ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 573–582

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of nifedipine in human plasma by square wave adsorptive stripping voltammetry

Nuran Özaltin *, Ceren Yardimci, Incilay Süslü

Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100, Sıhhıye, Ankara, Turkey

Received 23 February 2002; received in revised form 8 May 2002; accepted 15 May 2002

Abstract

A simple, sensitive and selective square-wave adsorptive stripping voltammetric method has been developed and validated for the determination of nifedipine (NIF) in plasma. The assay was performed after single extraction of NIF from alkalinised plasma into organic phase. The adsorption behaviour of NIF on a hanging mercury drop electrode (HMDE) was explored by square-wave and cyclic voltammetry. The drug was accumulated at HMDE and a well-defined peak was obtained at -730 mV versus Ag/AgCl in borate buffer of pH 9.0 including 0.01 M KCl. The linear concentration range was 2.89×10^{-9} M–3.61×10^{-7} M (1.00–125.01 ng ml^{-1}) when using 30 s accumulation time at -300 mV. Limit of detection and limit of quantification were 1.21×10^{-9} M (0.42 ng ml^{-1}) and 2.89×10^{-9} M (1.00 ng ml^{-1}) respectively. The intra-day relative standard deviation (RSD) ranged from 1.93 to 4.12% at three concentrations and the inter-day RSDs varied from 2.53 to 6.68%. The method was applied, to the plasma of pregnant women suffering from pregnancy induced hypertension, for the determination of NIF. The percentage recoveries varied from 96.26 to 99.49%. It has been shown that NIF could be determined in the presence of its main metabolite (dehydronifedipine) by the developed method.

Keywords: Nifedipine; Square-wave adsorptive stripping voltammetry; Plasma; Extraction method

1. Introduction

Nifedipine (NIF), 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester (Fig. 1a) is a calcium channel blocker, used in the treatment of angina pectoris, arterial hypertension and Reynold's phenomenon [1–3]. Following the usual therapeutic dose of NIF (30 mg) the plasma drug concentrations range from 10 to 100 ng ml^{-1} [4]. NIF is photosensitive and thermally unstable. On exposure to visible light the nitroso-pyridine derivative is formed in solution, while under UV light the nitro-pyridine derivative is formed [5]. In human body NIF is predominantly metabolised by oxidative mechanism to its nitro-pyridine derivative (dehydronifedipine) (Fig. 1b). For clinical and pharmacokinetic studies, sensitive assay methods are required. Various methods for the determination of NIF in biological fluids have been reported, involving mainly gas chromatographic

* Corresponding author. Fax: +90-312-311-4777
E-mail address: nozaltin@hacettepe.edu.tr (N. Özaltin).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00311-4

(GC) and high performance liquid chromatographic (HPLC) methods. In GC methods, electron-capture [6,7], N–P ionisation detection [8], and mass spectrometer [9,10] have been employed. In HPLC methods either UV [4,5,11,12] or electrochemical detector [13,14] have been used. The early fluorometric [15] and thin-layer chromatographic [16] methods can not be used for quantitative analysis in biological fluids because of lack of sensitivity and specificity.

The presence of nitro phenyl group in NIF, offered the possibility of development of an analytical procedure based on electroreduction [17]. Squella et al. described the reduction of NIF and its photodecomposition products by differential pulse polarography [18,19]. There is no voltammetric method in literature for determination of NIF in plasma.

The aim of this study was to develop a sensitive and specific square-wave adsorptive stripping voltammetric (SWASV) method for the determination of NIF in ng ml^{-1} level and to apply the method both to plasma including spiked NIF and to plasma of patients orally administered NIF.

2. Experimental

2.1. Apparatus

Square-wave adsorptive stripping voltammetric studies were carried out using BAS 100B/W electrochemical workstation coupled with a BAS controlled growth mercury electrode (CGME). An Ag/AgCl reference electrode and a platinum wire counter electrode were used. A magnetic stirrer and stirring bar provided the convective transport during pre-concentration. The peak heights were automatically or manually measured using the 'tangent fit' capability of the instrument. All voltammograms were recorded using a HP Hewlett Packard deskjet 640 printer.

