Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

# SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF CARVEDILOL AND HYDROCHLORTHIAZIDE IN THEIR PHARMACEUTICAL FORMULATION

**KAREEM M. YOUNES[1, 2]***

[1]*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt, [2]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Benghazi University, Benghazi, Libya. Email: kareemchem99@yahoo.com

*Received: 25 Feb. 2014 Revised and Accepted: 10 Mar 2014*

**ABSTRACT**

**Objective**: Four different methods were used to determine carvedilol and hydrochlorothiazide simultaneously in their pharmaceutical dosage forms.

**Methods**: The described methods are namely, first-derivative of ratio spectra; bivariate, thin layer chromatography and high performance liquid chromatography.

**Results**: The derivative ratio spectra method was based on measuring the peak amplitudes for carvedilol at 239 and 246 nm using 8.0µg.ml$^{-1}$ hydrochlorthiazide as a divisor, and measuring the peak amplitudes for hydrochlorthiazide at 260.4 and 291.8 nm using 10.0µg.ml$^{-1}$ carvedilol as a divisor over a concentration range of 2.0-12.0 µg.ml$^{-1}$ for both drugs. Bivariate method is used for simultaneous determination of both drugs over a concentration range of 2.0 – 12.0 µg.ml$^{-1}$ for both drugs. The method was based on measuring the absorbance at the selected wavelengths. A TLC separation with densitometric detection of both drugs was achieved using ethyl acetate: methanol: chloroform [8:2:2, v/v/v] as developing solvent. The method allowed determination of carvedilol and hydrochlorthiazide in concentration ranges of 2.0-8.0 and 1.0-6.0 µg.spot$^{-1}$, respectively. Furthermore, a high performance liquid chromatographic procedure with ultraviolet detection at 270 nm was developed for the separation and determination of the studied drugs using a C18 column over a concentration range of 5.0-30.0 and 7.5 – 20.0 µg.ml$^{-1}$ for carvedilol and hydrochlorthiazide, respectively. The mobile phase is composed of 0.01 M phosphate buffer: acetonitrile [60: 40, v/v], pH was adjusted to 3.5 by orthophosphoric acid.

**Conclusion**: The proposed methods are accurate, precise and can be applied successfully for the determination of the studied drugs in their mixtures and in pharmaceutical formulations containing them.

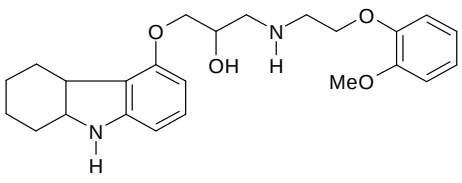
**Keywords**: *Ratio spectra, Bivariate, TLC, HPLC, Carvedilol, Hydrochlorothiazide*

## INTRODUCTION

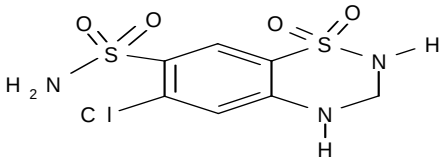
Carvedilol (CV), Fig. 1, is designated chemically as (±)-1-(carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino] -2- propanol [1]. It is a non-selective β adrenergic antagonist with no intrinsic sympathomimetic activity and is widely used to treat essential hypertension and angina pectoris. [2] Carvedilol is also indicated for the treatment of mild to severe chronic heart failure, Left ventricular dysfunction following myocardial infarction in clinically stable patients.

It also has multiple spectra of activities such as antioxidant property, inhibition of smooth muscle proliferation and calcium antagonistic blocking activity [3].

Hydrochlorothiazide (HCZ), Fig. 1, is designated chemically as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiazine-7-sulfonamide-1, 1-dioxide [1]. It is a diuretic that is widely used in antihypertensive pharmaceutical formulations. It decreases active sodium re-absorption and reduces peripheral vascular resistance [4]. It is used in association with other drugs in the treatment of hypertension. It is also indicated for the treatment of edema and management of diabetes insipidus [5].




**Carvedilol**




**Hydrochlorthiazide**

**Fig. 1: It shows Chemical structures of the studied drugs**

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 carvedilol and hydrochlorothiazide in tablets. These methods include spectrophotometric [6-9], HPLC [10-11] and capillary electrophoresis [12]. 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 CV and HCZ in bulk form or in their pharmaceutical formulation. 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 F254 [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 [100µL] were used.

