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Voltammetric determination of isradipine in dosage forms and spiked human plasma and urine

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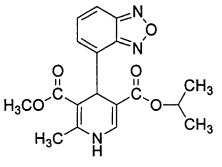
Abstract

The voltammetric behavior of isradipine was studied using direct current (DC_t), differential-pulse (DPP) and alternating current (AC_t) polarography. Isradipine exhibited well-defined cathodic waves over the whole pH range in Britton–Robinson buffer (BRb). At pH 5, the analytical pH, the diffusion-current constant (Id) was 8.27 ± 0.52. The current-concentration plots were rectilinear over the range 1–20 and 0.1–18 μg/ml using the DC_t and DPP modes, respectively, with minimum detectability of 0.01 μg/ml (2.7×10^{-8} M) using the latter technique. The current has been characterized as being diffusion-controlled, although adsorption phenomenon played a limited role in the electrode process. The proposed method was applied to commercial tablets and capsules. The percentage recoveries were in good agreement with those given by the manufacturer. The method was further extended to the in-vitro determination of the drug in spiked human urine and plasma, the percentage recoveries were ($n = 4$) 100.12 ± 1.42 and 103.88 ± 5.13, respectively. The number of electrons involved in the reduction process was accomplished and a proposal of the electrode reaction was presented.

Keywords: Isradipine; Voltammetry; Polarography; Dosage forms; Urine; Plasma

1. Introduction



Isradipine, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecarboxylic, methyl, 1-methylethyl ester, is a calcium-channel blocking agent with properties similar to that of nifedipine. It is used in the treatment of hypertension and angina pectoris [1].

Isradipine is the subject of a monograph in both, the British Pharmacopoeia, BP [2] and the US Pharmacopoeia, USP [3]. The BP [2] recommends spectrophotometric measurement for the raw material and liquid chromatography for the formulations. The USP [3] on the other hand, described

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liquid chromatography for both the drug and its formulations.

Reviewing the literature revealed that all the reported methods for the determination of isradipine in dosage forms and biological fluids rely on the use of chromatographic techniques such as Thin layer chromatography (TLC) [4,5], GC [6–9] and HPLC [4–17]. El-Jammal et al. [18] studied the cyclic voltammetry of calcium antagonists dihydropyridines (including isradipine) in aqueous medium. Although chromatographic methods offer a high degree of specificity, yet, sample clean-up and the instrumentation limitations preclude their use in routine clinical studies. The proposed method was developed as an alternative substitute to the chromatographic methods, and the results obtained were promising. The presence of the reducible furazan ring structure in the molecular formula of isradipine initiated the present study. Compared with the reported chromatographic methods, which require lengthy extraction and clean-up procedures, the proposed method does not require a prior extraction step. Just dilution of the urine with the buffer solution eliminates its potential interference. The plasma, however, needs precipitation of the proteins; dilution of the centrifugate with the buffer solution eliminated its interference.

2. Experimental

2.1. Reagents and materials

Isradipine was kindly provided by Novartis Pharma, Cork, Ireland, Batch #19525 D and was used as received. Tablets and capsules containing isradipine were obtained from commercial sources in the local market. Lomir SRO Capsules: Batch #B1016 (5 mg of isradipine per capsule). Lomir Tablets: Batch #T1001 (2.5 mg of isradipine per tablet). Both are products of Novartis Pharma, AG, Basle, Switzerland. Plasma was obtained from King Khalid University Hospital, Riyadh, KSA and kept frozen until use after gentle thawing. Urine was collected from healthy volunteers (males, around 35-years-old). Britton–Robinson buffers (BRb) 0.08 M covering the pH range 2.1–12 [19].

A stock solution of isradipine (1.0 mg/ml) was prepared in methanol, and was further diluted with the same solvent to give the appropriate working standard solutions. The solution was stable for 3 days if kept in the refrigerator.

