Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4 (1):529-537
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous determination of valsartan and hydrochlorothiazide in their pharmaceutical formulations

Mamdouh R. Rezk[a], Naema M. El Remali[b], Abdel-Aziz El Bayoumi Abdel Aleem[a]

[a]*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt*
[b]*Analytical Chemistry Department, Faculty of Pharmacy, Benghazi University, Benghazi, Libya*

ABSTRACT

Four methods namely, first-derivative of ratio spectra, bivariate, thin layer chromatography and high performance liquid chromatography were used to determine valsartan and hydrochlorothiazide simultaneously in their pharmaceutical dosage forms. The derivative ratio spectra method was based on measuring the peak amplitudes for valsartan at 233 nm and 253 nm using 0.4µg mL^{-1} hydrochlorothiazide as a divisor. Bivariate method is used for simultaneous determination of both drugs by measuring the absorbance at the selected wavelengths. A TLC separation with densitometric detection of both drugs was achieved using chloroform: methanol: ammonia [8:2:0.1, v/v/v] as developing solvent. Furthermore, a high performance liquid chromatographic procedure with ultraviolet detection at 225 nm was developed for the separation and determination of the studied drugs using a C_{18} column. The mobile phase is composed of 0.02 M phosphate buffer (pH 2.9): acetonitrile: methanol [50: 40: 10, v/v/v]. The proposed methods were successfully applied for the determination of the studied drugs in their mixtures and in pharmaceutical formulations containing them.

Keywords: Valsartan; Hydrochlorothiazide; Ratio spectra; Bivariate; TLC & HPLC.

INTRODUCTION

Valsartan [VAL],is *N*-[*p*-(*o*-1*H*-Tetrazol-5-ylphenyl)benzyl]-*N*-valeryl-L-valine [1]. It is an orally active angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients [2], figure 1.

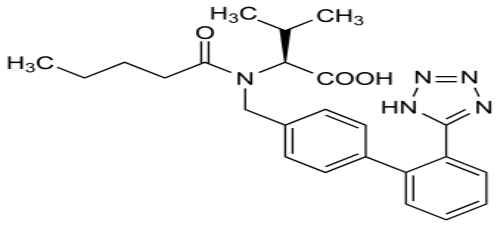


Fig.1. Structural formula of valsartan [VAL]
M.W. [$C_{24}H_{29}N_5O_3$ = 435.5]

Hydrochlorothiazide [HCZ], is 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [1]. It is a diuretic that is widely used in antihypertensive pharmaceutical formulations, figure 2. It decreases active sodium re-absorption and reduces peripheral vascular resistance [2]. It is used in association with other drugs in the treatment of hypertension.

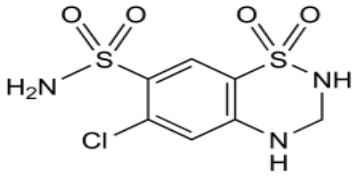


Fig.2. Structural formula of hydrochlorothiazide [HCZ]
M.W. [$C_7H_8ClN_3O_4S_2$ = 297.7]

Simultaneous determination of both drugs is highly desirable and could be more cost-effective than separate assays. Many methods were developed for the simultaneous determination of valsartan and hydrochlorothiazide in tablets. These methods include spectrophotometric methods [3-5], HPLC [6-8] and TLC [9]. In modern analytical laboratory, there is always a need for simple, rapid and accurate methods for simultaneous determination of drug combinations that could be used for routine analysis. The present work aimed to develop simple instrumental methods for the quantification of VAL and HCZ in bulk form or in their pharmaceutical formulations. These methods include spectroscopic methods and chromatographic methods; *namely*, TLC densitometry and HPLC. The present work describes simple, applicable and validated methods for simultaneous determination of these drugs in tablets.

MATERIALS AND METHODS

Instruments

A dual-beam UV-visible spectrophotometer [Shimadzu, Japan] model UV-1601 PC, with 1cm quartz cells, connected to an IBM compatible computer was used. Bundled, UV-PC personal spectroscopy software version 2.21 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2nm with wavelength-scanning speed of 2800 nm min^{-1}.