The chromatographic apparatus, a Shimadzu instrument, Model LC-10 AD *VP*, equipped with a variable wavelength UV-visible detector, Model SPD-10 AD *VP,* Degasser Model DGU-12 A and a 20-µl volume Rheodyne injector. The separation was performed on Lichrosorb RP-18 (5µm, 250mm x 4.6 mm I.D) column. The samples were injected by the aid of a 100µL Hamilton® analytical syringe.

**Materials**

**Samples**

Reference CV and HCZ standards were kindly supplied by Chemipharm Pharmaceutical Industries S.A.E, 6th October City Egypt. The purity of CV was found to be 99.40 ± 0.76 (n=6), while that of HCZ was found to be 99.75 ± 0.63 (n=6) according to their official methods [13-14], respectively.

Pharmaceutical dosage form [Co-Dilatrol® tablets] batch No. 701050., claimed to contain 25 mg carvedilol, expressed as base and 12.5 mg hydrochlorothiazide per tablet, respectively, was kindly supplied by Chemipharm Pharmaceutical Industries S.A.E, 6th October City, Egypt.

**Reagents**

All chemicals and reagents were of pure analytical grade.

-Methanol, ethyl acetate and chloroform were obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

-For HPLC work, de-ionized water, acetonitrile, 0.01 M phosphate buffer and o-phosphoric acid (E-Merck, Darmstadt, Germany) were of HPLC grade.

**Standard solutions**

Stock standard solutions of CV and HCZ [0.2mg.$mL^{-1}$] in methanol were prepared for the spectroscopic methods. Stock standard solutions of 0.2 mg.$mL^{-1}$ of CV and 0.1mg.$mL^{-1}$ of HCZ were prepared in the mobile phase for the HPLC method. Stock standard solutions of CV and HCZ [0.1mg.$mL^{-1}$] 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 CV and HCZ were transferred into a series of 100-mL volumetric flasks. The volume was completed with methanol to prepare solutions in concentration ranges of 2.0-12.0µg.$mL^{-1}$ CV and HCZ. The spectra of the prepared solutions were scanned from 200 nm to 400 nm and stored in the computer. The stored spectra of CV were divided (amplitude at each wavelength) by the spectrum of 8.0µg.$mL^{-1}$of HCZ. The first derivative of the ratio spectra ($^1$DD) with Δλ= 4 nm and a scaling factor = 10 was obtained. The amplitudes of the first derivative peaks of CV were measured at 239 nm and 246nm. The stored spectra of HCZ were divided (amplitude at each wavelength) by the spectrum of 10.0µg.$mL^{-1}$ of CV. The first derivative of the ratio spectra ($^1$DD) with Δλ= 4 nm and a scaling factor = 10 was obtained. The amplitudes of the first derivative peaks of HCZ were measured at 260.4 nm and 291.8 nm. Calibration graphs were constructed relating the peak amplitudes of ($^1$DD) to the corresponding concentrations of CV and HCZ. The regression equations were then computed at the specified wavelengths and used for determination of unknown samples of CV and HCZ.

**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 225.6 nm and 270.6 nm. The concentrations of CV 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:

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

$$C_{HCZ} = A_{AB1} - eA_{B1} - m_{B1}\,C_{CV} / m_{A1}$$

Where, $A_{AB1}$ and $A_{AB2}$ are the absorbance of A and B at 225.6 nm and 270.6 nm, respectively, $e_{AB1}$ and $e_{AB2}$ the sum of the intercepts of the linear calibration at the two wavelengths ($e_{AB1} = e_{A1} + e_{B1}$), $m_A$ 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 CV and HCZ. The concentrations were obtained from the corresponding regression equations from which percentage recoveries were calculated.

**Chromatographic Methods**

**TLC-Densitometric method**

Aliquots equivalent to 2.0-8.0µg.$spot^{-1}$ of CV standard solution and 1.0-6.0µg.$spot^{-1}$ of HCZ standard solution [each 0.1mg.$mL^{-1}$] were applied in the form of bands on TLC plates. The band length was 4 mm and dosage speed was 150nL $S^{-1}$, 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 ethyl acetate: methanol: chloroform [8:2:2, v/v/v] for 30 minutes at room temperature. The developed plates were air-dried and scanned at 254 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 CV and HCZ were constructed. The regression equations were then computed for the studied drugs and used for determination of unknown samples.

