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Adsorptive stripping voltammetric determination of antihypertensive agent: diltiazem☆

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Abstract

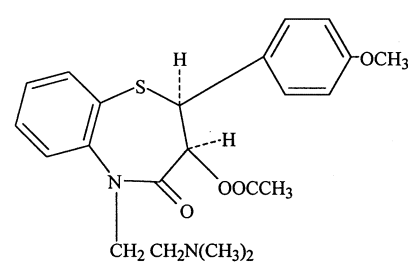
Adsorptive stripping voltammetry was used to determine the antihypertensive agent diltiazem in phosphate buffer (pH 7.0). The adsorptive cathodic peak was observed at -1.72 V vs. Ag/AgCl. The peak response was characterized with respect to pH, preconcentration time, possible interferences, accumulation potential and supporting electrolytes. The obtained results were analyzed and the statistical parameters were calculated. The proposed method was applied to determine the mentioned drug in pharmaceutical formulation (capsule) and urine. The detection limit is 1×10^{-8} M (4.5 ng ml^{-1}) using 180 s preconcentration time, whereas the lower limit of detection is 6×10^{-9} M (2.7 ng ml^{-1}).

Keywords: Diltiazem; Adsorptive stripping voltammetry; Urine; Diltiazem capsule

1. Introduction

Diltiazem[(2S-*cis*)-3-(Acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,-5-benzolthiazepin-4(5H)-one) [1] (I), is a calcium ion influx inhibitor. It has generally been indicated for the treatment of angina and hypertension [2]. In the solid state, diltiazem is reported to be highly stable. Storage of the drug substance under condition of room temperature and 79% relative humidity for 57 days did not cause any

(I)



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physical or chemical degradation. Diltiazem was stable after 3 weeks of storage under condition 40°C/75% relative humidity with exposure to UV light did not affect the chemical stability of diltiazem [3].

Diltiazem undergoes hydrolysis to desacetyl diltiazem in aqueous buffer solutions pH (1–7) diltiazem is most stable at pH 5. The extrapolated room temperater shelf-life was 42 days or 15.8 days at pH 5 or 2 respectively [4], as well as a stock solution of the drug was prepared daily during the measurements and protect from the light. Its therapeutic and toxic effects require very sensitive methods for determination of trace levels. Various analytical methods have been employed for this purpose e.g. high performance liquid chromatography in human cardiac tissue [5], in blood [6] and in human plasma [7–10], spectrophotometry [11] and voltammetry [12,13].

Cathodic adsorptive stripping voltammetry is a technique in which the analyte is pre-concentrated first by adsorption on to a working electrode surface followed by the voltammetric reduction of the electroactive species. Furthermore, stripping voltammetry is an important technique for trace determination of many inorganic and organic substances [14]. The cathodic stripping technique has been used successfully for the determination of subanogram level of several drugs [15–19].

The aim of this work is the quantitative determination of diltiazem using linear sweep stripping voltammetric technique (LSSV). This method is simple, rapid, sensitive and easy to apply in pharmaceutical formulation and in biological samples without previous treatment.

2. Experimental

2.1. Apparatus

Stripping and cyclic voltammograms were obtained using an EG&G Princeton Applied Research Corporation (PAR) model 264A polarographic analyzer/stripping voltammeter, coupled with a PAR Model 303A static mercury drop electrode (SMDE), (drop size, medium; area of the drop 0.014 cm^2). The polarographic cell (PAR Model K0060) was fitted with Ag/AgCl saturated KCl and used as reference electrode and a platinum wire as an auxiliary electrode. A PAR 305 stirrer was connected to the 303A SMDE. A