TLC plates [20 cm x 10 cm, 0.25 mm] coated with silica gel 60 F_{254} [Merck, Germany] were used.

Camag TLC scanner 3 S/N 130319 with WinCATS software and Camag Linomat 5 auto sampler [Muttenz, Switzerland] with Camag micro syringe [100 µL] were used.

The chromatographic apparatus (Shimadzu class-vp V6.12 SP4) consisted of a model 1050 solvent delivery system and a UV detector. The separation was performed on a Supelcosil™ (150 mm x 4.6 mm; *i.d.* 5 µm) column. The samples were injected by the aid of a 100 µL Hamilton® analytical syringe.

Materials and reagents

Reference VAL and HCZ standards were kindly supplied by Global Napi Co., [6 October City, Egypt]. The purity of VAL was found to be 100.02 ± 0.45% (n=6), while that of HCZ was found to be 99.6 ± 0.38 % (n=6) according to the official USP method [10]. Acetonitrile and methanol were HPLC grade and were supplied by Sigma Aldrich. Ammonia, chloroform and potassium dihydrogen phosphate were of analytical grade. Pharmaceutical dosage form [Co-Tareg 80/12.5 & Co-Diovan160/25] tablets were kindly supplied by Novartis Pharma, Cairo, Egypt.

Standard solutions

Stock standard solutions of VAL and HCZ [0.1 mg mL^{-1}] in methanol were prepared for the spectroscopic methods. Stock standard solutions of 0.4 mg mL^{-1} of VAL and 0.1 mg mL^{-1} of HCZ were prepared in the mobile phase for the HPLC method. Stock standard solutions of 1 mg mL^{-1} VAL and 1 mg mL^{-1} of HCZ were prepared in methanol for the TLC method. All solutions were freshly prepared on the day of analysis.

Procedures

Spectroscopic methods

Derivative ratio spectrophotometric method

Aliquots from standard stock solutions of VAL and HCZ were transferred into a series of 100-mL volumetric flasks. The volume was completed with methanole to prepare solutions in concentration ranges of 1-9 µg mL^{-1} VAL and 0.1-3.6 µg mL^{-1} HCZ. The spectra of the prepared solutions were scanned from 200 nm to 400 nm and stored in the computer. The stored spectra of VAL were divided (amplitude at each wavelength) by the spectrum of 0.4 µg mL^{-1} of HCZ. The first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$= 4 nm and a scaling factor = 10 was obtained. The amplitudes of the first derivative peaks of VAL were measured at 233 nm and 253 nm. Calibration graphs were constructed relating the peak amplitudes of (^{1}DD) to the corresponding concentrations. The regression equations were then computed at the two specified wavelengths and used for determination of unknown samples of VAL.

Bivariate method

Several dilutions of the two drugs were made from the stock solutions and were used for the bivariate calibration. Spectra of the obtained solutions were recorded and stored into the computer. The regression equations were computed at 225.8 nm and 269.6 nm. The concentrations of VAL and HCZ were calculated using the parameters of the linear regression functions evaluated individually for each component at the same wavelength and substituting in the following equations:

$$C_{VAL} = m_{A2}\,(A_{AB1} - e_{AB1}) + m_{A1}\,(e_{AB2} - A_{AB2}) / m_{A2}\,m_{B1} - m_{A1}\,m_{B2}$$

$$C_{HCZ} = A_{AB1} - e_{AB1} - m_{B1}\,C_{VAL} / m_{A1}$$

Where, A_{AB1} and A_{AB2} are the absorbance of A and B at λ1 and λ2, respectively, e_{AB1} and e_{AB2} the sum of the intercepts of the linear calibration at two wavelengths λ1 and λ2 ($e_{AB1} = e_{A1} + e_{B1}$), m_A and m_b are the slopes of the linear regressions and C is the concentrations [µg mL^{-1}]. The accuracy of the results was checked by applying the proposed bivariate method for determination of different samples of pure VAL and HCZ. The concentrations were obtained from the corresponding regression equations from which percentage recoveries were calculated.