**HPLC method**

Aliquots from stock standard solutions [0.2mg.$mL^{-1}$] of CV and [0.1mg.$mL^{-1}$] 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 5.0-30.0 µg.$ml^{-1}$ for CV and 7.5-20.0µg.$mL^{-1}$ for HCZ. The samples were then chromatographed using the following chromatographic conditions: stationary phase: a 150 mm x 4.6 mm i.d.C18 Lichrosphere 5µm analytical column. The mobile phase consisted of 0.01M phosphate buffer: acetonitrile (60: 40, v/v), pH adjusted to 3.5 by orthophosphoric acid. The mobile phase was prepared daily, filtered & sonicated before use and delivered at a flow rate of 1.0 mL.$min^{-1}$. [isocratically at ambient temperature (~25ºC)] with UV detection at 254 nm. The injection volume was 50µL. The regression equations were computed and calculations were performed following the external standard technique, concentrations of unknown samples of CV and HCZ were determined using the obtained regression equations.

**Assay of laboratory-prepared mixtures**

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

**Assay of pharmaceutical formulation (Co-Dilatrol® tablets)**

Ten tablets were weighed from the 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**

**Derivative Ratio Spectra Method**

The derivative-ratio spectroscopy is a useful tool in quantification of drugs. It could be applied for the simultaneous determination of CV and HCZ. The zero – order absorption spectra of CV and HCZ are overlapped (Fig.2). CV can be assayed in presence of HCZ by dividing the absorption spectra of different concentrations of CV in the range of 2.0-12.0 µg.ml$^{-1}$ by the absorption spectrum of (8.0µg.ml$^{-1}$) HCZ and then the first derivative of ratio spectra ($^1$DD) were recorded (Fig. 3).

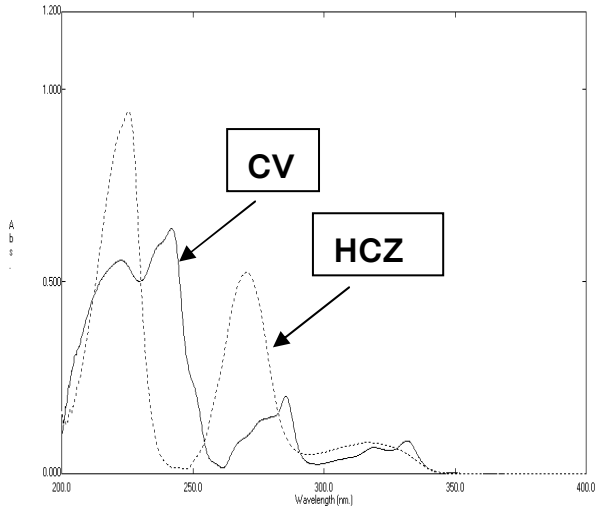



**Fig. 2: It shows zero-order spectra of 5.0µg.mL$^{-1}$ HCZ (.....) and 5.0µg.mL$^{-1}$ CV (—).**

It was found that upon dividing by 8.0µg.mL$^{-1}$ of HCZ, best results were obtained in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graph was obtained for CV in concentration range of 2.0-12.0µg.mL$^{-1}$ by recording the peak amplitudes at 239 and 246 nm using 8.0µg.mL$^{-1}$ of HCZ as a devisor. The regression equations were computed and found to be:

$^1$DD = 0.594 C – 0.171 ($r^2$ = 0.9998), at 239 nm

$^1$DD = 0.485 C + 0.005 ($r^2$ = 0.9992), at 246 nm.

Where, $^1$DD is the peak amplitude of the first derivative ratio curve for (CV/HCZ), C the concentration of CV (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 99.65 ± 0.78 at 239 nm and 99.55 ± 0.43 at 246 nm. The linearity ranges and analytical data for the calibration graphs are listed in table 2. Results for analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 3.

HCZ can be assayed in presence of CV by dividing the absorption spectra of different concentrations of HCZ in the range of 2.0-12.0µg.ml$^{-1}$ by the absorption spectrum of (10.0µg.ml$^{-1}$) CV and then the first derivative of ratio spectra ($^1$DD) were recorded (Fig. 4). It was found that upon dividing by 10.0µg.mL$^{-1}$ of CV, best results were obtained in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graph was obtained for HCZ in concentration range of 2.0-12.0µg.mL$^{-1}$ by recording the peak amplitudes at 260.4 nm and 291.8 nm using 10.0µg.mL$^{-1}$ of CV as a devisor. The regression equations were computed and found to be:

$^1$DD = 0.039 C + 0.07 ($r^2$ = 0.9995), at 260.4 nm

$^1$DD = 0.017 C + 0.029 ($r^2$ = 0.9994), at 291.8 nm.

