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Enantioselective assay for the determination of perhexiline enantiomers in human plasma by liquid chromatography

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Abstract

Effective use of the antianginal agent perhexiline is difficult because saturable metabolism by the polymorphic cytochrome P450 2D6 (CYP2D6) isoform produces elevated plasma perhexiline concentrations that have been associated with serious hepatic and neurological toxicity. Perhexiline is marketed for therapeutic use as a racemate and there is evidence for differences in the disposition of its enantiomers. The current study describes an enantioselective HPLC-fluorescent method utilising pre-column derivatization with (*R*)-(−)-1-(1-napthyl)ethyl isocyanate. Following derivatization, the enantiomers are resolved on a C18 column with gradient elution using a mobile phase composed of methanol and water. The method described is suitable for the quantification of (+)- and (−)-perhexiline in human plasma following clinical doses and demonstrates sufficient sensitivity, accuracy and precision between 0.01 and 2.00 mg/l for each enantiomer, with intra-assay coefficients of variation and bias <20% at 0.01 mg/l and <10% at 2.00 mg/l, and inter-assay coefficients of variation and biases <15% at 0.03 mg/l and <10% at 0.40 and 0.75 mg/l. The application of this method to plasma samples collected from a patient treated with perhexiline revealed that (+)-perhexiline concentrations were higher than (−)-perhexiline concentrations, confirming the stereoselective disposition of perhexiline. The current study describes an enantioselective method that utilises pre-column formation of fluorescent diastereomers that are resolved on a C18 HPLC column using a gradient of methanol and water.

Keywords: Enantiomer separation; HPLC; Perhexiline

1. Introduction

Perhexiline (2-(2,2-dicyclohexylethyl)piperidine) maleate was introduced over 30 years ago as monotherapy for the prophylaxis of exertional angina [1]. The only product on the market containing perhexiline (Pexsig®, Sigma Pharmaceuticals, Clayton, Victoria, Australia) is formulated with a racemic mixture of the (+) and (−) enantiomers (Fig. 1A). Although clinically effective, its application was limited by serious hepatotoxicity [2] and peripheral neurotoxicity [3] associated with elevated concentrations of perhexiline in plasma. Perhexiline undergoes extensive oxidative metabolism to produce several mono- and dihydroxylated products [4]. Formation of the primary metabolite, *cis*-4-monohydroxyperhexiline, is catalysed by CYP2D6 [5]. As a result of polymorphic [5,6] and saturable [7] metabolism of perhexiline attributable to CYP2D6, there is a poor relationship between steady-state plasma perhexiline concentration and dose [8]. Consequently, the use of perhexiline is limited to patients with intractable angina who are unsuitable for surgical revascularization and refractory or intolerant to conventional pharmacological therapy. Therapeutic drug monitoring is necessary to guide individual dosages in order to maintain plasma perhexiline concentrations within the range of 0.15–0.60 mg/l [9,10].

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Previous studies of the stereoselective metabolism of perhexiline concluded that the clearance of (−)-perhexiline showed the greatest polymorphic effect [11,12]. These studies used single doses of pure enantiomers and an achiral analytical method. There is, therefore, the potential for the relative concentrations of the enantiomers to differ significantly between patients, and to be affected by changes in the dose of perhexiline. The current study describes a validated enantioselective HPLC-fluorescent method utilising pre-column derivatization with the ChiraSelect® derivatizing reagent (*R*)-(−)-1-(1-napthyl)ethyl isocyanate (NEIC). It is suitable for the quantification of the individual enantiomers of perhexiline in plasma from angina patients undergoing treatment with racemic perhexiline.

2. Experimental

2.1. Chemicals

The ChiraSelect® derivatizing reagent (*R*)-(−)-1-(1-napthyl)ethyl isocyanate (NEIC), racemic perhexiline maleate, (*S*)-(+)- and (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate ((+)-BNPA and (−)-BNPA, respectively) and the internal standard prenylamine lactate (Fig. 1) were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). HPLC grade solvents (acetone, ethyl acetate, methanol and *n*-hexane) and sodium hydroxide were purchased from Merck (Kilsyth, Victoria, Australia). All other reagents were of analytical grade.

