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Note

Sensitive high-performance liquid chromatographic determination of propranolol in human plasma with ultraviolet detection using column switching combined with ion-pair chromatography

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High-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is the most widely used method for the determination of drugs in biological fluids. Unfortunately, propranolol (PL), a β-adrenergic blocking agent, shows strong UV absorption only in the short-wavelength region (molar absorptivity at 215 nm is 42 000), which lacks selectivity and is not usually suitable for the sensitive determination of drugs in biological fluids. PL has a native fluorescence, which allows its sensitive and selective detection with a simple pretreatment. Therefore, PL in biological fluids has been mainly determined by HPLC with fluorimetric detection [1–6].

We recently reported that the column-switching technique combined with ion-pair chromatography offers high selectivity and sensitivity for tertiary [7,8] or primary amines [9] whose elution can be delayed by ion-pair formation, even with a poorly selective short-wavelength UV detection. Although fluorimetric detection will be usually the method of choice, we made investigations to show that this technique, preceded by a simple pretreatment, can offer high sensitivity and selectivity for PL, as an example of a secondary amine, in plasma with UV detection and to demonstrate its wide applicability to basic compounds.

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EXPERIMENTAL

Reagents and materials

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

Extraction from plasma

To 1.0 ml of human plasma were added 50 μl of 1 *M* sodium hydroxide and the mixture was extracted twice with 4 ml of diethyl ether. The organic layer was evaporated to dryness under a stream of nitrogen. 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.

Instrument and conditions

The HPLC system consisted of two LC-6A pumps, two SPD-6A UV detectors, a CTO-6A column oven, an FCV-2AH six-port switching valve and an SIL-6A autoinjector, all of which were controlled automatically by an SCL-6A controller (all from Shimadzu, Kyoto, Japan). An C-R3A integrator (Shimadzu) was used for the measurement of the peak height of the analyte. Two ODS columns were used: YMC ODS (A type, 5 μm, 70 mm×4.6 mm I.D.) for column 1 (C1) and YMC ODS (A type, 5 μm, 100 mm×4.6 mm I.D.) for column 2 (C2) (both from Yamamura Chemical, Kyoto, Japan). The mobile phase for C1 (MP1) was 0.02 *M* potassium dihydrogenphosphate–acetonitrile (72:28, v/v) containing 5 m*M* sodium octane sulphonate (SOS), adjusted to pH 3.5 with 10% phosphoric acid. The mobile phase for C2 (MP2) was the same as MP1 but without SOS. The temperature and the flow-rate for both

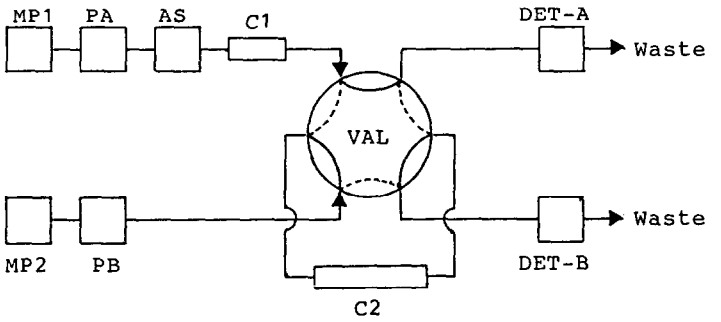

Fig. 1. Schematic diagram of the HPLC system. PA and PB = pumps A and B; AS = autosampler; VAL = six-port valve; C1 and C2 = columns 1 and 2; DET-A and DET-B = detectors A and B; MP1 and MP2 = mobile phases 1 and 2. The solid and dotted lines in the six-port valve indicate the valve positions A and B,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

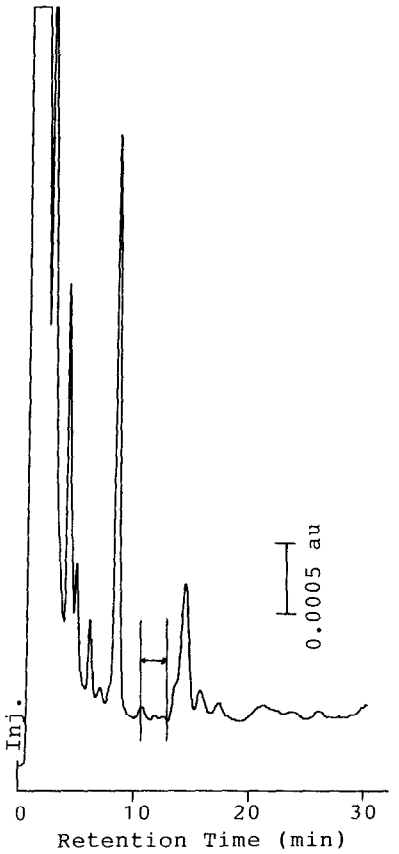

Fig. 2. Typical chromatogram of drug-free plasma obtained with C1 only without column switching, monitored by detector A. The marked zone indicates the heart-cut fraction.

columns were 40 °C and 1.0 ml/min, respectively. UV detection was carried out at 215 nm.