Chromatographic methods

TLC-densitometric method

Aliquots of 1-30 µg $spot^{-1}$ of VAL standard solution and of 0.5-9 µg $spot^{-1}$ of HCZ standard solution [each, 1mg mL^{-1}] were applied in the form of bands on TLC plates. The band length was 4 mm and dosage speed was 150 nL S^{-1}, the bands were applied 12.8 mm apart from each other and 15 mm from the bottom edge of the plate. Linear ascending development was performed in a chromatographic tank previously saturated with chloroform: methanol: ammonia [8:2:0.1, v/v/v] for 30 minutes at room temperature. The developed plates were air-dried and scanned at 225 nm using deuterium lamp, absorbance mode at 3 mm x 0.45 mm slit dimension and scanning speed of 20 mm S^{-1}. Calibration curves relating the optical density of each spot to the corresponding

concentration of VAL and HCZ were constructed. The regression equations were then computed for the studied drugs and used for determination of unknown samples.

High performance liquid chromatographic method

Linearity

Aliquots from stock standard solutions [0.4mg mL^{-1}] of VAL and [0.1 mg mL^{-1}] of HCZ were transferred into a series of 10-mL volumetric flasks. The contents of each flask were completed with the mobile phase to volume to get a concentration range of 12-36 µg ml^{-1} for VAL and 2-9 µg mL^{-1} for HCZ.

The samples were then chromatographed using the following chromatographic conditions: stationary phase: a 150 mm x 4.6 mm i.d. C18 Supelcosil 5 µm analytical column. The mobile phase consisted of 0.02M phosphate buffer (pH 2.9): acetonitrile: methanol (50: 40: 10, v/v/v). The mobile phase was prepared daily, filtered & sonicated before use and delivered at a flow rate of 1.4 mL min^{-1}. [isocratically at ambient temperature (~25 ◦C)] with UV detection at 225 nm. The injection volume was 50 µL. The regression equations were computed and calculations were performed following the external standard technique, concentrations of unknown samples of VAL and HCZ were determined using the obtained regression equations

Analysis of laboratory prepared mixtures

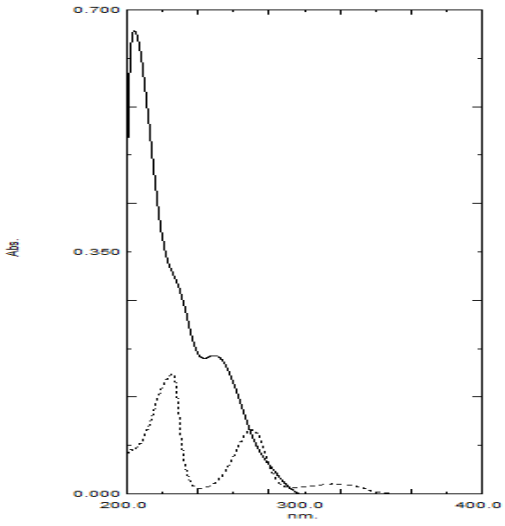
Laboratory prepared mixtures containing different ratios of VAL and HCZ were analyzed using the suggested methods, aliquots of VAL and HCZ were mixed to prepare different mixtures and proceed as mentioned under each method, the concentrations from the corresponding regression equations were then calculated.

Assay of pharmaceutical formulations [Co-Tareg 80/12.5 & Co-Diovan160/25 tablets]

Twenty tablets were weighed from each dosage form and the average weight was calculated, tablets were crushed to furnish a homogenous powder and certain amount of powdered tablets were dissolved by the aid of an ultrasonic bath for 2 hours and filtered. The solutions were diluted to the same concentration of the appropriate working solutions and proceed as described under each method.

