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Simultaneous Determination of Carvedilol and Hydrochlorothiazide in Tablets by Derivative Spectrophotometric and HPLC Methods

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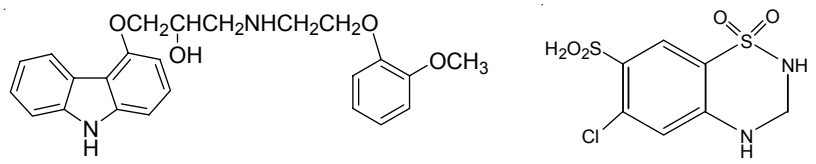
Two new simple and selective assay methods have been presented for the resolution and determination of the binary mixtures of carvedilol (CRV) and hydrochlorothiazide (HCTZ) in pharmaceutical formulations. The first method depends on first derivative ultraviolet spectrophotometry (^{1}D) with zero-crossing technique of measurements at 248 and 285 nm for CRV and HCTZ, respectively. The assay was linear over the concentration ranges 0.05-1.5 μg mL^{-1} for CRV and 0.5 - 15 μg mL^{-1} for HCTZ. The determination limit for CRV and HCTZ were found to be 0.025 and 0.25 μg mL^{-1}, respectively; while the detection limit were 0.005 μg mL^{-1} for CRV and 0.1 μg mL^{-1} for HCTZ. The second method was based on isocratic reversed-phase liquid chromatography (HPLC) on Nucleosil LC_{18} column (25 cm × 4.6 mm), using a mobile phase acetonitrile-10 mM potassium dihydrogen phosphate (pH 3.0), (30:70 v/v) at flow rate of 1 mL/min. Losartan was used as an internal standard and the substances were detected at 230 nm. The linearity range were 0.05-1.00 and 0.1-1.0 μg mL^{-1} for CRV and HCTZ, respectively. The determination and detection limit were found to be 0.02 and 0.005 μg mL^{-1} for CRV and 0.05 and 0.01 μg mL^{-1} for HCTZ, respectively. The proposed methods were successfully applied for the determination of these drugs in synthetic mixtures and commercially available tablets. The results were compared statistically at 95 % confidence level with each other. There was no significant difference between the mean percentage recoveries and precision of the two methods.

Key Words: Carvedilol, UV-Derivative spectrophotometry, HPLC, Hydrochlorothiazide.

INTRODUCTION

Carvedilol (±)-1-(carbazol-4-yloxy)-3-[(2-(O-methoxyphenoxy)ethyl) amino)-2-propanol (Fig. 1a) is a non-cardio selective β-blocker[1]. It also has vasodilating properties. It is used in the management of hypertension and angina pectoris and as an adjunct to standard therapy in symptomatic heart failure[1,2]. It is administered alone or together with antihypertensive, diuretic hydrochlorothiazide (HCTZ). Combined therapy of CRV and HCTZ had a significantly greater blood pressure reduction than with the same dosage of the drug alone[3].



(a) (b)

Fig. 1. Chemical structure of carvedilol (a) and hydrochlorothiazide (b)

Hydrochlorothiazide, 6-chloro-3,4-dihydro-2*H*-1,2,4-benothiadiazine -7-sulfonamide-1, 1-dioxide, (Fig. 1b) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical preparations which decreases active sodium reabsorption and reduces peripheral vascular resistance[1].

Both drugs are used often to treat heart trouble in the clinic. Therefore in view of the pharmacological importance, it is important that the method of simultaneous determination of CRV and HCTZ is developed.

European pharmacopoeia[4], describes a nonaqueous titration procedure for the determination of carvedilol in bulk. Several methods are described for the determination of CRV including high-performance liquid chromatography and capillary electrophoresis in human serum, plasma, human tissues and rat plasma[5-9]. Few methods are reported to the analysis of CRV in tablets including fluorimetry[10,11] and differential pulse voltammetry[12]. On the other hand, the British Pharmacopoeia[13] describes a non-aqueous titration procedure for the determination of HCTZ in bulk and a spectrophotometric method for its determination in tablets.

