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High-performance liquid chromatographic determination of phenylpropanolamine in human plasma and urine, using column switching combined with ion-pair chromatography

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SUMMARY

A highly sensitive and selective high-performance liquid chromatographic method with ultraviolet detection at 205 nm, without prior derivatization, is described for the determination of phenylpropanolamine in human plasma and urine. The method involved extraction of plasma or urine at a basic pH with diethyl ether, and chromatographic analysis using column switching combined with ion-pair chromatography. In the first octadecylsilica column, the drug was preseparated from endogenous substances in samples by ion-pair chromatography. After column switching, in the second octadecylsilica column, the heart-cut fraction containing the analyte was further separated by reversed-phase chromatography. The detection limits were 0.4 ng/ml in plasma and 8 ng/ml in urine, both at a signal-to-noise ratio of 3. The method was applied to the determination of the drug in plasma and urine after oral administration of 25 mg of phenylpropanolamine hydrochloride in water to a healthy human volunteer.

INTRODUCTION

Phenylpropanolamine (PPA), a sympathomimetic amine, has been widely used as an "over-the-counter" medicine. It does not undergo extensive metabolism [1,2]. Therefore, various methods for the determination of the unchanged drug in biological fluids have been developed for pharmacokinetic studies. The drug has been determined by gas chromatography (GC) with electron-capture detection (ECD) after derivatization with pentafluorobenzalde-

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hyde [3], heptafluorobutyric anhydride [4] or trifluoroacetic anhydride [5]. High-performance liquid chromatography (HPLC) has been also used after reaction with sodium naphthoquinone-4-sulphonate [6], *o*-phthalaldehyde [7–9] or phenyl isothiocyanate [10]. However, all these methods require laborious or time-consuming derivatization procedures. GC determination with a nitrogen-selective detector without a derivatization step was reported [11], but the detection limit of the analyte was not presented.

HPLC with ultraviolet (UV) detection without prior derivatization is the most widely used method for the determination of drugs in biological fluids. However, PPA shows UV absorption only in the short-wavelength region (molar absorptivity at 205 nm = 8000), which means that this technique has poor sensitivity and selectivity for PPA in biological fluids [7,8,10]; for example, UV absorbance at both 210 nm [12] and 220 nm [13] showed low sensitivity.

We recently reported [14,15] that the column-switching technique combined with ion-pair chromatography offers high selectivity and sensitivity for ionic compounds in biological fluids, even with short-wavelength UV detection. PPA is a primary amine and its elution was predicted to be delayed by ion-pair formation. The aim of this study was to use this technique to develop a highly sensitive and selective HPLC method for the determination of PPA in human plasma and urine by UV detection at 205 nm without prior derivatization.

EXPERIMENTAL

Reagents and materials

PPA hydrochloride was obtained from Takeda (Osaka, Japan). Acetonitrile was of HPLC grade (Wako, Osaka, Japan). All other reagents were of analytical-reagent grade and were used without further purification.

Extraction from plasma and urine

To 1.0 ml of human plasma were added 50 μl of 1 *M* sodium hydroxide solution. The mixture was extracted twice with 4 ml of diethyl ether. The organic layer was evaporated to dryness under a stream of nitrogen at 40 °C. The residue was dissolved in 300 μl of the mobile phase for column 1 (MP1), which will be described later. An aliquot of 200 μl was injected into the HPLC system.

To 100 μl of human urine was added 1 ml of 0.1 *M* sodium hydroxide solution. The mixture was treated in the same way as that for plasma. An aliquot of 100 μl was injected into the HPLC system.

Instrument and conditions

The HPLC system consisted of two LC-6A pumps, two SPD-6A UV detectors, a CTO-6A column oven and a FCV-2AH six-port switching valve and a SIL-6A autosampler, all of which were controlled automatically by a SCL-6A controller (all from Shimadzu, Kyoto, Japan). A C-R3A integrator (Shi-

