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Note

High-performance liquid chromatographic method for the measurement of nicardipine in plasma or serum

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Nicardipine hydrochloride, 2-(N-benzyl-N-methylamino)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride (Fig. 1), is a calcium ion antagonist structurally related to nifedipine. Nicardipine causes blockade of the transmembrane inward calcium movement, resulting in coronary and peripheral vasodilatation. Clinical studies have demonstrated its benefits in patients with angina pectoris and hypertension.

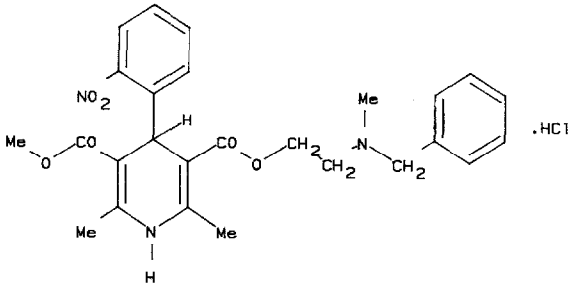

Fig. 1. Structure of nicardipine hydrochloride.

Nicardipine is rapidly and extensively metabolised with two main classes of metabolites, namely those in which the dihydropyridine ring remains intact and those in which oxidation occurs. The major urinary metabolites are glucuronide conjugates of alcohol metabolites [1,2]. In humans, intravenous or oral administration of nicardipine results in urinary recovery of $<0.03\%$ unchanged nicardipine. Expected concentrations of nicardipine in plasma range from 10 to 150 μg/l following single-dose administration of 40 mg daily [3] and 50 to 500 μg/l following chronic therapy, 40 mg three times daily [4]. The drug is well tolerated, with few serious side-effects [4].

The published methods for the measurement of nicardipine in plasma [5–10] involve lengthy extraction procedures which limit their application for the analysis of either single-patient samples or large numbers of samples resulting from

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clinical studies. The high-performance liquid chromatographic (HPLC) method described here overcomes these problem by means of a rapid extraction procedure followed by the direct injection of the extract onto the chromatographic system.

EXPERIMENTAL

Materials and reagents

Nicardipine hydrochloride and its pyridine metabolite (M5) were supplied by Syntex Pharmaceuticals (Maidenhead, U.K.). The internal standard, butriptyline hydrochloride, was supplied by Wyeth Labs. (Amersham, U.K.) and was used as an aqueous solution equivalent to 5.0 mg/l free-base. Methanol and methyl *tert.*-butyl ether were HPLC reagent grade (Rathburn, Walkerburn, U.K.). Tris and sodium hydroxide was obtained from BDH (Poole, U.K.) and ammonium perchlorate was obtained from Aldrich (Gillingham, U.K.). Tris was used as a 2 *M* solution in glass-distilled water, adjusted to pH 11.0 using 1 *M* sodium hydroxide.

Instrumentation

A constant-flow reciprocating pump (Knauer Model 64, Biotech Instruments, Luton, U.K.) was used with a syringe-loading valve (Rheodyne 7125, 100-μl loop). The analytical column was a stainless-steel tube (125 mm × 5 mm I.D.) packed with Spherisorb S5SCX silica (5 μm average particle size, Hichrom, Reading, U.K.), which was used at ambient temperature. The mobile phase was methanol containing 20 m*M* ammonium perchlorate, delivered at a flow-rate of 2.0 ml/min. The column effluent was monitored by electrochemical detection using a V25-grade glassy-carbon electrode in a wall jet assembly (Model TC100, Thames Chromatography, Maidenhead, U.K.) at an applied potential of 1.1 V against an Ag/AgCl reference electrode, range 100 nA. Peak heights were measured using a Shimadzu C-R4A recording integrator (Dyson Instruments, Houghton-le-Spring, U.K.).

Sample preparation

The sample (200 μl) and Tris solution (50 μl) were pipetted into a 1.5-ml capped polypropylene microcentrifuge vial (Elkay Labs., Basingstoke, U.K.). Internal standard solution (50 μl) and methyl *tert.*-butyl ether (20 μl) were added using Hamilton gas-tight syringes fitted with Hamilton repeating mechanisms. The contents of the vial were vortex-mixed for 30 s, following which the vial was centrifuged (9950 *g*, 2 min; Beckman microcentrifuge E). A portion of the extract was then used to fill the sample loop of the injection valve.

