Full Paper

Electrochemical Behavior of Carvedilol and Its Adsorptive Stripping Determination in Dosage Forms and Biological Fluids

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Abstract
Carvedilol is used in the management of hypertension and angina pectoris and as an adjunct to standard therapy in symptomatic heart failure. The electrochemical oxidation of carvedilol was investigated using cyclic, linear sweep voltammetry at a glassy carbon electrode. In cyclic voltammetry, in all values of pH, the compound shows two irreversible oxidation peaks. These two peaks are related to the different electroactive part of the molecule. First and second peak currents were found as diffusion and adsorption controlled, respectively. Using second oxidation step, two voltammetric methods were described for the determination of carvedilol by differential pulse adsorptive stripping voltammetry (AdSDPV) and square-wave adsorptive stripping voltammetry (AdSSWV) at a glassy carbon electrode. Accumulation of carvedilol was found to be optimized in 0.2 M H_2SO_4 solution following 275 second accumulation time at open circuit condition. Under optimized conditions, the current showed a linear dependence with concentration in the range between 2×10^{-7} M and 2×10^{-5} M in supporting electrolyte and in the range between 2×10^{-7} M and 1×10^{-5} M in spiked human serum samples for both methods. These methods were successfully applied for the analysis of carvedilol pharmaceutical dosage forms and spiked human serum samples. The repeatability and reproducibility of the methods for all media were determined. Precision and accuracy were also found. No electroactive interferences from the tablet excipients and endogenous substances from biological material were found.

Keywords: Carvedilol, Oxidation mechanism, Tablets, Serum, Determination, Adsorptive stripping voltammetry

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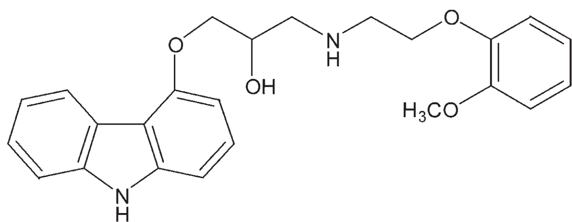
1. Introduction

Carvedilol is a nonselective (β)-adrenergic blocking agent with α_1-blocking activity. It has vasodilating properties that are attributed mainly to its blocking activity at α_1 receptor; at higher doses calcium-channel blocking activity may contribute. Carvedilol is used in the management of hypertension and angina pectoris and as an adjunct to standard therapy in symptomatic heart failure [1–3].

Few analytical methods were described for the determination of carvedilol in biological media or in pharmaceutical dosage forms including liquid chromatography [4–8], capillary electrophoresis [9], fluorimetry [10] and anodic voltammetry [11]. However, no attempt was made up to date to assay and detailed electrochemical behavior and redox properties of carvedilol itself using any of the electroanalytical techniques. Most of the reported methods required time-consuming sample pretreatment and solid-phase extraction steps prior to the drug analysis, expensive reagents and equipment, which are not economically feasible for routine use biological media studies. Commonly used separation techniques are undoubtedly superior, when carvedilol is to be determined in the presence of impurities from manufacture or of metabolites. But it may be answered, whether such procedures present the fastest, most accurate and most sensitive analytical methods for determination of the drug in pharmaceutical dosage forms. The choice of the appropriate technique to solve an analytical-pharmaceutical problem is often controlled by the sample matrix, and the amount of preparation that is required before the analytical measurement can be made. Under specific circumstances, electrochemical methods can offer optimal solution. Voltammetry is a powerful and versatile analytical technique that offers high sensitivity, precision, and accuracy as well as a wide linear range, with relatively low-cost instrumentation. Although voltammetric techniques represent a rather specialized area of instrumental analysis, it is a powerful tool for the pharmaceutical and clinical analysts. Voltammetric procedures have been used extensively to elucidate the redox properties of biological and pharmaceutical compounds.



Scheme 1. Chemical structure of Carvedilol.

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Adsorptive stripping analysis is an extremely sensitive electrochemical technique for measuring trace amount of compounds. Its remarkable sensitivity is attributed to the combination of an effective preconcentration step with advanced measurement procedures that generates an extremely favorable signal-to-background ratio. In this technique, trace amount of compounds can be measured at low concentrations, utilizing relatively inexpensive instrumentation. Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. This technique has been widely used in studying the redox mechanisms of many pharmaceutically and biologically significant molecules. The results of such investigations into the redox chemistry of biomolecules and drugs might have profound effects on the understanding of their in-vivo redox processes or pharmaceutical activity. This technique is often the first experiment performed in an electroanalytical study. [12 – 14].

The voltammetric determination of carvedilol has been studied by differential pulse voltammetry [11]. In this literature, Radi et al. was worked on the anodic voltammetric behavior of carvedilol using CV technique. They used the CV method for obtaining some important parameters such as diffusion or adsorption controlled process, reversible or irreversible procedure etc. This CV studies were realized in the short potential ranges. For this reason they could not obtained the second peak potential. They also proposed DPV procedure only based on the application of carvedilol pharmaceutical dosage form using glassy carbon electrode. The nature of the processes manifested in current peaks observed in Britton – Robinson buffers was not identified. Furthermore, in protic solvents where electron transfers are accompanied by proton transfers, the important role of pH and nature of the buffer, and the oxidation mechanisms of carvedilol were not discussed. Resulting empirical analytical method may be prone to unexpected matrix effects. The authors [11] only indicated that electrode processes involved are irreversible and diffusion controlled.

This work aimed to study the detailed voltammetric behavior and sensitive assay of carvedilol at a glassy carbon electrode using cyclic, linear sweep, DPV, SWV, AdSDPV and AdSSWV techniques. The adsorption nature of the drug at the glassy carbon electrode surface form the bases for the electroanalytical determination of carvedilol in pharmaceutical dosage forms and biological fluids such as human serum. In addition the purposed method can be considered as a stability-indicating and fully validated assay.

2. Experimental

All voltammetric measurements such as cyclic, linear sweep, differential pulse, square-wave, adsorptive stripping differential pulse, adsorptive stripping square-wave voltammetry were carried out using a BAS 100W electrochemical analyzer. A three electrode cell system incorporating the glassy carbon disc electrode as working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M KCl) reference electrode and standard one-compartment three-electrode cell of 10 mL capacity were used in all experiments.

