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High-performance liquid chromatographic determination of nifedipine, nicardipine and pindolol using a carbon fibre flow-through amperometric detector*

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Abstract. The electrochemical properties of the calcium-channel blockers, nifedipine and nicardipine, and the β-blocking agent, pindolol, have been exploited for the determination of their concentrations in plasma samples. High-performance liquid chromatography (HPLC) separation was carried out on a cyanopropyl modified column and the drugs were detected in a flow-through carbon fibre microelectrode cell. The chromatographic system was coupled to a column-switching arrangement in order to perform on-line solid-phase extraction of the drugs from spiked human plasma. Preliminary investigations showed the response of the method to be linear over a range of 20–500 ng ml^{-1} in plasma with a limit of detection of approximately 15 ng ml^{-1} for each compound.

Keywords: *HPLC; carbon fibre electrode; nifedipine; nicardipine; pindolol; column switching; on-line solid-phase extraction.*

Introduction

Nifedipine (I) and nicardipine (II) (Fig. 1) are calcium-channel blocking drugs which are widely used in the treatment of angina pectoris due to their potent vasodilating actions and their ability to reduce cardiac output in a dose-dependent fashion [1]. Pindolol (II) belongs to the β-blocking group of drugs which are used in the treatment of hypertension and cardiac arrythmias. Although they are not vasodilators, β-blockers may also be used in the treatment of angina, since they lower myocardial oxygen demand at rest and during exercise due to their haemodynamic effects (decreased heart rate, blood pressure and contractility). The combined therapy of a calcium-channel blocker and a β-adrenoceptor antagonist seems to be more effective in prolonging exercise duration and tolerance than either type of drug used alone in the treatment of ischaemic heart disease [1].

Drug level monitoring has become an important adjunct to routine patient care, especially where drugs have narrow therapeutic ranges and when accurate dosing is essential to produce the desired pharmacological response with the minimum of toxic or other undesirable effects. Since calcium-channel blockers can produce unwanted side-effects, it is often necessary to determine their concentration in the body, and a suitable analytical method should be capable of measuring these drugs in the presence of possible co-administered β-blocking drugs. Calcium-channel blocking drugs have frequently been determined by liquid chromatographic methods with UV [2,3] or electrochemical [4] detection and the electroactive characteristics of nicardipine have been exploited by Wang *et al.* [5] for its determination in diluted urine samples using carbon paste electrodes.

Electrochemical detection combined with HPLC offers the capability of the simultaneous, selective and sensitive determination of electroactive drug species, with little interference from the biological matrix. Carbon fibre microelectrodes are finding increasing use in bioanalytical applications, and several such

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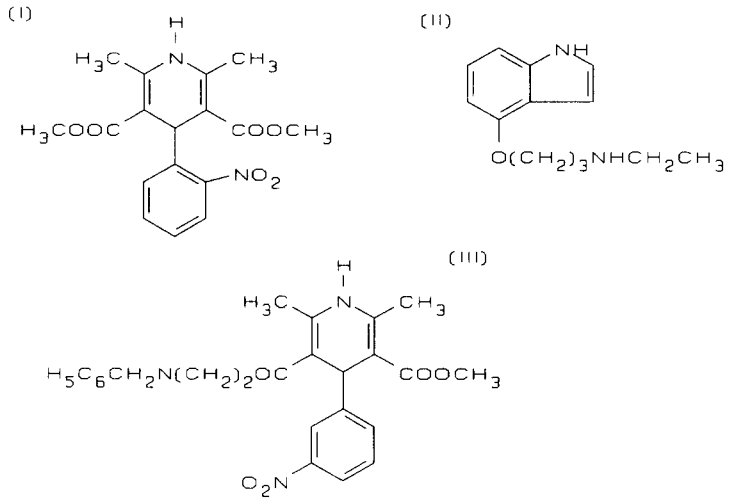

Figure 1
Chemical structures of nifedipine (I), pindolol (II) and nicardipine (III).

systems for detection in HPLC have been described [6, 7]. In most cases, the electrodes are positioned around the column outlet [8], or are joined via an epoxy resin to suitable connecting tubing [9].