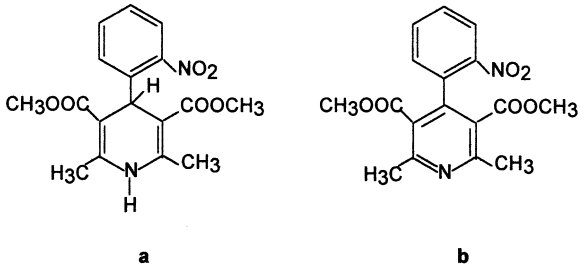

Fig. 1. Chemical structures of (a) nifedipine and (b) dehydronifedipine.

Cyclic voltammograms were obtained with the same instrument (scan rate 100 mV s^{-1}). For photodegradation studies a UV lamp 366 nm was used. All studies were performed by using dark coloured electrochemical cell.

2.2. Reagents

NIF was kindly supplied from FAKO AS and was used without further purification. The stock solution of NIF (1000 μg ml^{-1}) was prepared in methanol. Standard solutions were prepared daily by dilution of the stock solution with methanol:water (1:2). A total of 0.2 M borate buffer pH 9.0 including 0.01 M KCl was used as supporting electrolyte. Because of the photosensitivity of NIF all the solutions were adequately protected from light. The supporting electrolyte and all other chemicals used were extra pure quality and dissolved in Milli-Q water. The pH was adjusted by means of 0.1 N NaOH.

2.3. Procedure

2.3.1. Analysis of nifedipine

Five millilitres of the supporting electrolyte was added to the electrochemical cell and de-aerated with oxygen-free nitrogen for 12 min. The preconcentration potential was then applied to the new drop for 30 s while stirring at 400 rpm. After an equilibrium time of 10 s, a negative-going scan was performed using the following parameters: preconcentration and initial potential: − 300 mV; frequency: 120 Hz; pulse amplitude: 25 mV; scan increment: 3 mV.

After the voltammogram of supporting electrolyte had been recorded, aliquots of the NIF standard were introduced by micropipette and adsorptive stripping cycle was repeated using a new mercury drop. All data were obtained at room temperature.

2.3.2. Analysis of nifedipine in human plasma

The calibration curves were prepared for each assay by spiking 10, 20, 40, 60, 80, 100 ng NIF into tubes containing 1 ml of blank human plasma. A total of 100 μl of 20% sodium metabisulphite and 200 μl of 1 M sodium hydroxide were added into this mixture. The mixture was shaken on a vortex mixer for 30 s. A total of 5 ml of *n*-hexane-dichloromethane (7:3, v/v) were added for extraction and the mixture was shaken on a vortex mixer for 1 min. After centrifugation for 10 min at 3000 rpm, the organic layer was transferred into another tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.5 ml methanol:water (1:2). An appropriate aliquot of 50–100 μl was added into the electrochemical cell including supporting electrolyte. Analysis was performed as mentioned in Section 2.3.1.

2.3.3. Precision, accuracy and recovery studies

Intra-day accuracy and precision were evaluated by analysis of plasma samples containing NIF at levels of 20, 60 and 100 ng ml^{-1} on the same day. These levels were chosen to demonstrate accuracy and precision of the method at low, moderate and high concentrations of the calibration curve. To assess the inter-day accuracy and precision, analysis of plasma samples containing NIF at the same levels was performed on seven different days. Accuracy and precision were expressed as bias and relative standard deviation (RSD), respectively.

The extraction recovery from the liquid–liquid extraction was estimated at three levels of 20, 60 and 100 ng ml^{-1} NIF. These levels were spiked into tubes containing 1 ml of water and 1 ml of blank human plasma. Each solution was analysed as mentioned in Section 2.3.2.

3. Results and discussion

Recently square-wave adsorptive stripping voltammetry has been utilised successfully for the quantitation of nanomolar concentrations, because its wave form allows very rapid and sensitive determinations. Electrochemical behaviour of NIF was investigated by using polarographic techniques [17–19]. NIF exhibits only one polarographic wave through the whole pH range (pH 2–12). This wave is due to the four electron reduction of nitro group to a hydroxylamine derivative and is electrochemically irreversible [18].

