Separation of propafenone enantiomers by liquid chromatography with a chiral counter ion

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ABSTRACT

Propafenone is a class Ic antiarrhythmic drug with complex pharmacological and pharmacokinetic profiles due to the existence of an asymmetric carbon that is only available as a racemic mixture. A liquid chromatographic method for the determination of propafenone enantiomers using N-benzyloxycarbonylglycyl-L-proline as the chiral selector in the mobile phase is described. Studies on the retention behaviour of both propafenone and propranolol (internal standard) enantiomers with different mobile phase compositions permitted the determination of the optimum conditions. The best resolution for propafenone enantiomers was 2.3. Application to plasma samples with a simple sample preparation is also presented. The method was linear up to 2500 ng/ml of racemic propafenone and the limit of detection was 100 ng/ml for each enantiomer.

INTRODUCTION

Propafenone (PFN) is a class Ic antiarrhythmic drug with weak β-blocking action; its weak calcium-blocking action has also been described [1–4]. PFN contains a chiral centre and is available only as a racemate in therapeutic formulations. The major difference in the pharmacological action of (R)-$(-)$-PFN and (S)-$(+)$-PFN is their affinity to β-receptors, which is 50–100 times greater for the latter [5,6]. The pharmacokinetic profile of the two enantiomers is also different, as hydroxylation, which is the major route of metabolism, is stereoselective and favours

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(R)-$(-)$-PFN [5]. The hydroxylation of PFN is related to debrisoquin oxidation polymorphism, and stereoselectivity seems to be abolished in poor metabolizers [7]. This issue is important as 5-hydroxy-PFN is an active metabolite which possibly contributes to the clinical activity of the drug [4].

Few chromatographic methods have been devised to separate and determine PFN enantiomers and these methods were based on chiral derivatization [5,8]. A derivatization procedure with $(-)$-naphthylethyl isocyanate afforded a low detection limit (below 6.25 ng/ml) [8] and Kroemer *et al.* [5] obtained a detection limit of 100 ng/ml after a multi-step extraction procedure and derivatization with 2,3,4,5-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate. However, even with highly optically pure reagents, racemization may occur before or
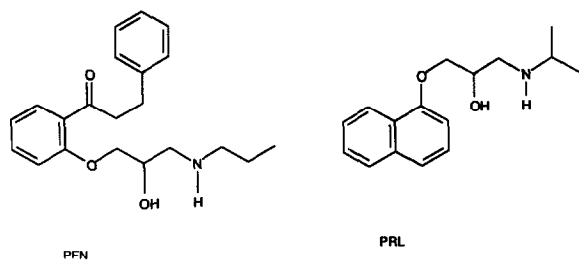
Fig. 1. Structures of propafenone (PFN) and propranolol (PRL).

during the reaction, leading to erroneous results [9–11].

As the structure of PFN is similar to that of β-blocking agents (Fig. 1), a high-performance liquid chromatographic (HPLC) method described by Pettersson and Josefsson [12] for amino alcohols was assessed. This method was reported to give high enantioselectivity for propranolol ($\alpha = 1.35$) and related structures, and is based on the formation of diastereoisomeric ion pairs with N-benzyloxycarbonylglycyl-L-proline (ZGP) in the mobile phase. Our results on PFN enantiomer separation are presented and discussed in this paper.

EXPERIMENTAL

Instrumentation

A Shimadzu LC 6A pump, a Rheodyne Model 7125 valve injector with a 20-μl sample loop, a Shimadzu SPD 6A detector operating at 300 nm and a Shimadzu CR 5A integrator, purchased from Touzart et Matignon (Vitry, France), were used.

Chemicals

Racemic PFN hydrochloride was obtained from Sigma (St. Louis, MO, USA), (+)- and (−)-propranolol (PRL) hydrochloride, (Z)-glycyl-L-proline (ZGP), 0.4-nm molecular sieves and optically active di-O,O'-p-toluyltartaric acids were obtained from Fluka (Buchs, Switzerland). Dichloromethane without preservative (OSI, Paris, France) was stored desiccated at room temperature; the water content, as measured by the Karl Fisher method, was 120 ppm. Ethanol (Carlo Erba, Rueil, France), 1-pentanol (Merck, Darmstadt, Germany) and triethylamine (TEA) (Prolabo, Paris, France) were of analytical-reagent grade and used without further purification.   N-Succinimido-(Z)-glycyl-L-proline

was a gift from Eurobio (Les Ulis, France). Silica thin-layer chromatographic (TLC) plates (2.5 × 7.5 cm) were obtained from Whatman (Maidstone, UK).