2.2. Preparation of (+)- and (−)-perhexiline

The individual enantiomers of perhexiline were resolved by the use of a previously reported method [13]. Racemic perhexiline maleate was dissolved in a sodium hydroxide solution and extracted with ether. The combined ethereal extracts were washed with water, dried over magnesium sulfate and the solvent removed to give racemic perhexiline. Treatment of racemic perhexiline with (+)-BNPA resulted in precipitation of the diastereomeric salt, (−)-perhexiline (+)-BNPA, which was purified by selective fractional crystallization from methanol/acetone (twice). Treatment of a chloroform solution of the purified salt with ammonia solution resulted in isolation of (−)-perhexiline. Passing dry hydrogen chloride gas through an *n*-hexane solution of (−)-perhexiline resulted in precipitation of the corresponding hydrochloride salt. (+)-Perhexiline was obtained in a similar fashion by use of (−)-BNPA. The purity of the prepared enantiomers was assessed by NMR, optical rotation and HPLC.

2.3. Stock solutions

A stock solution containing the equivalent of 100 mg/l racemic perhexiline-base was prepared by dissolving the appropriate amount of perhexiline maleate in methanol. This solution was diluted in 0.1 M HCl to give working standard concentrations of 10 and 0.5 mg/l. Solutions of prenylamine lactate were prepared in a similar manner to yield concentrations of 100 and 10 mg/l. These solutions were stored at −20 °C between assays.

2.4. Sample preparation procedure

The extraction of perhexiline from plasma was the same as described in a previously published achiral HPLC method [8], except that the samples were spiked with 50 μl of 10 mg/l prenylamine lactate as the internal standard. Briefly, 500 μl plasma samples (calibrators, controls or patient specimens) were made basic by the addition of 50 μl of 2 M NaOH before the addition of 4 ml of a solution of 10% ethyl acetate in *n*-hexane. These were shaken on a horizontal mixer for 15 min at 100 oscillations per minute followed by centrifugation at 2500 rpm for 10 min at 10 °C. The phases were separated by snap freezing the aqueous layer in a dry ice/ethanol bath and decanting the organic layer, which was then evaporated to dryness in a vacuum centrifuge at ambient temperature. Following this initial extraction, 200 μl of a freshly prepared solution of NEIC in acetone (0.05%) was added to the dried residue and vortexed briefly before incubating at ambient temperature for 5 min. Samples were made basic by the addition of 200 μl of 0.5 M NaOH before the addition of 3 ml of a solution of 10% ethyl acetate in *n*-hexane. This mixture was vortexed for 5 min, followed by centrifugation at 2500 rpm for 3 min at 10 °C. The phases were separated by snap freezing the aqueous layer in a dry ice/ethanol bath and decanting the organic layer, which was then evaporated to dryness in a vacuum centrifuge at ambient temperature. The dried residue was reconstituted in 150 μl of a mixture composed of 80% methanol and 20% water and 100 μl injected onto the HPLC column.

2.5. Chromatography

Analyses were conducted on an Agilent 1100 series HPLC apparatus (Agilent Technologies, Forest Hill, Victoria,

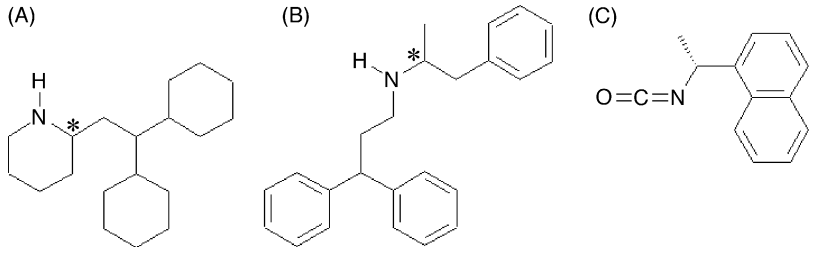

Fig. 1. Perhexiline (A), prenylamine (B) and (*R*)-(−)-1-(1-napthyl)ethyl isocyanate (C). Chiral carbons are marked with an asterisk.

Australia) operated by Chemstation for LC 3D software. The hardware consisted of a model G1322A degasser, a model G1311A pump operating at 1 ml/min, a model G1313A autosampler and a model G1321A fluorescence detector with excitation and emission wavelengths of 218 and 334 nm, respectively. Resolution of the diastereomers was achieved using a Merck Purospher RP-18E column (5 μm, 125 mm × 4 mm) at ambient temperature. The mobile phase was composed of 80% methanol and 20% water for the first 12 min, at which time the methanol was increased to 86% in a linear gradient over 1 min and maintained at that concentration for the remaining 16 min of each sample run time.