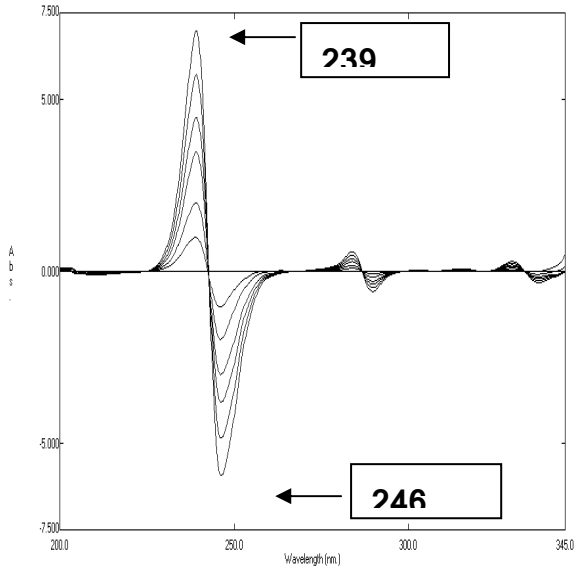



**Fig .3: It shows first-order of ratio spectra of different concentrations of CV (using 8.0µgmL$^{-1}$ HCZ as divisor).**

Where, $^1$DD is the peak amplitude of the first derivative ratio curve for (HCZ/CV), C the concentration of HCZ (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 99.45 ± 0.84 at 260.4 nm and 99.76 ± 0.43 at 291.8 nm. The linearity ranges and analytical data for the calibration graphs are listed in table 2. Results for analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 3.

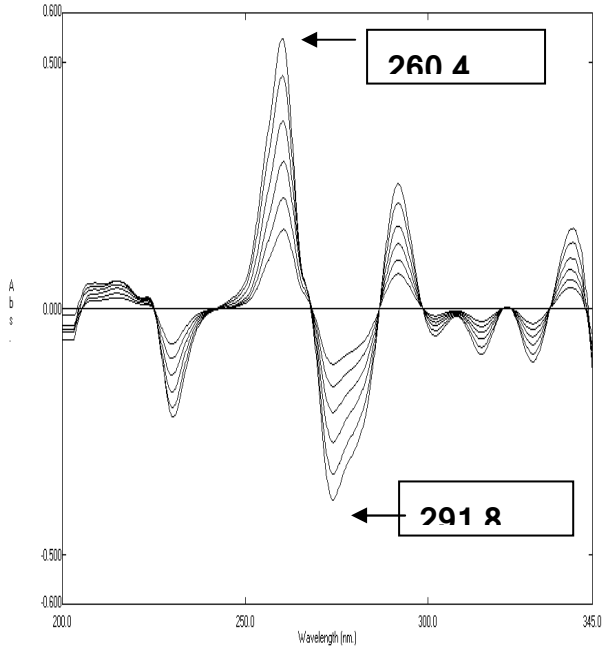



**Fig. 4: It shows first-order of ratio spectra of different concentrations of HCZ (using 10.0µgmL$^{-1}$ CV as divisor).**

**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 CV and HCZ, we first select the signals of the two components located at six wavelengths; 220, 225.6, 270.6, 285.6, 319, and 331.6 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 [15]. The determinants of these matrices were calculated and the wavelength set was selected for which the highest matrix determinant value was obtained, table 1. For the bivariate method determination of CV and HCZ was done using 225.6 nm and 270.6 nm. The linearity ranges are listed in table 2. Results of analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 3.

**Table 1: It shows Application of Kaiser method for the selection of the wave length set for carvedilol (CV) - hydrochlorothiazide (HCZ) mixture**

| λ / λ | 220 | 225.6 | 270.6 | 285.6 | 319 | 331.6 |
|---|---|---|---|---|---|---|
| 220 | 0 | 3.79 | 70.1 | 49.9 | 3.0 | 15.4 |
| 225.6 | | 0 | 71.4 | 53.1 | 3.6 | 16.5 |
| 270.6 | | | 0 | 41.4 | 9.9 | 15.2 |
| 285.6 | | | | 0 | 5.3 | 2.8 |
| 319 | | | | | 0 | 1.5 |
| 331.6 | | | | | | 0 |