2.2. Apparatus

The polarographic study and the differential pulse polarographic (DPP) measurements were carried out using the Polarecord E 506 Metrohm (Herisau, Switzerland). The drop-time of 1 s was electronically-controlled using a 505 Stand from the same company. The polarograms were recorded using a potential scan-rate of 10 mV/s. A three-electrode system, a Dropping Mercury Electrode (DME) as the working electrode, an Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode, was used. Phase selective alternating current (AC_t) polarography of 1.5×10^{-4} M solutions were recorded at different pH values, using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

2.3. Procedures

2.3.1. Recommended procedure

Transfer aliquots of isradipine working standard solution into a set of 25 ml volumetric flasks, so that, the final concentration is in the range cited in Table 2. Add sufficient methanol so that its content should be always 20% (v/v). Complete to volume with BRb of pH 5. Pass nitrogen gas for 5 min. Record the polarograms over the range of −0.4 to −1.2 V. Plot the produced current (in μA) in both the Direct current (DC_t) and DPP modes versus the final concentration of the drug (in μg/ml) to get the calibration graph. Alternatively, derive the corresponding regression equation.

2.3.2. Analysis of tablets and capsules

Empty the contents of 10 capsules or weigh and pulverize 20 tablets. Mix the powder. Weigh accurately a quantity of the powder equivalent to

25 mg of isradipine and transfer into a 50 ml volumetric flask. Add about 40 ml of methanol (in case of the tablets) or acetone (in case of the capsules) and sonicate for half an hour. Filter into a 50 ml volumetric flask. Wash the residue and flask with the same solvent and transfer the washing into the same volumetric flask then complete to the mark with the same solvent. Transfer aliquot volumes containing isradipine over the concentration range in Table 2 into a 25 ml volumetric flask. Adjust the volume to 5 ml with methanol. Complete to the mark with BRb of pH 5. Determine the nominal content of the tablets or capsules using either the calibration graph or the corresponding regression equation adopting both DC_t and DPP modes.

2.3.3. Construction of calibration curve for urine

Transfer 1 ml aliquots of urine into a series of 25 ml volumetric flasks. Add aliquots of isradipine working standard solution so that the final concentration is in the range of 0.08–0.64 μg/ml. Mix well and complete to the mark with BRb of pH 5. Transfer the whole contents of the flask into the polarographic cell, pass nitrogen gas for 5 min and measure the current adopting the DPP mode. Derive the corresponding regression equation. Plot the current (ip) versus the corresponding concentration to get the calibration graph.

For analysis of urine samples: Transfer 1 ml aliquot of the spiked urine into 25 ml volumetric flask and complete to the mark with BRb of pH 5 then mix well. Transfer the whole contents of the flask into the polarographic cell. Pass nitrogen gas for 5 min. Record the polarogram in the DPP mode. Measure the current and determine the concentration of isradipine in the sample adopting the corresponding regression equation.

2.3.4. Construction of calibration curve for plasma

Transfer 1 ml aliquots of plasma into a series of centrifugation tubes. Add aliquots of isradipine working standard solution so that the final concentration is in the range of 0.08–0.64 μg/ml. Mix well using a vortex mixer. Adjust the volume of methanol to 5 ml by addition of methanol centrifuge at 2500 rpm for 5 min. Transfer 2.5 ml of the clear centrifugate into 25 ml volumetric flask. Complete to the mark with BRb of pH 5. Transfer the whole contents of the flasks into the polarographic cell. Pass nitrogen gas for 5 min. Record the ip in the DPP mode and multiply the value by two. Plot the values of the ip versus the final concentration to get the calibration graph. Alternatively, derive the regression equation.

For analysis of plasma, transfer 1.0 ml of spiked plasma into a centrifugation tube. Add 4 ml of methanol, mix well and centrifuge at 2500 rpm for 5 min. Transfer 2.5 ml of the clear supernatant into 25 ml measuring flask. Proceed as described under Section 2.3.4. Determine the concentration of the sample adopting the corresponding regression equation.

3. Discussion

3.1. Influence of pH on the reduction waves

Fig. 1 shows typical DC_t and DPP polarograms of isradipine in BRb of pH 5. The effect of pH on the development of the reduction waves is shown in Fig. 2. Well-defined cathodic waves were obtained all over the pH range. The waves showed cathodic shift upon increasing the pH of the medium. The relation between the half-wave potentials ($E_{1/2}$) of the reduction wave and pH is given by the following regression equation:

$$E_{1/2}\ (\text{mV}) = -613.8 - 68.1\ \text{pH} \quad (R = 0.9935)$$

Logarithmic analysis of the reduction waves obtained in BRb of different pH values resulted in straight lines with different slopes. Assuming that the rate-determining step involves the transfer of two electrons (a free-radical, one electron-transfer is not likely to occur) the values of the slopes suggest that the reduction process is irreversible in nature. The αn_a values were calculated according to the treatment of Meites and Israel [20] and are listed in Table 1. The number of protons (Z_{H+}) involved in the rate-determining step of the reduction process was also calculated according to the following formula [21]:

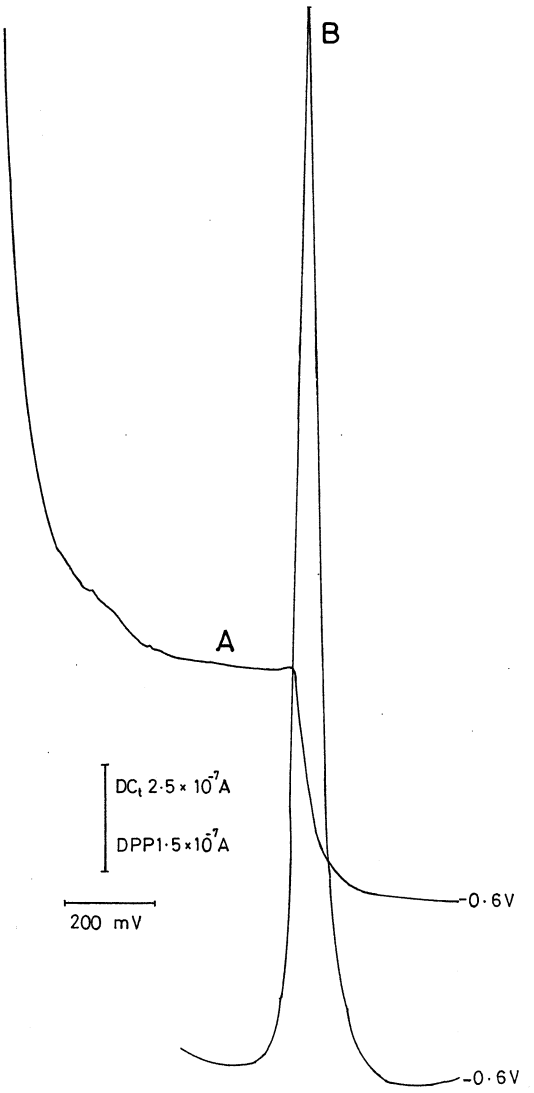


Fig. 1. Typical DC_t (A) and DPP (B) polarograms of isradipine (10 μg/ml) in BRb of pH 5. Potential scan rate 10 mV/s and drop time: 1 s.

$$\frac{\Delta E_{1/2}}{\Delta \mathrm{pH}} = -0.059 \frac{Z_{\mathrm{H}+}}{\alpha n_{\mathrm{a}}}$$

where α is the transfer coefficient and n_a is the number of electrons transferred in the rate-determining step.

The small figures obtained for Z_{H+} (Table 1) point out to the irreversibility of the reduction process.

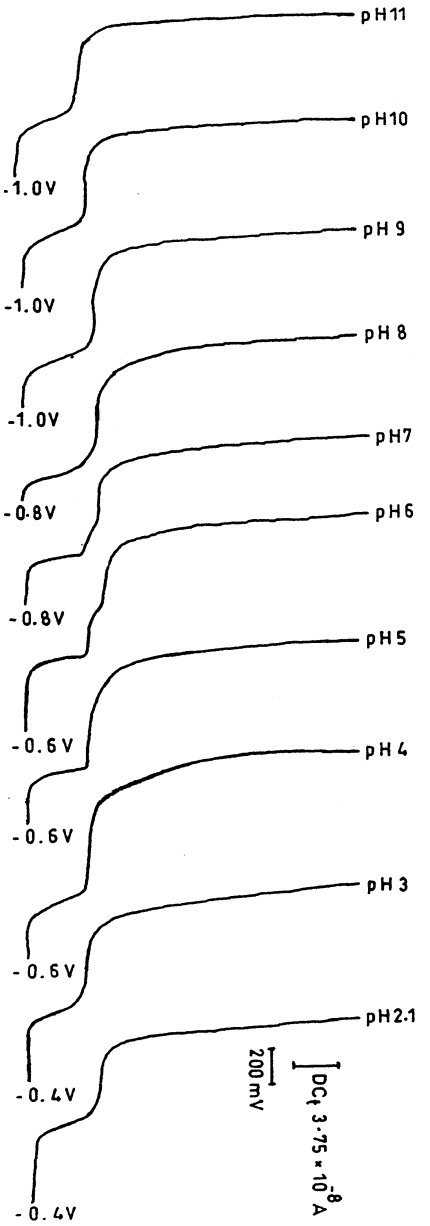


Fig. 2. Effect of pH on the development of the polarographic waves of isradipine (10 μg/ml) in BRb. Potential scan rate 10 mV/s and drop time: 1 s.