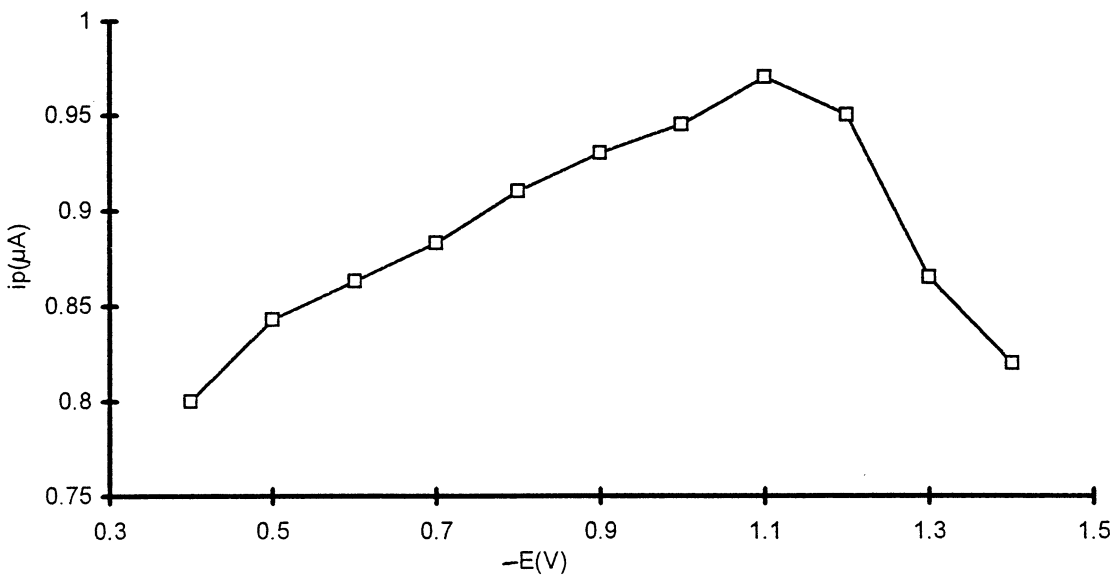

Fig. 1. Effect of accumulation potential on the peak current of 1×10^{-6} M diltiazem in the presence of 0.01 M phosphate buffer (pH = 7.0) at 45 s accumulation time and scan rate = 100 mV s^{-1}.

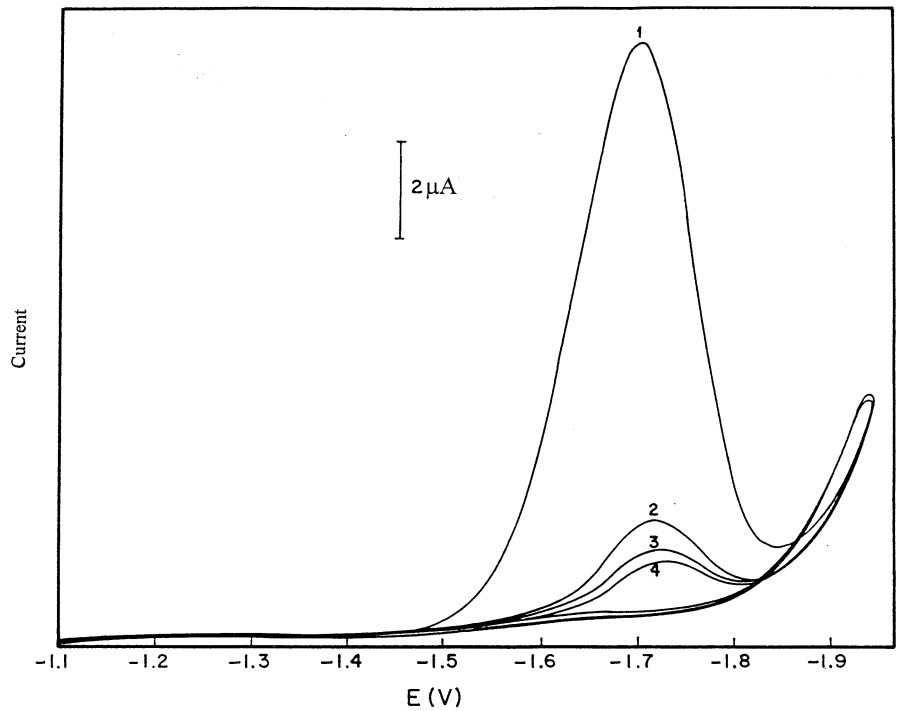

Fig. 2. Repetitive cyclic voltammograms for 1×10^{-6} M diltiazem in the presence 0.01 M phosphate buffer (pH = 7.0), accumulation potential -1.1 V, accumulation time 30 s and scan rate 100 mV s^{-1}: (a) first cycle; (b) second cycle; (c) third cycle; (d) fourth cycle.