Three liquid chromatographic columns were used: Nucleosil diol (7 μm) (250 × 4.6 mm I.D.) and Nucleosil cyano (5 μm) (250 × 4.6 mm I.D.) columns were obtained from SFCC (Neuilly Plaisance, France) and a Brownlee Labs. Spheri 5 cyano column (250 × 4.6 mm I.D.) was obtained from Touzart et Matignon.

Preparation of PFN enantiomers

PFN enantiomers were obtained by fractional crystallization of diastereoisomeric salts with D- and L-di-O,O'-p-toluyltartaric acids [13]. Each salt was recrystallized three times. The yields were 68 and 56% for (−)- and (+)-PFN (expressed as bases), respectively.

Column preparation

Before use, each column was washed with 100 ml of dry dichloromethane. Before each manipulation, the mobile phase was recirculated through the column using a volume equal to 100 times the dead volume of the system. Then a new mobile phase was introduced and arranged for recirculation. Separations were carried out at room temperature.

Composition of the mobile phases

Mobile phases were prepared extemporaneously with dried dichloromethane containing $2 \cdot 10^{-4}$–$5 \cdot 10^{-3}$ M ZGP and $2 \cdot 10^{-4}$–$2 \cdot 10^{-3}$ M TEA, with various amounts of 1-pentanol and water to control retention. The flow-rate was always 1 ml/min.

Calculation

The efficiency of the column was calculated using the peak width at half-height (ω) given by the integrator according to [14]

$$N = 5.54 \frac{t_R^2}{\omega^2}$$

where t_R is the retention time. The resolution (R_s) was expressed as a classical function of the capacity factor of the second-eluted peak (k_2'), enantioselectivity (α) and efficiency (N) according to [14]

$$R_s = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2'}{k_2' + 1} \cdot \sqrt{N}$$

Determination of PFN in plasma

Plasma samples (2 ml) were spiked with 60 μl of 10^{-4} M (S)-(−)-PRL as the internal standard in centrifuge tubes and 200 μl of 1 M sodium hydroxide solution and 4 ml of toluene–n-butanol (95:5) were added. The tubes were agitated on a horizontal shaker (Toulemonde, Paris, France) for 30 min and centrifuged at 2500 g for 15 min. The organic layer, after transfer into a new glass tube and addition of 10 μl of acetic acid, was evaporated to dryness in a block heater (45°C) under a stream of air. The residue was dissolved in 100 μl of chloroform before analysis.

RESULTS AND DISCUSSION

Optical purity of PFN enantiomers: TLC and HPLC

For assessment of optical purity, a rapid semi-quantitative TLC system was used. Samples were derivatized with a 100% excess of N-succinimido-ZGP in dimethylformamide (12 h at room temperature) and were then spotted on silica gel plates and developed at room temperature with dichloromethane–ethanol (10:1) as mobile phase. Spots were revealed with a 254-nm UV lamp or by spraying with 2,4-dinitrophenylhydrazine. Under these conditions, the R_F values for (+)- and (−)-PFN were 0.62 and 0.64, respectively. Thus the optical purity was evaluated as more than 90% in both instances.

The HPLC method with a chiral counter ion allowed the measurement of optical purity. After three crystallizations it was 98.7% for (−)-PFN and 98.6% for (+)-PFN (Fig. 2).

Choice of stationary phase

The three columns were checked for efficiency in the normal-phase system. Nucleosil diol, Spheri 5 cyano and Nucleosil cyano gave 6000, 18 000 and 18 000 theoretical plates, respectively, for nitrobenzene.

The Nucleosil diol column demonstrated poor efficiency and yielded highly asymmetric peaks; the best R_s was 0.78 for PRL enantiomers despite a selectivity factor of 1.42. The system failed to separate PFN enantiomers.