2.6. Method validation

Calibration standards were prepared by adding the appropriate volumes of the working standards of perhexiline to 500 μl aliquots of drug-free heparinized plasma to yield final concentrations of enantiomers in the range of 0.01–2.00 mg/l. Calibration curves of the concentrations of each enantiomer against the peak area ratios between the analyte and the first eluting peak of the internal standard were constructed using linear regression. Precision and accuracy were evaluated using the coefficient of variation (CV) and the bias of the measured concentration versus the known spiked concentration, respectively. The method was validated within a single run by assaying six replicates of the 0.01, 0.03, 0.40, 0.75 and 2.00 mg/l calibrators. Inter-assay quality control was assessed in 11 analytical runs by analysing aliquots from three separate plasma pools spiked with 0.03, 0.40 and 0.75 mg/l of perhexiline enantiomers. A CV and bias of ±15% was considered acceptable, except at the limit of quantification (LOQ), where ±20% was accepted [14]. Due to the lack of UV absorbance and fluorescence of underivatized perhexiline and the unavailability of pure derivatized perhexiline, it was not possible to determine the absolute extraction efficiency of the method. Nevertheless, the relative recovery of each perhexiline enantiomer and the first eluting peak of the internal standard from plasma was determined by analysing six replicate aliquots each from four separate plasma pools spiked with 0.03, 0.40 and 0.75 mg/l of perhexiline enantiomers and 1.0 mg/l of prenylamine and the peak areas compared against those for six replicates for each non-extracted standard. A total of 57 randomly selected clinical specimens were used to compare the summed concentrations of the enantiomers determined by the enantioselective assay with the total concentration from an established achiral HPLC method [8]. Drugs commonly coadministered to patients receiving treatment with perhexiline were screened for possible chromatographic interference by analysis of plasma from patients admitted to the cardiology unit who were not taking perhexiline.

2.7. Clinical study

The method was used to measure concentrations of the enantiomers of perhexiline in plasma samples obtained from a 74-year-old female patient who was receiving 100 mg of racemic perhexiline maleate twice per day. The study was approved by the Ethics of Human Research Committee of The Queen Elizabeth Hospital and the patient was informed about the study and gave written consent to participate. Blood samples (5 ml) were taken during the course of one dosing interval from an indwelling venous catheter at times 0, 1, 2, 3, 4, 6 and 12 h. Patency of the catheter was maintained by instilling 1.5 ml of heparinized saline (15 U heparin) into the catheter after each sample was drawn. The first 1.5 ml of blood drawn was discarded to prevent dilution by the heparinized saline. Samples were collected in EDTA tubes and were immediately centrifuged to separate the plasma, which was stored at −20 °C until assayed.

3. Results and discussion

3.1. Characterisation of perhexiline-BNPA salts and pure perhexiline enantiomers

3.1.1. (−)-Perhexiline (+)-BNPA salt

m.p. > 290 °C (decomposes). $[\alpha]_D = -7.2°$ (c = 2.78 g/100 ml, $CHCl_3$, 22.9 °C), −14.1° (c = 2.78 g/100 ml, EtOH, 22.5 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 0.79–1.63 [m, 30 H], 2.49–2.57 [m, 2 H], 2.95 [br s, 1 H], 3.35 [br d, 1 H], 7.23 [ddd, J = 9.6, 9.0, 1.5 Hz, 2 H], 7.37–7.41 [m, 4 H], 7.54 [dd, J = 9.0, 1.5 Hz, 2 H], 7.88 [dd, J = 9.0, 1.5 Hz, 2 H], 7.92 [d, J = 9.0 Hz, 2 H], 8.94 [br q, J = 9.6 Hz, NH], 9.70 [br d, J = 9.6 Hz, NH]. ^{13}C NMR (75 MHz, $CDCl_3$): 21.93, 22.56, 26.36, 26.52, 26.75, 26.77, 26.88, 27.02, 27.44, 29.04, 30.15, 31.09, 31.92, 39.07, 40.02, 44.62, 44.81, 56.69, 121.93 [d, J_{CP} = 2.3 Hz], 122.31 [d, J_{CP} = 2.3 Hz], 124.64, 125.88, 127.09, 128.11, 130.06, 131.09, 132.62, 149.30 [d, J_{CP} = 9.4 Hz].