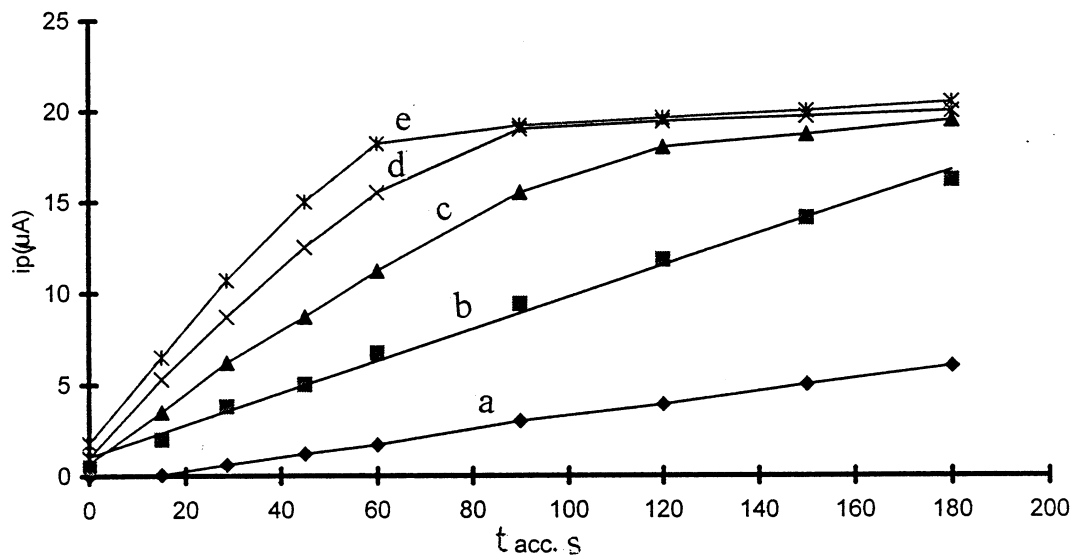

Fig. 3. Peak current vs. accumulation time in the presence of 0.01 M phosphate buffer (pH = 7.0), accumulation potential -1.1 V and scan rate 100 mV s^{-1} for: (a) 1×10^{-7} M; (b) 3×10^{-7} M; (c) 5×10^{-7} M; (d) 7×10^{-7} M; and (e) 9×10^{-7} M diltiazem.

Table 1
Characteristic of current–time curves established using different diltiazem concentration in 0.01 M phosphate buffer (pH = 7.0)

Diltiazem (M)	Equation[a]	Linearity Range (S)	RSD for slope	RSD for Intercept	Correlation coefficient
1×10^{-8}	$Y=0.0024$ $X-0.1131$	180–300	0.0065	0.0014	0.9980
3×10^{-8}	$Y=0.0088$ $X-0.4213$	60–300	0.017	0.0011	0.9942
5×10^{-8}	$Y=0.0250$ $X-0.0653$	30–300	0.0096	0.0045	0.9988
7×10^{-8}	$Y=0.0253$ $X+0.6682$	30–300	0.0037	0.0008	0.9927
9×10^{-8}	$Y=0.0332$ $X+0.3763$	30–300	0.0058	0.0015	0.9961
1×10^{-6}	$Y=0.3510$ $X+3.1609$	0–45	0.086	0.0073	0.9979

[a] Peak height (Y) in μA, concentration (X) in molar.