The two cyano-bonded silica columns exhibited similar selectivities for PFN (between 1.10 and 1.20). However, with the Spheri 5 cyano column, short retention times were only obtained with large amounts
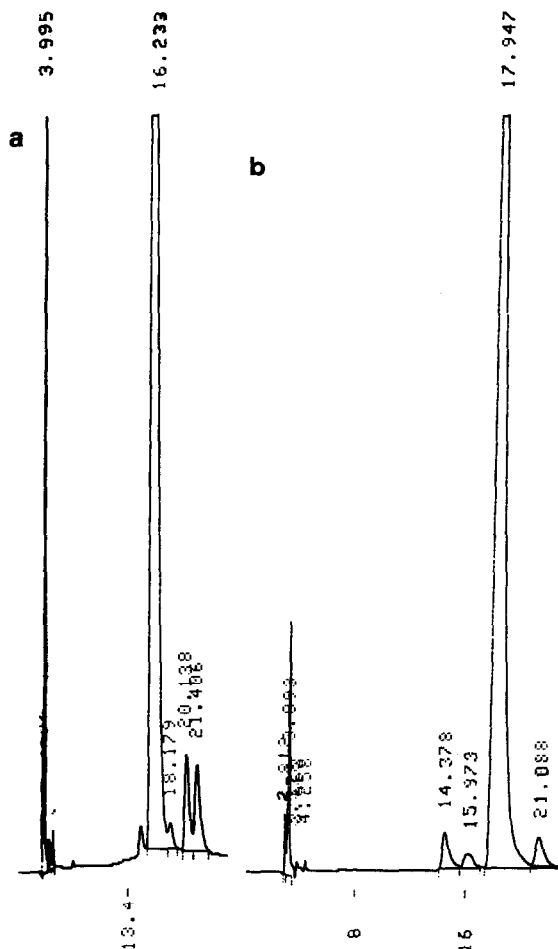

Fig. 2. Optical purity after three recrystallizations. Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–1-pentanol (100:1, v/v)–2.5 · 10^{-3} M ZGP–1.5 · 10^{-3} M TEA; solutes, PFN enantiomers (10^{-4} M); detection, 0.32 a.u.f.s.; detection wavelength, 300 nm; flow-rate, 1 ml/min. (a) $k'_{(+)\text{-PFN}}$ = 4.78 (retention time 16.2 min); $k'_{(-)\text{-PFN}}$ = 5.47 (retention time 18.2 min); enantiomeric excess (ee) = 97.3. (b) $k'_{(+)\text{-PFN}}$ = 4.68 (retention time 16 min); $k'_{(-)\text{-PFN}}$ = 5.39 (retention time 17.9 min); ee = 97.5. Peaks at 14.3, 20 and 21 min are unidentified.

of 1-pentanol in the mobile phase leading to poor efficiency and incomplete resolution (cf., Table I). In contrast, on the Nucleosil cyano support, short retention times without 1-pentanol in the mobile phase yielded good separations of PFN enantiomers (R_s up to 2.3). The resolutions of PFN enantiomers by methods based on chiral derivatization were similar. Therefore, this stationary phase was used for further studies.

TABLE I

EFFECT OF 1-PENTANOL ON RETENTION AND RESOLUTION OF PFN ENANTIOMERS

Stationary phase, Spheri 5 cyano; mobile phase, dichloromethane–1-pentanol (variable ratio)–2.5 · 10^{-3} M ZGP–10^{-3} M TEA.

Parameter	Dichloromethane–1-pentanol			
	95:5	93.5:7.5	90:10	85:15
k'^a	25	15	14.3	7.5
α	1.13	1.14	1.13	1.14
R_s	1.19	1.2	1.09	0.61

^{a} k' = Capacity factor of the last-eluted enantiomer.

Optimization of separation on Nucleosil cyano column

Because application to biological fluids requires internal standardization, PRL and PFN enantiomer separations were investigated concomitantly. The effect of the proportion of each mobile phase component on retention, selectivity and resolution was also evaluated.

Effect of 1-pentanol. Addition of a small percentage of 1-pentanol to the mobile phase always led to shorter retention times associated with a decrease in column efficiency. Unexpectedly, the effects on enantioselectivity differed for PFN and PRL (Fig. 3). The results for resolution were opposite, with an increase for PFN enantiomers and a decrease for PRL enantiomers. The hydrogen donor capacity of 1-pentanol may explain these results: on retention by masking the free silanols of the stationary phase; on selectivity by displacing PRL from ion pairs with ZGP. The absence of a decreased PFN enantioselectivity may be due to steric hindrance.

Effect of ZGP concentration. The concentration of ZGP in the mobile phase affected mainly the enantioselectivity, efficacy of the column and thus resolution. On the other hand, only a minor effect on retention was observed. This unusual behaviour implies a complex elution mechanism of diastereoisomeric ion pairs in this system.