Before each measurement the glassy carbon electrode was polished manually with aqueous slurry of alumina powder (∅: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad). All measurements were realized at room temperature.

The pH was measured using a pH meter Model 538 (WTW, Austria) using a combined electrode (glass electrode-reference electrode) with an accuracy of ± 0.05 pH.

Voltammetric analyses were carried out in 0.2 M H_2SO_4. The accumulation potential (usually open circuit condition) was applied for a selected deposit time (275 s) while the solution was stirred at 600 rpm. The stirrer was then stopped and after 10 s rest period, the compound was removed by stripping anodically using differential pulse and square-wave voltammetry. All data were obtained at ambient temperature.

Operating conditions for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate; 20 mV s^{-1}; for the SWV were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV; for Adsorptive stripping DPV were: scan rate 20 mV s^{-1}, pulse amplitude 50 mV; pulse width; 50 ms, pulse period: 200 ms, and for Adsorptive stripping SWV were: step potential: 4 mV, amplitude 25 mV, frequency, 15 Hz.

2.1. Reagents

Carvedilol and Diladrent tablets were kindly supplied by Roche Pharm. Ind. (Istanbul, Turkey). Model compounds, etodolac, melatonin, tamsulosin, L-dopa, and mebeverin HCl were kindly supplied from different pharmaceutical company. Indol-3-acetic acid and indol-3-butiric acid were also used as model compounds and they were supplied from Sigma. All chemicals for preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma).

Stock solutions of carvedilol (1×10^{-3} M) were prepared in methanol and kept in the dark in a refrigerator. Carvedilol working solutions under voltammetric investigations were prepared by dilution of the stock solution and contained 20% methanol. The stock solutions of all other compounds were also prepared in methanol or bi-distilled water and kept in the dark in a refrigerator.

Four different supporting electrolytes, namely sulfuric acid (0.1; 0.2; 0.3 and 0.5 M), phosphate buffer (0.2 M; pH 2.0 – 12.0), acetate buffer (0.2 M; pH 3.5 – 5.7), Britton – Robinson buffer (0.04 M, pH 2.0 – 12.0) were prepared in doubly distilled water.

The calibration curve for AdSDPV and AdSSWV analysis was constructed by plotting the peak current against the carvedilol concentration. The ruggedness and precision were checked at different days, within day ($n = 5$) and between days ($n = 5$) for two different concentrations. Relative standard deviations were calculated to check the ruggedness and precision of the method. [15, 16].

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The accuracy and precision of the developed methods are described in a quantitative fashion by the use of relative errors (Bias%). One example of the Bias% is the accuracy, which describes the deviation from the expected results. All solutions were protected from light and were used within the same day after preparation to avoid decomposition. However, current-potential curves of carvedilol solutions recorded three weeks after preparation did not show any appreciable change in assay values.

2.2. Diladrent Tablet Assay Procedure

Ten tablets of Diladrent (Roche Pharm Ind., Istanbul), containing 25 mg carvedilol per tablet, were accurately weighed and crushed to a homogeneous fine powder in a mortar. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-3} M, was weighed, transferred into a 50 mL calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to achieve complete dissolution. Analyzed solutions were prepared by taking aliquots of the clear supernatant and diluting with the selected supporting electrolyte. Voltammograms were recorded according to the AdSDPV and AdSSWV parameters and as in pure carvedilol.

2.3. Recovery Experiments

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies is used. To study the accuracy and reproducibility of the proposed techniques, recovery experiments were carried out using the standard addition method.

In order to know whether the excipients show any interference with the analysis, known amounts of pure carvedilol were added to the pre-analyzed tablet formulation and the mixtures were analyzed by the proposed method. The recovery results were determined based on five parallel analyses.

2.4. Analysis of Spiked Human Serum Samples

Serum samples, obtained from healthy subjects (after obtaining their written consent) were stored frozen until assay. After gentle, thawing, an aliquot volume of sample was spiked with carvedilol dissolved in methanol to achieve final concentration of 1×10^{-3} M and treated 1.0 mL acetonitrile (about 1:1; v/v) as serum denaturing and precipitating agent, then the volume was completed to 2 mL with the same serum sample. The sample tubes were vortexed for 10 min and then centrifuged for 10 min at $5000 \times$ g for removing of protein residues. The supernatant was taken carefully. The concentration of carvedilol was varied in the range of 2×10^{-7} to 1×10^{-5} M in human serum samples. These solutions were analyzed in the one compartment voltammetric cell containing 0.2 M H_2SO_4 and constant amount of methanol (20%)in order to obtain a final solution of 20:80 methanol: 0.2 M H_2SO_4. The amount of carvedilol in spiked human serum samples for the recovery studies was calculated from the related linear regression equation.

3. Results and Discussion

No previous detailed electrochemical data were available concerning the electrode behavior of carvedilol. Carvedilol is manifested on current-voltage curves recorded by CV and LSV on a glassy carbon electrode by two anodic peaks (I, II) (at less positive potentials) and one wave (III) (at more positive potentials than the peaks) depending on pH (Fig. 1). All anodic responses will be discussed in this contribution. The peak currents and peak potentials were determined in supporting electrolytes containing 20% methanol (v/v) to maintain solubility. Therefore, several measurements with different electrochemical techniques (cyclic, linear sweep, differential pulse and square-wave voltammetry) were performed using various supporting electrolytes and buffers in order to obtain such information. Carvedilol was electrochemically oxidized in a broad pH range (1.3 – 12.00) using glassy carbon disc electrode.

The scan rate studies were realized between 5 and 1000 mV s^{-1} range. The less positive peak (i_{p1}) corresponds to one electrooxidation step of carvedilol and is diffusion controlled. This is confirmed by a linear dependence of peak current i_{p1} on concentration of carvedilol and on its linear dependence on $v^{1/2}$ according to the following equation, which is obtained in Britton – Robinson buffer at pH 11.00:

$$i_{p1}\ (\mu A) = 0.26\ v^{1/2}\ (mV\ s^{-1}) - 0.61\quad r = 0.997\ (n = 10)$$

A 73 mV positive shift in the first peak (i_{p1}) potential confirmed the irreversibility of the oxidation process with absence of the cathodic peak or wave.

