenient solution for a number of well defined analytical problems, such as resolutions of multicomponent systems, elimination of interference from sample turbidity and matrix background, and enhancement of spectral details [5]. Salinas et al. developed a spectrophotometric method based on the use of the first derivative of the ratio spectra for resolving binary mixtures when the spectra of the components are overlapped [6]. The method was extended to the resolution of ternary mixtures in combination with zero-crossing methods [7]. On the other hand, owing to the increase in the resolving power of analytical instrumentation and easier access to microcomputers with appropriate software, the use of multivariate calibration data of analytical signal depending on two or more variables, has become more general. Multivariate methods such as classical least squares (CLS) and principal component regression (PCR) have been used in quantitative spectral analysis to obtain selective information from unselective data. The main advantage of multicomponent analysis by using multivariate calibration is the speed of the determination for the components in mixtures and avoiding the need for prior separation, that is, otherwise necessary owing to the overlapping of the absorption spectra. The CLS method is the easiest of the multivariate methods and is based on calibration by multiple linear regression. Its main disadvantage is that it is a rigid model that needs the knowledge of all the components in the mixture and their concentrations and that there should be no chemical or physical interaction between the components in the mixture. While the PCR method is more flexible and does not need these requirements for its application [8].

The present work presents more sensitive second derivative (^{2}D); second derivative of the ratio spectra (^{2}DD); classical least squares (CLS) and principal component regression (PCR) of the UV absorption (A) and first derivative (^{1}D) spectra for simultaneous determination of (**I**) and (**II**) in tablets.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–vis spectrophotometer, model UV–1601PC connected to an IBM compatible computer and a HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.

The absorption spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–280 nm. The first and second derivatives of the measured spectra were obtained using the accompanying software with $\Delta\lambda = 4$ nm and scaling factor of 10.

2.2. Materials and reagents

Pharmaceutical grade of benazepril hydrochloride and hydrochlorothiazide (Ciba–Geigy, Switzerland) were kindly supplied by Swisspharma (Cairo, Egypt) and certified to contain 99.70 and 99.99 %, respectively. Methanol and hydrochloric acid used were of analytical grade.

The commercial Cibadrex tablets used (Batch No. 007), was manufactured by Swisspharma (Cairo, Egypt) under license from Ciba–Geigy Ltd., Basle, Switzerland. Each tablet contains 10 and 12.5 mg of benazepril hydrochloride and hydrochlorothiazide, respectively, in addition to tablet excipients consisting of hydrogenated castor oil, lactose, crospovidone, hydroxypropyl mythylcellulose, macrogol 8000, talc, titanium dioxide and red iron oxide. No interferences resulted from tablet excipients during the analytical procedures.

2.3. Standard solutions and calibration

Stock standard solutions of each of (**I**) and (**II**) were prepared separately by dissolving 100 mg of each drug in 100 ml methanol.

The standard solutions were prepared by dilution of the stock solutions with 0.1 M hydrochloric acid to reach concentration range of 4–20 μg ml^{-1} for (**I**) and (**II**).

2.3.1. For ^2D method

The values of the ^2D amplitudes were measured at 214.8 nm (zero-crossing of **II**) and 227.4 nm (zero-crossing of **I**) for the determination of (**I**) and (**II**), respectively.

2.3.2. For ^2DD method

For (**I**), UV absorption spectra of standard solutions of (**I**) were divided by a normalized spectrum of (**II**) (a spectrum of unit concentration) The second derivative was calculated for the obtained spectra with $\Delta\lambda = 4$ nm. The second derivative of the ratio spectra obtained were smoothed with 16 experimental points. The amplitudes at 241.2 nm were measured and found to be proportional to the concentration of (**I**).

For (**II**), UV absorption spectra of standard solutions of (**II**) were divided by a normalized spectrum of (**I**). The second derivative was calculated for the obtained spectra with $\Delta\lambda = 4$ nm. The second derivative of the ratio spectra obtained were smoothed with 16 experimental points. The amplitudes at 273.2 nm were measured and found to be proportional to the concentration of (**II**).