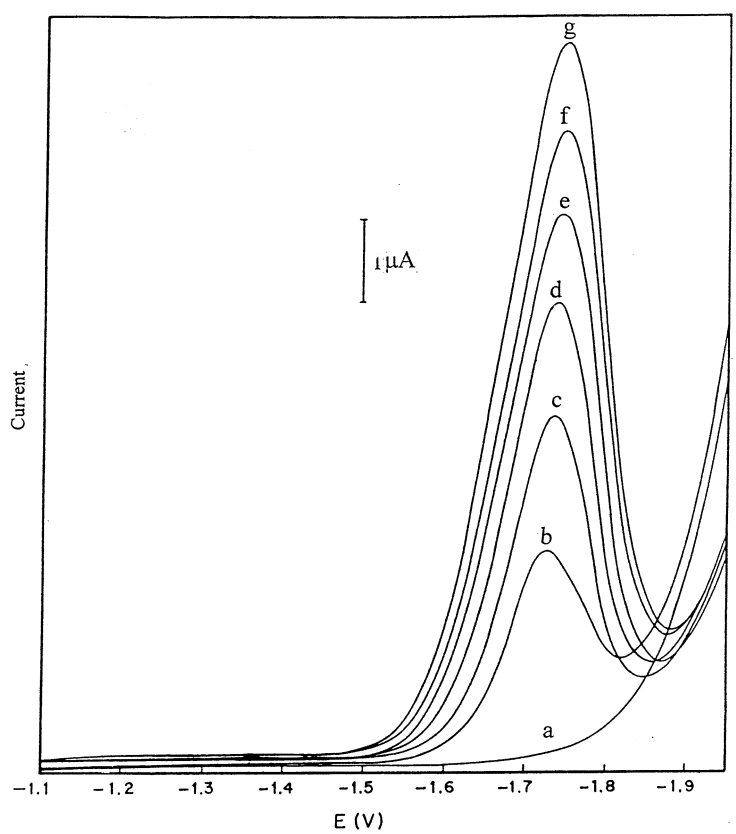

Fig. 4. LSS voltammograms for different concentration of diltiazem in the presence of 0.01 M phoshate buffer (pH = 7.0), accumulation potential −1.1 V, accumulation time 60 s and scan rate 100 mV s^{-1}; (a) residual; (b) 1×10^{-7} M; (c) 3×10^{-7} M; (d) 4×10^{-7} M; and (e) 5×10^{-7} M; (f) 6×10^{-7} M; and (g) 7×10^{-7} M diltiazem.

