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Note

Determination of pyridinol carbamate in plasma using high-performance liquid chromatography

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2,6-Pyridinedimethanol N-ethylcarbamate (pyridinol carbamate) inhibits platelet aggregation[1,2] and decreases the permeability of capillaries[3], and is therefore used to prevent and treat atherosclerosis[4–7].

In order to determine the pharmacokinetics of pyridinol carbamate in man, it is necessary to use a highly specific and sensitive technique. The previously described methods for the determination of pyridinol carbamate in biological fluids were not able to determine plasma levels accurately for a sufficiently long period after the administration of a single therapeutic oral dose. In the technique proposed by Maezawa and co-workers[6,8], pyridinol carbamate is extracted from whole blood with chloroform. The extract is evaporated to dryness, the residue dissolved in hot water and the absorbance of the resulting aqueous solution measured at 264 nm. Schettino and La Rotonde[9] used a chromatographic method, with densitometry on thin-layer chromatographic plates after chloroform extraction. Mallein *et al.*[10] used the Technique of Maezama and co-workers but extracted serum instead of whole blood. For the determination of pyridinol carbamate in urine, they used the densitometric technique of Schettino and La Rotonde with another system of solvents for the thin-layer chromatography. To our knowledge, no other chromatographic methods have been used.

In this paper, we describe a technique for the determination of pyridinol carbamate that can easily be applied in pharmacokinetics studies. As the molecule pyridinol carbamate and its derivatives are not heat-stable and the former is characterized by a molar absorptivity of 4230 at 254 nm, we preferred to use high-performance liquid chromatography (HPLC) with UV detection instead of gas chromatography.

EXPERIMENTAL

Apparatus

An analytical liquid chromatograph (Chromatem 38, Touzard and Matignon,

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Paris, France) with automatic correction for solvent viscosity was used. The effluent was monitored with a UV-sensitive detector (Schoeffel) set at 254 nm. The volume of the flow cell was 8 μl with an optical pathlength of 10 mm. The system was connected to a 15 cm $\times$ 4.5 mm I.D. stainless-steel analytical column packed with LiChrosorb Si 60 (5 μm). The column was equilibrated with *n*-hexane; its efficiency was 19,000 theoretical plates as measured with anthracene, eluted with isooctane.

Reagents

The reagents and solvents used were of guaranteed reagent grade (Merck, Darmstadt, G.F.R.): dichloromethane, isopropanol and ammonia solution. Pyridinol-carbamate was obtained from Laboratoires Allard (Paris, France). Chlorpromazine[2-chloro-10-(3-dimethylaminopropylphenothiazine] from Specia (Lyon, France) was used as an internal standard. Because it is not usually found in patients treated with pyridinol carbamate. Its retention time is near that of pyridinol carbamate and its molar absorptivity is large enough to be monitored at 254 nm.

Stock solutions of pyridinol carbamate and the internal standard (chlorpromazine) were prepared as 500 and 1.25 μg/ml solutions, respectively, in dichloromethane.

Calibration graph

Plasma samples to which had been added 2.5, 5, 10, 15 and 20 μg/ml of pyridinol carbamate and 0.124 μg/ml of chlorpromazine were extracted and carried through the procedure. The peak area ratio of pyridinol carbamate to chlorpromazine for each injected sample was plotted against the amount of drug injected. The graph was linear in the range 0–20 μg/ml ($y = 0.130\,x + 0.028$; $r = 0.999$).

Extraction and analysis procedure

A 1-ml volume of plasma was placed in a 50-ml centrifuge tube together with 100 μl of the stock solution of chlorpromazine and 15 ml of dichloromethane. The tube was shaken for 15 min and the aqueous phase discarded after centrifugation. The organic layer was transferred into a second tube and evaporated to dryness under a gentle stream of nitrogen in a water-bath at 25°. The residue was dissolved in 25 μl of dichloromethane and a 2-μl aliquot injected into the chromatograph.

The solvent system used for chromatography was dichloromethane–isopropanol–ammonia solution (90:10:0.1), delivered at a pressure of 45 bar with a flow-rate through the column of 1.75 ml/min.

The sensitivity range of the UV detector was set at 0.2–0.04 absorbance unit full scale (a.u.f.s.), depending on the sample concentration.

RESULTS AND DISCUSSION

With the extraction procedure proposed, the recoveries obtained were 85–90% for pyridinol carbamate and 95% for chlorpromazine.