In this report, a simple method is described in which the carbon fibre electrode is inserted into the flowing eluent stream; thus the use of sealants is avoided so that contamination arising from contact between the sealants and the mobile phase is prevented. This novel low-cost electrochemical detection scheme was combined with the separating power of HPLC and the rapid efficient technique of on-line solid-phase extraction to develop a rapid and sensitive technique for the determination of these drugs in human plasma.

Experimental

Materials and reagents

Analytical grade disodium hydrogen phosphate was obtained from Merck (Darmstadt, Germany), orthophosphoric acid (Analar grade) from BDH (Poole, UK) and HPLC grade methanol was supplied by Labscan Analytical Sciences (Dublin, Ireland). Dried human plasma, obtained from the Blood Transfusion Board (Dublin), was dissolved in Milli-Q (Millipore Corporation, MA, USA) water and used within 7 days of reconstitution. The drugs were kindly donated by the Institute of Clinical Pharmacology, Dublin.

Stock solutions equivalent to 0.2 mg ml^{-1} of the drugs in methanol–water (1:1, v/v) were freshly prepared. These solutions were diluted and added to drug-free plasma aliquots to provide spiked plasma standards in the concentration range 20–500 ng ml^{-1}.

Instrumentation and operating conditions

The drugs were separated on a Spherisorb (Phase Separations, Clywd, UK) 10-μm cyanopropyl (CN) modified column (250 × 4.6 mm i.d.). The mobile phase consisted of methanol–phosphate buffer (0.05 M; pH 6.7) (40:50, v/v) filtered through a 0.45-μm membrane and degassed by sonication. It was delivered by a Waters (Milford, MA, USA) Model 501 HPLC pump at a flow rate of 1.0 ml min^{-1}. The sample was introduced via a Rheodyne Model 7010 injection valve (Cotati, CA, USA) fitted with a 20-μl loop for direct injection. For the purposes of extraction by column switching, the injector was fitted with a 1-ml loop, and a second pump (pump A) and the concentration column were connected to the analytical assembly via a Rheodyne Model 7000 six-port switching valve. The 10 × 1.5 mm i.d. concentration column was dry-packed with pellicular cyano material supplied by Supelco (Bellefonte, PA, USA); the loading/washing eluent delivered by pump A was filtered and degassed Milli-Q water. The operation of this instrument arrangement has been previously described [10].

The eluted components were detected electrochemically using a carbon fibre working electrode constructed in-house as follows: a 8-μm carbon fibre (Sigre Electrographit, Meit-

inger, Germany) was inserted into a polyethylene tubing block (15 × 1.0 mm i.d.). A syringe was then used to puncture a hole and to introduce a length of fibre sufficiently long to extrude from either end of the tubing. The holes formed by the syringe were then sealed by gentle heating and the exposed portions of the fibre were covered with a layer of silver epoxy to facilitate electrical connection. An auxiliary electrode composed of stainless steel tubing (20 mm ×0.5 mm i.d. and 1.5 mm o.d.) was mounted downstream of an Ag/Ag_3PO_4 reference electrode. This was constructed from a 0.1-mm silver wire (Johnson Matthey, UK) inserted into a polyethylene tubing block in the same manner as for the working electrode. The Ag/Ag_3PO_4 layer was coated on the surface of the silver wire by the application of an anodic potential of +1.5 V across the reference and auxiliary electrodes for 2 min with 1.0 M phosphoric acid flowing through the cell at 0.1 ml min^{-1}. This flow-through system was then connected at a distance of 30 mm from the column outlet; the distances between the working and references electrodes and the reference and auxiliary electrodes were approximately 10 mm. In order to reduce the noise levels in the system, the flow-through cell was covered with an earthed aluminium film. The performance and operation of such an electrode assembly has been described previously in greater detail [9].