In both instances retention reached a maximum for a ZGP concentration of 10^{-3} M (Table II). The column efficiency increased with the increasing concentration of ZGP, as usual in ion-pair chroma-
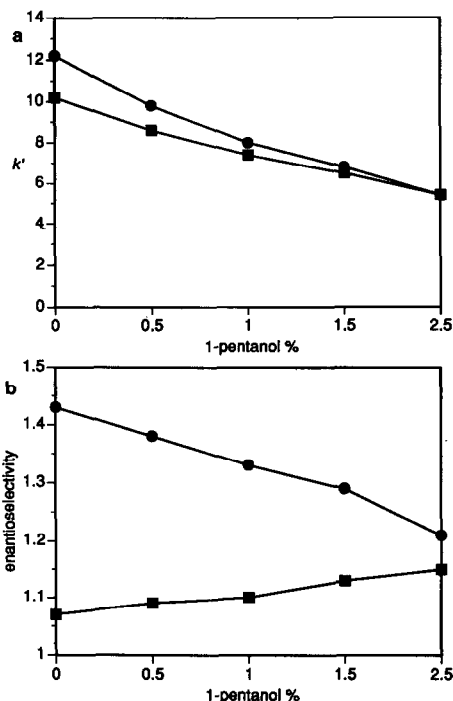

Fig. 3. Effects of 1-pentanol on (a) retention and (b) enantioselectivity. ■ = PFN; ● = PRL. Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–1-pentanol (variable ratio)–2.5 · 10^{-3} M ZGP–5 · 10^{-4} M TEA. k' = Capacity factor of the last-eluted enantiomer.

tography. However, the effects on enantioselectivity were different for PRL and PFN, as described by Petterson and Josefsson [12] for alprenolol and 8-hydroxy-2-(di-n-propylamino)tetralin. For PRL, the enantioselectivity increased with increasing concentration of ZGP and no separation occurred when the ZGP concentration was lower than that of TEA, showing a competitive action of TEA towards ion pairs. For PFN, the enantioselectivity and resolution increased for concentrations of ZGP up to 10^{-3} M and then decreased. These results remain unexplained.

Effect of triethylamine. As for 1-pentanol, TEA led to shorter retention times (Fig. 4), but addition of TEA never resulted in a decrease in the efficiency of the system. However, the effects on enantioselectivity were still opposite; for PRL enantiomers the selectivity decreased when the ZGP/TEA ratio decreased, whereas for PFN enantiomers TEA did not significantly affect the selectivity over a wide range

TABLE II

EFFECTS OF ZGP CONCENTRATION ON RETENTION AND SEPARATION OF PFN AND PRL ENANTIOMERS

Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–ZGP (variable)–10^{-3} M TEA–200 ppm water.

Enantiomer	Parameter	ZGP concentration (M)						
		$2.5 \cdot 10^{-4}$	$5 \cdot 10^{-4}$	$1 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	$3.75 \cdot 10^{-3}$	$5 \cdot 10^{-3}$
(−)-PFN	k'^{a}	7.1	7.7	8.9	7.8	7.2	5.6	4.7
	N	2030	2580	4080	4110	4660	4420	4050
	α	1.13	1.17	1.19	1.15	1.13	1.09	1.07
	R_s	1.14	1.63	2.29	1.85	1.72	1.16	0.86
(−)-PRL	k'	9.85^{b}	11.0^{b}	12.8	10.7	9.0	6.4	5.0
	N	—	—	—	1360	1600	2300	2140
	α	1	1	1.05	1.21	1.32	1.42	1.44
	R_s	—	—	—	1.46	2.18	3.07	2.95

^{a} k' = Capacity factor of the last-eluted isomer.
^{b} Enantiomers not resolved.

of concentrations. Hence TEA is suitable for retention adjustment.

Effect of water content. In both instances, amounts of water in the mobile phase ranging from 200 to 1500 ppm resulted in slightly decreased retention times and increased column efficiency (Table III).

The selectivity still differed between the two compounds; no effects on the separation of PFN enantiomers was observed, whereas a slight decrease in enantioselectivity of PRL enantiomer separation was noted.

Comments

It seems that the two racemic compounds studied behave differently in this chromatographic system. PRL enantiomers give highly selective diastereoisomeric ion pairs; PFN enantiomers do not allow enantioselectivity greater than 1.2, probably because their side-chain in the *ortho* position causes steric hindrance.