3.2. Study of wave characteristics

Increasing the mercury reservoir height (h) resulted in a corresponding increase in waveheight (W); a plot of W versus $\sqrt{h}$ gave a straight line. A

Table 1
Effect of pH on the development of cathodic waves of isradipine

pH	$-E_{1/2}$ (mV)	$\Delta E_{1/2}/\Delta$pH	id/C	$W_{1/2}$ (mV)	αn_a	Z_{H+}
2.1	763		9.32	110	0.85	
		70				0.8
3	826		11.13	150	0.71	
		71				0.9
4	897		9.32	110	0.76	
		29				0.5
5	926		9.32	70	1.01	
		84				1.8
6	1010		9.32	70	1.24	
		53				1.5
7	1063		9.18	80	1.66	
		113				1.4
8	1176		11.13	110	0.72	
		89				1.1
9	1265		10.58	160	0.72	
		48				0.7
10	1313		8.35	140	0.87	
		19				0.2
11	1332		8.35	130	0.77	

plot of log W versus log h gave a straight with a slope of 0.66. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in waveheight. These two characteristics point out to a diffusion-controlled process. The AC_t behavior of isradipine (1.5×10^{-4} M solution) was studied in BRb of different pH values (5, 7 and 10) using a phase selective angle of 90°. The summit potentials (E_s) at pH 5, 7 and 10 were shifted 126, 97, and 17 mV more negative than the corresponding $E_{1/2}$ values, respectively. Fig. 3 demonstrates that at pH 5 and 7, both the drug and its reduction product are adsorbed to the mercury surface. At pH 10, neither the drug nor its reduction product are adsorbed. However, addition of methanol (20%, v/v) to the electrolyzed solution decreases the effect of the adsorption process. It is concluded that, the current is mainly diffusion-controlled and partially affected by adsorption phenomenon. The diffusion coefficient (D) of isradipine was determined in BRb of pH 5 according to Ilkovic equation [22] and was found to be 1.1×10^{-5} cm^2/s. This small value may be attributed to the bulky nature of the molecule.

3.3. Number of electrons involved in the reduction process

The number of electrons transferred during the reduction process could be accomplished through comparing the waveheight of isradipine with that obtained from an equimolar solution of an earlier studied compound of the same chemical group (1,4-dihydropyridine) and of nearly identical value of diffusion coefficient, that is nifedipine [23]. In BRb of pH 5, both compounds gave one wave of the same height corresponding to the consumption of four electrons (nifedipine gave an additional ill-defined cathodically-shifted wave). The first wave of nifedipine corresponds to the consumption of four electrons as a result of the reduction of the nitro group to the nitroso group (two electrons) then to the hydroxyl amine group (two more electrons). It is evident from the experimental results for isradipine that, a slow electron-transfer reaction is involved in the reduction process. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step of the reduction wave, and the shift in