3.1.2. (+)-Perhexiline (−)-BNPA salt

m.p. >290 °C (decomposes). $[\alpha]_D = +7.2°$ (c = 2.78 g/100 ml, $CHCl_3$, 23.6 °C), +14.6° (c = 2.78 g/100 ml, EtOH, 22.1 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 0.81–1.64 [m, 30 H], 2.52–2.59 [m, 2 H], 2.85 [br s, 1 H], 3.36 [br d, 1 H], 7.23 [ddd, J = 9.6, 9.0, 1.5 Hz, 2 H], 7.37–7.42 [m, 4 H], 7.54 [dd, J = 9.0, 1.5 Hz, 2 H], 7.88 [dd, J = 9.0, 1.5 Hz, 2 H], 7.92 [d, J = 9.0 Hz, 2 H], 8.95 [br q, J = 9.6 Hz, NH], 9.69 [br d, J = 9.6 Hz, NH]. ^{13}C NMR (75 MHz, $CDCl_3$): 21.95, 22.57, 26.37, 26.53, 26.75, 26.78, 26.89, 27.02, 27.51, 29.06, 30.17, 31.12, 31.92, 39.09, 40.03, 44.63, 44.83, 56.73, 121.91 [d, J_{CP} = 2.3 Hz], 122.29 [d, J_{CP} = 2.3 Hz], 124.67, 125.91, 127.10, 128.12, 130.09, 131.12, 132.62, 149.20 [d, J_{CP} = 9.2 Hz].

3.1.3. (−)-Perhexiline hydrochloride salt

m.p. 234–236 °C (lit. m.p. 243–245 °C [15]). $[\alpha]_D = -18.4°$ (c = 2.78 g/100 ml, EtOH, 25.1 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 0.79–2.06 [m, 31 H], 2.75–2.95 [m, 2 H], 3.45 [br d, 1 H], 9.19 [br q, J = 9.6 Hz, NH], 9.63 [br d, J = 9.6 Hz, NH]. ^{13}C NMR (75 MHz, $CDCl_3$): 22.23, 22.52, 26.47, 26.49, 26.66, 26.72, 26.86, 26.98, 28.37, 29.46, 29.94, 31.42, 31.90, 32.24, 39.07, 40.03, 44.75, 44.84, 57.40. MS (EI); m/z: 278 ($[M + H]^+$, 2%), 277 (M^+, 0.3%), 194 (M^+ - C_6H_{11}, 1%), 84 ($C_5H_{10}N^+$, 100%).

3.1.4. (+)-Perhexiline hydrochloride salt

m.p. 233–236 °C (lit. m.p. 243–245 °C [15]). $[\alpha]_D = +18.2°$ ($c = 2.78$ g/100 ml, EtOH, 24.3 °C). ^{1}H NMR (300 MHz, $CDCl_3$): 0.82–2.06 [m, 30 H], 2.75–2.95 [m, 2 H], 3.45 [br d, 1 H], 9.20 [br q, $J = 9.6$ Hz, NH], 9.64 [br d, $J = 9.6$ Hz, NH]. ^{13}C NMR (75 MHz, $CDCl_3$): 22.21, 22.52, 26.46, 26.48, 26.65, 26.70, 26.85, 26.97, 28.36, 29.45, 29.93, 31.40, 31.89, 32.23, 39.06, 40.02, 44.74, 44.83, 57.39. MS (EI); *m/z*: 278 ($[M + H]^+$, 2%), 277 (M^+, 0.4%), 194 (M^+ - C_6H_{11}, 1%), 84 ($C_5H_{10}N^+$, 100%).

The perhexiline-BNPA salts were characterised by NMR spectroscopy. In the ^{1}H NMR spectrum, key resonances were observed for the six aromatic hydrogens of the BNPA, the three aliphatic hydrogens adjacent to the nitrogen ($H2_{ax}$ and $H6_{ax}$ at ca. δ 2.5 and $H6_{eq}$ at δ 3.35) and the two quaternary ammonium hydrogens (δ 8.94 and 9.70) of both salts. The enantiomeric relationship between the (−)-perhexiline (+)-BNPA and (+)-perhexiline (−)-BNPA salts was established from the observation that the optical rotation of the salts was equal in magnitude but opposite in sign.