RESULTS AND DISCUSSION

Spectroscopic methods



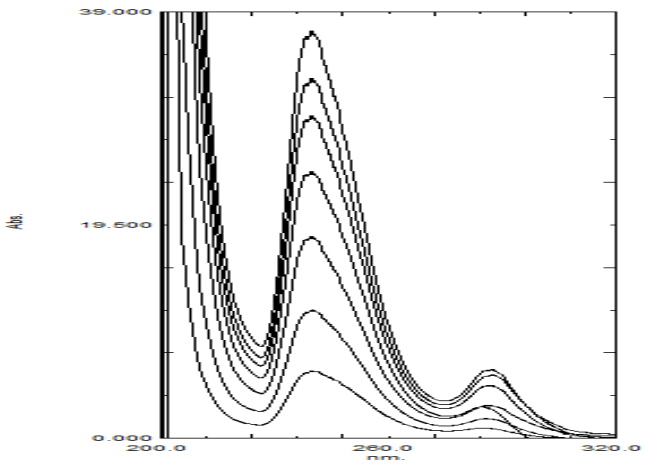
Wavelength nm

Fig.3. Zero-order spectra of 12.5 µg mL^{-1} HCZ (…..) and 80 µgmL^{-1} VAL (——).

Derivative-ratio spectra method

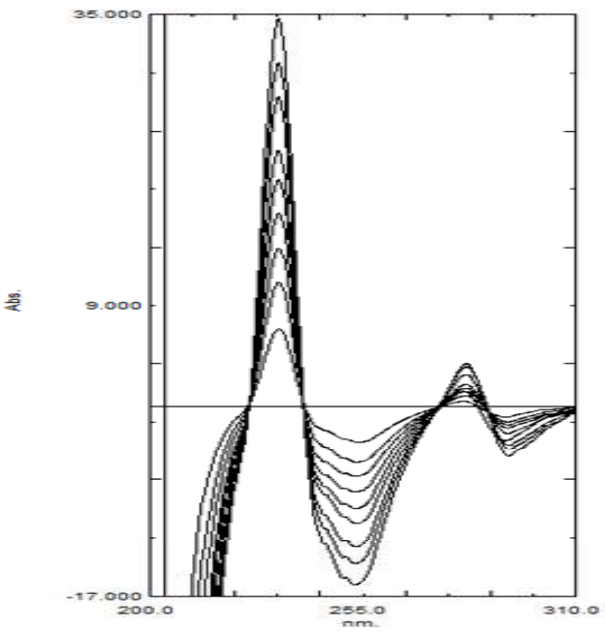
The derivative-ratio spectroscopy is a useful tool in quantification of drugs. It could be applied for the determination of VAL in presence of HCZ. The absorption spectrum of VAL is overlapped with that of HCZ (Fig.3) and the small content of HCZ and the high content of VAL in the commercial tablets (1:6) increase the difficulty of simultaneous determination of VAL and HCZ.

It could determine VAL in the presence of very low concentration HCZ than the first derivative method does. The zero-order of the ratio spectra is shown in figure 4 while the first order of the ratio spectra is presented in figure 5.



Wavelength (nm)

Fig.4. Zero-order ratio spectra of different concentration of VAL (using 0.4µgmL^{-1} HCZ as divisor)



Wavelength (nm)

Fig.5. first-order of ratio spectra of different concentration of VAL (using 0.4µgmL^{-1} HCZ as divisor)

It was found that upon dividing by 0.4 µgmL^{-1} of HCZ, best results were obtained in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graph was obtained for VAL in concentration range of 1-9 µgmL^{-1} by recording the peak amplitudes at 233 and 253 nm using 0.4 µg mL^{-1} of HCZ as a devisor. The regression equations were computed and found to be:

^{1}DD = 3.6077 C + 1.9225 (r^2 = 0.999), at 233 nm
^{1}DD = 1.68 C + 0.722 (r^2 = 0.999), at 253 nm.