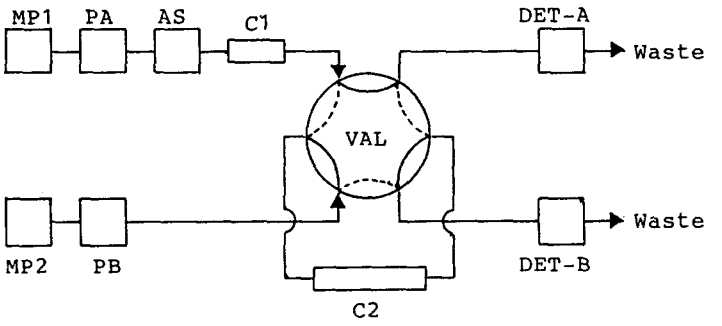

Fig. 1. Schematic diagram of the HPLC system. PA, PB = Pumps A and B; AS = autosampler; VAL = six-port valve; C1, C2 = columns 1 and 2; DET-A, DET-B = detectors A and B; MP1, MP2 = mobile phases 1 and 2. The solid and dotted lines in the six-port valve indicate the valve positions A and B, respectively.

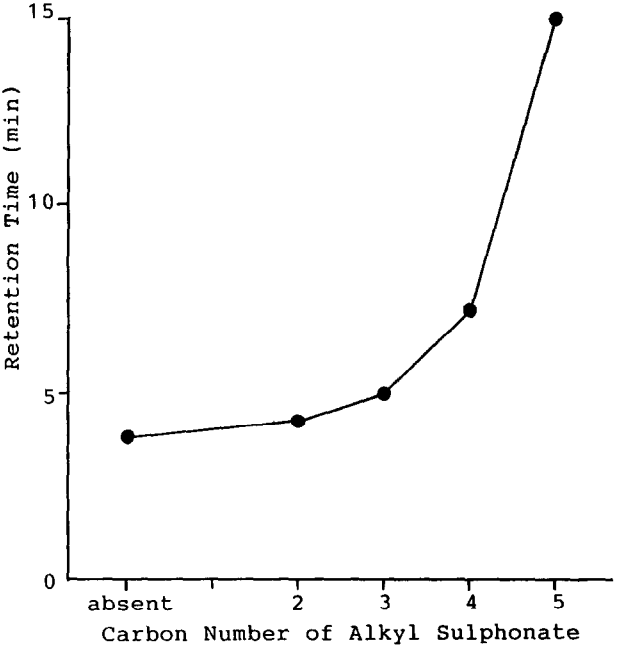

Fig. 2. Relation between the chain length of an alkyl sulphonate in MP1 and the retention time of PPA with C1 only, monitored by detector A. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* alkyl sulphonate ($C_nH_{2n+1}SO_3Na$). For other conditions, see text.

madzu) was used for quantitation. Two ODS columns were used: YMC ODS (A type, 5 μm particle size, 70 mm × 4.6 mm I.D.) for column 1 (C1) and YMC ODS (A type, 5 μm particle size, 100 mm × 4.6 mm I.D.) for column 2 (C2) (both from Yamamura, Kyoto, Japan). The mobile phase for C1 (MP1) was 0.02 *M* potassium dihydrogenphosphate (pH 3.5 with 10%, w/v, orthophos-

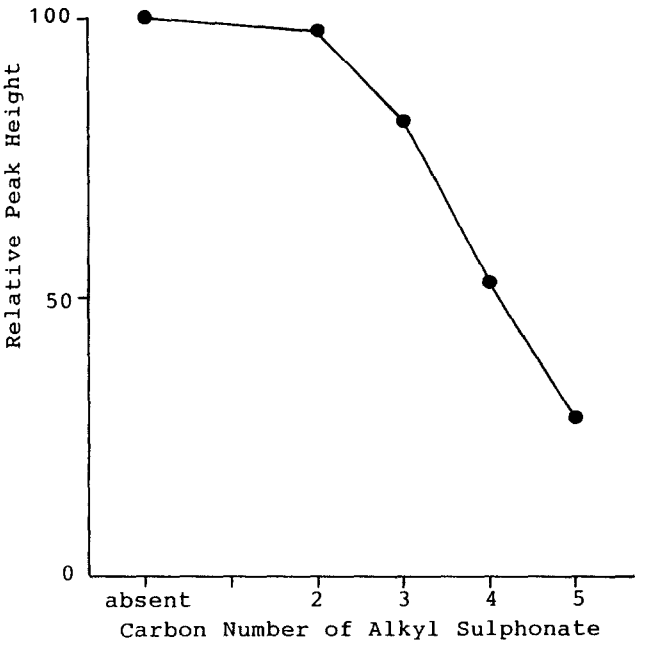

Fig. 3. Relation between the chain length of an alkyl sulphonate in MP1 and the peak height of PPA with C1 only, monitored by detector A. Peak height is expressed as a percentage of that obtained with MP1 containing no ion-pair reagent. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* alkyl sulphonate ($C_nH_{2n+1}SO_3Na$). For other conditions, see text.