A plot of logarithm of the first peak current ($\log i_{p1}$) versus logarithm of scan rate ($\log v$) gave a straight line with a slope of 0.65, close to the theoretical value of 0.5, which is expressed for an ideal reaction the diffusion controlled electrode process [18].

The equation obtained is:

$$\log i_{p1}\ (\mu A) = 0.65\ \log v\ (mV\ s^{-1}) - 1.05\quad r = 0.998\ (n = 10)$$

The peak potential of the first peak remains practically pH-dependent in all values. The first peak potential (E_{p1}) values are shifted to less positive potential values with increasing pH. It is a general rule that a conjugate base is oxidized at less positive potentials than the corresponding acid form. Thus the observed pH dependence indicates that the electroactive group which is corresponding to the first peak is in acid-base equilibrium with pKa about 8.0. Above pH 8.0 the E_{p1} still is shifted to less positive potential values with increasing pH (Fig. 2a). This intersection point of the

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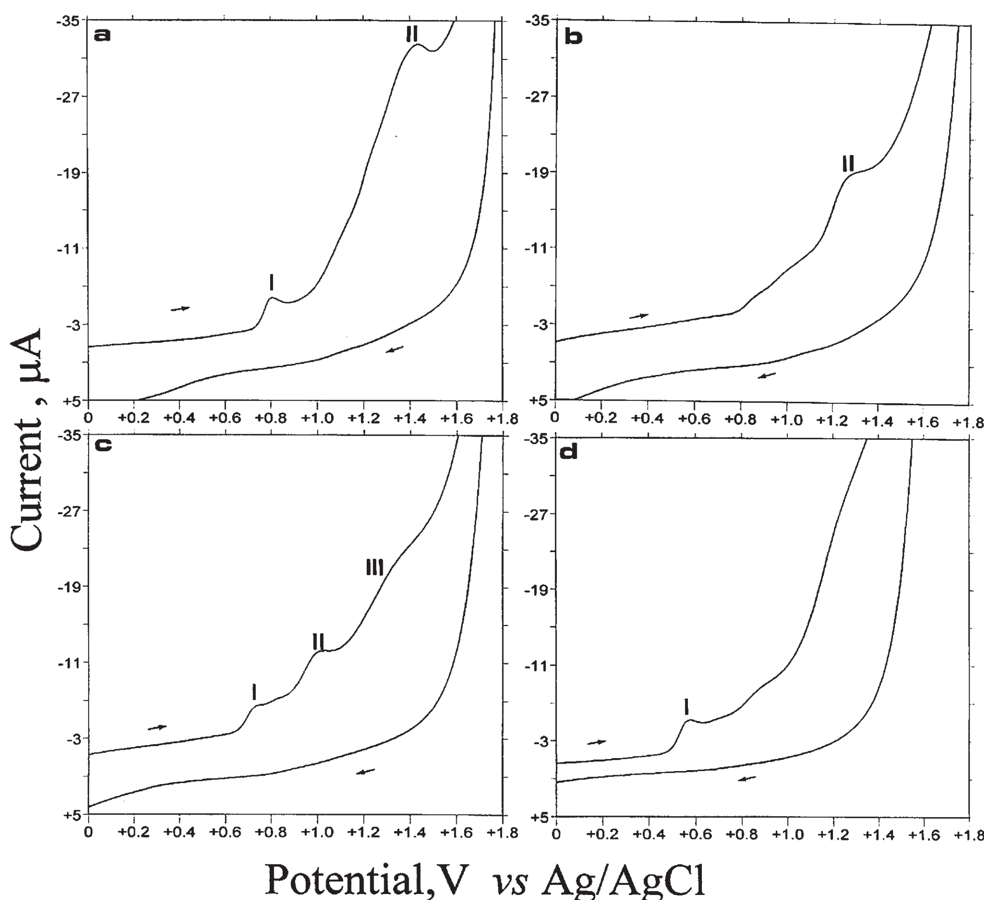

Fig. 1. Cyclic voltammograms of 1×10^{-4} M cervedilol in 0.2 M H_2SO_4 (a); phosphate buffer at pH 2.0 (b); at pH 6.0 (c); Britton–Robinson buffer at pH 10.0 (d) with constant amount methanol (20%). Scan rate: 100 mV s^{-1}.

curves is close to the pKa value of carvedilol which is pKa varying between 7.7 and 7.9 [17]. The influence of pH on the carvedilol first peak current was also studied. The i_{p1} versus pH plot (Fig. 2b) shows that peak current is maximum in the basic media. The experimental results showed that the shapes of the curves and peak current were better in Britton–Robinson buffer at pH 11.0. Our obtained results for the first peak are not agreement with the Radi et al. [11] E_{p1}-pH study. They obtained two intersection points for E_{p1}-pH graph and maximum peak current in Britton–Robinson buffer at pH 8.0. They did not realize the detailed study on the first peak such as nature of the buffer effect. Also they did not search and focus on the second peak, which is more important process for the analytical application. There is not any information about second peak in that article [11].

The linear segments can be expressed by the following equations in all buffers for the first peak using CV technique. In the first linear section, disharmony was obtained between Britton–Robinson (0.04 M) and phosphate buffer (0.2 M) in the pH range 2 to 5. This may be occurred due to the differences of the ionic strength of the buffers.

$$E_p(\text{mV}) = 941.5 - 33.41\text{pH} \quad r = 0.967 \quad (\text{between 2.0 and 8.0})$$

$$E_p(\text{mV}) = 1172 - 62.2\ \text{pH} \quad r = 0.998 \quad (\text{between 8.0 and 12.0})$$

The more positive peak (i_{p2}) corresponds to second and our focused electrooxidation step for the sensitive analytical application of carvedilol and the second process was found to be adsorption controlled. The effect of the potential scan rate between 5 and 250 mV s^{-1} on the second peak current and potential of carvedilol was evaluated. A 105 mV positive shift in the second peak potential was also confirmed the irreversibility of second oxidation process with absence of the cathodic wave. When the scan rate varied from 5 to 250 mV s^{-1} in 6×10^{-5} M solution of carvedilol, a linear dependence of the peak intensity i_{p2} (μA) upon the scan rate v (mV s^{-1}) was found, confirmed an adsorptional behavior. This equation is noted below in 0.2 M H_2SO_4:

$$i_{p2}\ (\mu\text{A}) = 0.108\ v\ (\text{mV s}^{-1}) + 1.49 \quad r = 0.995 \quad (n = 7)$$

A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.87, close to the theoretical value of 1.0, which is expressed for an ideal reaction the adsorption controlled electrode process [18].