The perhexiline hydrochloride salts were characterised by NMR spectroscopy and mass spectrometry. In the ^{1}H NMR spectrum, key resonances were observed for the three aliphatic hydrogens adjacent to the nitrogen ($H2_{ax}$ and $H6_{ax}$ at ca. δ 2.8 and $H6_{eq}$ at δ 3.45) and the two quaternary ammonium hydrogens (δ 9.19 and 9.63) of both salts. In the mass spectrum, observation of a weak (M + H) ion at *m/z* 278 together with fragment ions at *m/z* 194 and 84 (corresponding to loss by simple cleavage of C_6H_{11} and $CH(C_6H_{11})_2$, respectively) was consistent with the structure of perhexiline

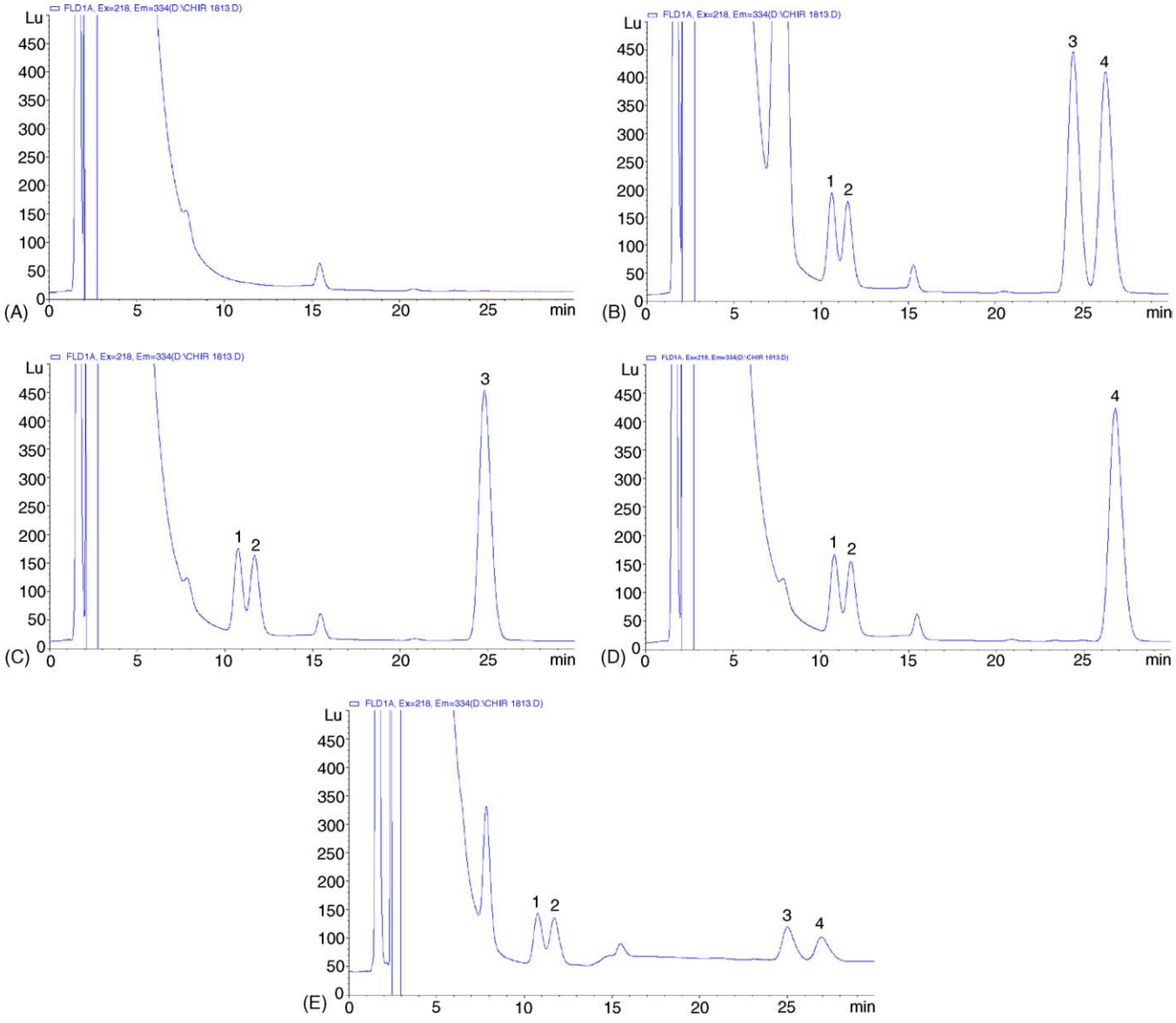

Fig. 2. Representative chromatograms of blank plasma (A), blank plasma spiked with 2.0 mg/l of *rac*-perhexiline hydrochloride (B), 1.0 mg/l of (+)-perhexiline (C) or (−)-perhexiline (D), and a patient sample containing 0.46 mg/l of (+)-perhexiline and 0.36 mg/l of (−)-perhexiline (E). Peaks (1) and (2) correspond to the peaks of the diastereomers of derivatized prenylamine. Peak (1) was used in quantification. Peaks (3) and (4) correspond to the diastereomers of derivatized (+)-and (−)-perhexiline, respectively.

hydrochloride. The enantiomeric relationship between the salts was again established from the observation that the optical rotation of the salts was equal in magnitude but opposite in sign.

3.2. Chromatography

Perhexiline displays very poor UV absorbance and fluorescence and requires derivatization to enhance its detection by conventional HPLC. Reaction with the (*R*)-(−) enantiomer of 1-(1-napthyl)ethyl isocyanate made resolution and detection of the enantiomers possible by the formation of highly fluorogenic diastereomeric napthylethyl ureas. Racemic perhexiline produced two peaks with equal areas (Fig. 2B) and no racemization of the enantiomers occurred during the assay procedure (Fig. 2C and D). Several compounds were evaluated for use as an internal standard. Hexadiline, a dehydro derivative of perhexiline used routinely by this laboratory for quantification of *rac*-perhexiline during therapeutic drug monitoring [8], proved unsuitable because it has two chiral centres that resulted in four diastereomeric derivatives that could not be separated from the two diastereomers formed from perhexiline. Prenylamine was chosen as an internal standard, although a gradient was required for its elution due to its significantly lesser retention compared to perhexiline. Prenylamine is also a chiral compound but has only