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Short communication

Selective extraction of β-blockers from biological fluids by column-switching high-performance liquid chromatography using an internal-surface phenylboronic acid precolumn

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Abstract

A column-switching HPLC method using an internal-surface phenylboronic acid precolumn for the selective extraction of β-blockers from biological fluids has been developed. Filtered urine and plasma samples (50 μl) were injected onto the precolumn equilibrated with methanol–0.05 *M* disodium hydrogenphosphate (5:95, v/v). After the precolumn had been washed briefly, the selectively retained β-blockers were eluted with methanol–0.05 *M* phosphate buffer (pH 2.0) and transferred to a reversed-phase analytical column, on which they were then separated. Even after exposure to at least 160 injections of non-treated urine and plasma samples, the retention efficiency of the precolumn was maintained with no increase in back pressure. Quantitative recoveries and good reproducibility were demonstrated with pindolol.

1. Introduction

Recently, direct injection techniques such as micellar chromatography, restricted-access stationary phases (RASPs) and column switching have been finding increasing application in high-performance liquid chromatography (HPLC) for the determination of drugs in biological samples because they combine shorter analysis times with high reproducibility. Of these techniques, micellar chromatography is easy to perform, but has disadvantages such as low column efficiency and relatively poor peak symmetry [1,2]. The use of RASPs has attracted the most attention, and a number of phases have been developed [3–10]. However, the separation of hydrophilic drugs from biological components is not sufficient with the commercially available reversed-phase type RASPs, because the capacity factors of analytes on these columns are much lower than those on conventional reversed-phase columns [11]. A further problem is that the requirement to both remove plasma proteins without causing precipitation in the RASP columns and at the same time elute hydrophobic drugs leads to contradictory variations in the composition of the organic modifier [12]. In addition, the separation of drugs from endogenous low-molecular-mass components may in some cases be unsatisfactory, because size-exclusion and reversed-phase partition are the main modes of their retention in

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most RASP columns. This is a serious problem at low drug concentrations.

The above disadvantages can be overcome by using a column-switching technique in which highly selective RASPs are used as precolumns. So far, an internal-surface phenylboronic acid (ISPBA) precolumn [13] and a copper phthalocyanine trisulphonic acid-immobilized porous-glass precolumn [14] have been developed for the determination of catecholamines and 1-hydroxypyrene, respectively.

It has been reported that, in the determination of β-blockers by gas chromatography, their hydroxypropylamino or hydroxyethylamino moieties form cyclic boronates [15], and recently Martin et al. found that several β-blockers were retained on a solid-phase extraction cartridge containing phenylboronic acids [16]. In the study described in this paper, we have developed a method for the selective extraction of β-blockers from urine and plasma using an ISPBA precolumn.

2. Experimental

2.1. Chemicals

β-Blockers and the other drugs were obtained from Sigma (St. Louis, MO, USA), Wako Pure Chemicals Industries (Osaka, Japan) and Aldrich (Milwaukee, WI, USA). Methanol was of HPLC grade, and other chemicals were of analytical-reagent grade. Deionized water (obtained with a Millipore RX-Q system) was used throughout.

Stock solutions (10 m*M*) of the β-blockers were prepared in methanol, and diluted with water to the desired concentrations. The stock solutions were stable in the dark at 4°C for at least six months.

The experimental product of ISPBA precolumn was kindly provided by Yokogawa Analytical Systems (Tokyo, Japan). The gel was prepared by coupling carboxymethylcellulose to the external surface of glycidoxymethacrylate gel, followed by coupling *m*-aminophenylboronic acid to its internal surface [13]. The amount of *m*-aminophenylboronic acid immobilized, determined by elemental analysis of nitrogen, was about 0.9 mmol/g.

2.2. Column-switching HPLC procedure

The column-switching HPLC system consisted of a Model NP-S-464 pump (Nihon Seimitsu Kagaku, Tokyo, Japan), a Model LC-6A pump (Shimadzu, Kyoto, Japan), a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model E1E 002 six-port switching valve (Senshu Kagaku, Tokyo, Japan), a Model SPD-6A UV detector (Shimadzu), a Model C-R6A integrator (Shimadzu), an ISPBA precolumn (10 × 4.6 mm I.D.) (C1) and an analytical column, Capcell Pak C_{18} SG120 (150 × 4.6 mm I.D.) (Shiseido, Tokyo, Japan) (C2). Methanol–0.05 *M* disodium hydrogenphosphate (5:95, v/v) (S1) was delivered at 1 ml/min for sample loading. After injection of the sample (50 μl), C1 was washed with S1 for 5 min, and then the valve was changed. Methanol–0.05 *M* phosphate buffer (pH 2.0) (different compositions in the range 20:80–50:50, v/v) (S2) was delivered at 1 ml/min for back-flushing of the components retained on C1 and for successive isocratic elution from C2. For the next run, the valve was changed, and C1 was re-equilibrated with S1 for at least 5 min.

The wavelengths of the UV detector were selected in the range 255–305 nm depending on the analytes. For the detection of pindolol in plasma a Model 820-FP fluorescence detector (JASCO, Tokyo, Japan) was operated at 255 nm excitation and 315 nm emission.