Modifiers seem to induce opposite effects. Enhancing the solubility of ion-pair components in the mobile phase or coating of the stationary phase silanols increased the efficiency, whereas changing the ion-pair equilibria led to lower selectivity. The resulting effect depends on the lability of ion pairs
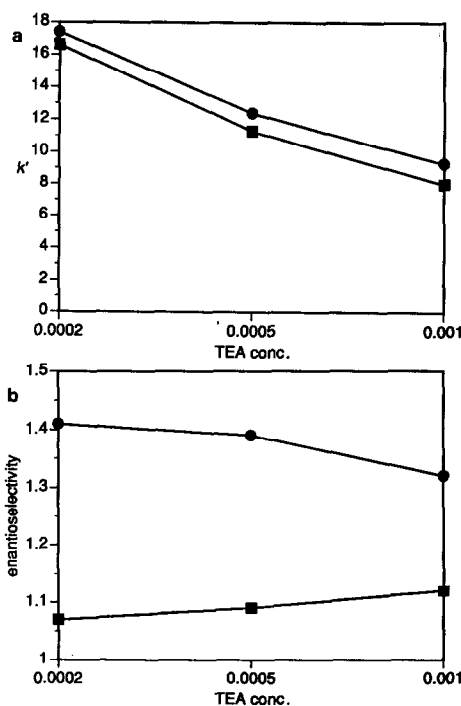

Fig. 4. Effects of TEA concentration (M) on (a) retention and (b) enantioselectivity. ■ = PFN; ● = PRL. Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–$2.5 \cdot 10^{-3}$ M ZGP–TEA (variable). k' = Capacity factor of the last-eluted enantiomer.

TABLE III

EFFECTS OF SMALL AMOUNTS OF WATER ON RETENTION AND SEPARATION OF PFN AND PRL ENANTIOMERS

Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–2.5 · 10^{-3} M ZGP–10^{-3} M TEA–water (variable).

Enantiomer	Parameter	Water content (ppm)				
		0	200	500	1000	1500
(−)-PFN	k'^a	8.1	7.2	6.9	6.4	6.2
	N	3460	4660	4620	6180	6690
	α	1.10	1.13	1.13	1.12	1.12
	R_s	1.19	1.72	1.69	1.77	1.83
(−)-PRL	k'	10.6	9.0	8.0	7.1	6.6
	N	1060	1600	2230	3560	4250
	α	1.39	1.32	1.35	1.29	1.26
	R_s	2.08	2.18	2.7	2.93	2.87

^{a} k' = Capacity factor of the last-eluted isomer.

and on steric effects. With PRL, the modifiers used to regulate retention always led to a decrease in enantioselctivity. With PFN, the observed effects seem to be due mainly to interactions with the stationary phase.

Application to biological samples

Preliminary studies on the extraction of PFN showed a loss of PFN during evaporation of the organic layer which resulted in irreproducible yields. Therefore, the extracted PFN base was acidified with acetic acid before evaporation. Under these extraction conditions, a yield of at least 85% was obtained. The calibration graphs were linear up to 2500 ng/ml of the racemate in plasma. The equation of the mean four-point calibration graph ($n = 3$) was (y = PFN-to-PRL area ratio; x = PFN concentration in ng/ml) $y = 2.46 \cdot 10^{-4} x + 0.019$ with $r = 0.998$ (range 0.997–0.999) for (+)-PFN and $y = 2.48 \cdot 10^{-4} x + 0.018$ with $r = 0.998$ (range 0.9980–0.9988) for (−)-PFN. The limit of detection (signal-to-noise ratio = 3) was less than 100 ng/ml for each enantiomer in plasma. Typical chromato-

grams are shown in Fig. 5. Further, in our experience, the acetic acid remaining after incomplete evaporation of the organic phase led to a system peak during elution of (+)-PFN and thus peak compression as shown in Fig. 6. Such interference may be used to enhance the limit of detection.