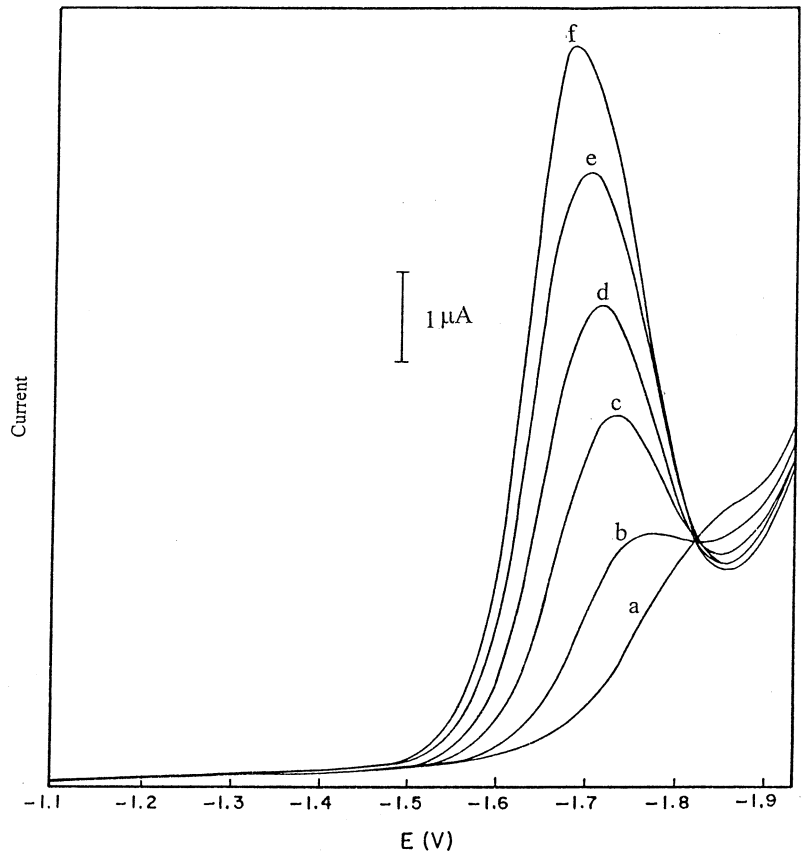

Fig. 5. LSS voltammograms for diluted urine (1:1000) spiked with different levels of diltiazem in the presence of 0.01 M phosphate buffer (pH = 7.0), accumulation potential −1.1 V, accumulation time 60 s and scan rate 100 mV s^{-1}: (a) Urine sample; (b) 5×10^{-8} M; (c) 15×10^{-8} M; (d) 15×10^{-8} M; (e) 20×10^{-8} M; and (f) 25×10^{-8} M diltiazem.

PAR model RE 0089 *X–Y* recorder was used to collect the experimental data.

2.2. Materials and reagents

A stock solution of 1×10^{-3} M of diltiazem hydrochloride (Sigma Chemical Co.) was prepared daily by dissolving the appropriate amounts of drug in double distilled water.

Solutions of 1×10^{-3} M metal ions i.e. copper (II), nickel (II), cadmium (II), lead (II) and zinc (II) nitrates (Merck) and the amino acids glycine, L-aspartic acid and L-ascorbic acid were prepared and used in the interference studies. Urine samples were taken from healthy donors.

2.3. Supporting electrolytes

Solutions of 0.1 M sodium nitrate, sodium chloride, sodium acetate–acetic acid buffer, borax, Britton–Robinson and phosphate buffers were prepared and used as the supporting electrolytes. A standard sodium hydroxide solution (0.1 M) was used to adjust the pH of the supporting electrolyte using an Orion 601A Precision Research Ionalyzer Digital pH meter. All solutions were prepared using double distilled water.

2.4. Procedure

A phosphate buffer (1 ml, 0.1 M), (pH = 7.0) as

supporting electrolyte was transferred and diluted to 10 ml in the voltammetric cell. The cell content was deaerated by passing pure nitrogen for 16 min. The accumulation potential of (−1.1 V) was selected to a fresh mercury drop, the scan rate used was 100 mV s^{-1}, drop size medium, drop time 1 s and pulse amplitude 25 mV. After the accumulation time and further 15 s equilibrium time the voltammogram was recorded (quiescent solution) and a potential was terminated at −1.95 V. The drug sample was added using an automatic pipettor (10–100 μl). The solution was stirred while purging nitrogen, then proceeded through the accumulation and stripping step as before. All results were obtained at room temperature (25 ± 1)°C with a nitrogen atmosphere maintained over the solution surface.

2.5. Analysis of capsule

The whole content of one capsule (Delaytiazem® SR) 120 mg diltiazem hydrochloride (Amoun–Cairo) was dissolved in water and the insoluble components after washing with water were separated by filtration. The filtrate and washings were collected quantitatively in 25 ml measuring flask. The linear sweep stripping voltammogram was recorded after 60 s pre-concentration time.

2.6. Analysis of urine

Ten microlitres of a urine sample taken from a healthy person was added to the cell containing (0.01 M phosphate buffer) pH = 7.0. The resulting voltammogram was recorded at constant deposition time then different concentration of the standard solution of diltiazem was introduced to the cell and the voltammogram was recorded after each addition.

3. Results and discussion

3.1. Effect of supporting electrolyte

The influence of different supporting electrolytes i.e. sodium acetate–acetic acid buffer, sodium nitrate, potassium chloride, sodium borate, borax, Britton–Robinsson and phosphate buffers was examined for 1×10^{-6} M diltiazem using linear sweep stripping voltammetry (LSSV). It was observed that a highest and sharp peak was obtained in the presence of phosphate buffer. The effect of the supporting electrolyte (phosphate buffer) concentrations (0.01, 0.04, 0.06 and 0.1) M were studied. The highest peak current was obtained at 0.01 M phosphate buffer. Therefore 0.01 M phosphate buffer is used in subsequent experiments. The effect of different pH's was studied over the range from 5.3 to 11.3. It was noticed that the peak potential was shifted to positive values by increasing the pH, a sharp cathodic peak was observed at pH = 7.0. This peak is due to the reduction of the oxo group.