Instrument calibration

Standard solutions containing nicardipine hydrochloride at concentrations

equivalent to 5, 10, 25, 50, 100, 200, 300 and 500 μg/l analyte free-base were prepared in nicardipine-free human plasma by serial dilution from a methanolic stock solution containing nicardipine hydrochloride at a concentration equivalent to 1.0 g/l free-base. Internal quality control samples containing nicardipine hydrochloride at concentrations equivalent to 20, 75 and 250 μg/l analyte free-base were also prepared in nicardipine-free human plasma, using an independently prepared stock solution. On analysis of the extracts, the ratios of the peak heights of the nicardipine standards to the peak heights of the internal standard, when plotted against the nicardipine concentration, were linear and passed through the origin of the graph. The mean (± S.D.) slope, intercept and correlation coefficient for the linear regression line were 0.0155 ± 0.003, −0.00522 ± 0.06265 and 0.99 ± 0.0097, respectively (n = 10). There was no significant difference between the slope of the standard curve for standards made in human plasma compared with those made in human serum (data not included).

The concentration range for the standards was chosen after consideration of the pharmacokinetic data available (summarized in ref. 4).

RESULTS AND DISCUSSION

No endogenous sources of interference were noted when extracts of human plasma from subjects who had not received nicardipine were analysed (Fig. 2). There was minimal interference from other drugs prescribed routinely (Table I). Oxprenolol and nadolol were the only drugs tested that interfered with the analysis of nicardipine under the conditions of the assay. The nicardipine metabolite (M5) was fully resolved from the parent compound following analysis of a mixture of nicardipine and metabolite M5 in methanol (Fig. 2). The M5 metabolite is the only metabolite seen in extracted plasma samples with this assay.

The only other published method which uses normal-phase chromatography [10] uses UV detection at 254 nm and suffers some problems with interference from endogenous substances; no data for the retention times of commonly prescribed drugs were reported using this system. The high sensitivity for the method (reported detection limit 2 μg/l) is achieved by use of a 1-ml sample volume and a complex extraction procedure.

Visor *et al.* [9] used electrochemical detection for the measurement of nicardipine but the limit of accurate measurement was 8 μg/l, despite a sample volume of 400 μl. Again, retention data for other drugs were not recorded and the data reported relate only to samples from rats given nicardipine.

We were unable to retain nicardipine on a unmodified silica column but, because of its secondary amine group, it was possible to retain it using a strong cation-exchange column. Silica columns and ionic methanolic eluents have been used sucessfully for the separation of a number of basic cardioactive drugs [11].

Whilst nicardipine has a usable UV spectrum ($E_{1\%}$ is approximately 500 with UV maxima at 239 and 338 nm) for the measurement of the plasma concentra-

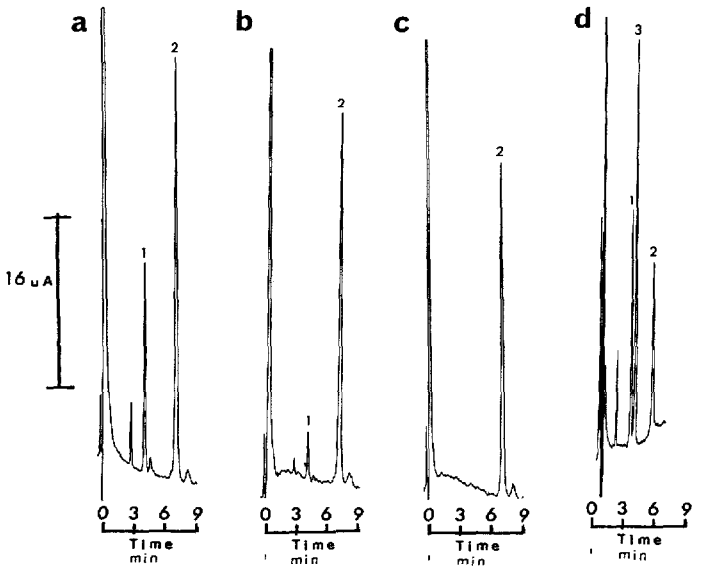

Fig. 2. Chromatograms of (a) an extract of a nicardipine plasma standard (300 μg/l), (b) an extract of a patient plasma sample (the measured concentration of nicardipine was 92 μg/l), (c) an extract of nicardipine-free plasma and (d) a methanolic solution of nicardipine and its metabolite M5. The internal standard was aqueous butriptyline at an initial concentration of 5 mg/l. Peaks: 1 = nicardipine; 2 = internal standard, butriptyline; 3 = metabolite M5.

tions expected following single-dose administration, electrochemical detection was considered to be both more selective and sensitive. Nicardipine does not fluoresce under the assay conditions used here.