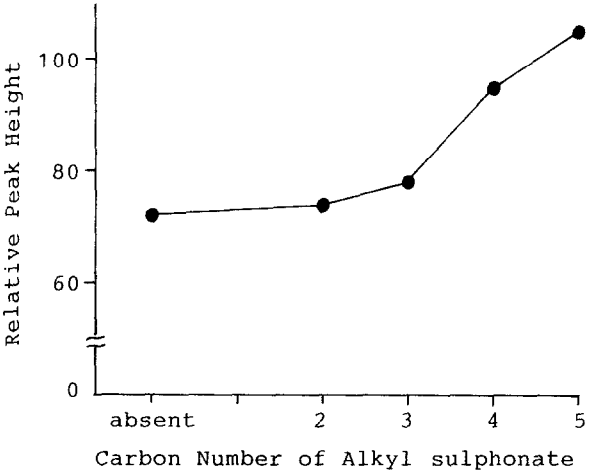

Fig. 4. Relation between the chain length of an alkyl sulphonate in MP1 and the peak height of PPA after column switching monitored by detector B. Peak height is expressed as a percentage of that obtained with C2 and MP2 without column switching. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* alkyl sulphonate ($C_nH_{2n+1}SO_3Na$). For other conditions, see text.

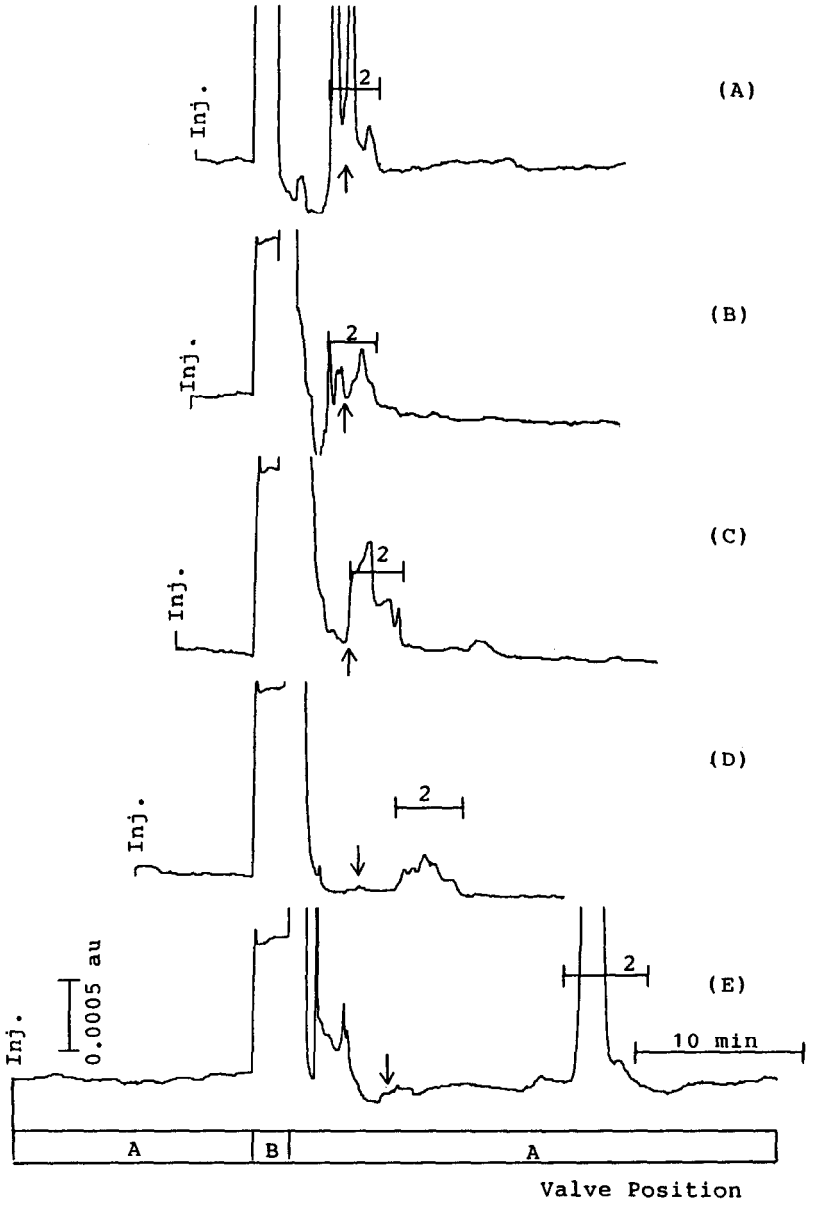

Fig. 5. Effect of the chain length of an alkyl sulphonate in MP1 on the chromatograms of drug-free plasma with column switching monitored by detector B. The arrow indicates the retention time of PPA. Peak 2 = coeluted substances from C1. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* alkyl sulphonate ($C_nH_{2n+1}SO_3Na$). (A) No ion-pair reagent; (B) $n=2$; (C) $n=3$; (D) $n=4$; (E) $n=5$. For other conditions, see text.

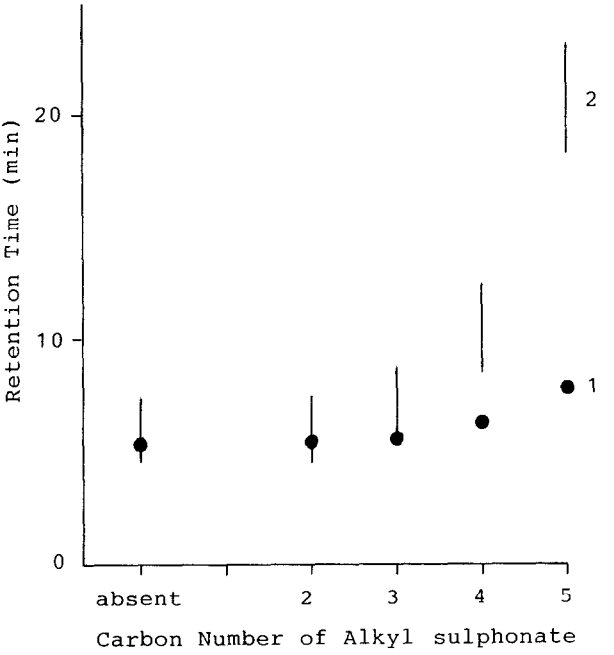

Fig. 6. Effect of the chain length of an alkyl sulphonate in MP1 on the retention times of PPA and coeluted substances from C1 after column switching monitored by detector B. Retention time zero indicates the time when the valve position is switched to B. Retention times: 1 = PPA; 2 = coeluted substances from C1. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* alkyl sulphonate ($C_nH_{2n+1}SO_3Na$). For other conditions, see text.

phoric acid)-acetonitrile (95:5, v/v) containing 5 m*M* sodium butanesulphonate (SBS). The mobile phase for C2 (MP2) was the same as MP1 but without SBS. The temperature and the flow-rate for both columns were 40°C and 1.0 ml/min, respectively. Detection was carried out at 205 nm.

Analytical system and procedure

The analytical system and the procedure were similar to those reported previously [14,15]. A schematic diagram of the HPLC system is shown in Fig. 1. Before start-up, the retention time of PPA in C1 was checked daily by detector A to determine the time programme for column switching. The injected sample was first analysed on C1 with MP1 (valve position A). The eluate fraction containing PPA was transferred from C1 to C2 by switching the valve position from A to B. After the elution of PPA from C1 to C2, the valve position was switched back to A and the heart-cut fraction was further analysed on C2 with MP2 and monitored by detector B with UV absorbance at 205 nm. The valve