The equation obtained is:

$$\log i_{p2}\ (\mu\text{A}) = 0.87 \log v\ (\text{mV s}^{-1}) - 0.61 \quad r = 0.997 \quad (n = 7)$$

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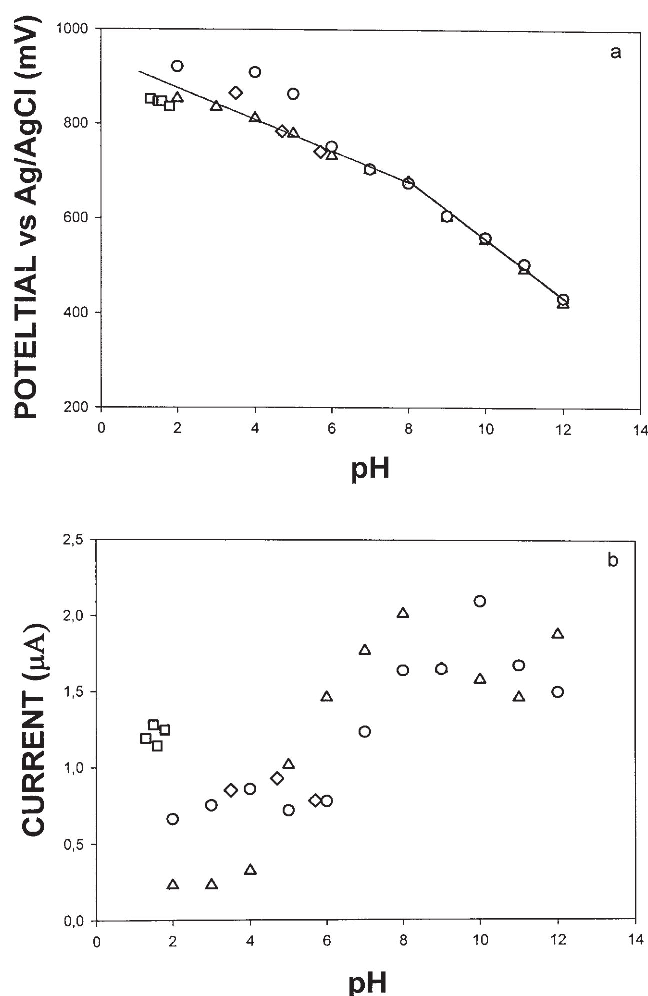

Fig. 2. Effect of pH on carvedilol first peak potential (a) and peak current (b); carvedilol concentration 1×10^{-4} M with constant amount methanol (20%). (□) H_2SO_4 (0.1 M; 0.2 M; 0.3 M and 0.5 M); (○) Britton – Robinson (0.04 M); (△) phosphate (0.2 M) and (◇) acetate buffers (0.2 M).

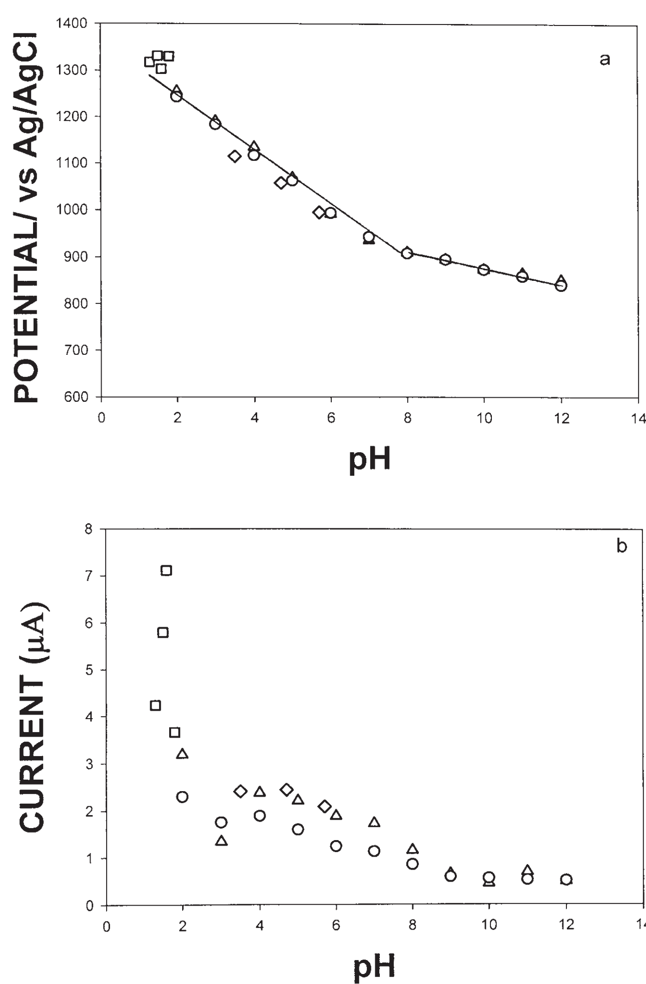

Fig. 3. Effect of pH on carvedilol second peak potential (a) and peak current (b). Carvedilol concentration 1×10^{-4} M with constant amount methanol (20%). (□) H_2SO_4 (0. 1 M; 0.2 M; 0.3 M and 0.5 M); (○) Britton – Robinson (0.04 M); (△) phosphate (0.2 M) and (◇) acetate buffers (0.2 M).

The peak potential of the second oxidation process (E_{p2}) moved to less positive potential with a giving two linear segments.