Where, ^{1}DD is the peak amplitude of the first derivative ratio curve for (VAL/HCZ), *C* the concentration of VAL (µg mL^{-1}) and r^2 is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 99.83 ± 0.54 at 233 nm and 100.32±0.57 at 253 nm. The linearity ranges and analytical data for the calibration graphs are listed in table 1. Results for analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 2.

Bivariate method

The bivariate calibration method may be competitive and in some cases even superior to commonly use derivative spectrophotometric methods as applied for the resolution of binary mixtures. The advantage of bivariate calibration method is its simplicity and the fact that derivatization procedures are not necessary. Unlike other chemometric techniques, there is no need for full spectrum information and no data processing is required. Calibration function was calculated (r > 0.9990), *mi*- and *ei*-values were taken for the bivariate algorithm. In order to apply the bivariate method to the resolution of binary mixture of VAL and HCZ, we first select the signals of the two components located at six wavelengths; 207, 225.8, 241.8, 250, 269.6, and 286.6 nm. The calibration curve equations and their respective linear regression coefficients are obtained with the aim of ensuring that there is a linear relationship between the absorbance values and the concentrations. All the calibration curves at the selected wavelengths showed satisfactory linear regression coefficients (r > 0.9990). The slope values of the linear regression were estimated for both components at the selected wavelengths and used for determination of the sensitivity matrices *K*, proposed by Kaiser's method [11].The determinants of these matrices were calculated and the wavelength set was selected for which the highest matrix determinant value was obtained. For the bivariate method determination of VAL and HCZ was done using 225.8 nm and 269.6 nm.

The linearity ranges are listed in table 1. Results of analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 2.

Chromatographic methods

TLC-densitometry

A TLC-densitometric method could be used for the simultaneous determination of VAL and HCZ without prior separation. Different solvent systems were tried for the separation of both drugs. Satisfactory results were obtained by using a mobile phase composed of chloroform: methanol: ammonia [8:2:0.1, v/v/v] where Rf = 0.13 and 0.51 for VAL and HCZ, respectively. The separation allowed the determination of VAL and HCZ with no interference, fig. 6. The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 225 nm over a range of 1-30 µg spot^{-1} for VAL and over a range of 0.5-9µg spot^{-1} for HCZ, where a linear response was obtained, regression equations were found to be:

A= 2.68 C + 3.90 r = 0.9995 for VAL
A= 0.982 C +0.447 r = 0.9995 for HCZ

Where, A is the integrated area under the peak x10^{-4} for VAL and HCZ, C is the concentration in µg $spot^{-1}$ and r is the correlation coefficient.

The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recovery was found to be 99.94 for VAL and 100.74 for HCZ.

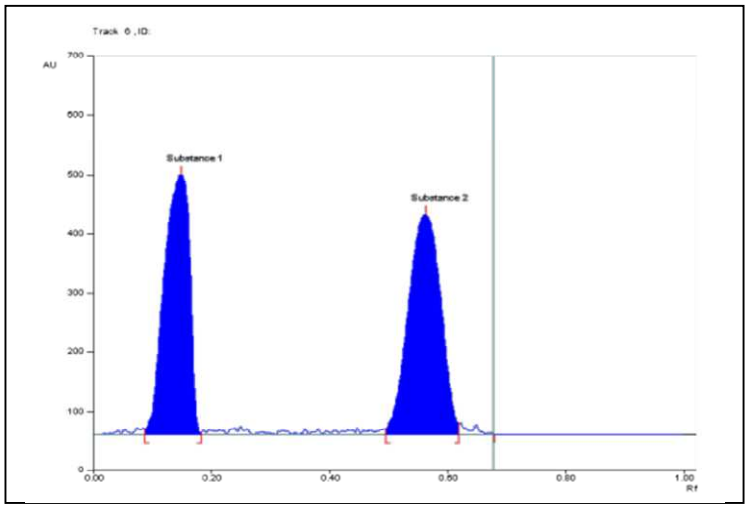
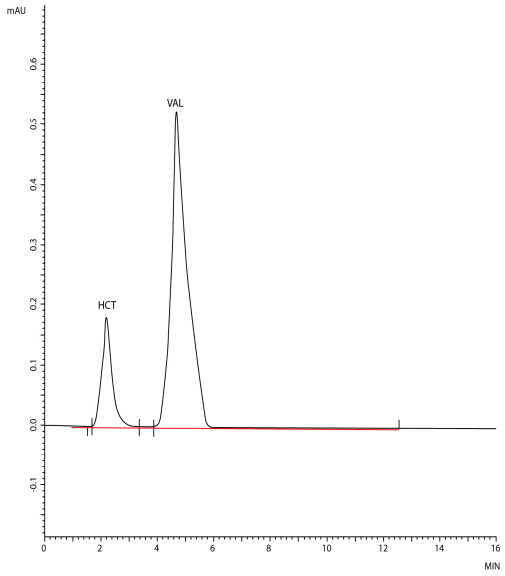