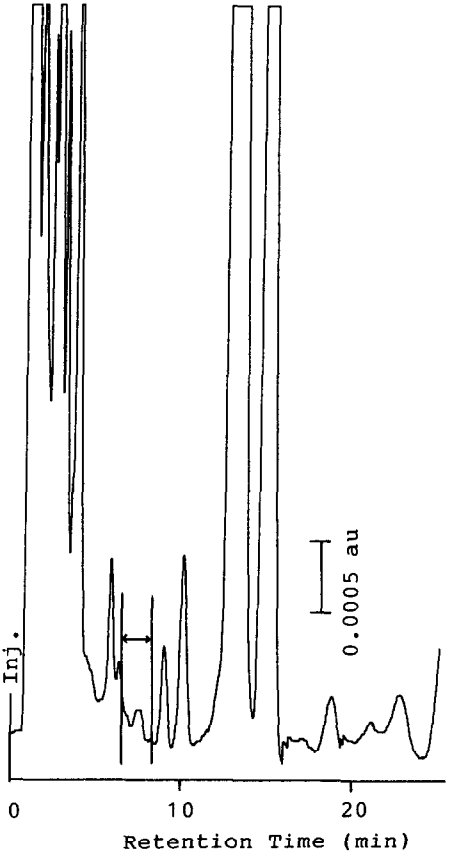

Fig. 7. Typical chromatogram of drug-free plasma obtained with C1 only without column switching, monitored by detector A. The marked zone indicates the heart-cut fraction. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)–acetonitrile (95:5, v/v) containing 5 m*M* SBS. For other conditions, see text.

operations were carried out automatically by the SCL-6A controller according to the predetermined time programme.

RESULTS AND DISCUSSION

Chromatography

The elution of PPA, a primary amine, was predicted to be delayed by ion-pair formation. Various alkyl sulphonates ($C_nH_{2n+1}SO_3Na$, $n=2$–5) were investigated as an ion-pair reagent in MP1. The relations between the chain length of the alkyl sulphonate contained in MP1, and the retention time of

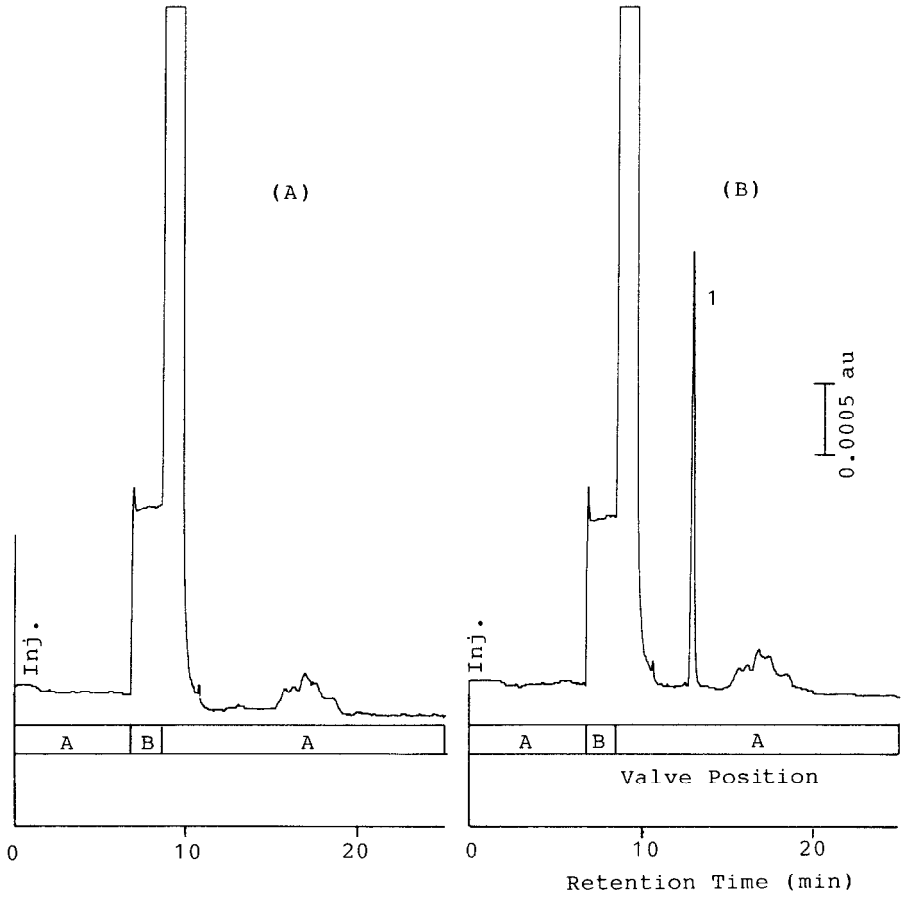

Fig. 8. Typical chromatograms of (A) drug-free plasma and (B) plasma spiked with PPA (20 ng/ml) obtained with column switching monitored by detector B. Peak 1 = PPA. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* SBS. For other conditions, see text.