These two linear segments can be expressed by the following equations in all buffer solutions using by CV technique.

$$E_p \text{ (mV)} = 1353.7 - 58.92 \text{ pH } r = 0.983 \text{(between 2.0 and 8.0)}$$

$$E_p \text{ (mV)} = 1028.6 - 15.0 \text{ pH } r = 0.991 \text{(between 8.0 and 12.0)}$$

The observed pH dependence with E_{p2} indicates that the second electroactive group which is corresponding to the second peak is in acid-base equilibrium with also pKa about 8.0. Above pH 8.0, E_{p2} slightly shifted to less positive potential values with increasing pH (Fig. 3a). This intersection point of the curves is also close to the pKa value of carvedilol [17]. This can also be explained by changes in protonation of the acid-base functions in the molecule.

The influence of pH on the carvedilol second peak current (i_{p2}) at glassy carbon disc electrode was also studied. The i_{p2} versus pH graph (Fig. 3b) shows that peak current is maximum in the acidic media and the shape of the curve and the i_{p2} were better in 0.2 M H_2SO_4. For this reason 0.2 M H_2SO_4 was chosen with respect to sharp response and better peak shape for the analytical application using adsorptive stripping techniques.

According to the obtained results from the i_{p1} and i_{p2}, the first and second peaks were found diffusion and adsorption controlled process, respectively. For this reason, the second peak was chosen for the analytical application because of the possibility using more sensitive adsorptive stripping techniques.

The Tafel plots (log i versus E) was obtained with a scan rate of 5 mV s^{-1} beginning from a steady-state potential in Britton – Robinson buffer at pH 11.00 for the first peak and in 0.2 M H_2SO_4 for the second peak. The α_n value of the first anodic reaction (i_{p1}) from the slope of the linear part of the Tafel plot was found to be as 0.266 and for the second anodic

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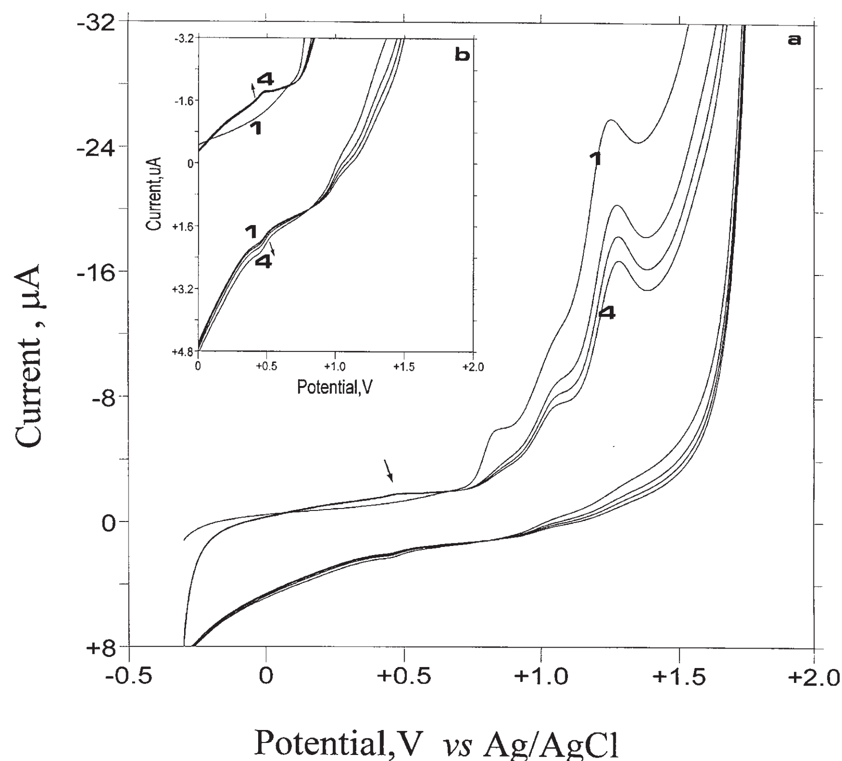

Fig. 4. Repetitive cyclic voltammograms of 1×10^{-4} M carvedilol in phosphate buffer at pH 3.0 with constant amount methanol (20%). Scan rate 100 mVs^{-1}. The numbers indicate the number of scan. a) The full scale is shown and b) is zoomed to specific potential range indicated with arrow.

reaction (i_{p2}) is 0.1097. The exchange current density (i_o) is 1.91×10^{-8} A cm^{-2} and 8.13×10^{-8} A cm^{-2} for the first (i_{p1}) and second (i_{p2}) anodic process, respectively. These values together with the absence of cathodic waves in CV (Fig. 1) indicated the irreversibility of the oxidation reaction.

Voltammetric methods especially cyclic voltammetry are most suitable for investigating the redox behavior of the new pharmaceutical compound; this can give insights into its metabolic fate [19]. Cyclic voltammetric measurements from the redox properties of active compounds [20–29] and biomolecules [30–32] might have profound effects on the understanding of the redox mechanism related to the activity of the carvedilol compound. The anodic oxidative behavior of carvedilol is comparable to indole and alkoxybenzene oxidations that were reported our previous study and literature assay [20–29]. To support the working hypothesis that it is the carbazole ring (similar oxidation way with indole ring) and methoxy group on the phenyl ring in carvedilol that undergoes oxidation, the behavior of anodic peaks i_{p1} (related to carbazole) and i_{p2} (related to methoxy group) of carvedilol was compared with that some model compounds. There are two main groupings present in the structure of carvedilol, which might be considered as undergoing electro-oxidation: the carbazole ring and alkoxybenzene ring.

As a model substances for oxidation of the carbazole ring (similar with indole oxidation), were used different compounds namely etodolac, melatonin, fluvastatin, indole-3-acetic acid and indole-3-butiric acid. The electrooxidation of the indole moiety of all compounds have already reported [22, 25–29]. Our obtained results revealed a good agreement with the redox mechanism postulated for the model compounds could be oxidized electrochemically by oxidation on the nitrogen atom in the carbazole ring of the carvedilol molecule, which is electroactive in both acidic and basic media. These results strongly indicate that in carvedilol first oxidation step is related to the nitrogen atom on the carbazole ring. Carbazole ring can be converted for instance into 3-hydroxycarbazole or carbazole-1,4-dione [33] under the action of specific circumstances. The oxidation mechanism of carbazole ring is similar to the oxidation of other N-containing compounds such as indole group.