According to Wang et al. [12], various basic electrolytes were evaluated as suitable media (not mentioned) and the best results were obtained in 0.05 M sodium hydroxide solution. However, they used the differential pulse waveform. In the present study the use of phosphate buffer brought about an improved signal over sodium hydroxide solution using LSSV. Furthermore, the linear scan (ramp) is faster ten times or more than differential pulse waveforms.

By using sodium hydroxide solutions no significant difference in the peak current was observed on changing the pre-concentration potential over the range −0.4 to −1.2 V; a potential of −0.9 V was used throughout [12]. However, the influence of accumulation potential from −0.4 to −1.4 V on the peak height for 1×10^{-6} M diltiazem was tested using LSSV technique in the presence of 0.01 M phosphate buffer (pH = 7.0), an enhanced adsorption peak at initial potential −1.1 V as shown in Fig. 1 The increase of the peak height is observed on going to negative direction till −1.1 V; after this potential, the peak height decreases. Therefore, −1.1 V was used as an accumulation potential in subsequent experiments.

Fig. 2 shows cyclic voltammograms for 1×10^{-6} M of diltiazem in a medium of 0.01 M phosphate buffer (pH = 7.0) which obtained after 30 s accumulation time and accumulation potential −1.1 V. A distinct cathodic peak is observed (at about −1.72 V) during the negative going scan. The

repetitive cyclic voltammograms shows that peak current decrease sharply in the second and third cycles indicating the rapid desorption of the analyte. No peaks was observed in the positive direction, indicating that the electrode process is irreversible.

3.2. Effect of scan rate

A concentration of 1×10^{-6} M diltiazem was studied and the voltammograms were recorded at different scan rates (v) ranging from 5 to 200 mV s^{-1}, after pre-concentration time 45 s and accumulation potential -1.1 V. A log i_p vs. log v plot is a linear over the range 5–200 mV s^{-1} with a slope of 0.846 which is in close proximity to a slope of 1.0 that is expected for ideal reaction of surface species [20]. A 22 mV negative shift in the peak potential was observed upon increasing the scan rate in the range given. This is further evidence for the adsorption of the drug onto the electrode surface [21]. The plot of E_p vs. log v is also linear (correlation coefficient 0.9980).

3.3. Effect of accumulation time

Fig. 3 shows the plots of peak current versus pre-concentration time for 1×10^{-7}, 3×10^{-7}, 5×10^{-7}, 7×10^{-7} and 9×10^{-7} M diltiazem in the presence of 0.01 M phosphate buffer (pH = 7.0) and initial potential -1.1 V where straight lines with slope 0.036 and 0.099 μA s^{-1} were obtained for 1×10^{-7} and 3×10^{-7} M diltiazem, respectively. However at higher concentrations, the straight lines break at 90, 60 and 45 s for 5×10^{-7}, 7×10^{-7} and 9×10^{-7} M diltiazem, respectively. The breaks at certain stirring accumulation times mean that surface coverage was attained. The slope of the straight lines are 0.175, 0.253 and 0.294 μA s^{-1} for 5×10^{-7}, 7×10^{-7} and 9×10^{-7} M diltiazem, respectively and the increase in the slope due to the increase in the concentration. The collected data are illustrated in Table 1.

To reveal the adsorption of the analyte without stirring, some experiments were carried out with 1×10^{-7} M diltiazem in quiescent solution. A linear dependence of the peak current vs. the square root of the pre-concentration time was observed. This behaviour is expected for mass transport controlled by adsorption [21].

3.4. Effect of concentration

A series of solution containing different concentrations of diltiazem ranged 1, 3, 5, 7 and 9×10^{-7} M were examined. Fig. 4 shows a typical voltammograms of different concentrations at accumulation time 60 s pre-concentration potential -1.1 V and scan rate 100 mV s^{-1}. A concentration of 4.5 ng ml^{-1} was detected in phosphate buffer (pH = 7.0) after 180 s pre-concentration time. The general definition given in the literature ($\gamma\beta + 3\sigma\beta$) was used for the estimation of the limit of detection (L.O.D.) [22–24]. The calculated L.O.D. of diltiazem in aqueous solutions is 6×10^{-9} M which is equal to 2.7 ng ml^{-1} of diltiazem.