The necessary conditions for the adsorptive accumulation of NIF were studied at the mercury electrode surface using cyclic voltammetry (CV). The CV behaviour of NIF shows a single irreversible cathodic peak over the whole pH range (2.0–12.0). No oxidation peak is observed in the anodic direction, which is indicative of the irreversibility of the NIF reduction.

A study of the influence of the scan rate (v) on the peak current (i_p) and peak potential (E_p) within the range 10–500 mV s^{-1} was carried out. When an accumulation time 30 s was applied, plotting $\log i_p$ versus $\log v$ gave a straight line with a slope of 0.91 ($r = 0.9988$), close to 1, which is the expected slope for an ideal reaction of surface species [20]. So in this case, the process appears to have an important adsorptive component. Furthermore, the effect of the adsorption time [t_s (s)] on the morphology of the CV peak reflects the strong adsorption and the accumulation of NIF molecules at the mercury electrode surface (Fig. 2). The peak potential shifts to a more negative value on increasing the scan rate which confirms the irreversibility of the reduction process.

pH, the nature and the concentration of the supporting electrolyte, all influence the voltammetric response. Among the four buffers tested (acetate, borate, phosphate, BR) borate buffer pH 9.0 resulted in the highest signal. The presence of KCl increases the ability of the analyte to adsorb on to the electrode surface [21]. Therefore the optimal conditions for studying the square-wave adsorptive stripping voltammetry of NIF is a mixture of 0.02 M borate buffer and 0.01 M KCl at pH 9.0.

In order to obtain the best adsorptive square-wave voltammetric peak of NIF, an optimisation of the frequency, pulse amplitude and the scan rate were attempted at pH 9.0 and 30 s accumulation time. The optimum conditions chosen for

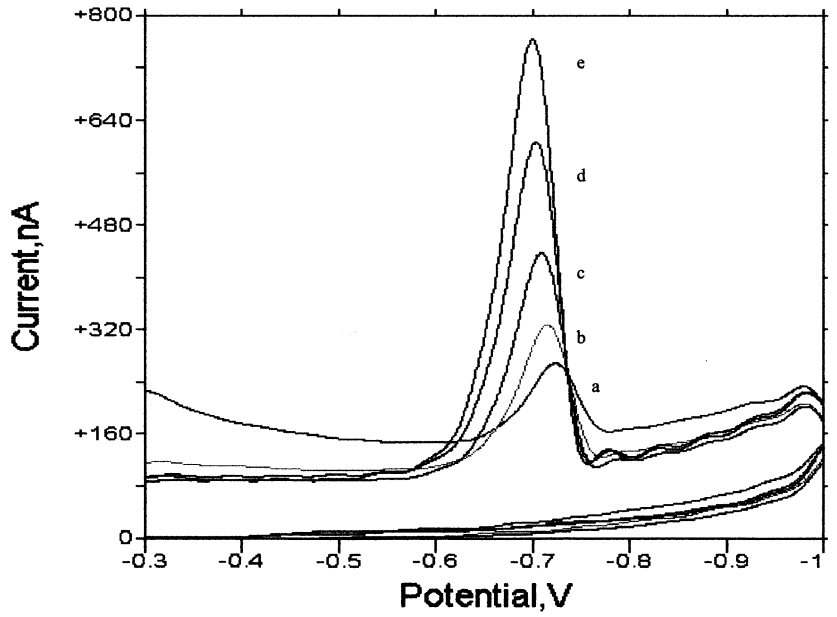

Fig. 2. Cyclic voltammograms of 1.3×10^{-6} M (450.19 ng ml^{-1}) nifedipine solution obtained in borate buffer pH 9.0 using a scan rate 100 mV s^{-1} and accumulation potential of -300 mV at different accumulation time. (a) 0 s, (b) 10 s, (c) 30 s, (d) 60 s and (e) 90 s.

NIF determination were: accumulation potential: -300 mV; frequency: 120 Hz; pulse amplitude: 25 mV.