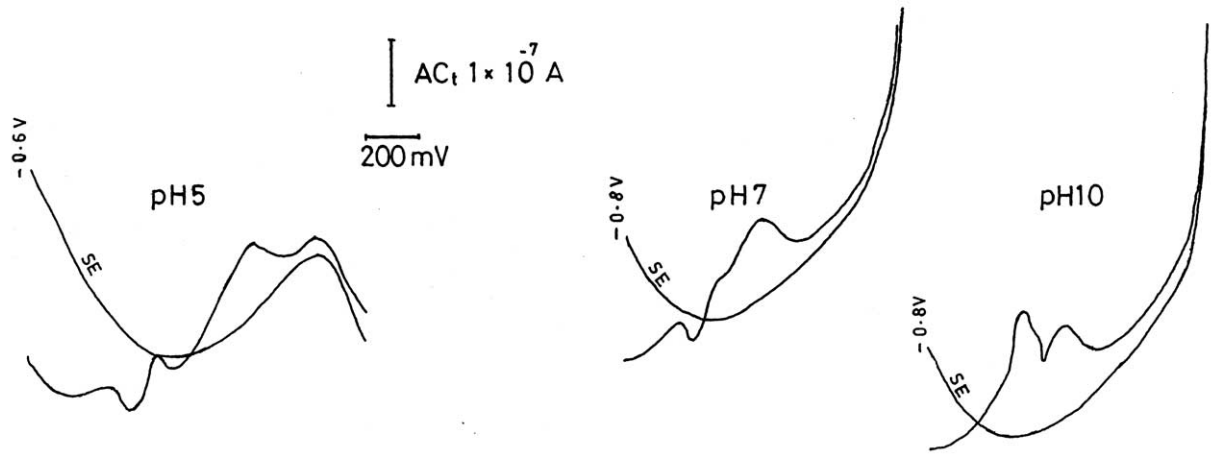
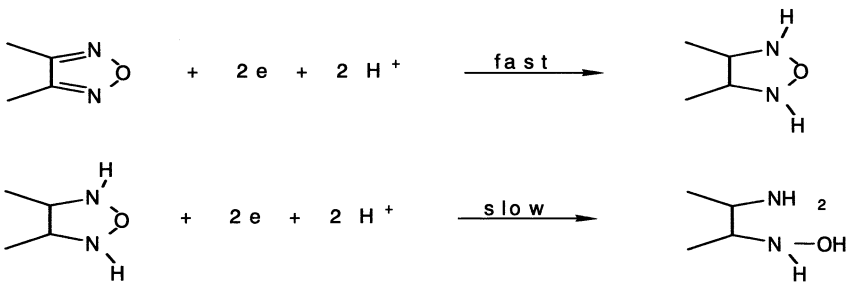


Fig. 3. AC_t behavior of isradipine (1.5×10^{-4} M) in BRb of different pH values. Superimposed alternating voltage: 15 mV; frequency 75 Hz, phase angle: 90°: SE, supporting electrolyte.

$E_{1/2}$ potentials with increasing pH indicates that two protons are consumed in this step. Based on these facts, and depending on the presence of the furazan ring structure, the following pathway for the electrode reaction may be postulated:



3.4. Validation

The method was tested for linearity, specificity, precision and reproducibility. By using the above polarographic procedure, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the current intensity on the concentration in both DC_t and DPP modes over the ranges given in Table 2. The table also shows the detection limits and the results of the statistical analysis of the experimental data such as the slopes, the intercepts, the correlation coefficients obtained by the linear least squares treatment of the results along with standard deviation (S.D.) of the slope (S_b) and intercept (S_a) on the ordinate and the S.D.of the residuals ($S_{y/x}$). The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficients and S.D. The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets or capsule mass. It was shown that, these compounds do not interfere with the proposed method (Table 3) co-administered drugs such as hydrochlorothiazide did not interfere.

To examine the ruggedness of the procedure, the within-day and between-day precisions were evaluated by analysis of 0.2 μg/ml sample of isradipine in urine six times a day and for 5 consecutive days. As shown in Table 4, the precision of the proposed method is comparatively fair. The robustness of the proposed method is demonstrated by the

Table 2
Analytical performance data of the proposed cathodic polarographic methods

Parameter	DC_t mode	DPP mode
Concentration range (μg/ml)	1.0–20.0	0.1–18
Regression equation	id = 0.0065 + 0.0269C	ip = 0.0044 + 0.0274C
Correlation coefficient	0.9963	0.9966
Id	8.27 ± 0.52	
Minimum detectability		0.01 μg/ml (2.7×10^{-7} M)
$S_{y/x}$	0.0166	1.33×10^{-3}
S_a	6.17×10^{-4}	7.47×10^{-4}
S_b	8.91×10^{-3}	5.65×10^{-5}
Applications	Dosage forms	Dosage forms and biological fluids

versatility of the experimental factors that affect the measured current.

3.5. Analytical applications

Polarograms of isradipine in BRb of pH 5 exhibit very well-defined cathodic waves. No polarographic maxima was developed, therefore, no maximum suppressor was needed. The current is mainly diffusion-controlled and proportional to the concentration over a convenient range. At that pH value, the DC_t wave was the steepest and the peak (in the DPP mode) had the least half-peak width ($W_{1/2}$) as shown in Table 1.