2.3.3. For CLS and PCR methods

A training set of 16 synthetic mixtures with different concentrations of (**I**) and (**II**) in the range of 4–20 μg ml^{-1} within concentration ratio ranged from 1:0.66 to 1:1.56 for (**I**) and (**II**), respectively, were prepared in 0.1 M hydrochloric acid (Table 3).

The UV absorption and first derivative were recorded over the range 200–280 nm. The data points of the spectra were collected at every 1 nm. The data was then exported into excel®. The computations were made in Microsoft® Excel 97-Arabic edition using macros written in VISUAL BASIC for application. The multivariate calibration models were applied to these mixtures to calculate the concentrations of each component.

2.4. Sample preparation

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 10 mg of (**I**) and 12.5 mg of (**II**) was weighed accurately, dissolved and diluted to 100 ml with methanol. The sample solution was filtered. Further dilution was carried out with 0.1 M hydrochloric acid to provide a solution of 10 μg ml^{-1} of (**I**) and 12.5 μg ml^{-1} of (**II**). The general procedures for ^2D, ^2DD, CLS and PCR described under calibration were followed and the concentrations of (**I**) and (**II**) were calculated.

2.5. Percent recovery study

This study was performed by addition of known amounts of (**I**) and (**II**) to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained were compared with expected results (Table 4).

3. Results and discussion

3.1. ^2D method

The UV absorption spectra of weakly absorbing compound (**I**) and strongly absorbing compound (**II**) in 0.1 M hydrochloric acid were produced in Fig. 1a. The two spectra clearly display considerable overlap; hence, the direct UV absorption measurement for assaying binary mixture seems to be impossible. The second order derivative spectra (^2D) present spectral features which can be used for the simultaneous determination of (**I**) and (**II**) (Fig. 1b). The zero crossing method is the most common procedure for the preparation of analytical calibration graph [9]. (**I**)

Table 1
Characteristic parameters for the regression equations of second derivative (^2D) and second derivative of the ratio spectra (^2DD) methods for determination of benazepril hydrochloride (**I**) and hydrochlorothiazide (**II**)

Parameters	^2D		^2DD	
	I	**II**	**I**	**II**
Linearity (μg ml^{-1})	4–20	4–20	4–20	4–20
Regression equation (Y)[a]: Slope (b)	5.50×10^{-3}	4.02×10^{-2}	14.64×10^{-2}	4.86×10^{-2}
S.D. of the slope (S[b])	3.01×10^{-5}	1.30×10^{-4}	2.33×10^{-5}	1.18×10^{-4}
R.S.D. of the slope (%)	0.55	0.32	0.02	0.37
Confidence limit of the slope[b]	5.40×10^{-3}–5.60×10^{-3}	3.96×10^{-2}–4.07×10^{-2}	14.60×10^{-2}–14.70×10^{-2}	4.81×10^{-2}–4.90×10^{-2}
Intercept (a)	-2.70×10^{-3}	5.20×10^{-3}	8.68×10^{-2}	2.1×10^{-3}
S.D. of the intercept (S[a])	3.8×10^{-4}	1.5×10^{-3}	4.1×10^{-4}	2.2×10^{-3}
Confidence limit of the intercept[b]	-1.77×10^{-3}–(-3.63×10^{-3})	1.5×10^{-3}–8.8×10^{-3}	7.67×10^{-2}–9.68×10^{-2}	-3.29×10^{-3}–7.59×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	4.5×10^{-4}	3.8×10^{-5}	4.8×10^{-4}	3.0×10^{-3}

[a] $Y=a+bC$, where C is the concentration of drug in μg ml^{-1} and Y is the amplitude at the specified wavelength.
[b] 95% confidence limit.