The effect of accumulation time (preconcentration time) t_s (s), on the peak height was studied at two concentration levels of NIF. The i_p–t_s relation shows a curve with a saturation point at an adsorption time of 60 s (Fig. 3).

The effect of accumulation potential was also evaluated. A total of -300 mV was chosen as the accumulation potential which has given the best defined peak and the highest peak current.

The calibration curves for NIF were established using the optimum conditions chosen, following different accumulation times. It was observed that the peak current increases with increase of accumulation time. Optimum accumulation time was chosen 30 s which give results of adequate sensitivity and more extensive linearity range than the others (Fig. 4). The regression equation of the calibration curve was $y = 25.541x - 0.033$ where y is the peak current in nA and x is the concentration of NIF in ng ml^{-1}. Standard errors of slope and intercept were 0.35 and 0.0012, respectively ($n = 9$). The correlation coefficient was 0.9999. The linearity range was 1.00–125.01 ng ml^{-1}. Fig. 5 shows the SWAS voltammograms of NIF at different concentrations.

Limit of detection (LOD) (0.42 ng ml^{-1}) was calculated from the calibration curves as 3 s_1/m, where s_1 is the standard deviation (S.D.) of the intercept and m is the slope.

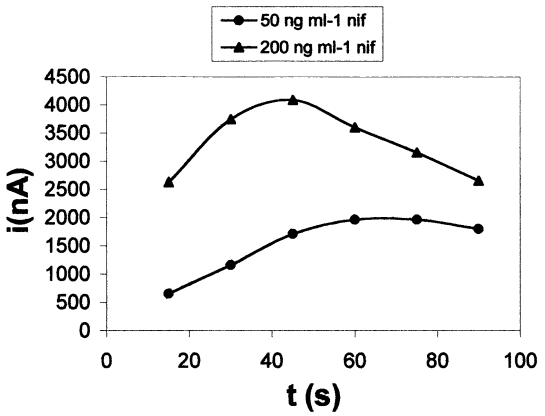

Fig. 3. The influence of accumulation time on the peak current of nifedipine.

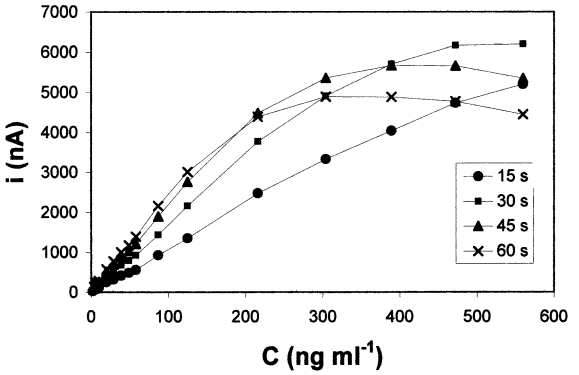

Fig. 4. The calibration curves of nifedipine at different accumulation times.

Limit of quantification (LOQ) (1.00 ng ml^{-1}) was determined by analysing seven different standard solutions containing the lowest concentration on the calibration curve. The RSD of 1 ng ml^{-1} was 11.1%.

Reproducibility was evaluated by performing a seven measurements on a 10 ng ml^{-1} solution after 30 s accumulation time. A mean value of 226.98 nA was found with a range of 223.75–229.40 nA and a RSD of 1.13%.

Precision of the method was investigated by intra- and inter-day determination of NIF at three different concentrations ($n = 7$) in the linear range (Table 1).

Accuracy of the method was expressed as bias % for within and between day were less than 5% at low and high concentrations (Table 1).

3.1. Application to human plasma

NIF is highly bound to plasma proteins. This necessitates the development of the extraction procedure to effectively recover the drug from plasma to exclude the matrix interferences. In order to find the best solvent for liquid–liquid extraction of NIF from human plasma, a number of solvents including acetonitrile, ethyl-acetate, toluene, *n*-pentane-dichloromethane (7:3, v/v) and *n*-hexane-dichloromethane (7:3, v/v) were tested.