Fig.6 TLC-densitometric resolution of VAL (substance 1) and HCZ (substance 2)

To assess the specificity, accuracy and selectivity of the TLC method for assay of both drugs without interference from one another, synthetic mixtures of VAL and HCZ at various concentrations within the linearity range were prepared and analyzed.



Retention time (min.)

Fig.7. Liquid chromatographic separation of HCZ (2.19 min.) from VAL (4.69 min.).

HPLC method

A simple isocratic high-performance liquid chromatography method was developed for the determination of VAL and HCZ in pure form and in pharmaceutical formulations using a 150 mm x 4.6 mm, i.d. C_{18} Supelcosil^{TM} 5µm analytical column. The mobile phase consisted of 0.02M phosphate buffer (pH 2.9): acetonitrile: methanol [50: 40: 10, v/v/v]. The mobile phase was prepared daily, filtered & sonicated before use and delivered at a flow rate of 1.4 mL min^{-1} [isocratically at ambient temperature (~25 ◦C)] with UV detection at 225 nm. The injection volume was 50 µL. HCZ and VAL were well separated and the average retention time for HCZ was 2.19 min. while that of VAL was 4.69 min. as shown in figure 7.

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the external standard versus concentration. The linearity ranges and analytical data for the calibration graphs are listed in table 1. Calibration graphs were obtained by plotting the peak area ratios of drug to that of external standard versus concentrations of VAL and HCZ. Linearity ranges were found to be 12-36 µg mL^{-1} for VAL and 2-9µg mL^{-1} for HCZ using the following regression equations:

A = 0.0845 C + 0.017 [r=0.9995] for VAL
A = 4.36 C + 0. 140 [r=0.9995] for HCZ

Where, A is the peak area ratio, C is the concentration of VAL and HCZ [µg mL^{-1}] and r is the correlation coefficient. The precision of the method was evaluated by repeating three experiments on the same day (within-day precision) and over 3 days (day-today precision). The variability in the peak area ratios on the concentration of 24 µg mL^{-1} of VAL and 4µg mL^{-1} of HCZ was determined as the precision of the assay. The relative standard deviation values from intra-day and inter-day analysis were found to be 1.22 and 1.59% for VAL, and 0.52 and 1.04% for HCZ, respectively. Results for HPLC analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 4. The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH [4-4.5], small changes in phosphate buffer / methanol / acetonitrile ratio in the mobile phase and changing the analytical column using a 150 mm x 4.6mm i.d.C_{18} Zorbax™ 10 µm analytical column, Agilent [USA]. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peak symmetry were conserved.