one centre of asymmetry (Fig. 1B) and thus produced two diastereomers following derivatization. The peak retention times were 10.6 and 11.8 min for prenylamine and 24.8 and 26.7 min for (+)- and (−)-perhexiline, respectively (Fig. 2). No interference was detected from endogenous compounds in plasma (Fig. 2A). It was noted during method development that blood samples collected in Greiner Bio-one plasma tubes with lithium heparin and gel (Interpath Services, Australia) produced significant chromatographic interference with the internal standard following prolonged storage. Thus, all blood samples were routinely drawn into gel-free tubes.

3.3. Method validation

The stability of perhexiline in plasma has previously been reported by this laboratory [16]. Specimens were stable at room temperature for up to 22 days and at 47 °C for up to 24 h. Derivatization reactions were studied using 0.05, 0.1 and 0.2% NEIC in chloroform, acetonitrile and acetone at ambient temperature and at 40 °C for between 5 min and 20 h. A 5 min incubation at ambient temperature with a 0.05% solution of NEIC in acetone produced sufficient sensitivity and reproducibility. Quantitation using the area of the first eluting peak of the internal standard produced a lower coefficient of variation when compared with using the area of the second eluting peak or the combined areas. Linear relationships were obtained between response and concentration for the enantiomers of perhexiline in the range of 0.01–2.00 mg/l, with correlation coefficients (r^2) of 0.991–1.000 for both (+)- and (−)-perhexiline ($n = 11$). Intra- and inter-assay CV and bias were within acceptable limits [14] (Table 1). The LOQ for both enantiomers was 0.01 mg/l, with intra-assay CV and bias of 10.2 and 13.9% for (+)-perhexiline and 9.4 and −17.6% for (−)-perhexiline, respectively (Table 1). The relative extraction efficiency from plasma for both enantiomers of perhexiline was in the range of 65.7–70.1% and for the first eluting peak of the internal standard was 74.3%. A good correlation was observed between the summed concentrations of the enantiomers of perhexiline in plasma as determined by the enantioselective assay and an established achiral HPLC method [8] for total perhexiline (Fig. 3). There was no chromatographic interference from samples of plasma collected from patients administered a range of drugs for ischaemic heart disease or unrelated conditions that are likely to be coadministered to patients undergoing antianginal