**Chromatographic Methods**

**TLC-Densitometric method**

TLC densitometry overcomes the problem of overlapping absorption spectra of mixture of drugs by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram. The TLC densitometric method has the advantage of simultaneously determining the active ingredients in multi-component dosage forms [16]. A TLC-densitometric method could be used for the simultaneous determination of CV 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 ethyl acetate: methanol: chloroform [8:2:2, v/v/v] where $R_f$ = 0.3 and 0.7 for CV and HCZ, respectively. The separation allowed the determination of CV and HCZ with no interference, figs. [5-6]. The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 254 nm over a range of 2.0-8.0µg.spot$^{-1}$ for CV and over a range of 1.0-6.0µg.spot$^{-1}$ for HCZ, where a linear response was obtained, regression equations were found to be:

A= 4.81 C + 0.98 r = 0.9995 (for CV).

A= 5.61C + 5.22 r = 0.9997 (for HCZ).

Where A is the area integrated under the peak x $10^{-3}$ for CV 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 98.57± 0.62 for CV and 98.92 ± 1.19 for HCZ. To assess the specificity, accuracy and selectivity of the TLC method for assay of both drugs without interference from one another, synthetic mixtures of CV and HCZ at various concentrations within the linearity range were prepared and analyzed.

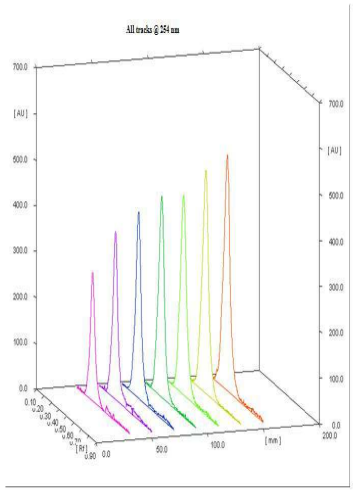


**Fig. 5: It shows a 3 D linearity graph of CV ($R_f$ = 0.30) over a concentration range (2.0- 8.0 µg. spot$^{-1}$).**

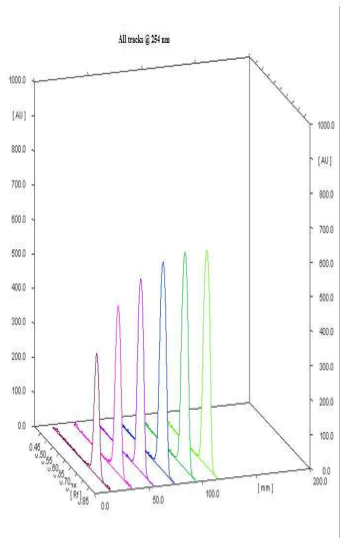


**Fig. 6: It shows a 3D linearity graph of HCZ ($R_f$ = 0.70) over a concentration range (1.0-6.0 µg. spot$^{-1}$).**

**HPLC method**

A simple isocratic high-performance liquid chromatography method was developed for the determination of CV and HCZ in pure form and in pharmaceutical formulations using a 150 mm x 4.6 mm, i.d. C18 Lichrosphere 5µm analytical column. The mobile phase consisted of 0.01M phosphate buffer: acetonitrile [60: 40, v/v]. The mobile phase was prepared daily, filtered & sonicated before use and delivered at a flow rate of 1.0 mL.min$^{-1}$ [isocratically at ambient temperature (~25 ⍰C)] with UV detection at 254 nm. The injection volume was 50µL. HCZ and CV were well separated and the average retention time for HCZ was 2.84 min. while that of CV was 9.87 min. as shown in figure 7.

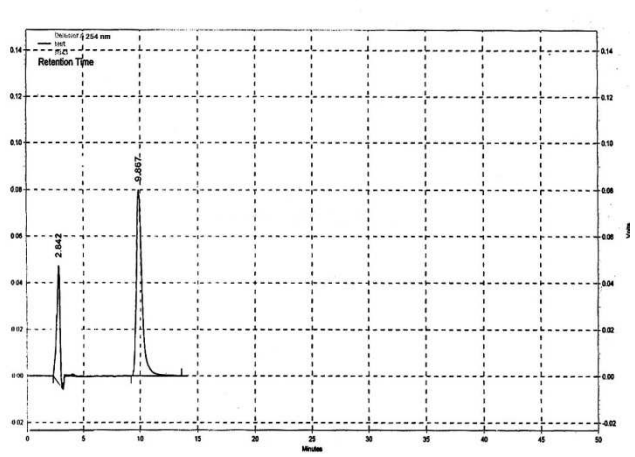



**Fig.7: It shows liquid chromatogram of HCZ at $R_t$ 2.84 min and CV at $R_t$ 9.87 min.**

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the internal standard versus concentration. The linearity ranges and analytical data for the calibration graphs are listed in table 2.