Fig. 1 shows a chromatogram of a solution of pyridinol carbamate (P) and chlorpromazine (C) in dichloromethane. The retention times are as follows: solvent peak, $t_{R0} = 1.36$ min (2.36 ml), pyridinol carbamate and $t_{RP} = 3.88$ min (6.79 ml), chlorpromazine, $t_{RC} = 5.28$ min (9.24 ml). The capacity factors are $k'_P = 1.85$ and

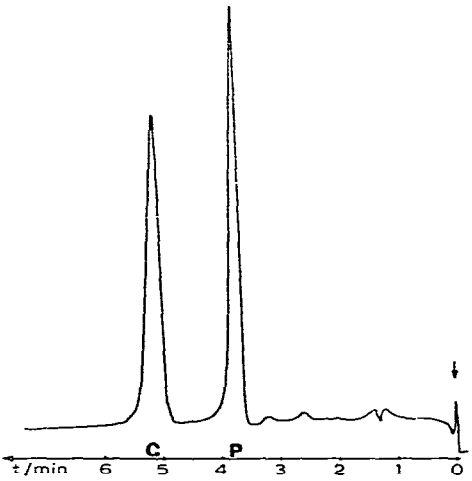

Fig. 1. Chromatogram of a solution containing pyridinol carbamate (P) and internal standard, chlorpromazine (C).

$k'_C = 2.88$ for pyridinol carbamate and chlorpromazine respectively. The selectivity factor (k'_P/k'_C) is thus 1.56.

The peak widths are 0.34 and 0.42 min for pyridinol carbamate and chlorpromazine, respectively. The resolution factor, R_S, is

$$RS = \frac{2\,(t_{RC} - t_{RP})}{W_P + W_C} = 3.68$$

where W_p and W_c are the widths of the peak bases.

The column efficiency measured with pyridinol carbamate is HETP = 0.072 mm.

Fig. 2a shows the chromatogram of a plasma extract before administration of pyridinol carbamate. Peak S, with a retention time of 2.5 min, is always found in the extracts, but it did not interfere with the peaks of pyridinol carbamate and chlorpromazine.

Fig. 2b is the chromatogram of a plasma sample obtained 6 h after an oral administration of pyridinol carbamate. It contains peaks corresponding to pyridinol carbamate (P), chlorpromazine internal standard (C), an unidentified metabolite of the drug (M) and the peak S as in Fig. 2a.

Reproducibility

On analysing ten times each plasma to which different concentrations of pyridinol carbamate had been added, and injecting each concentration in triplicate, the method exhibited excellent reproducibility (Table I).

Sensitivity

The minimum detectable concentration of pyridinol carbamate was 50 ng/ml. This concentration is lower than the therapeutic blood concentration observed 24 h after the oral administration of 1 g of pyridinol carbamate.

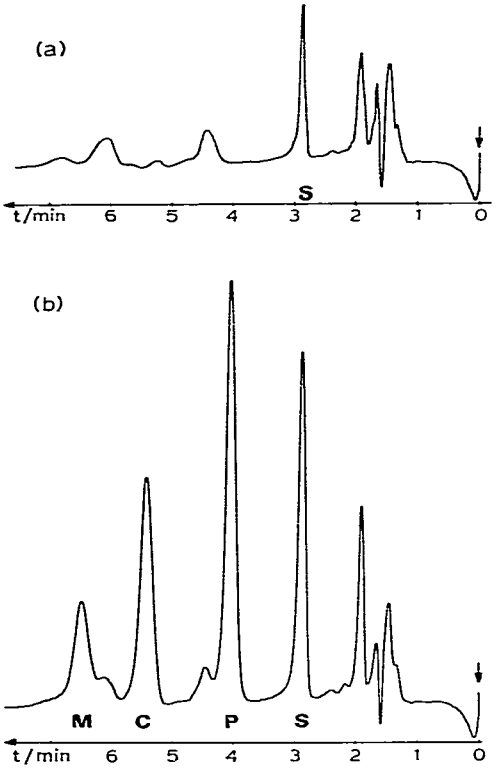

Fig. 2. (a) Chromatogram of a plasma extract before administration of pyridinol carbamate. (b) Chromatogram of a plasma extract 6 h after administration of pyridinol carbamate.

TABLE I

REPRODUCIBILITY OF THE METHOD

Pyridinol carbamate concentration (μg/ml)	*Ratio of peak areas of pyridinol carbamate to chlorpromazine (mean* ± standard error)*
2.5	0.35 ± 0.005
5	0.68 ± 0.009
10	1.37 ± 0.028
15	1.94 ± 0.028
20	2.64 ± 0.036

* $n = 10$.

CONCLUSION

The method described is simple, rapid, highly sensitive and reproducible. In addition, it separates pyridinol carbamate from its main metabolite, which is a necessary condition for pharmacokinetic studies.

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