Oxidative amperometric measurements were performed using a Princeton Applied Research Model 400 potentiostat (Princeton, NJ, USA) connected to the flow cell by crocodile pins. The drugs were detected amperometrically by applying a positive potential of 1.4 V at the working electrode. The resultant signals were recorded using a Philips Model PM 8252 chart recorder (Eindhoven, The Netherlands) at a chart speed of 300 mm h^{-1}. The peak height of each drug as a function of concentration were then measured for quantitative analysis.

Under these chromatographic conditions, the observed retention times for nifedipine, pindolol and nicardipine were 4.2, 6.4 and 11.0 min, respectively.

Extraction procedure

Aliquots of drug solutions in plasma (1 ml) were injected via the injector port and swept on to the concentration column by the aqueous wash solution. The drugs were selectively retained on the concentration column while the plasma components were removed by elution. Upon switching of the valve, the analytical mobile phase was re-routed onto the concentration column, from whence the drugs were desorbed and swept onto the analytical column where separation occurred.

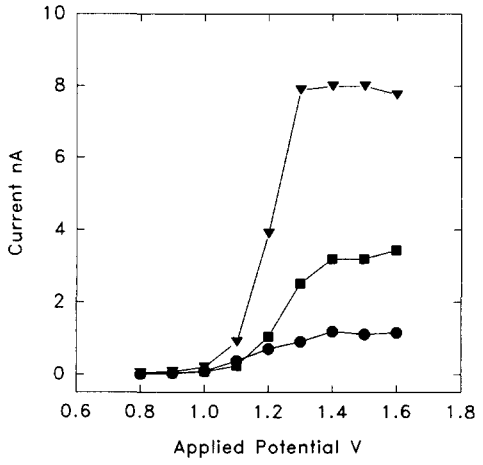

Figure 2
Hydrodynamic voltammograms of pindolol (▼), nifedipine (■) and nicardipine (●), obtained by injection of a mixture of the three drugs.

Results and Discussion

In order to select the optimum working potential, hydrodynamic voltammograms for the drugs were obtained by directly injecting 20 µl of a solution containing 22.5, 14.5 and 27.5 µg of nifedipine, pindolol and nicardipine, respectively, directly onto the column and varying the detector working electrode potential. As shown by the results (Fig. 2), the optimum response was observed when the working potential was increased to +1.4 V. If the working electrode potential was increased to +1.7 V the noise level was higher, an effect caused by higher background current. Accordingly, an anodic potential of +1.4 V was used in all subsequent experiments.

A cyano column was chosen for the separation of these drugs after it had been found to offer superior chromatographic characteristics than an octadecyl-bonded phase. It was found that the retention time of pindolol was most strongly affected by eluent pH; this was adjusted to 6.7 to optimize separation of the three analytes (Fig. 3).

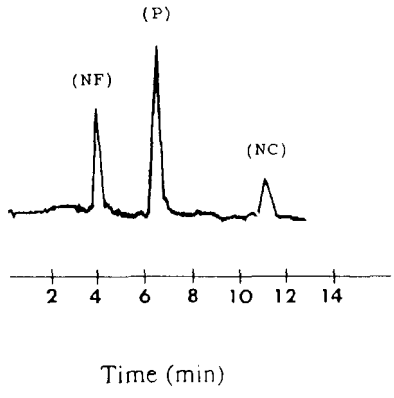

Figure 3
Chromatograms obtained following direct injection of 20 μl of an authentic standard solution containing 90 pg ml^{-1} of nifedipine (NF), 110 pg ml^{-1} of pindolol (P) and 60 pg ml^{-1} of nicardipine (NC). Chromatographic conditions as described in the text. Detector sensitivity was 2 nA.