Analytical system and procedure

A schematic diagram of the HPLC system is shown in Fig. 1. The analytical system and the procedure were similar to those reported previously [7–9]. Before start-up, the retention time of PL on C1 was checked daily by detector A to determine the time programme for the column switching. The injected sample was first analysed by C1 with MP1 (valve position A). The eluate fraction containing PL (2.0 ml) was transferred from C1 to C2 by switching the valve position from A to B. After the elution of PL from C1 to C2, the valve position was switched back to A and the heart-cut fraction was further analysed by C2 with MP2 and monitored by detector B with UV absorbance at 215 nm. The

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

RESULTS AND DISCUSSION

The elution of PL, a secondary amine, was predicted to be delayed by ion-pair formation. Therefore, the column-switching technique, which combines ion-pair and non-ion-pair chromatography [7–9], was considered to offer high selectivity and sensitivity for PL even with a poorly selective UV detection, with a suitable ion-pair reagent in MP1. The investigation of various sodium

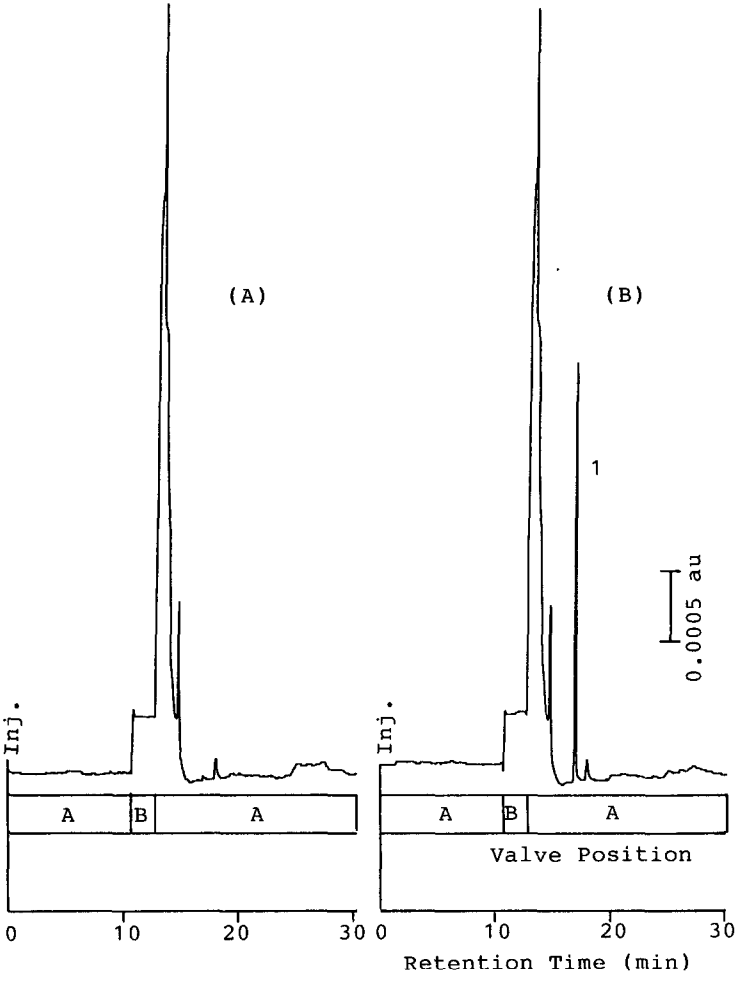

Fig. 3. Typical chromatograms of (A) drug-free plasma and (B) plasma spiked with PL (3 ng/ml) obtained with column switching, monitored by detector B. Peak 1 = PL.

alkyl sulphonates ($C_nH_{2n+1}SO_3Na$) as the ion-pair reagent in MP1 indicated that SOS ($n=8$) was the most suitable for the present study, on the basis of the selectivity and the total analysis time. Fig. 2 shows a typical chromatogram of drug-free plasma obtained on C1 with MP1 without column switching. Some interferences were observed at the retention time of PL. Further, the peak height of PL obtained on C1 with MP1 (capacity factor $k'=8.5$) was only about a quarter of that obtained on C2 with MP2 ($k'=2.3$), with the delay of the elution by SOS. Therefore, high sensitivity was not obtainable on C1 alone, without column switching.

On the other hand, the peak height of PL obtained after column switching was almost the same as that obtained by C2 with MP2 without column switching. This suggests that the difference of the elution ability between MP1 containing SOS and MP2 containing no ion-pair reagent was enough to enrich the analyte on the top of C2 after the introduction of the eluate from C1, and resulted in no peak broadening after column switching. In addition to no peak broadening, no interferences were observed at the retention time of PL after column switching (Fig. 3).

The calibration graph was obtained by analysing spiked plasma samples over the PL concentration range 0.3–40 ng/ml. The least-squares regression fit showed good linearity passing through the origin (correlation coefficient=0.999). The mean recovery of PL from spiked plasma samples was 101.7%, with a coefficient of variation of 3.6% (3 ng/ml, $n=5$). Though the use of an internal standard for quantification of analytes is preferable, it is in principle difficult to find a suitable one for the proposed heart-cut column-switching method. However, repeated extraction from plasma and the use of an autoinjector with a good reproducibility offered satisfactory recovery with only small variation without an internal standard. The lower detection limit was 0.06 ng/ml in plasma at a signal-to-noise ratio of 3.

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