United States Pharmacopoeia[14] however recommended high performance liquid chromatography for the analysis of HCTZ in bulk and in tablets. Several other methods have been reported for the determination of HCTZ either individually or jointly with other pharmaceutical substances, including electrochemical[15], spectrophotometric[16,17], HPLC-densitometric[18] and HPLC[19,20], methods.

There is no reported method available in literature for the simultaneous analysis of CRV and HCTZ in pharmaceutical preparations. This led us to search for simple, precise, accurate and reliable method that can be applied in quality control laboratories for simultaneous determination of the two drugs. For this purpose, a novel methods for simultaneous determination of CRV and HCTZ by UV-derivative spectrophotometric and liquid chromatographic assay methods have been developed in this study. The two methods have been applied for the determination of both components in laboratory-made mixtures and in tablets.

EXPERIMENTAL

Reference standard samples of CRV, HCTZ and losartan, as an internal standard were obtained from Drug Control Center, Riyadh, Saudi Arabia. Their purity were checked according to the United States Pharmacopoeia[14] and European Pharmacopoeia[4] methods. Synthesized tablets were prepared in laboratory to contain 25 mg CRV and 12.5 mg HCTZ/tablet, as the commercial tablets (Co-dilatrend, Roche) can't be obtained from local markets. The excipients were lactose monohydrate, providone, sucrose, magnesium stearate and colloidal silicon dioxide. Analytical grade KH_2PO_4 and HPLC grade CH_3CN were purchased from Merck (Germany), water was doubly distilled.

Spectrophotometric analysis was carried out on a Schimadzu 1601 double beam spectrophotometer with fixed slit width (2 nm) connected to an IBM PC computer loaded with Lexmark printer.

The high-performance liquid chromatography system consisted of a Jasco model PV-980 pump with a 7725 Rheodyne value injector 20 µL fixed loop, equipped with a Jasco UV-975UV/Vis detector. The detector was set at 230 nm and peak areas were integrated automatically by computer using Borwin software programme.

Chromatographic conditions: Separation was achieved at room temperature, using a Nucleosil LC_{18} (25 cm × 4.6 mm i.d.), column with guard column (Nucleosil, Mecherey-Nagel, Germany). The chromatographic separation were accomplished by using 10 mM potassium dihydrogen phosphate, (adjusted to pH 3.0 with phosphoric acid): acetonitrile (70:30 v/v) as a mobile phase at a flow rate of 1 mL min^{-1}. All solvents were filtered through 0.45 µm Millipore filter to use and degassed in an ultrasonic bath.

Solutions: CRV and HCTZ stock solutions (0.5 mg mL^{-1} in methanol) were freshly prepared. Standard solutions were obtained by diluting the stocks solutions for the preparation of calibration curves in the concentration range of 0.05-1.50 µg mL^{-1} (CRV) and 0.5-15.0 µg mL^{-1} (HCTZ) for derivative spectrophotometric method. Meanwhile the final concentration range was 0.05-1.00 µg mL^{-1} of both drugs for HPLC method. These solutions also contained losartan as internal standard at 0.25 µg mL^{-1}.

Assay procedure: The two developed methods were applied to the determination of CRV and HCTZ in laboratory made tablets in clinically recommended ratio (25 mg CRV and 12.5 mg HCTZ) Ten tablets were separately weighed and powdered. About 60 mL of MeOH was added to accurately weighed amount of the tablet powder equivalent to *ca.* 25 mg of CRV and 12.5 mg of HCTZ in a 100 mL calibrated flask. The mixture was shaken for 0.5 h on a shaker, diluted to volume with MeOH and then filtered. Appropriate dilutions were made with MeOH.

First derivative spectra of the solutions were recorded against MeOH. Derivative absorbance (^{1}D) values of the spectra at 248 and 285 nm were measured for the determination of CRV and HCTZ, respectively.