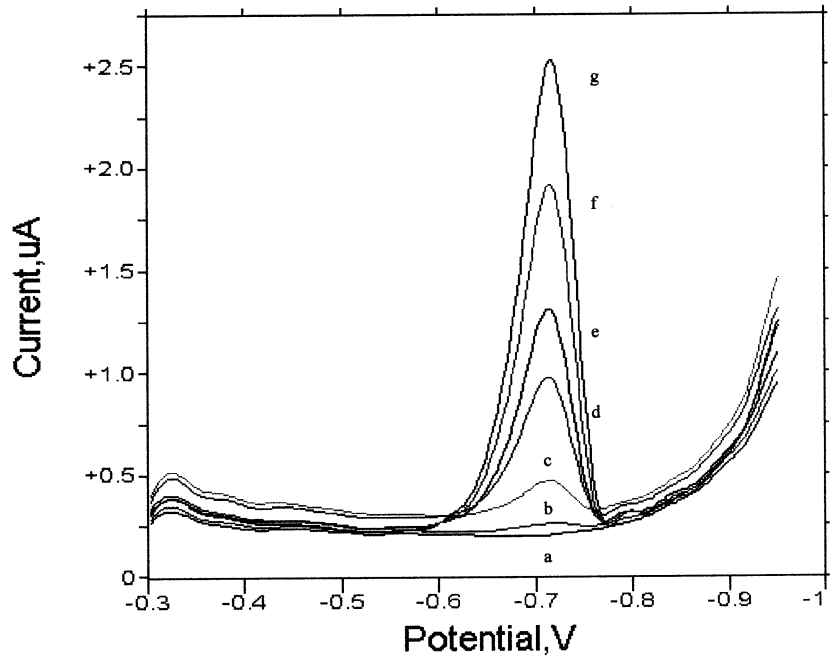

Fig. 5. The effect of nifedipine concentrations on the peak current obtained in borate buffer pH 9.0 at frequency 120 Hz, pulse amplitude 25 mV, scan increment 3 mV and deposition time 30 s. (a) Supporting electrolyte, (b) 2 ng ml^{-1}, (c) 8 ng ml^{-1}, (d) 30 ng ml^{-1}, (e) 50 ng ml^{-1}, (f) 90 ng ml^{-1}, (g) 125 ng ml^{-1}.

Table 1
Analytical precision and accuracy of nifedipine determination by SWASV ($n = 7$)

Concentration (ng ml^{-1})	Intra-day				Inter-day			
	Measured concentration (ng ml^{-1})	S.D.	Accuracy bias %	Precision RSD%	Measured concentration (ng ml^{-1})	S.D.	Accuracy bias %	Precision RSD %
8.0	7.76	0.15	−3	1.93	7.62	0.18	−4.75	2.53
50.0	50.73	1.67	1.46	3.29	49.97	3.27	−0.06	6.54
100.0	99.68	4.11	−0.32	4.12	99.67	6.66	−0.33	6.68

Bias% = [(Measured concentration − added concentration)/added concentration] × 100; RSD% = relative standard deviation; S.D. = standard deviation.