Calibration graphs were obtained by plotting the peak area ratios of drug to that of internal standard versus concentrations of CV and HCZ. Linearity ranges were found to be 5.0-30.0µg.mL$^{-1}$for CV and 7.5-20.0µg.mL$^{-1}$for HCZ using the following regression equations:

A= 0.180 C + 0.45 r = 0.9998 (for CV).

A= 0.087 C+ 0.19 r = 0.9995 (for HCZ).

Where, A is the peak area ratio, C is the concentration of CV 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 to day precision). The variability in the peak area ratios on the concentration of 20 µg.mL$^{-1}$ of CV and 12.5µg.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 0.38 and 0.81% for CV, and 0.57 and 0.62% for HCZ, respectively. Results for HPLC analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in table 3. 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 [2-4], small changes in phosphate buffer / acetonitrile ratio in the mobile phase and small changes in mobile phase flow rate [0.8 – 1.6 ml.min$^{-1}$]. It was found that the method was robust when the mobile phase ratio and flow rate were varied. During these investigations, the retention times were modified, however the areas and peak symmetry were conserved.

**Table 2: It shows assay parameters and validation of the proposed methods for determination of CV and HCZ**

| Parameters | The proposed methods | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bivariate method | | | | Derivative ratio method | | | | TLC method | | HPLC method | |
| | CV | | HCZ | | CV | | HCZ | | CV | HCZ | CV | HCZ |
| | 225.6 nm | 270.6 nm | 225.6 nm | 270.6 | 239 nm | 246 nm | 260.4 nm | 291.8 nm | | | | |
| LOD (µg.ml$^{-1}$) | 0.65 | 0.65 | 0.26 | 0.26 | 0.11 | 0.14 | 0.17 | 0.20 | 0.12 (µg.spot$^{-1}$) | 0.14 (µg.spot$^{-1}$) | 0.67 | 0.85 |
| LOQ (µg.ml$^{-1}$) | 1.95 | 1.95 | 0.77 | 0.77 | 0.32 | 0.41 | 0.52 | 0.61 | 0.36 (µg.spot$^{-1}$) | 0.42 (µg.spot$^{-1}$) | 2.03 | 2.58 |
| Range (µg.ml$^{-1}$) | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-12.0 | 2.0-8.0 (µg.spot$^{-1}$) | 1.0-6.0 (µg.spot$^{-1}$) | 5.0-30.0 | 7.5-20.0 |
| Slope | 0.115 | 0.021 | 0.143 | 0.089 | 0.594 | 0.485 | 0.039 | 0.017 | 4.81 | 5.61 | 0.18 | 0.09 |
| Intercept | 0.08 | 0.05 | 0.38 | 0.17 | -0.17 | 0.005 | 0.07 | 0.03 | 0.98 | 5.22 | 0.45 | 0.19 |
| Mean ± S.D. | 99.55 ± 0.5 | 99.55 ± 0.5 | 99.43 ± 0.4 | 99.43 ± 0.4 | 99.38 ±0.60 | 99.82 ±0.68 | 99.44 ±0.85 | 99.70 ±0.50 | 99.06 ± 0.93 | 98.90 ± 0.96 | 99.96 ±1.17 | 99.72 ±0.63 |
| Correlat. Coeff. (r) | 0.9992 | 0.9993 | 0.9994 | 0.9998 | 0.9998 | 0.9992 | 0.9995 | 0.9994 | 0.9995 | 0.9997 | 0.9998 | 0.9995 |
| RSD% [a*] | 1.52-1.63 | 1.52-1.63 | 0.62-0.67 | 0.62-0.67 | 0.22-0.26 | 0.25-0.28 | 0.28-0.31 | 0.34-0.36 | 0.34-0.47 | 0.26-0.37 | 0.38-0.70 | 0.57-0.84 |
| RSD% [b*] | 1.82-1.89 | 1.82-1.89 | 0.87-0.92 | 0.87-0.92 | 0.64-0.67 | 0.72-0.75 | 0.76-0.79 | 0.84-0.86 | 0.41-0.52 | 0.31-0.44 | 0.81-1.12 | 0.62-0.93 |