As model substances for oxidation of methoxybenzene moiety, were used three drugs, Tamsulosin, Formaterol, Mebeverin HCl and two model compounds 4-methoxyphenol and anisole. The electrooxidation of the methoxybenzene of Tamsulosin and formaterol have already been reported to our previous studies [20, 21]. Tamsulosin and Mebeverin are oxidized over most of the pH range in a single peak at potentials by about 0.12 V and 0.26 V less positive than those of second peak of carvedilol. The anodic behavior of carvedilol is comparable to alkoxybenzene oxidation also, which was reported in our previous study [20, 21]. As a comparative study anisole and 4-methoxyphenol were also performed by cyclic voltammetry at the glassy carbon electrode, as a function of pH in order to identify the

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oxidation process of carvedilol (not shown). Anisole and 4-methoxyphenol are both converted to quinone [24]. Our obtained results revealed a good agreement with the redox mechanism postulated for similar compounds such as formaterol fumarate, mefexamide, sulpride, Tamsulosin and model compounds such as anisole and 4-methoxyphenol and suggested that carvedilol can be determined electrochemically by oxidation of methoxybenzene group. Anodic oxidation of methoxybenzenes in aqueous acidic medium also leads to loss of the methoxy substituent, this time through ipso-substitution on the radical-cation by water [24]. Carvedilol oxidizes in all supporting electrolyte via initial two electron oxidation, including fast chemical reactions with water to give the benzoquinone, which is responsible for the redox couple (Fig. 4). This cationic radical intermediate is well known in anodic oxidation of aromatic ethers. This redox couple can be easily seen in Figure 4.

Taking into account all the studies performed so far and the appearance of a new redox couple at lower potential, likely corresponding to the reversible behavior of a liberated dihydroxyphenyl species, we suggested that the second oxidation process of carvedilol may be occurring on the methoxybenzene groups of the molecule, which is electroactive in both acidic and basic media. The second oxidation step and related mechanism and parameters were not indicated and explained in Radi et al. study [11].

With the above results we can confirm that the electroactive center corresponding to the first and second anodic peak was the nitrogen atom on the carbazole ring and methoxybenzene group, respectively.

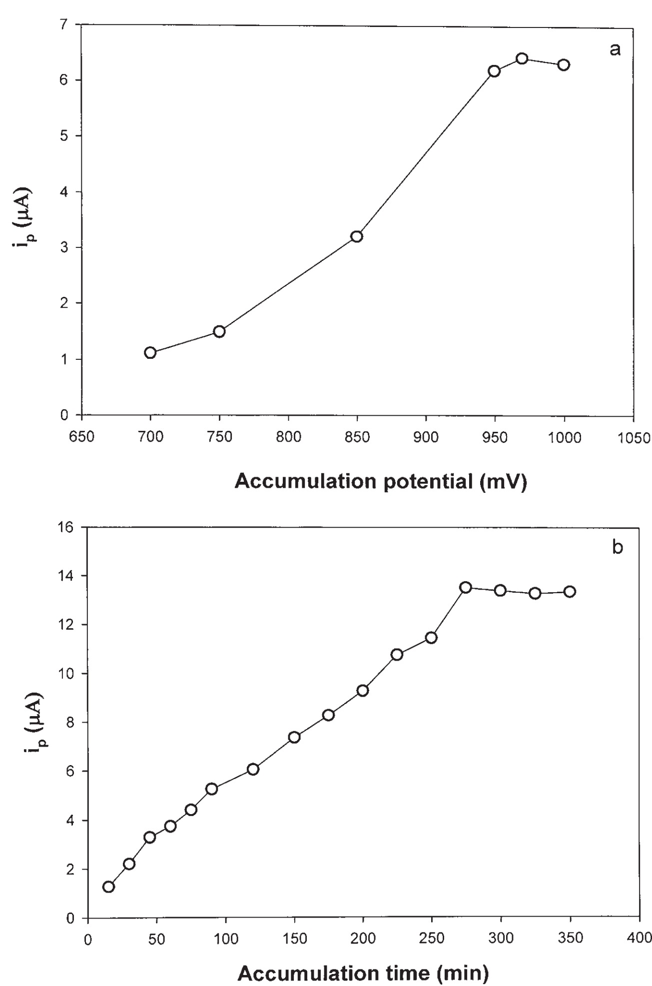

Fig. 5. Effect of accumulation potential on the peak current at 60 s accumulation time (a); and effect of accumulation time on the peak current, accumulation potential +0.95 V of 6×10^{-5} M carvedilol in the presence of 0.2 M H_2SO_4 using AdSDPV method.

3.1. Analytical Parameters and Validation of the Developed Methods

Pulse voltammetric techniques such as DPV, SWV, adsorptive pulse techniques are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits [12, 13]. Especially, adsorptive stripping analysis greatly enhances the scope of stripping measurements toward numerous low amount compounds. Short adsorption times (1–5 min) result in a very effective interfacial accumulation. This technique has been shown to be highly suitable for measuring organic drug compounds.

To develop a rugged and suitable voltammetric methods for the quantitative determination of carvedilol, various supporting electrolyte and different methanol ratios were employed. After our preliminary trials using different percentage of methanol, 20% of methanol ratio gave the best response. Different supporting electrolytes, such as sulfuric acid, phosphate, acetate and Britton–Robinson buffers were examined. The peak current-potential curve is the most useful analytical signal for both differential pulse and square-wave techniques and also their adsorptive stripping modes in 0.2 M H_2SO_4 with constant amount methanol as 20%. Also all adsorptive stripping parameters such as accumulation time, potential etc. were investigated in Britton–Robinson buffer at pH 11.0, phosphate buffer at pH 2.0 and 6.0. After all detailed investigation the best results with respect to signal enhancement (Fig. 3b) accompanied by sharper response was obtained with 0.2 M H_2SO_4. This supporting electrolyte was chosen for subsequent determination experiments.