Also LOQ (limit of qauntitation) was estimated using the following equation LOQ = 10 σ/S where σ was the standard deviation and S was the slope [25]. The results are given in Table 2.

The reproducibility of the results can be attributed to the reproducible area and self-cleaning control provided by the instrument used. Also, the repeatability of the data can be achieved by five successive measurements of 5×10^{-7} M diltiazem with 1.76% relative standard deviation.

3.5. Interferences

3.5.1. Effect of metal ions

Interferences by several metal ions were examined for 1×10^{-7} M diltiazem in the presence of 0.01 M phosphate buffer, pH = 7.0 and 60 s accumulation time. On the addition of 1×10^{-7}–1×10^{-6} M of Co(II), Ni(II), Cd(II), Pb(II), Cu(II) and Zn(II) ions, individually or in admixture, no change in the peak current of the drug was observed.

3.5.2. Effect of some amino acids

The presence of some amino acids i.e. glycine, L-ascorbic acid and L-aspartic acid were studied. The addition of 1×10^{-7}–1×10^{-5} M of these amino acids individually or in admixture in the

Table 2
Statistical parameters for different diltiazem concentration

Concentration linearity (M)	Accum. Time (s)	Slope ($\mu A/10^{-8}$ or 10^{-7} M)	Intercept (μA)	Standard deviation	Correlation coefficient	Confidence at 95% significant level	LOQ (PPb)
$(3–9)\times10^{-8}$	60	0.3167	−0.8997	0.7637	0.9987	0.6111	24.11
$(2–9)\times10^{-8}$	120	0.5250	−0.8833	1.3706	0.9989	1.0966	26.11
$(1–9)\times10^{-7}$	30	1.3857	−0.7357	3.6677	0.9996	2.7170	26.47
$(1–7)\times10^{-7}$	60	2.4086	−0.5621	5.2038	0.9999	3.8550	21.61

cell containing 0.01 M phosphate buffer (pH = 7.0) and accumulation time 30 s had no effect on the 1×10^{-7} M drug concentration.

3.6. Application to a pharmaceutical formulation

3.6.1. Capsule

Ten microlitres from the filtrate of the whole diltiazem capsule solution was introduced into the cell containing 0.01 M phosphate buffer (pH = 7.0). The LSS voltammogram was recorded after 60 s pre-concentration time. The content of the capsule in the cell was determined by the standard addition method. Five aliquots each 10 μl were added from 1×10^{-4} M standard drug solution. A plotting of peak height vs. diltiazem concentration, a straight line is obtained with the RSD for slope of 0.147, RSD for intercept of 0.087 and the correlation coefficient of 0.9985, from which the recovery of this drug is 90% ($n = 5$) of the amount claimed with standard deviation 2.99%.

3.7. Application to biological samples

3.7.1. Urine

The measurement of diltiazem in urine sample was demonstrated using (LSSV) technique. The sample was diluted (1:1000) with supporting electrolyte (0.01 M phosphate buffer) and increasing diltiazem concentration. At 5×10^{-8} M drug, a well defined peak was observed at -1.73 V corresponding to diltiazem alone. The peak current increases with increasing diltiazem concentration from 5×10^{-8} M up to 25×10^{-8} M. The voltammograms are shown in Fig. 5, a linear relation was observed with slope of 2.82 and correlation coefficient of 0.9996 from which 19.6 ng ml^{-1} in the original sample can be analysed using 60 s accumulation time. The recovery is 87.5%.

4. Conclusion

The linear sweep stripping voltammetric method (LSSV) for the quantitative determination of diltiazem was found to be simple and highly sensitive over the reported data [26,27] for the determination of diltiazem. It can be used successfully to stability-indicating assay the drug in dosage form as well as in urine at trace levels.

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