The optimal applied potential was decided upon by use of a hydrodynamic voltammogram (Fig. 3). Although a higher voltage would have maximised the response to nicardipine, the selectivity and sensitivity of the assay would have been diminished, due to increased oxidation of the eluent and electrode; this would have produced a higher background current and decreased the signal-to-noise ratio [11].

The mean recoveries for nicardipine from human plasma, at concentrations of 25, 200 and 500 μg/l, were 99, 101 and 108%, respectively, when compared with methanolic solutions containing nicardipine of the same concentrations (n = 10).

The within- and between-assay coefficients of variation (C.V., %) for replicate analyses of standard solutions of nicardipine hydrochloride prepared in human plasma are summarised in Table II, together with the minimum and maximum measured concentrations for the prepared standards. The lower limit of accurate measurement for nicardipine was taken as 5 μg/l. This limit could be improved by either operating the detector at a more sensitive attenuation or by using a larger sample volume, since the drug is well resolved from the solvent front and such a procedure would be within the capabilities of the detector.

TABLE I

RETENTION TIMES OF NICARDIPINE AND SOME OTHER COMPOUNDS RELATIVE TO BUTRIPTYLINE (INTERNAL STANDARD)

See text for chromatographic conditions.

Compound	Relative retention time	Compound	Relative retention time
Nitrazepam	Not retained	Lignocaine	0.81
Diazepam	Not retained	Nortriptyline	0.84
Mexiletine	Not retained	Maprotyline	0.89
Desalkylflurazepam	Not retained	Verapamil	0.95
Lignocaine metabolite		Desipramine	0.95
(glycine xylidide)	Not retained	*Butriptyline*	1.00
M5 Metabolite	0.33	Amitriptyline	1.14
Propranolol	0.46	Mepivacaine	1.18
Hydroxypropafenone	0.49	Imipramine	1.19
Oxprenolol	0.53	Trimipramine	1.21
Nicardipine	0.54	Fluazepam	1.23
Nadolol	0.56	Chlorpromazine	1.25
Metoprolol	0.61	Ajamaline	1.26
Propafenone	0.63	Phenazone	1.30
Prilocaine	0.67	Doxepin	1.37
Pindolol	0.68	Dothiepin	1.40
Timolol	0.70	Amiodarone	1.47
Acebutalol	0.70	Fenethazine	1.54
Desethylamiodarone	0.70	N-Acetylprocainamide	1.72
Tocainide	0.74	Mianserin	1.79
Norverapamil	0.75	Lignocaine metabolite	
Dextropropoxyphene	0.77	(monoethylglycine xylidide)	1.96
Protriptyline	0.77	Quinidine	2.35
Disopyramide	0.77	Quinine	2.53
Bupivicaine	0.81		

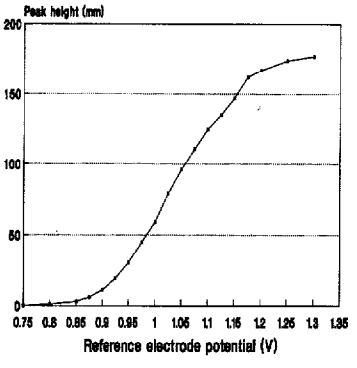

Fig. 3. Hydrodynamic voltammogram of a methanolic solution of nicardipine.

TABLE II

REPRODUCIBILITY OF NICARDIPINE ASSAY

Concentration added (μg/l)	Concentration found (mean ± S.D.) (μg/l)	Coefficient of variation (%)
Within-assay variation		
5	4.20 ± 0.51	12.14
50	51.30 ± 2.71	5.30
200	181.30 ± 7.78	4.30
500	475.60 ± 16.58	3.50
Between-assay variation		
10	9.04 ± 1.11	12.28
50	50.10 ± 4.68	9.30
200	196.30 ± 6.82	3.50
500	496.40 ± 3.78	0.80

The HPLC method described here for the determination of plasma nicardipine concentrations has sufficient sensitivity and selectivity for the analysis of samples both as a guide to therapy and following single-dose pharmacokinetic studies. It has been used successfully in clinical pharmacokinetic studies in which volunteers received the relatively low dose of 40 mg daily.

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