Adsorption of the analyte was confirmed by the results obtained with cyclic voltammetry. The plot of logarithm of peak current versus logarithm of scan rate has a slope of 0.87, close to the theoretical value of 1.0 which is expressed for an ideal reaction of the adsorption controlled electrode process. The spontaneous accumulation of carvedilol can be exploited for effective preconcentration prior to the voltammetric scan. Figure 5a displays the resulting peak current versus accumulation potential for 6×10^{-6} M carvedilol. An adsorption potential of 950 mV was adopted for analytical determination of carvedilol.

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Table 1. Regression and necessary validation data of the calibration lines of carvedilol by AdSDPV and AdSSWV in supporting electrolyte and human serum.

	AdSDPV		AdSSWV	
	Supporting electrolyte	Serum	Supporting electrolyte	Serum
Measured potential (V)	1.22	1.22	1.26	1.26
Linearity range (M)	$2\times10^{-7}-2\times10^{-5}$	$2\times10^{-7}-1\times10^{-5}$	$2\times10^{-7}-2\times10^{-5}$	$2\times10^{-7}-1\times10^{-5}$
Slope (μA M^{-1})	1.74×10^{6}	2.77×10^{6}	2.84×10^{6}	4.43×10^{6}
Intercept (μA)	−0.241	−0.129	−0.577	−0.830
Correlation coefficient	0.999	0.999	0.999	0.999
SE of slope	1.565×10^{4}	2.85×10^{4}	3.08×10^{4}	4.06×10^{4}
SE of intercept	0.118	0.134	0.231	0.191
LOD (M)	2.06×10^{-9}	5.99×10^{-8}	2.37×10^{-9}	5.95×10^{-8}
LOQ (M)	6.86×10^{-9}	2.00×10^{-7}	7.88×10^{-9}	1.98×10^{-7}
Repeatability of peak current (RSD%)	0.24	0.71	0.81	0.68
Repeatability of peak potential (RSD%)	0.15	0.14	0.28	0.20
Reproducibility of peak current (RSD%)	0.68	0.91	0.998	0.70
Reproducibility of peak potential (RSD%)	0.18	0.23	0.35	0.28

Figure 5b shows the resulting peak current vs. preconcentration time profile for 6×10^{-6} M carvedilol. The rapid increase of the current observed at short preconcentration time, is followed by a leveling-off. The plots do not pass through the origin possibly because of the strong adsorption of the analyte at the electrode surface at the equilibrium time, which was fixed at 10 s. Hence to maximum sensitivity, a 275 s accumulation time was used for subsequent quantitative determinations with both AdSDPV and AdSSWV methods. However, the ultimate choice of accumulation time should depend on the concentration range studies. The obtained AdSDPV and AdSSWV curves for accumulation potential and time effects were found similar. For this reason, only AdSDPV curves were given as Figure 5.

The peak current-potential curve is the most useful analytical signal for both AdSDPV and AdSSWV techniques. The two calibration curves obtained from both techniques for carvedilol determination following preconcentration for selected time were established applying the developed procedures. A linear relation in the concentration range between 2×10^{-7} and 2×10^{-5} M was found, indicating that the response was adsorption controlled in this range. Above this concentration (3×10^{-5} M) a loss of linearity was observed. The characteristics of the calibration plots are summarized in Table 1.

Validation of the procedures for the quantitative assay of carvedilol was examined via evaluation of the limit of detection (*LOD*), limit of quantification (*LOQ*), repeatability (within-day), reproducibility (between-day), specificity, recovery, precision and accuracy. The low values of SE of slope and intercept and greater than 0.999 correlation coefficient, established the precision of the proposed methods. Several approaches are given in the ICH guideline to determine the *LO*D and *LOQ values.*

In this study *LOD* and *LOQ* values for carvedilol following preconcentration time period were calculated (Table 1) using the following equations [15, 16]:

$$LOD = 3\,s/m \quad LOQ = 10\,s/m$$

The abbreviation of *s* is the standard deviation of the peak currents (three runs) and *m* is the slope of the related calibration curve. Both *LOD* and *LOQ* values confirmed the sensitivity of the proposed methods. The precision of the method was evaluated by repeating five experiments on the same day in the same standard solutions (repeatability) and over two weeks from the different standard solutions (reproducibility) repeating the experiments for five times [15, 16]. To study these experiments the chosen concentration was 8×10^{-6} M. The results were given in Table 1. The within day and between day precision, accuracy and reproducibility were determined as the RSD% and mean value and the results were shown in Table 1. Precision, accuracy; and reproducibility results shown in Table 1 demonstrate good precision, accuracy and reproducibility.

The stability of the reference substance and sample solutions was checked by analyzing a prepared standard solution of carvedilol in supporting electrolyte aged at $+4\,^{\circ}$C, in the dark against a sample freshly prepared. The results demonstrated that the working reference solutions were stable for up to 3 weeks. The carvedilol response for the assay reference solutions over 3 weeks did not change considerably.

When working on standard solutions, and according to the obtained validation parameters, results encourage the use of the proposed methods described for the assay of carvedilol in pharmaceutical dosage forms and spiked human serum samples.

3.2. Determination of Carvedilol Tablets

On the basis of above results, both AdSDPV and AdSSWV methods were applied to the direct determination of carvedilol tablets, using related calibration straight lines without any sample extraction or filtration and after an adequate dilutions. The results obtained from the analysis of tablet dosage forms are summarized in Table 2. These

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Table 2. Results of the assay from the dosage forms and the recovery analysis of carvedilol in tablets.

	AdSDPV	AdSSWV
Labeled (mg)	25.00	25.00
Amount found (mg) [a]	25.04	25.06
RSD %	1.03	1.16
Bias %	−0.16	−0.24
Added (mg)	10.00	10.00
Found (mg) [a]	9.99	10.03
Recovery %	99.93	100.26
Bias %	0.10	−0.30
RSD % of recovery	0.28	0.35

[a] Each value is the mean of five experiments.

Table 3. Application of the AdSDPV and AdSSWV methods to the determination of carvedilol in spiked serum samples.

	AdSDPV	AdSSWV
Added (M)	8×10^{-6}	8×10^{-6}
n	5	5
Found (M)	7.99×10^{-6}	7.97×10^{-6}
Average recovery %	99.88	99.63
RSD %	0.65	0.49
Bias %	0.13	0.38

results show that the proposed methods were successfully applied for the assay of carvedilol in its tablet dosage forms.