Solutions of isradipine in methanol were found to be stable for at least 1 week if kept away from light in the refrigerator. In BRb of pH 5 (the analytical pH) the solutions were found to be stable for at least 3 h. The relation between each of the limiting diffusion-current (id) in the DC_t mode and the peak current (ip) and the concentration of isradipine (μg/ml) is rectilinear over the ranges cited in Table 2. The analytical performance data of the proposed method (regression equations, correlation coefficients, minimum detectability, and Id) are compiled in the same table.

Statistical evaluation of the experimental data regarding S.D. of the residuals ($S_{x/y}$) S_b and S_a gave the values cited in Table 2. The small values point out to the high precision of the method [24]. The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients (close to one in both cases). To establish the reproducibility of the electrode response, six replicate concentrations were tested at isradipine concentrations of 4, 8, 12 and 16 μg/ml adopting the DC_t mode. Mean current values of: 0.1478 ± 0.0022; 0.2981 ± 0.0029; 0.4527 ± 0.0020 and 0.0822 ± 0.0088 μA, respectively, were obtained. The precision of these measurements is expressed by the

Table 4
Within-day and between-day precision of the proposed method for the determination of isradipine in urine

Percentage recovery	
Within-day	Between-days
97.91	105.65
95.47	105.65
104.18	99.29
99.65	92.22
100.00	105.65
102.09	
$\bar{X}$ = 99.88	$\bar{X}$ = 101.69
R.S.D. = 2.793	R.S.D. = 5.154

Table 3
Application of the proposed polarographic method to the analysis of commercial tablets and capsules

	Preparations	percentage recovery ± S.D.	
		DC_t	DPP
1	Lomir Tablets (2.5 mg isradipine per tablet)	100.35 ± 0.66	99.65 ± 1.21
2	Lomir SRO capsules (5 mg isradipine per capsule)	99.63 ± 1.85	100.77 ± 0.99

Both tablets and capsules are products of Novartis Pharma, AG, Basle, Switzerland.

relative standard deviations (R.S.D.) of 1.52; 0.965, 0.445 and 0.815, respectively. These small values indicate a highly precise electrode response.

Both DC_t and DPP modes were successfully applied to the determination of isradipine in commercial tablets and capsules. The percentage recoveries based on eight separate determinations are abridged in Table 3. The results are in good

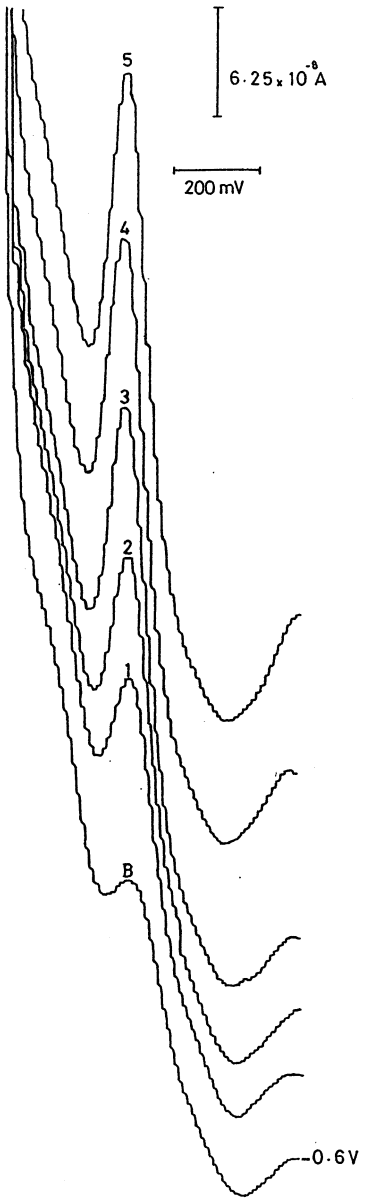


Fig. 4. DPP polarograms of isradipine in spiked human urine in BRb of pH 5. B, Blank. 1, 0.08; 2, 0.16; 3, 0.32; 4, 0.48; 5, 0.64 μg/ml.