For HPLC measurements appropriate dilutions were made with 10 mM KH_2PO_4/CH_3CN (7:3, v/v) for both studied drugs and the internal standard (losartan), the final concentration of the internal standard was 0.25 μg mL^{-1}.

The quantity of the drugs were calculated using the regression equations of the corresponding calibration curves constructed for both the methods.

Assay validation: Synthetic mixtures, prepared by adding known amount of CRV and HCTZ, were analyzed by both developed methods using the procedure outlined above. The mean percentage recoveries and relative standard deviations (RSD) were calculated.

RESULTS AND DISCUSSION

Derivative UV-spectrophotometry: Direct UV-absorption measurements were found to be inapplicable for the analysis of CRV and HCTZ in binary mixtures because of the spectral interference (Fig. 2). Derivative spectrophotometry is a favourable technique to solve this problem. Therefore, first, second and third derivative spectra of methanolic solutions of the two drugs were recorded. The first derivative spectra presented spectral features which can be used for the simultaneous determination of CRV and HCTZ (Fig. 3). Zero crossing wavelengths at 248 and 285 nm were selected for determination of CRV and HCTZ, respectively, since, reproducible readings were obtained at these wavelengths and is less affected by the concentration of any other component. Other operating conditions were determined as mentioned above. Calibration curves were constructed by plotting ^{1}D, (dA/dλ), values at selected wavelengths against corresponding concentrations in the range of 0.05-1.50 μg mL^{-1} for CRV and 0.5-15.0 μg mL^{-1} for HCTZ. The regression equations of linear calibration graph for CRV and HCTZ were calculated as $\Delta^1D = 0.0504 + 5.4369\ C$ ($r = 0.9995$) and $\Delta^1D = 3.60 \times 10^{-5} + 0.5038\ C$ ($r = 0.9999$), respectively. The high values of the correlation coefficients and the values of Y-intercepts close to zero indicate the good linearity of the calibrations[21]. Repeatability of the analytical method was tested by analyzing six samples at 100 % standard concentrations of both drugs, *i.e.*, 1.5 μg mL^{-1} for CRV and 15.0 μg mL^{-1} for HCTZ. All validation parameters, such as correlation coefficient, concentration ranges and detection limit are summarized in Table-1. Accuracy and precision was determined with three replicates of quality control samples (Table-2). The recoveries ranged from 99.51 to 100.51 % for CRV and 99.97 to 100.80 % for HCTZ with relative standard deviation of 0.33 and 0.30 %, respectively. Both the intra-and inter-day RSD of quality control standard were less than 3 % over the selected range (Tables 4 and 5)

indicating that the method is sufficiently accurate and precise. The proposed method was found to be selective for the estimation of the two drugs in the presence of various tablets excipients. For this purpose, a powder blend using typical tablet excipients was prepared along with the drug and then analyzed. The recoveries were not affected by the excipients and the excipient blend did not show any absorption in the range of analysis (Table-6).

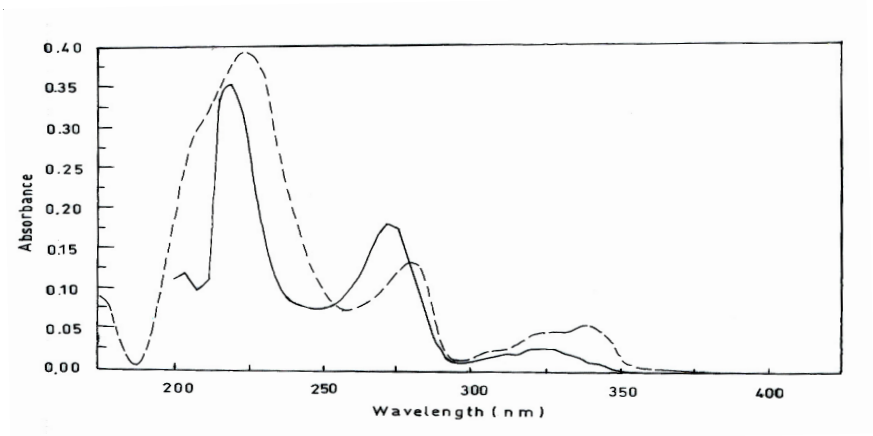


Fig. 2. Zero-order spectra showing overlapping bands for CRV (-----) and HCTZ (——).