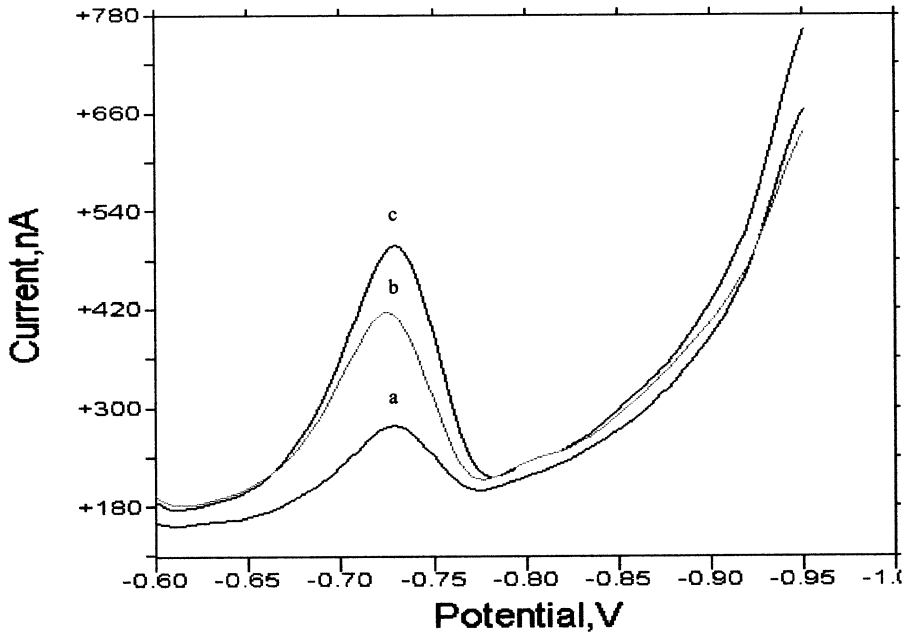

Fig. 6. Square-wave adsorptive stripping voltammograms of spiked nifedipine in plasma. (a) 20 ng ml^{-1}, (b) 60 ng ml^{-1}, (c) 100 ng ml^{-1}.

Table 2
Precision, accuracy and recovery of nifedipine in spiked human plasma ($n = 7$)

Added concentration (ng ml^{-1})	Recovery % ± S.E.	Intra-day		Inter-day	
		Bias %	RSD %	Bias %	RSD %
20	96.26 ± 4.76	0.30	6.26	4.15	−10.51
60	99.49 ± 4.89	1.70	2.12	0.85	5.12
100	98.08 ± 1.23	−1.84	1.76	0.96	4.50

S.E. = standard error.

Best results were obtained using *n*-hexane-dichloromethane (7:3, v/v) as the extraction solvent.

The optimum extraction procedure was developed as mentioned in Section 2.3.1. Sodium metabisulphite was used for the stability of NIF in plasma and sodium hydroxide was used to improve the degree of extraction of NIF. Under alkaline medium, NIF would be in the unionised form thereby dispersing better into organic layer than into the aqueous layer [4]. Fig. 6 shows the square-wave adsorptive stripping voltammograms of NIF, extracted from 1.0 ml plasma samples.

3.1.1. Linearity

The linear regression analysis of NIF was made by plotting the peak current versus spiked concentration in the range of 10–150.0 ng ml^{-1}. The following equation was obtained: $y = 2.433x - 0.04$ $r = 0.9988$.

3.1.2. Precision

The intra-day precision was evaluated by seven replicate analysis of plasma samples containing NIF at three concentrations (low, medium and high). The intra-day precision showed a RSD of 1.76–6.26% (Table 2). The inter-day precision was

similarly evaluated on seven different days. The inter-day relative standard deviations varied from 4.50 to (− 10.51)% (Table 2).

3.1.3. Accuracy

The accuracy of the method was determined as bias %. As shown in Table 2 the accuracies ranged from 0.30 and 4.15%.

3.1.4. Extraction recovery

Percentage recovery from the liquid–liquid extraction was determined by dividing the peak current of NIF extracted from spiked plasma to the peak current of NIF extracted from spiked water at the same concentration and multiplying by 100. Recoveries were determined at 20, 60 and 100 ng ml^{-1} spiked NIF concentrations (Table 2).

3.1.5. Selectivity

Selectivity of the method was investigated by comparing the voltammograms of NIF and its decomposition product dehydronifedipine (which is the main metabolite of NIF). In order to obtain the decomposition product, NIF solution was exposed to UV radiation (366 nm) for 14 h [22]. In the voltammogram of this solution the reduction peak of nitro group shifted to more positive potential comparing with NIF (Fig. 7). The voltammogram of the plasma of pregnant women, administered NIF and suffered from pregnancy induced hypertension (PIH), shows both NIF and dehydronifedipine peaks (Fig. 8). Peak potentials of NIF and dehydronifedipine were shifted more positive values in NIF administered human plasma. As shown Fig. 8, addition of dehydronifedipine increased the peak current at − 550 mV. This was proved to be the peak at − 550 mV was due to the metabolite of NIF. So it has been concluded that NIF could be determined in the presence of its metabolite by the proposed method.