*[a,b] Intra-day and inter-day (n=3) relative standard deviations of samples of concentrations 4.0µg.ml$^{-1}$ of CV and 3.0µg.ml$^{-1}$ of HCZ for Bivariate method, 6.0 µg.ml$^{-1}$ of CV and 8.0µg.ml$^{-1}$ of HCZ for derivative ratio method, 6.0 µg.Spot$^{-1}$ of CV and 3.0 µg.Spot$^{-1}$ of HCZ for TLC method, 20.0µg.ml$^{-1}$ and 12.5µg.ml$^{-1}$ of HCZ for HPLC method.

**Table 3: It shows determination of CV and HCZ in laboratory prepared mixtures by the proposed methods.**

| Drug | Derivative ratio method | | Bivariate method | | TLC method | HPLC method |
|---|---|---|---|---|---|---|
| | 239 nm | 246 nm | 225.6 nm | 270.6 nm | | |
| CV | 99.65 ± 0.78 | 99.55 ± 0.43 | 99.65 ± 0.31 | 99.23 ± 0.61 | 98.57± 0.62 | 99.89 ± 0.61 |
| | 260.4 nm | 291.8 nm | 225.6 nm | 270.6 nm | | |
| HCZ | 99.45 ± 0.84 | 99.76 ± 0.43 | 99.44 ± 0.49 | 98.31 ± 0.58 | 98.92 ± 1.19 | 99.56 ± 0.72 |

A statistical comparison of the results obtained by the proposed methods and the official method for each pure CV [13] and pure HCZ [14] is shown in table 4. The values of the calculated T and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods and the official ones [17].

**Analysis of Laboratory-prepared mixtures**

The validity of the proposed methods for the simultaneous determination of CV and HCZ was assessed by analysis of laboratory prepared mixtures containing different ratios of CV and HCZ and calculating the concentrations from the corresponding regression equations. The results are shown in table 3.

**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-Dilatrol® tablets (labeled to contain 25 mg of CV and 12.5 mg of HCZ per tablet), the results are present in table 5.

**Table 4: It shows statistical analysis of the results obtained by applying the proposed methods and the official ones for the analysis of pure CV and HCZ.**

| Values | The proposed methods | | | | | | | | | | Official methods** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bivariate method | | [1]DD method | | | | TLC method | | HPLC method | | | |
| | CV | HCZ | CV | | HCZ | | CV | HCZ | CV | HCZ | CV | HCZ |
| | | | 239 | 246 | 260.4 | 291.8 | | | | | | |
| Mean | 99.55 | 99.43 | 99.38 | 99.82 | 99.44 | 99.70 | 99.06 | 98.90 | 99.96 | 99.72 | 99.56 | 99.34 |
| S.D. | 0.54 | 0.45 | 0.60 | 0.68 | 0.85 | 0.50 | 0.93 | 0.96 | 1.17 | 0.63 | 0.72 | 0.65 |
| N | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Variance | 0.29 | 0.20 | 0.36 | 0.46 | 0.72 | 0.25 | 0.86 | 0.92 | 1.37 | 0.40 | 0.52 | 0.42 |
| T[2.23]* | 0.03 | 0.28 | 0.47 | 0.65 | 0.23 | 1.08 | 0.44 | 0.93 | 0.71 | 1.03 | ……. | ……. |
| F[5.05 ]* | 1.79 | 2.1 | 1.44 | 1.13 | 1.71 | 1.68 | 1.65 | 2.19 | 2.63 | 1.05 | ……. | ……. |

*The figures in parenthesis are the corresponding tabulated values at P=0.05. ** Official methods are Eur. Ph. non-aqueous titration method for CV [13] and USP HPLC method for HCZ [14].

**Table 5: It shows determination of CV and HCZ in Co-Dilatrol® tablets (B.N:701050) by the proposed methods.**

| Drug | Derivative ratio method | | Bivariate method | | TLC method | HPLC method |
|---|---|---|---|---|---|---|
| | 239 nm | 246 nm | 225.6 nm | 270.6 nm | | |
| CV | 99.15 ± 0.42 | 99.34 ± 0.65 | 99.65 ± 0.31 | 99.23 ± 0.61 | 99.52 ± 1.16 | 99.15 ± 0.42 |
| | 260.4 nm | 291.8 nm | 225.6 nm | 270.6 nm | | |
| HCZ | 98.97 ± 0.34 | 98.95 ± 0.45 | 99.44 ± 0.49 | 98.31 ± 0.58 | 99.55 ± 0.94 | 98.97 ± 0.34 |

**CONCLUSION**

The proposed methods are accurate and precise and could be used for determination of CV & HCZ in their mixtures and in their pharmaceutical formulation 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.