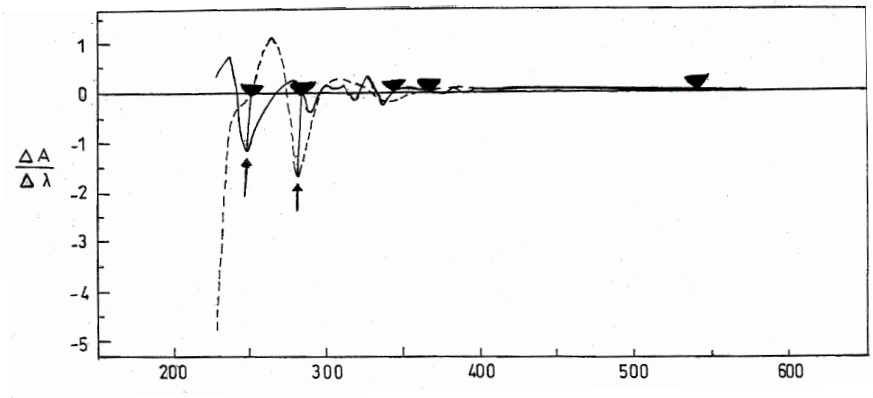


Fig. 3. First derivative spectra of CRV, (——), (0.15 μg mL^{-1}) and HCTZ (-----), (2.5 μg mL^{-1})

Chromatographic method: Drug content analysis is undertaken during various phases of pharmaceutical development, such as formulation and stability studies, quality control and pharmacological testing in animals and humans. All these investigations require reliable and validated

TABLE-1
FIRST DERIVATIVE UV AND HPLC METHOD VALIDATION PARAMETERS FOR THE CALIBRATION GRAPHS OF CARVEDILOL AND HYDROCHLOROTHIAZIDE

Drug	Conc. range (μg/mL)	Linear regression			RSD (%)	LOQ (μg/mL)	LOD (μg/mL)
		Intercept (a) ± S_a	Slope (b) ± S_b	CC*			
First derivative spectrophotometry							
CRV	0.05-1.50	0.0504 ± 0.89×10^{-3}	$5.4369 \pm 1.13 \times 10^{-2}$	0.9995	0.836	0.025	0.005
HTCZ	0.50-15.00	$3.6 \times 10^{-5} \pm 1.03 \times 10^{-2}$	$0.5038 \pm 1.63 \times 10^{-3}$	0.9999	0.421	0.250	0.100
High performance liquid chromatography							
CRV	0.05-1.00	802.585 ± 89.600	145605.03 ± 1122.50	0.9999	1.132	0.020	0.005
HTCZ	0.10-1.00	357.683 ± 18.700	99770.23 ± 123.90	0.9998	1.643	0.050	0.010

CC* = Correlation coefficient (r).
S_a = Standard deviation of the intercept (n = 5).
S_b = Standard deviation of the slope (n = 5).
RSD = Relative standard deviation (n = 5).

TABLE-2
RECOVERY DATA OBTAINED FOR DIFFERENT MIXTURES BY DERIVATIVE METHODS

Mixture no.	Carvedilol			Hydrochlorothiazide		
	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)*	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)*
1	0.10	0.1005	100.51	0.5	0.4998	99.97
2	0.30	0.3006	100.21	1.5	1.5120	100.80
3	0.60	0.5971	99.51	0.3	0.3017	100.57
4	0.80	0.8041	100.26	2.0	2.0088	100.44
5	1.00	1.0051	100.51	5.0	5.0000	100.00
6	1.50	1.4937	99.58	8.0	8.0192	100.24
$\bar{X}$ ± RSD %			100.17 ± 0.33			100.34 ± 0.30

*Each is the mean of 3 replicate determinations.

analytical methods in order to measure drugs in pharmaceutical formulations and biological samples. Hence, HPLC method was developed to provide a specific procedure suitable for rapid QC analysis of binary mixture containing CRV and HCTZ and as a reference method for first derivative UV procedure.