Table 2
Determination of benazepril hydrochloride (**I**) and hydrochlorothiazide (**II**) in laboratory prepared mixtures using second derivative (^2D) and second derivative of the ratio spectra (^2DD) methods

Mixture No.	Mixture composition (μg ml^{-1})		% Recovery			
	I	**II**	^2D		^2DD	
			I	**II**	**I**	**II**
1	10	10	99.8	99.4	99.9	99.6
2	10	15	100.3	100.8	100.3	100.5
3	8	10	99.4	100.1	99.2	100.4
4	10	12.5	100.1	100.2	100.2	100.2
5	14	20	99.5	100.0	99.7	100.7
6	18	17.5	100.8	100.5	100.5	100.6
7	10	7.5	99.9	99.3	99.9	99.2
Mean			99.97	100.04	99.96	100.17
±S.D			0.48	0.54	0.43	0.56

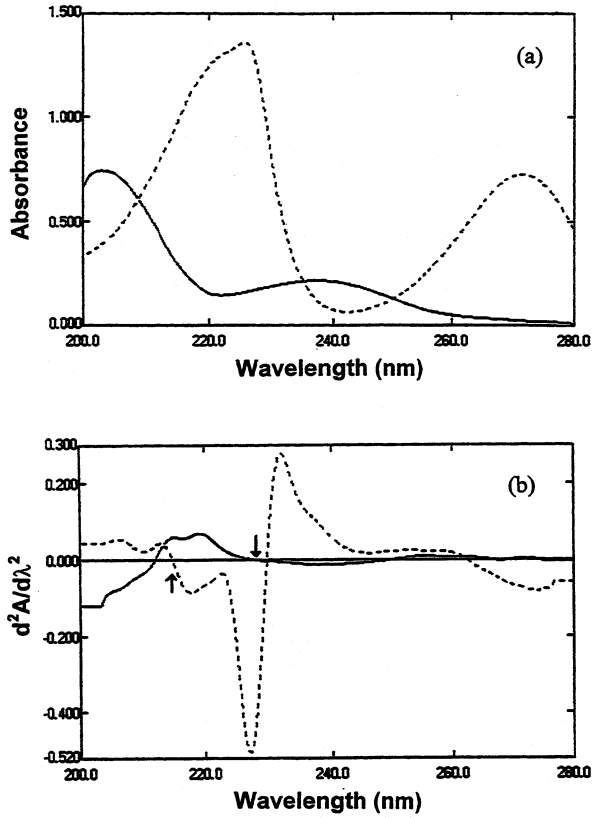

Fig. 1. UV absorption spectra (a) and second derivative spectra (b) of 10 μg ml^{-1} benazepril hydrochloride (—) and 12.5 μg ml^{-1} hydrochlorothiazide (- - - -) in 0.1 M hydrochloric acid.

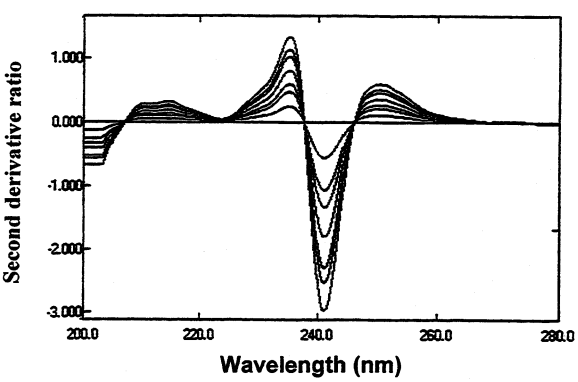

Fig. 2. Second derivative of the ratio spectra for different concentrations (4, 7, 9, 12, 15.5, 17, 20 μg ml^{-1}) of benazepril hydrochloride, using normalized spectrum of hydrochlorothiazide as a devisor.

was determined by measurement of its second derivative amplitude at the zero-crossing point of (**II**) (at 214.8 nm). While (**II**) was determined by measurement of its second derivative amplitude at the zero-crossing point of (**I**) (at 227.4 nm). The plots of the absolute values of second derivative at 214.8 and 227.4 nm against concentration of (**I**) and (**II**), respectively, showed linear relationship.

3.2. ^2DD method

The main advantage of the derivative of the ratio spectra method may be the chance of doing measurements in correspondence of peaks, hence there is a potential for greater sensitivity and accuracy. While 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. This may be particularly dangerous when the slope of the spectrum is very high with consequent loss of accuracy and precision, and the working wavelength is in proximity of the base of the spectrum which causes poor sensitivity [5]. Fortunately, in the present case, the above circumstances did not occur.