Table 1
Intra- and inter-assay precision and accuracy data

Spiked perhexiline concentration (mg/l)	*n*	Bias (%)	CV (%)
Intra-assay			
0.01 (+)-Perhexiline	6	+13.9	10.2
0.01 (−)-Perhexiline	6	−17.6	9.4
0.03 (+)-Perhexiline	6	+8.5	6.3
0.03 (−)-Perhexiline	6	−13.6	4.4
0.40 (+)-Perhexiline	6	+6.3	3.1
0.40 (−)-Perhexiline	6	−8.3	2.9
0.75 (+)-Perhexiline	6	+7.0	3.0
0.75 (−)-Perhexiline	6	−8.8	1.8
2.00 (+)-Perhexiline	6	+0.8	9.6
2.00 (−)-Perhexiline	6	+0.7	9.8
Inter-assay			
0.03 (+)-Perhexiline	11	+9.4	11.9
0.03 (−)-Perhexiline	11	+2.2	12.5
0.40 (+)-Perhexiline	11	+9.7	9.3
0.40 (−)-Perhexiline	11	+8.2	9.7
0.75 (+)-Perhexiline	11	+4.2	9.2
0.75 (−)-Perhexiline	11	+3.1	9.3

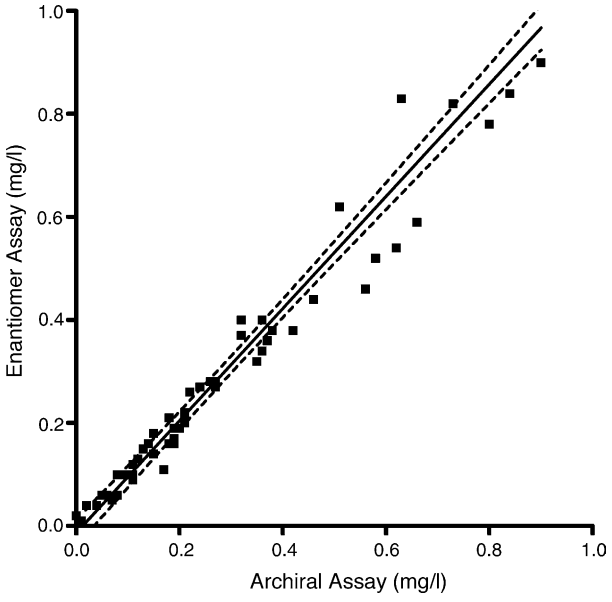

Fig. 3. Correlation between an established achiral HPLC method and the summed perhexiline concentrations measured using the enantioselective assay from 57 randomly selected clinical specimens ($y = 1.089x - 0.014$, $r^2 = 0.952$, 95% CI of slope 1.023–1.156).

Table 2
Drugs screened and found not to interfere with the method for measuring concentrations of the enantiomers of perhexiline in plasma