In order to resolve CRV and HCTZ peaks, different mobile phase's combinations at various flow rates were tried. The optimum wavelength for detection was set at 230 nm at which much better detector responses for both drugs were obtained. 10 mM potassium dihydrogen phosphate, (adjusted to pH 3.0 with phosphoric acid):acetonitrile (70:30 v/v) as a mobile phase at a flow rate of 1.0 mL min^{-1}, proved to be better than the others in terms of resolution and peak shape. As shown in Fig. 4, the retention times were 3.38 min for HCTZ and 17.95 min for CRV. Linear regression parameters are presented in Table-1.

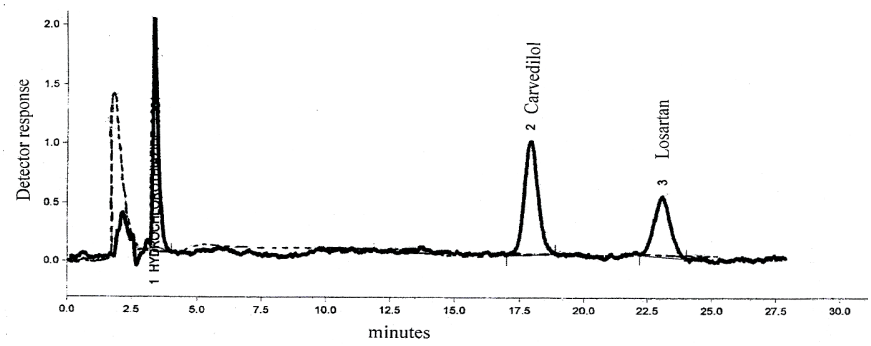


Fig. 4. Chromatograms demonstrating specificity of the proposed method. Doted chromatogram represents excipient injection without drug and show no interference with the two drugs as is evident in the bold chromatogram of mixture of CRV, HCTZ and losartan, (0.25 μg mL^{-1} each)

Peak area ratios (A/A_{is}) were plotted against corresponding concentrations in the 0.05-1.00 μg mL^{-1} (CRV) and 0.1-1.0 μg mL^{-1} (HCTZ) concentration range. Regression equations of the calibration curves for CRV and HCTZ were calculated as: A = 802.59 + 145605 C (r = 0.999) and A = 357.68 + 9977 C (r = 0.9998), respectively.

In order to access the validity and applicability of the developed method, recovery studies were performed by analyzing synthetic mixtures of each drug in different ratios. The mean percentage recoveries (± RSD) indicating good accuracy and precision were found to be 99.76 ± 0.72 for CRV; 100.61 ± 0.85 for HCTZ, (Table-3). Both the intra- and inter-day RSD of quality control standards were less than 2.5 % over the selected ranges (Tables 4 and 5). The data showed the reproducibility and precision for the analytes.

The proposed methods were applied to the determination of CRV and HCTZ in tablets and the results were statistically compared with each other at the 95 % confidence level with the aid of t- and F-tests[21].

TABLE-3
RECOVERY DATA OBTAINED FOR DIFFERENT MIXTURES BY HPLC METHODS

Mixture no.	Carvedilol			Hydrochlorothiazide		
	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)*	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)*
1	1.00	0.99000	99.00	0.50	0.4963	99.25
2	0.80	0.80000	100.00	0.40	0.4076	101.90
3	0.50	0.50210	100.42	1.00	1.0063	100.63
4	0.30	0.29630	98.75	0.15	0.1508	100.56
5	0.10	0.10070	100.75	0.10	0.1003	100.32
6	0.05	0.04982	99.63	0.20	0.2021	101.025
$\bar{X}$ ± RSD %			99.76 ± 0.72			100.61 ± 0.85

*Each is the mean of 3 replicate determinations.