The second derivative of the ratio spectra was preferred than the first derivative for a better resolution of the ratio spectra and more accurate and precise results. In this method, the UV absorption spectra of (**I**) were divided by a normalized spectrum [10] of (**II**) (obtained by dividing the spectra for several standards of different concentrations by their corresponding concentrations and subsequently averaging them, in order to obtain a spectrum of unit concentration). The second derivative was calculated for the ratio spectra obtained with $\Delta\lambda = 4$ nm. These spectra were smoothed with 16 experimental points due to the high noise of the signals obtained [10], (Fig. 2). The concentration of (**I**) was proportional to the amplitude at 241.2 nm, in the concentration range 4–20 μg ml^{-1}. Similarly for determination of (**II**), the UV absorption spectra of (**II**) were divided by a normalized spectrum of (**I**). From the

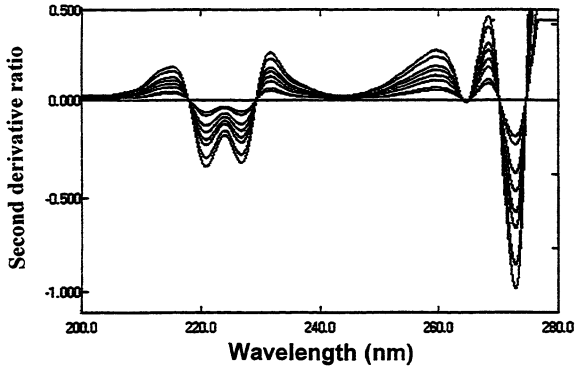

Fig. 3. Second derivative of the ratio spectra for different concentrations (4, 5, 7.5, 10, 12, 14, 17.5, 20 μg ml^{-1}) of hydrochlorothiazide, using normalized spectrum of benazepril hydrochloride as a devisor.

ratio spectra obtained, second derivative was calculated with $\Delta\lambda = 4$ nm. These spectra were also smoothed with 16 experimental points (Fig. 3). The concentration of (**II**) was proportional to the amplitude at 273.2 nm, in the concentration range 4–20 μg ml^{-1}.

For the ^{2}D and ^{2}DD methods, the characteristic parameters of regressions equations and correlation coefficients are given in Table 1.

The accuracy of the ^{2}D and ^{2}DD methods were checked by analysing seven laboratory-prepared mixtures of (**I**) and (**II**) at various concentrations ranged from 4 to 20 μg ml^{-1} (Table 2). Satisfactory recoveries with small standard deviations were obtained, which indicated the high repeatability and accuracy of the two methods. The ^{2}D and ^{2}DD methods were able to determine (**I**) and (**II**) within the concentration ratio ranged from 1:0.75 to 1:1.5, respectively.

Table 3
Recoveries of benazepril hydrochloride (**I**) and hydrochlorothiazide (**II**) in training samples determined by CLS and PCR applied to UV absorption (A) and first derivative (^{1}D) spectra using leave one out procedure