Table 1. Assay parameters and validation sheet for determination of VAL and HCZ

Parameter	Derivative ratio method		Bivariate method				HPLC		TLC -Densitometry	
	at 233 nm	at 253.7 nm	at 225.8 nm		at 269.6 nm					
Range (µg mL^{-1})	VAL	VAL	VAL	HCZ	VAL	HCZ	VAL	HCZ	VAL	HCZ
	1-9	1-9	0.5-9	0.2-3.6	0.5-9	0.2-3.6	12-36	2-9	1-30	0.5-9
Slope	3.608	1.68	0.04228	0.7325	0.0126	0.7227	0.0845	4.36	2.681	0.982
Intercept	1.923	0.722	0.019	-0.0042	0.0001	-0.0028	0.0173	0.14	3.9	0.477
Mean	100.34	99.81	99.99	99.25	99.96	99.90	99.99	99.55	99.96	100.48
SD	1.37	1.24	0.35	0.94	0.235	0.572	0.56	0.99	0.38	1.30
CV %	1.37	1.24	0.35	0.95	0.235	0.573	0.56	0.99	0.38	1.29
Correlation coefficient	0.999	0.999	0.9993	0.9996	0.9993	0.9996	0.999	0.999	0.9991	0.999

Analysis of tablets

The validity of the proposed methods for the analysis of the pharmaceutical formulations and the effect of possible interferences from common excipients were studied by assaying Co-Tareg

tablets (labeled to contain 80 mg of VAL and 12.5 mg of HCZ per tablet), and Co-Diovan tablets (labeled to contain 160 mg of VAL and 25 mg of HCZ per tablet).

Table 2. Determination of VAL and HCZ in laboratory prepared mixtures by the proposed methods.

Drug determined	Derivative ratio method		Bivariate method		TLC	HPLC
	at 233 nm	at 253 nm	at 225.nm	at 269.6 nm		
VAL	100.15 ± 0.72	100.13 ± 0.74	99.99 ± 0.69	99.96 ± 0.33	98.79 ±1.02	100.00 ± 0.59
HCZ			99.25 ± 0.98	99.21 ± 0.86	99.58± 0.97	99.90 ± 0.6

CONCLUSION

The proposed methods are accurate and precise and could be used for determination of VAL & HCZ in their mixtures and in their pharmaceutical formulations without prior separation. The most striking feature of the spectrometric methods is their simplicity and rapidity. For spectroscopic methods there was no need for time-consuming sample preparation steps such as filtration, degassing that are needed for the HPLC procedure. The HPLC method is a versatile reference method and may offer advantages over the derivative method for the selective determination. The TLC-method has some advantages over HPLC such as a short run time, large sample capacity and minimal volume use of solvent. With these two methods, one can gain the advantages of speed, low-cost, and environmental protection without sacrificing accuracy.

REFERENCES

[1] A.C. Moffat, M.D. Osselton and B. Widdop, Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press**, 2004**.
[2] J.A. Schoenberger, *J Hypertension*, ***1995***, *13, 43.*
[3] K Wróbel, K Wróbel, I M De La Garza Rodríguez, P L López-de-Alba, L López-Martínez, *J Pharm & Biomed Anal*, **2000**, 20, 99.
[4] E. Şatana, Ş. Altinay, N.G. Göğer, S.A. Özkan, Z. Şentürk, *J Pharm & Biomed Anal*, **2001**, 25, 1009.
[5] K.S. Lakshmi and S. Lakshmi, *Acta Pharm.* **2011**, 61, 37.
[6] D.F. Tian, X.L. Tian, T. Tian, Z.Y. Wang and F.K. Mo, *Ind. J. Pharm. Sci.* **2008**,70, 372.
[7] G. Carlucci, V. Di Carlo & P. Mazzeo, *Analytical Letters*, **2000**, 33, 2491.
[8] D. Jothieswari, D. Priya, S. Brito, E. Mohanambal, S. Wasim, *Intl J Novel Trends in Pharm. Sci. P. Analysis*, **2011**,1, 22.
[9] N.J. Shah**,** B.N. Suhagia**,** R.R. Shah**,** N.M. Patel, *Ind. J. Pharm. Sci*.**, 2009** , 71, 72.
[10] United States Pharmacopoeia. 27th ed. Washington DC: United States Pharmacopoeial Convention Inc., 2009.
[11] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman (Eds.) Chemometrics: A Textbook, Elsevier, Amsterdam, 1988, p. 124