PPA, and its peak height obtained on C1 with MP1 without column switching monitored by detector A are shown in Figs. 2 and 3, respectively. As the chain length increased, so did the retention (Fig. 2), but the peak height decreased (Fig. 3). However, as shown in Fig. 4, the peak height increased with increasing chain length after column switching. When SBS ($n=4$) or sodium pentanesulphonate (SPS, $n=5$) was used, almost the same peak height was obtained after column switching as that obtained with C2 and MP2 without column switching. This suggests that the difference between the elution ability of MP1

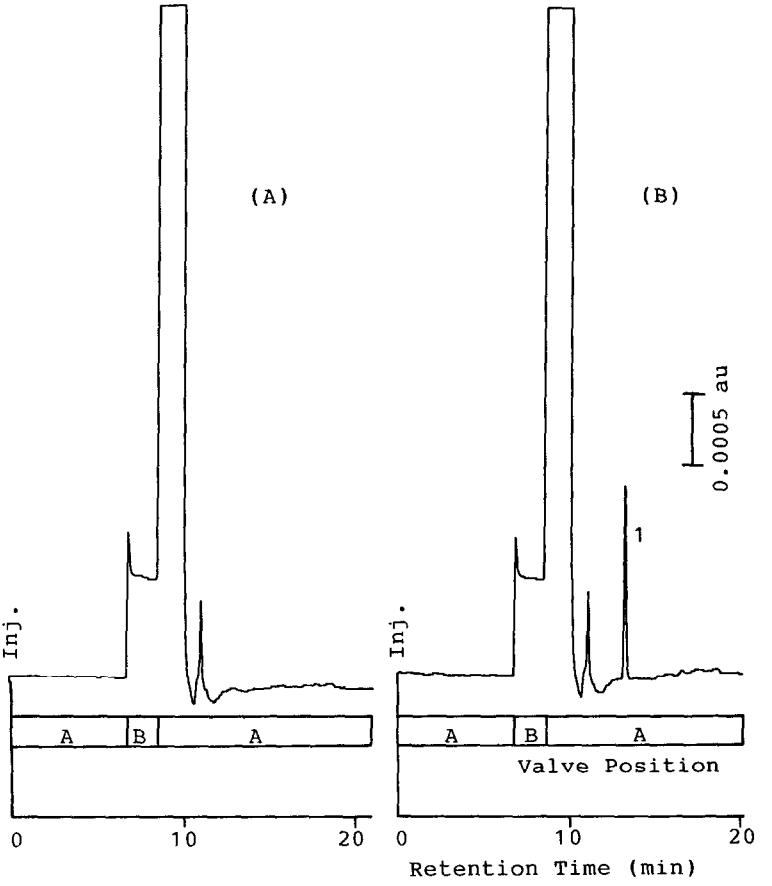

Fig. 9. Typical chromatograms of (A) drug-free urine and (B) urine spiked with PPA (200 ng/ml) obtained with column switching monitored by detector B. Peak 1 = PPA. MP1: 0.02 *M* potassium dihydrogenphosphate (pH 3.5)-acetonitrile (95:5, v/v) containing 5 m*M* SBS. For other conditions, see text.

containing SBS or SPS, and MP2 containing no ion-pair reagent was enough for the analyte to enrich on the top of C2 after the introduction of the eluate from C1, and resulted in no peak broadening after column switching.

The clean-up efficiency by the proposed column-switching method was first investigated for plasma samples. Fig. 5 shows the effect of an ion-pair reagent in MP1 on the chromatogram of drug-free plasma obtained with column switching monitored by detector B. The retention times of PPA and the coeluted substances from C1 in Fig. 5 are also summarized in Fig. 6. As shown in Figs. 5 and 6, PPA was successfully separated from the coeluted substances, with the increase of the chain length of alkyl sulphonates. This shows that the elution of PPA was delayed by a suitable alkyl sulphonate, whereas that of the

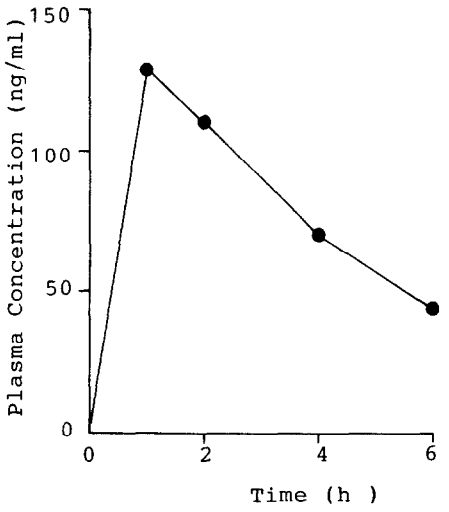

Fig. 10. Plasma levels of PPA after oral administration of 25 mg of PPA·HCl in water to a healthy human volunteer.