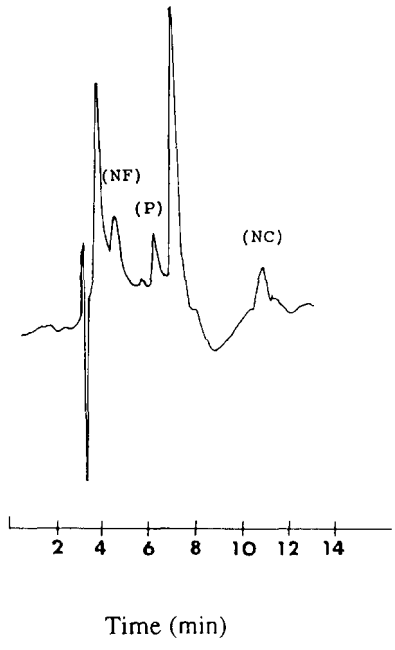

Figure 4
Chromatograms obtained following extraction of plasma samples containing 25.0 ng ml^{-1} nifedipine (NF), 20.0 ng ml^{-1} pindolol (P) and 21.0 ng ml^{-1} nicardipine (NC). The regression equations over the concentration range examined were: nifedipine $y = 5.16 \times 10^{-4}\ x - 5.32 \times 10^{-3}$ ($r = 0.999$); pindolol, $y = 6.34 \times 10^{-4}\ x + 1.21 \times 10^{-2}$ ($r = 0.999$); nicardipine, $y = 6.25 \times 10^{-4}\ x + 2.08 \times 10^{-3}$ ($r = 0.999$). Chromatographic conditions as described in the text. Detector sensitivity was 1 nA.

To determine the optimum wash time for the samples on the concentration column, an aqueous solution containing 100 ng ml^{-1} of each drug was injected onto the concentration column. The wash time (defined as the length of time between injection and switching of the valve) was varied between 1.3 and 4.0 min; it was found that drug peaks remained constant with a wash time of up to 2 min before they began to elute from the concentration column during the wash cycle. Five injections of 100 ng ml^{-1} of each drug dissolved in water yielded a relative standard deviation (RSD) of 6.2% for nifedipine and nicardipine, and a RSD of 12.1% for pindolol.

Drug-free aliquots of plasma were then spiked to produce solutions containing 20, 50, 100, 250 and 500 ng ml^{-1} of each drug in plasma. A 1 ml volume of each standard solution was then injected onto the concentration column and extracted using the procedure described. A linear response range of 20–500 ng ml^{-1} was established for pindolol and nifedipine; for nicardipine it extended to 460 ng ml^{-1}. For each drug, the lower limit of quantitation was taken to be 20 ng ml^{-1} while the limit of detection was 15 ng ml^{-1} (signal-to-noise ratio = 3). The regression equations for each of the drugs are presented in Fig. 4 together with sample chromatograms of extracted plasma samples. Correlation coefficients greater than 0.999 were obtained for all three compounds. The amount found at each concentration level was calculated by interpolation of the detector response (peak height) on the regression lines. These amounts were then compared with the amount added in order to estimate drug recoveries. The recoveries over the calibration range were calculated to be 92.6 ± 8.1, 100.8 ± 4.8 and 107.2 ± 18.9% for nifedipine, pindolol and nicardipine, respectively.

Conclusions

A new method employing a low-cost, easily constructed carbon fibre electrochemical detection scheme for the simultaneous determination of nifedipine, pindolol and nicardipine has been described. Preliminary investigations reveal that this new electrode may be used in conjunction with the column switching technique of plasma extraction to provide satisfactory clean-up and a low limit of detection. Further studies are required to produce a fully validated method based on this system and to establish the durability of the detector in the face of repeated injections of plasma extracts. However, should the electrode prove sensitive to poisoning by a

build-up of residual biological constituents, it may be readily replaced without undue inconvenience and expense because of its simple construction and low cost.

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