CONCLUSION

Propafenone and propranolol enantiomers, despite their similar structures, exhibited different behaviours in their chiral resolution when the chromatographic conditions were altered. Thus, chiral counter-ion chromatography, as described by Petterson and Josefsson [12], seems to be a method of general applicability for β-amino alcohols, but the conditions must be carefully defined in each instance. Once optimized, the method provides a simple way to separate propafenone enantiomers. Hence this chromatographic method with one-step sample preparation may be useful for evaluating PFN enantiomer hydroxylation and for assessing steady-state concentrations in patients undergoing PFN therapy.
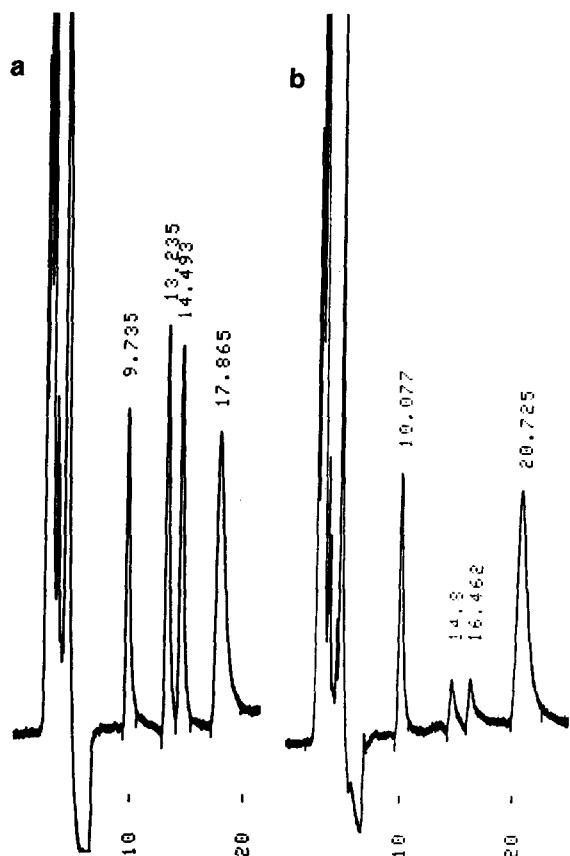
Fig. 5. Chromatograms obtained after extraction of spiked plasmas containing (a) $7.5 \cdot 10^{-6}$ M of racemic PFN (1250 ng/ml of each enantiomer) and (b) $8.8 \cdot 10^{-7}$ M of racemic PFN (150 ng/ml of each enantiomer). Internal standard, $(-)$-PRL $(3 \cdot 10^{-6}$ $M)$; stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–3 $\cdot 10^{-3}$ M ZGP–1.5 $\cdot 10^{-3}$ M TEA–250 ppm water; detection, 0.04 a.u.f.s.; detection wavelength, 300 nm; flow-rate, 1 ml/min. $k'_{(+)\text{-PFN}}$ = 4.37 (retention time 14.7 min); $k'_{(-)\text{-PFN}}$ = 4.97 (retention time 16.4 min); $k'_{(-)\text{-PRL}}$ = 6.57 (retention time 20.8 min). The peak at 10 min is an endogenous compound.

Fig. 6. Effect of system peak on elution of $(+)$-PFN. Stationary phase, Nucleosil 5 cyano; mobile phase, dichloromethane–3 $\cdot$ 10^{-3} M ZGP–1.5 $\cdot$ 10^{-3} M TEA–250 ppm water; solute: racemic PFN $(5 \cdot 10^{-5}$ $M)$ and $(-)$-PRL $(2 \cdot 10^{-5}$ $M)$; detection, 0.16 a.u.f.s.; detection wavelength, 300 nm; flow-rate, 1 ml/min. $k'_{(+)\text{-PFN}}$ = 4.09 (retention time 14.2 min), N = 16 400; $k'_{(-)\text{-PFN}}$ = 4.27 (retention time 14.7 min), N = 2710; $k'_{(-)\text{-PRL}}$ = 5.27 (retention time 17.6 min).

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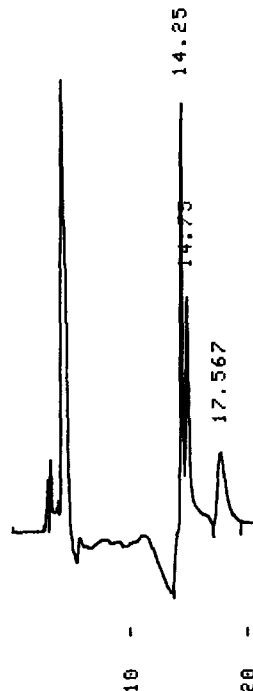6 K. Stoschitzky, W. Klein, G. Stark, U. Stark, G. Zernig, I. Graziadei and W. Lindner, *Clin. Pharmacol. Ther.*, 47 (1990) 740.
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