The accuracy of the method was determined by its recovery using the standard addition method. Recovery studies were realized after the addition of known amounts of the pure drug to various pre-analyzed formulation of carvedilol. Recovery experiments using the developed assay procedure further indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulation (Table 2). These results reveal that both methods had adequate precision and accuracy and consequently can be applied to the determination of carvedilol in tablets without any interference from the excipients.

3.3. Determination of Carvedilol in Spiked Human Serum Samples

Methanol and acetonitrile were tried as a serum denaturizing and precipitating agents. The best results were obtained using 1.0 mL acetonitrile for about 1 mL serum (1:1; v/v). No extraction steps other than centrifugal protein separation were required prior to the assay of drug. The measurements of carvedilol in human serum samples were performed as described in Section 2. The characteristics of calibration plots for assay of the spiked serum samples at selected accumulation duration are reported in Table 1. The necessary validation parameters such as *LOD*, *LOQ*, repeatability, reproducibility etc. was shown in Table 1. Figure 6 shows the response of some selected concentrations of carvedilol. Typical AdSDPV and AdSSWV curves of carvedilol are shown in Figures 6a and 6b, respectively. As can be seen in Figure 6, no oxidation compounds, and no extra noise and endogenous substances peaks present in biological material peak occurred in the potential range where the analytical peak appeared. Obtained recovery results of spiked human serum samples were given in Table 3. Analysis of drug compounds from biological samples usually requires extensive time-consuming sample preparation, use of expensive organic solvents and other chemicals.

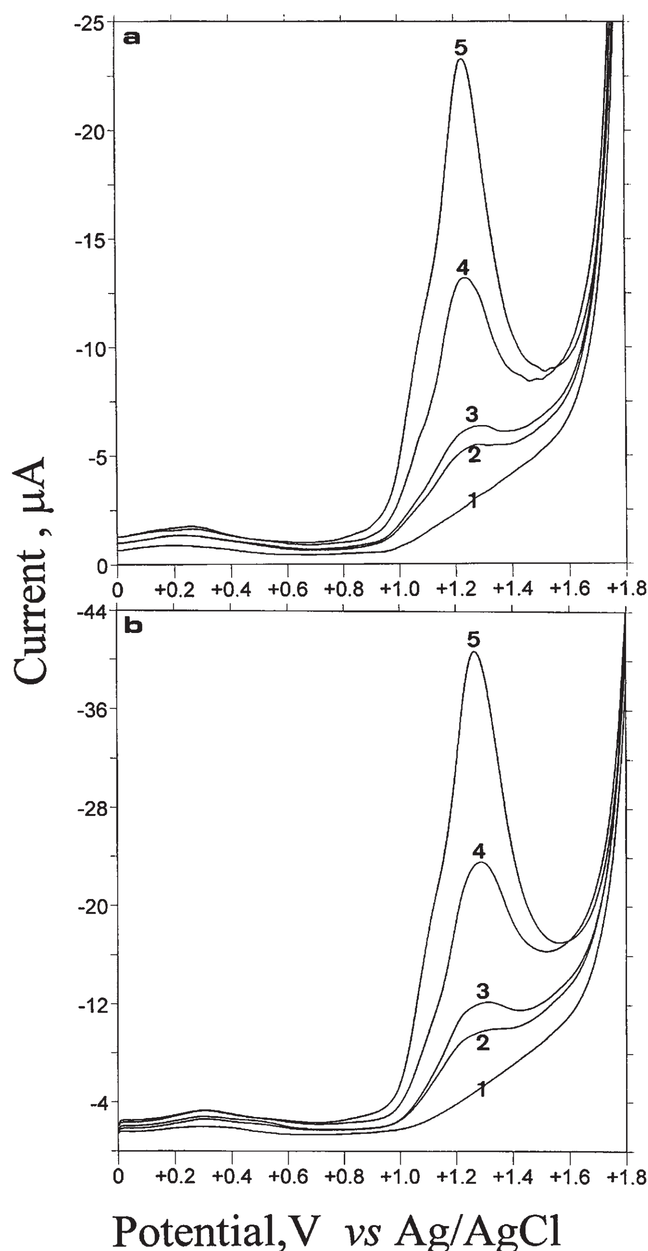

Fig. 6. AdSDPV (a) and AdSSWV(b) obtained for the determination in spiked serum. 1) Blank; 2) 6×10^{-7} M; 3) 1×10^{-6} M; 4) 4×10^{-6} M; 5) 8×10^{-6} M carvedilol sample in 0.2 M H_2SO_4 with constant amount methanol (20%).

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The serum proteins and endogenous substances were precipitated by the addition of acetonitrile. Then the samples centrifuged and the supernatant was taken and diluted with the supporting electrolyte (constant amount of methanol 20%) and directly analyzed.

Stability of serum samples kept in refrigerator (+4 °C) was tested by making five consecutive analysis of the sample over a period of approximately 6 h. There were no significant changes in the peak currents and potentials between the first and last measurements.

The proposed methods give reproducible results, is easy to perform, and is sensitive enough for the determination of carvedilol in human serum samples.

4. Conclusions

The voltammetric oxidation steps of carvedilol in different buffer solutions of pH 1.3 – 12.0 have been elucidated. The electrochemical oxidation of carvedilol molecule has two irreversible electrode process and both of them are pH dependent. The obtained results may possibly clarify and aid in understanding carvedilol molecule oxidation pathways.

Two adsorptive stripping voltammetric techniques have been developed for the determination of carvedilol in tablet dosage forms and biological samples. The both procedures and its applications represent a good alternative for the quality control, because the preparation of the sample is easy and the excipients and endogenous substances do not interfere with the determination and consequently, separations, evaporation or extraction procedures are not needed. These methods are rapid, requiring less than 7 min to run sample. There is no official method in any pharmacopoeias such as USP, BP or EP related to determination of pharmaceutical dosage forms of carvedilol. To prove the absence of the interference by experiments, recovery studies were carried out from the real tablet formulations. These recovery results reveal that the proposed methods had adequate precision, accuracy and consequently can be applied to the determination of carvedilol without any interference from tablet excipients.

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