3.2. Clinical application

The measured amount of NIF from the plasma of pregnant women administered NIF and suffered from PIH was tabulated in Table 3. The determination of NIF in samples were made by

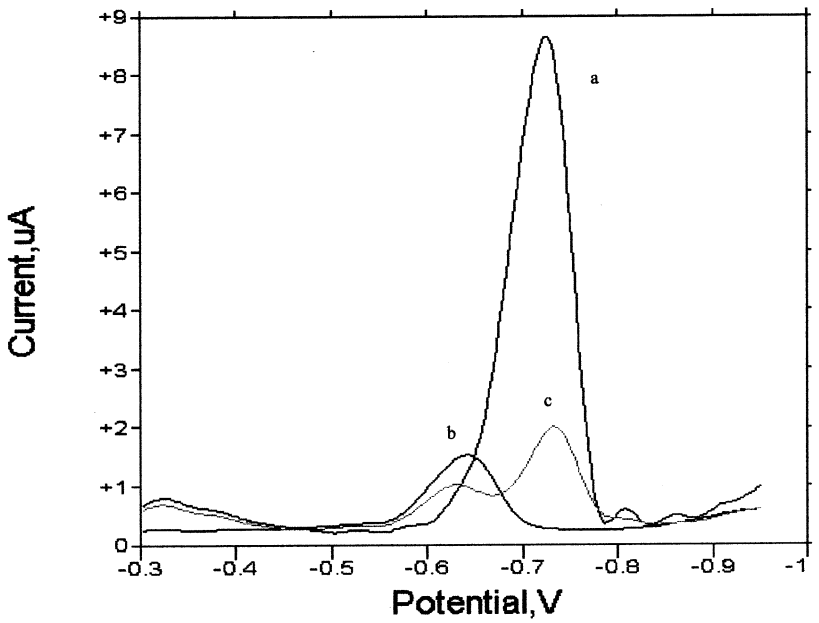

Fig. 7. The voltammograms of (a) nifedipine, (b) dehydronifedipine (decomposition product), (c) the mixture of nifedipine and dehydronifedipine.

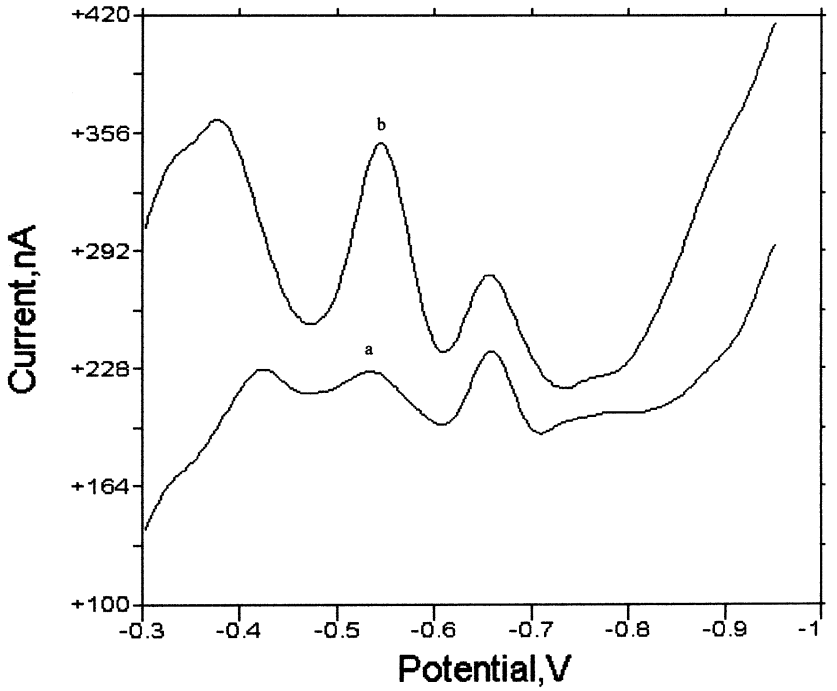

Fig. 8. The voltammograms of (a) the plasma of pregnant women administered 4×10 mg nifedipine, (b) addition of 100 μl 1000 ng ml^{-1} dehydronifedipine solution to (a).

plotting the calibration curves daily. The presence of main metabolite of NIF did not interfere with the method (Fig. 8).