TABLE-4
ESTIMATED INTRA-DAY AND INTER–DAY PRECISION AND ACCURACY FOR DETERMINATION OF CARVEDILOL USING DERIVATIVE SPECTROPHOTOMETRIC AND HPLC METHODS, (n = 6)

	Added (μg mL^{-1})	Derivative spectrophotometric method			HPLC method		
		Found (μg mL^{-1})	RSD (%)*	Error (%)*	Found (μg mL^{-1})	RSD (%)*	Error (%)*
Intra-day	0.10	0.0990	0.86	- 1.00	0.0989	0.35	- 1.10
	0.70	0.7074	1.03	1.06	0.7100	0.61	1.43
	1.20	1.2144	1.18	1.20	1.1970	0.45	- 0.25
Inter-day	0.10	0.0994	2.14	- 0.60	0.1010	1.01	1.00
	0.70	0.7120	2.07	1.71	0.6980	1.53	- 0.29
	1.20	1.2120	1.77	1.00	1.2200	1.36	1.67

*Of six replicate determinations.

TABLE-5
INTRA-DAY AND INTER-DAY PRECISION AND ACCURACY FOR DETERMINATION OF HYDROCHLOROTHIAZIDE USING DERIVATIVE SPECTROPHOTOMETRIC AND HPLC METHODS, (n= 6)

	Derivative spectrophotometric method				HPLC method			
	Added (μg/mL)	Found (μg/mL)	RSD (%)*	Error (%)*	Added (μg/mL)	Found (μg/mL)	RSD (%)*	Error (%)*
Intra-day	1.0	0. 993	0.47	- 0.73	0.1	0.0996	0.82	- 0.40
	4.0	3.973	0.97	- 0.67	0.4	0.4030	0.67	0.75
	8.0	7.949	1.12	- 0.64	0.8	0.8010	0.89	0.13
Inter-day	1.0	0. 993	1.86	- 0.70	0.1	0.1020	2.03	2.00
	4.0	3.954	2.66	- 1.15	0.4	0.3950	1.81	- 1.25
	8.0	8.131	2.20	1.64	0.8	0.7970	0.73	0.38

*Of six replicate determinations.

As it can be seen from the Table-6, there is no significant difference between the two methods with respect to mean values and standard deviations, since the calculated t- and F- values were less than the corresponding theoretical ones.

TABLE-6
ASSAY RESULTS OF TABLETS CONTAINING 25 mg CARVEDILOL AND 12.5 mg HYDROCHLOROTHIAZIDE

Compound	Statistical value	Derivative method (recovery, %)[a]	HPLC method (recovery, %)[a]
Carvedilol	$\bar{x}$	99.78	99.89
	SD	± 0.88	± 0.41
	t	0.254 (2.228)*	
	F	4.565 (6.390)*	
Hydrochlorothiazide	$\bar{x}$	99.84	99.60
	SD	± 0.46	± 0.76
	t	0.602 (2.228)*	
	F	2.728 (6.390)*	

[a]Each result is the average of three separate determinations.
*Tabulated values of t and F at 95% confidence level[21].

Conclusion

The proposed first derivative UV and HPLC methods are suitable techniques for simultaneous determination of carvedilol and hydrochlorothiazide in tablets without any interference from each other. All the parameters for both drugs met the criteria of ICH guidelines for bioanalytical method validation.

The derivative method is rapid, simple and cost effective. However, HPLC method may be considered more specific and sensitive than the derivative UV method but also is more expensive requiring sophisticated chromatographic instrumentation for its performance. Both the developed methods may be recommended for routine and quality control analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination of carvedilol and hydrochlorothiazide in pharmaceutical formulations.

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