Alendronate	Flucloxacillin	Oxybutynin
Allopurinol	Fluconazole	Oxycodone
Amiloride	Fluticasone	Oxymetazoline
Amiodarone	Fosinopril	Pantoprazole
Amisulpride	Frusemide	Paracetamol
Amitriptyline	Gabapentin	Paroxetine
Amlodipine	g-csf	Perindopril
Amoxycillin	Gemfibrozil	Pethidine
Amphotericin	Gentamicin	Phenytoin
Aspirin	Gliclazide	Pioglitazone
Atenolol	Glimepiride	Pravastatin
Atorvastatin	Glipizide	Prednisolone
Atropine	Glyceryl trinitrate	Prochlorperazine
Azathioprine	Haloperidol	Promethazine
Beclomethasone	Heparin	Prothiaden
Benztropine	Hydrochlorothiazide	Quinine
Biperiden	Hydrocortisone	Rabeprazole
Calcitriol	Imipramine	Ramipril
Carbadopa	Indapamide	Ranitidine
Carbamazepine	Insulin	Rifampimicin
Carvedilol	Ioperamide	Risedronate
Ceftazidime	Irbesartan	Roxithromycin
Ceftriaxone	Isosorbide dinitrate	Salbutamol
Celecoxib	Isosorbide mononitrate	Salmeterol
Cephalothin	Itraconazole	Sertraline
Chlorpromazine	Levodopa	Simvastatin
Ciprofloxacin	Lorazepam	Sodium fusidate
Clavulanic acid	Metalazone	Sodium valproate
Clonazepam	Metformin	Sorbitol
Clopidogrel	Methadone	Spironolactone
Clotrimazole	Methotrexate	Stem cell factor
Codeine phosphate	Metoclopromide	Sulfamethoxazole
Cyclosporin	Metoprolol	Tacrolimus
Darbepoetin alfa	Metronidazole	Temazepam
Dexamethasone	Midazolam	Theophylline
Diazepam	Morphine	Thyroxine
Digoxin	Mupiricin	Timolol
Diltiazem	Mycophenolate	Tiotropium
Diprydimole	Nicotine	Tramadol
Dothiepin	Nifedipine	Trandolapril
Doxepin	Nitrazepam	Trimethoprim
Doxycycline	Norfloxacin	Tropisetron
Enoxaparin	Nystatin	Valganciclovir
Ergocalciferol	Omeprazole	Vancomycin
Esomeprazole	Oxazepam	Verapamil
Famciclovir	Oxpentifylline	Warfarin
Fentanyl		

treatment with perhexiline (Table 2). This demonstrates that these drugs and their metabolites will not interfere with the determination of the enantiomers in plasma from patients being treated with perhexiline.

3.4. Clinical study

To demonstrate the application of the method, the plasma concentrations of (+)- and (−)-perhexiline were determined by the validated method in a patient who had been phenotyped as a CYP2D6 extensive metaboliser from the metabolic ratio of *cis*-4-monohydroxyperhexiline to perhexiline in plasma [8]. The concentrations of (+)-perhexiline were higher at all times during the dosing interval (Fig. 4), with a mean ratio of (+)- to (−)-perhexiline of 1.26.

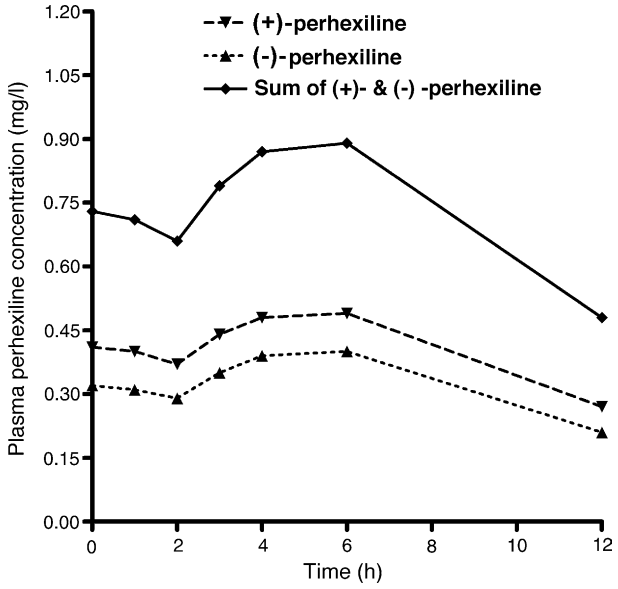

Fig. 4. Plasma perhexiline concentration–time profiles over the course of one dosing interval in a CYP2D6 extensive metabolizer patient who was receiving 100 mg of *rac*-perhexiline maleate twice per day. The perhexiline was administered after the 0 h sample was drawn.

4. Conclusion

The enantioselective HPLC-fluorescent method described was validated over a wide range of concentrations in human plasma with acceptable precision and accuracy, and proved to have the specificity necessary for complex plasma samples. The concentrations of perhexiline enantiomers in plasma over 24 h in a patient identified as a CYP2D6 extensive metaboliser were quantified and the concentration of (+)-perhexiline was higher at all time points, confirming the stereoselective disposition of perhexiline. This assay will subsequently be used to examine the pharmacokinetics of the enantiomers of perhexiline in CYP2D6 poor and extensive metabolisers.

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