Mixture No.	Mixture composition (μg ml^{-1})		% Recovery							
	I	**II**	CLS				PCR			
			A		^{1}D		A		^{1}D	
			I	**II**	**I**	**II**	**I**	**II**	I	II
1	4.00	5.00	101.5	100.0	100.5	99.9	100.0	100.1	100.8	99.6
2	4.00	6.25	101.3	100.4	100.2	99.6	100.0	100.4	100.0	99.5
3	6.00	4.00	101.8	100.1	99.9	99.8	100.2	100.0	99.6	99.8
4	6.00	6.25	101.2	100.2	100.9	100.2	100.0	100.1	100.1	99.8
5	6.00	7.50	101.6	100.0	100.6	100.0	100.1	100.0	100.0	100.0
6	8.00	8.75	100.5	99.9	100.1	100.2	100.5	99.8	99.9	100.2
7	8.00	10.00	100.0	100.0	99.8	100.0	100.0	100.0	99.8	100.0
8	10.00	8.75	100.6	100.0	98.4	99.2	100.0	100.0	100.0	99.9
9	10.00	10.00	99.3	99.9	99.4	100.0	99.6	99.5	99.6	99.4
10	10.00	12.50	98.7	100.0	99.4	100.0	99.8	100.2	99.4	100.2
11	10.00	15.00	100.0	100.6	99.7	100.9	100.2	100.6	100.5	100.8
12	14.00	12.50	100.0	100.2	99.6	100.2	100.3	100.8	100.1	100.9
13	14.00	15.00	99.2	100.4	98.9	100.4	100.0	100.0	99.7	100.0
14	18.00	17.50	100.4	100.0	100.0	99.4	100.7	99.4	100.9	99.4
15	20.00	17.50	100.5	99.9	99.8	99.6	100.6	99.4	100.7	99.4
16	20.00	20.00	100.5	100.5	99.9	100.5	100.6	100.6	100.2	100.7
Mean			100.44	100.13	99.82	99.99	100.16	100.05	100.08	99.98
±S.D.			0.89	0.22	0.62	0.42	0.31	0.41	0.44	0.49
RMSEP			0.081	0.042	0.066	0.062	0.060	0.065	0.06	0.075

Table 4
Determination of benazepril hydrochloride (**I**) and hyddrochlorothiazide (**II**) in commercial tablet using second derivative (^{2}D) and second derivative of the ratio spectra (^{2}DD); classical least squares (CLS) and principal component regression (PCR) applied to UV absorption (A) and first derivative (^{1}D) spectra; and HPLC methods

	Mean ± S.D[a]						
	^{2}D	^{2}DD	CLS		PCR		
			A	^{1}D	A	^{1}D	HPLC
Commercial tablets							
For (**I**)	100.6 ± 0.67	100.5 ± 0.59	100.1 ± 1.36	100.1 ± 0.83	100.1 ± 0.56	100.2 ± 0.54	100.3 ± 0.62
	t = 0.73	0.52	0.30	0.43	0.54	0.27	(2.31)[b]
	F = 1.17	1.10	4.81	1.79	1.23	1.32	(6.39)[b]
For (**II**)	99.7 ± 0.77	99.5 ± 0.63	100.2 ± 0.51	100.1 ± 0.66	100.2 ± 0.60	100.0 ± 0.64	99.6 ± 0.57
	t = 0.23	0.26	1.75	1.28	1.62	1.04	(2.31)[b]
	F = 1.82	1.22	1.25	1.34	1.11	1.26	(6.39)[b]
Recovery[c]							
For (**I**)	100.1 ± 0.56	1002 ± 0.41	99.9 ± 1.37	100.3 ± 0.94	100.1 ± 0.79	100.0 ± 0.58	
For (**II**)	99.9 ± 0.63	100.2 ± 0.78	100.2 ± 0.32	100.0 ± 0.67	99.9 ± 0.77	99.9 ± 0.68	

[a] Mean and S.D for five determinations, percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content (n = 5).

3.3. CLS and PCR methods

The multivariate methods (CLS and PCR) were applied with UV absorption and first derivative spectra. For application of these multivariate methods, a training (calibration) set of 16 binary samples with different concentrations of (**I**) and (**II**) in the range of 4–20 μg ml^{-1} were prepared. The multivariate models developed were validated using cross validation procedure (leave one out) [11]. Given the set of 16 training spectra corresponding to samples listed in table (3), the CLS and PCR calibrations on 15 training spectra were performed, and using these calibrations, the concentration of the left out sample during calibration was determined. This process was repeated 15 times until each training sample had been left once. The predicted concentrations of the components in each sample were compared with the known concentrations in this training sample and the root mean square error of prediction (RMSEP) was calculated for each method (Table 3). The RMSEP was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both of the precision and accuracy of predictions and plays the same role of standard deviation in indicating the spread of the concentration errors [12]. The results are shown in Table 3 indicating good accuracy and precision.

Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating an adequate model for CLS and PCR methods applied on UV absorption and first derivative spectra of (**I**) and (**II**).