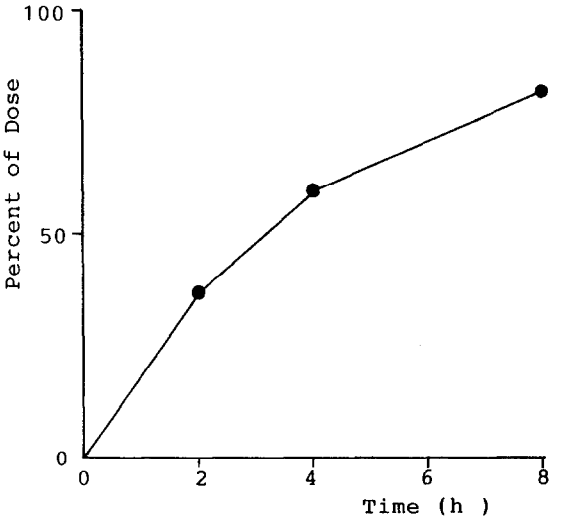

Fig. 11. Cumulative urinary excretion of PPA after oral administration of 25 mg of PPA·HCl in water to a healthy human volunteer.

coeluted substances from C1 was affected little if at all, which resulted in the high selectivity. In view of the selectivity and the total analysis time, SBS ($n=4$) was selected as an ion-pair reagent in MP1 for the present study.

Fig. 7 shows a typical chromatogram of drug-free plasma obtained with C1 only, without column switching, monitored by detector A. Fig. 8 shows typical

chromatograms of plasma samples obtained with column switching monitored by detector B. Figs. 7 and 8 demonstrate the satisfactory clean-up efficiency of the proposed column-switching technique. No peak broadening and no interferences at the retention time of the analyte, as shown in Fig. 8, allowed the highly sensitive determination of the drug in plasma. Similar chromatograms free from interferences were also obtained for urine samples, as shown in Fig. 9.

Recovery

The calibration graphs were obtained by analysing spiked plasma and urine samples over the drug concentration ranges 1–1000 and 40–20 000 ng/ml, respectively. The least-squares regression fit showed good linearity, passing through the origin (correlation coefficient = 0.9999) for both plasma and urine. The intra- and inter-assay recoveries of PPA from spiked plasma samples were 83.5% with a coefficient of variation (C.V.) of 2.6%, and 85.3% with a C.V. of 3.6% (both 20 ng/ml, $n=6$), respectively. The intra- and inter-assay recoveries of PPA from spiked urine samples were 78.1% with a C.V. of 4.0%, and 76.9% with a C.V. of 5.8% (both 400 ng/ml, $n=6$), respectively. The detection limits were 0.4 ng/ml in plasma and 8 ng/ml in urine, both at a signal-to-noise ratio of 3. The sensitivity obtained by the proposed method with a simple pretreatment and no prior derivatization was comparable with that obtained by GC–ECD, which required laborious derivatization procedures [3,5], and much higher than that by simple HPLC–UV methods [12,13]. The higher sensitivity allows a better determination of low drug levels and/or the use of smaller amounts of sample.

Application

Fig. 10 shows a plasma concentration profile after oral administration of 25 mg of PPA·HCl in water to a healthy human volunteer. A cumulative urinary excretion profile is shown in Fig. 11. These results were almost the same levels as those reported previously [1,2,5,7,8]. The method is thus sensitive enough for pharmacokinetic studies of the drug in humans.

CONCLUSION

The proposed column-switching method combined with ion-pair chromatography offers high selectivity and sensitivity for the determination of PPA in human plasma and urine with UV detection at 205 nm. This HPLC method, with a simple pretreatment and no prior derivatization, is suitable for pharmacokinetic studies of PPA in human subjects.

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