4. Conclusion

The SWASV method developed in this study is sensitive, selective, accurate, precise, cheap and easy to use for the determination of NIF in plasma. Because of the high sensitivity of the method for the determination of NIF in plasma, it might be preferred to chromatographic methods comparing the global cost of the methods.

Table 3
Analysis of plasma of pregnant women suffering from PIH (administered 4×10 mg) ($n = 7$)

Sample	Found (ng ml^{-1}) ± S.E.
1	26.96 ± 1.08
2	27.90 ± 0.43
3	37.64 ± 0.16
4	38.22 ± 0.20
5	33.64 ± 0.6
6	37.18 ± 0.65
7	43.20 ± 0.40

Acknowledgements

The authors thank to the FAKO AS for providing the analytical reference standard of NIF. The authors also thank Prof. Dr. Sinan Beksaç for his kind interest.

References

[1] S.R. Hamann, M.T. Piascik, R.G. McAllister Jr, Biopharm. Drug Dispos. 7 (1986) 1–5.
[2] J.A. Miller, K.A. McLean, D.J. Sumner, J.L. Reid, Eur. J. Clin. Pharmacol. 24 (1983) 315–322.
[3] J.L. Blackshear, C. Orlandi, G.H. Williams, N.K. Hollenberg, J. Cardiovasc. Pharmacol. 8 (1986) 37–45.

[4] P. Thongnopnua, K. Viwatwongsa, J. Pharm. Biomed. Anal. 12 (1994) 119–125.
[5] C.H. Kleinbloesem, J. van Harten, P. van Brummelen, D.D. Breimer, J. Chromatogr. 308 (1984) 209–213.
[6] P. Jakobsen, O. Lederballe-Pedersen, E. Mikkelsen, J. Chromatogr. 162 (1979) 81–87.
[7] B.J. Schmid, H.E. Perry, J.R. Idle, J. Chromatogr. 425 (1988) 107–119.
[8] M.T. Rosseel, M.G. Bogaert, J. Chromatogr. 279 (1983) 675–680.
[9] J. Martens, P. Banditt, F.P. Meyer, J. Chromatogr. B 660 (1994) 297–302.
[10] K.S. Patrick, E.J. Jarvi, A.B. Straughn, M.C. Meyer, J. Chromatogr. 495 (1989) 123–130.
[11] A. Jankowski, H. Lamparczyk, J. Chromatogr. A 668 (1994) 469–473.
[12] J.S. Grundy, R. Kherani, R.T. Foster, J. Chromatogr. B 654 (1994) 146–151.
[13] V. Horvath, A. Hrabeczy-Pall, Z. Niegreisz, E. Kocsi, G. Horvai, L. Godorhazy, A. Tolokan, I. Klebovich, K. Balogh-Nemes, J. Chromatogr. B 686 (1996) 211–219.
[14] H. Suzuki, S. Fujiwara, S. Konda, J. Sugimoto, J. Chromatogr. 341 (1985) 341–347.
[15] K. Schlo(mann, Arzneimittel-Forsch. Drug Res. 22 (1972) 60–62.
[16] S. Ebel, H. Schutz, A. Hornitschek, Arzneimittel-Forsch. Drug Res. 28 (1978) 2188–2193.
[17] M.M. Ellaithy, P. Zuman, J. Pharm. Sci. 81 (2) (1992) 191–196.
[18] J.A. Squella, E. Barnafi, S. Perna, L.J. Nunez-Vergara, Talanta 36 (3) (1989) 363–366.
[19] J.A. Squella, L.J. Nunez-Vergara, Bioelectrochem. Bioenergetics 23 (1990) 161–166.
[20] E. Laviron, J. Electroanal. Chem. 112 (1980) 1–9.
[21] A.M.M. Ali, J. Pharm. Biomed. Anal. 18 (1999) 1005–1012.
[22] J.A. Squella, A. Zanocco, S. Perna, L.J. Nunez-Vergara, J. Pharm. Biomed. Anal. 8 (1) (1990) 43–47.