3.4. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels — 50, 75, 100, 125 and 150% of label claim for each individual drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for each drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy

and precision of the proposed methods for determination of each drug. The recoveries ranging from 99.2 to 100.7% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.7%, the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [13] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [13], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the proposed methods were found to be 0.8, 0.3, 0.7 and 0.6 μg ml^{-1} for (**I**) and 0.1, 0.2, 0.5 and 0.4 μg ml^{-1} for (**II**), detected by ^{2}D, ^{2}DD, CLS and PCR methods, respectively. While the quantitation limits of the proposed methods were found to be 3, 2, 1.5 and 1.3 μg ml^{-1} for (**I**) and 2, 1.5, 1.2 and 1.4 μg ml^{-1} for (**II**), determined by ^{2}D, ^{2}DD, CLS and PCR methods, respectively.

The stability of benazepril hydrochloride and hydrochlorothiazide during the analytical procedures were studied and found to be stable. The two analytes were stable for at least 24 h in solution.

3.5. Tablets analysis

The proposed methods were applied to the determination of (**I**) and (**II**) in commercial tablets. Five replicates determinations were made. Satisfactory results were obtained for both drugs and were in a good agreement with the label claims (Table 4). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding (**I**) and (**II**) to the earlier analysed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of the commercial tablets and the recovery study (standard addition method) of both the drugs (Table 4) suggested that there is no interference from any excipients which are present in tablets.

The results of determination of (**I**) and (**II**) in tablets obtained from the ^{2}D, ^{2}DD, CLS and PCR methods were compared with those of the HPLC method, using a C_{18} microbore column with 0.025 M sodium dihydrogen phosphate (pH 4.8) acetonitrile (55:45, v/v) as a mobile phase [4]. Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and the F-ratio at 95% confidence level (Table 4). There is no significant difference between the proposed methods and reference HPLC method, with regard to accuracy and precision.

4. Conclusion

The proposed methods (^{2}D, ^{2}DD, CLS and PCR) can be used for simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in tablets. The ^{2}D method is more rapid and simple than ^{2}DD method. While the ^{2}DD method has greater sensitivity and accuracy. The easier treatment of the data by using CLS and PCR methods makes them preferable to the ^{2}DD method. The proposed methods are suitable for routine determination of benazepril hydrochloride and hydrochlorothiazide in their formulations, but they can not be considered as stability indicating assays.

References

[1] N. Erk, F. Onur, Analusis 25 (5) (1997) 161–163.
[2] N. Erk, J. Pharm. Biomed. Anal. 20 (1999) 155–167.
[3] I.E. Panderi, J. Pharm. Biomed. Anal. 21 (2) (1999) 257–265.
[4] I.E. Panderi, P.M. Parissi, J. Pharm. Biomed. Anal. 21 (5) (1999) 1017–1024.

[5] B. Morelli, Talanta 41 (5) (1994) 673–683.
[6] F. Salinas, J.J. Berzas Nevado, A. Espinosa Mansilla, Talanta 37 (3) (1990) 347–351.
[7] J.J. Berzas Nevado, C. Guiberteau Cabanillas, F. Salinas, Talanta 39 (5) (1992) 547–553.
[8] T. Galeano Diaz, A. Guiberteau, J.M. Ortiz Burguillos, F. Salinas, Analyst 122 (1997) 513–517.
[9] J.J. Berzas Nevado, J. Rodriguez Flores, M.J. Villasenor Llerena, Anal. Lett. 27 (5) (1994) 1009–1029.
[10] J.M. Garcia, O. Hernandez, A.I. Jimenez, F. Jimenez, J.J. Arias, Anal. Chim. Acta 317 (1995) 83–93.
[11] Y. Ni, X. Gong, Anal. Chim. Acta 354 (1997) 163–171.
[12] K.R. Beebe, J.P. Randy, M.B. Seasholtz, Chemometric: A Practical Guide, Weily-Interscience, 1998.
[13] The United States Pharmacopoeia, 24 revision, Asian Edition, United States Pharmacopoeial Convention, INC. Twinbrook Parkway, Rockville, MD, 2000, pp. 2149–2152.