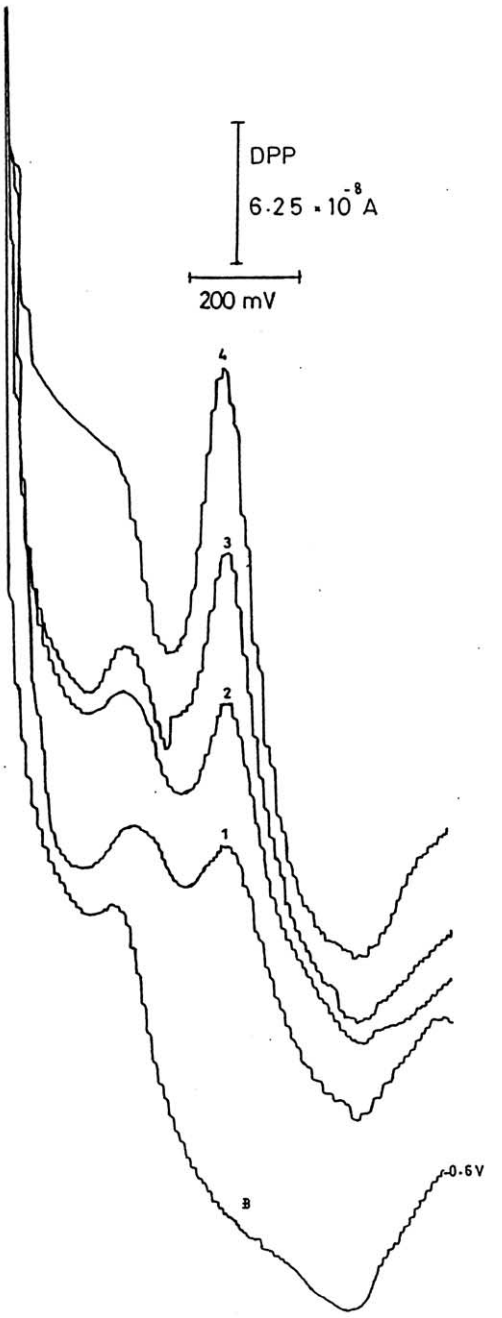


Fig. 5. DPP polarograms of isradipine in spiked human plasma in BRb of pH 5. B: Blank. 1, 0.08; 2, 0.16; 3, 0.32; 4, 0.64 μg/ml.

agreement with the label claim. Both DC_t and DPP modes proved to be equally useful; however, the DPP mode is more convenient.

The high sensitivity of the method allowed the detection and determination of isradipine in spiked human urine and plasma. Isradipine is orally administered in a dose of 5 mg daily. The anticipated concentrations in urine and plasma will be around 0.12 μg/ml, which is within the working range of the DPP mode. Linear regression analysis of the concentration of spiked isradipine in urine and plasma versus current gave the following equations:

$$ip = 0.003 + 0.049C \quad R = 0.9991$$

for urine and

$$ip = 0.0037 + 0.037C \quad R = 0.9921$$

for plasma

where ip is the current in μA in the DPP mode ... and C is the concentration of the drug (μg/ml). Figs. 4 and 5 show the DPP polarograms of spiked human urine and plasma, respectively. The proposed method was applied to the in vitro determination of isradipine in spiked human urine and plasma. The results are abridged in Table 5. The results are satisfactorily accurate and precise. The major advantage of the proposed method over the reported chromatographic methods as applied to urine and plasma is that, it does not require a prior extraction step, thus, it is more simple and time saving. Moreover, no sophisticated instrumentation is required.

Table 5
Application of the proposed method to the determination of isradipine in spiked human urine and plasma

Material	Added (μg/ml)	Found (μg/ml)	Percentage recovery
1. Urine	0.08	0.0811	101.38
	0.16	0.1602	100.13
	0.24	0.2428	101.17
	0.32	0.3129	97.79
$\bar{X}$			100.12
S.D.			1.42
2. Plasma	0.32	0.3382	105.69
	0.40	0.4457	111.43
	0.48	0.4780	99.58
	0.56	0.5532	98.79
$\bar{X}$			103.88
S.D.			5.13

4. Conclusion

A simple sensitive and reliable method has been developed for the determination of isradipine in dosage forms and spiked biological fluids. As applied to urine and plasma, the method has the advantage that no prior extraction step or clean-up procedure is required. The detection limit (2.7×10^{-8} M) is comparable to that reported by chromatographic methods. As applied to tablets or capsules, the method is very simple and time saving.

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