**ACKNOWLEDGMENT**

The author thanks, Chemipharm Pharmaceutical Industries Company, S.A.E, 6th October City Egypt, for providing Carvedilol and Hydrochlorthiazide standards and their dosage form as gift samples for this work.

**CONFLICT OF INTEREST**

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

**REFERENCES**

1. Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material**,** London: Pharmaceutical Press; 2004.
2. Anderson P, Knoben J, Troutman W. Handbook of Clinical Drug Data. New York: McGraw-Hill; 2002.
3. Mollendorff EV, Reiff K, Neugebauer G. Pharmacokinetics and Bioavailability of Carvedilol: a Vasodilating Beta-blocker**.** Eur. J. Clinical Pharm**.** 1987; 33: 511-13.
4. Schoenberger JA. Losartan with Hydrochlorothiazide in the Treatment of Hypertension**.** J. Hypertension. 1995; 13 Suppl 1: 143-47.
5. Palazzuoli A, Calabria P, Verzuri MS, Auteri A. A Carvedilol: Something Else than a Simple Beta-blocker ? Eur. Rev. Med. Pharmacol. Sci. 2002; Suppl 6: 115-26
6. Ansary A, Abdel-Moety MM, Abdel Gawad FM, Ether AM, Khater MM. Simultaneous Determination of Carvedilol and Hydrochlorothiazide in Tablets and Spiked Human Plasma using Derivative Spectrophotometry**.** Pharmaceut. Anal. Acta. 2012; 3 Suppl 9: 1-6.
7. Abdelwahab NS. Spectrophotometric Methods for Simultaneous Determination of Carvedilol and Hydrochlorthiazide in Combined Dosage Form Arab. J. Chem. 2011.
8. Sultan MA. Simultaneous Determination of Carvedilol and Hydrochlorothiazide in Tablets by Derivative Spectrophotometric and High-performance liquid chromatographic methods**.** Asian J. Chem. 2008; 20 Suppl 3: 2283-92.
9. Patel HM, Pancholi SS, Jivani NP. UV Spectrophotometric Method for Simultaneous Estimation of Carvedilol and Hydrochlorthiazide in Bulk and Pharmaceutical Dosage Form by Simultaneous Equation Method. Inventi Rapid: Pharm Analysis & Quality Assurance. 2013; 1.
10. Haggag RS, Shaalan RA, Belal TS. Validated HPLC Determination of The Two Fixed Dose Combinations (Chlordiazepoxide hydrochloride and Mebeverine hydrochloride; Carvedilol and Hydrochlorothiazide) in their Tablets. J. AOAC Int. 2010; 93 Suppl 4: 1192-200**.**
11. Belal TS, Shaalan RA, El Yazbi FA, Elonsy SM. Validated Stability-indicating HPLC-DAD Determination of Anti-hypertensive Binary Mixture of Carvedilol and Hydrochlorthiazide in Tablet Dosage Forms. Chromatographia 2013; 76: 1707- 20.
12. Alzoman NZ, Sultan MA, Maher HM, Al-Shehri MM, Ola IV. Validated Stability-Indicating Capillary Electrophoresis Method for the Separation and Determination of a Fixed-Dose Combination of Carvedilol and Hydrochlorothiazide in Tablets. J. AOAC Int. 2013; 96 Suppl 5: 951-59.

13. European Pharmacopoeia 5, Strasburg: Council of Europe; 2004: 1194.
14. Rockville MD United States Pharmacopoeia 29. National Formulary 24, USA: United States Pharmacopoeial Convention Inc; 2009: 1061.
15. Massart DL, Vandeginste BGM, Deming SN, Michotte Y, Kaufman L. Chemometrics: A Textbook. Amsterdam: Elsevier; 1988.
16. Grinberg N. Modern Thin Layer Chromatography New York: Marcel Dekker Inc; 1990.
17. Miller JN, Miller, JC. Statistics and Chemometrics for Analytical Chemistry 5th ed. Harlow, England: Pearson Education Limited; 2000.