The percentage retention of each drug on the ISPBA precolumn was calculated by the method described previously [17].

2.3. Preparation of urine and plasma samples

Pindolol was dissolved in human urine and plasma at a known concentration. The spiked urine and plasma were filtered through a Dismic-25CS filter (0.45 μm, Advantec, Tokyo) under positive pressure. A portion of the urine or plasma filtrate (50 μl or 20 μl, respectively) was injected directly into the column-switching HPLC system.

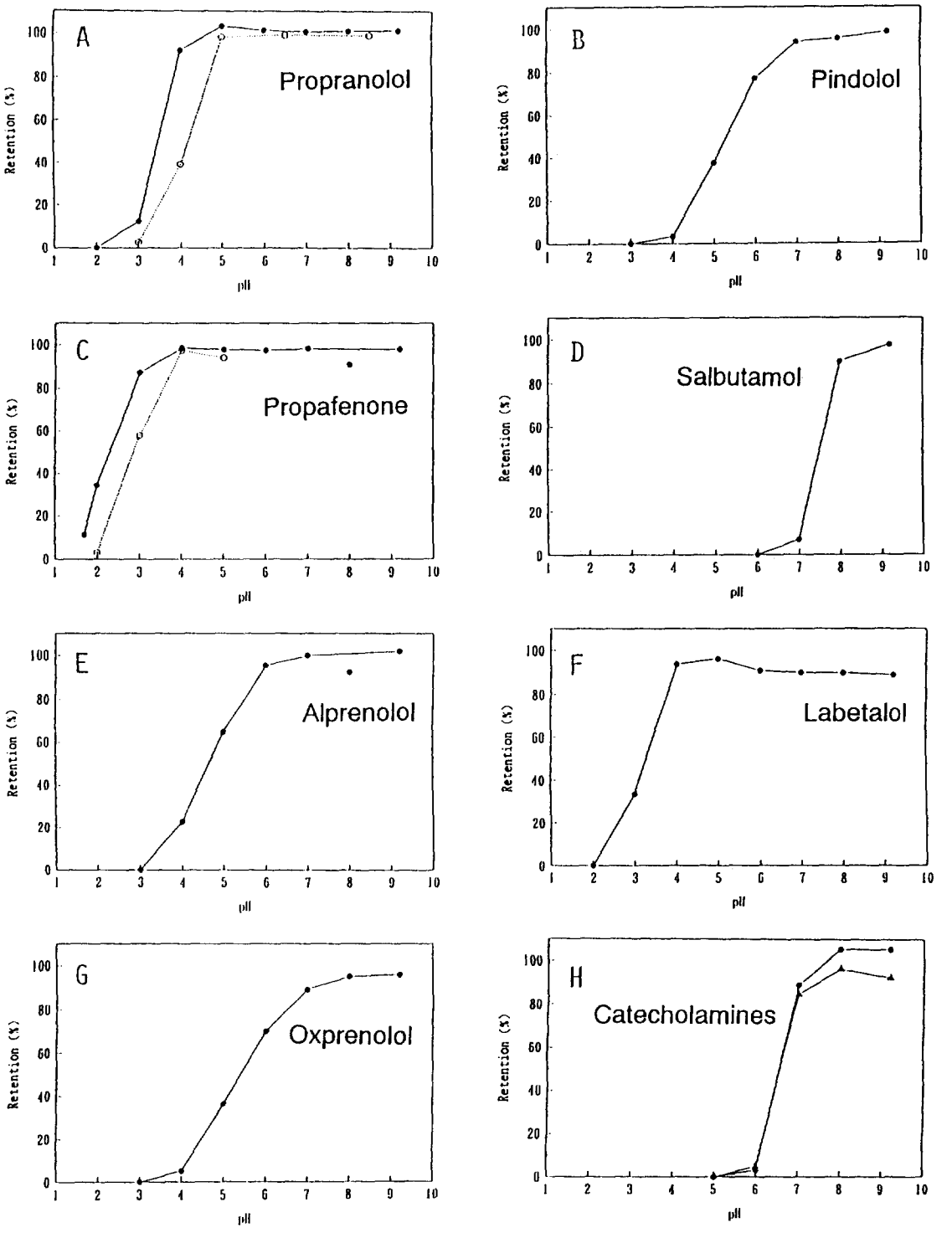

Fig. 1. Retention of β-blockers on the ISPBA precolumn as a function of pH. (A) Propranolol. (B) Pindolol. (C) Propafenone. (D) Salbutamol. (E) Alprenolol. (F) Labetalol. (G) Oxprenolol. (H) Dopamine (●) and epinephrine(▲). The dotted lines in (A) and (C) were obtained with the buffers containing 10% methanol.

3. Results and discussion

The pH dependence of the retention of various β-blockers by the ISPBA precolumn was investigated (Fig. 1A–G). For comparison, the retention behaviour of catecholamines such as dopamine and epinephrine was also examined under the same conditions (Fig. 1H). In contrast to the catecholamines, all the β-blockers except salbutamol were retained, even in the acidic pH region. In particular, propranolol, propafenone and labetalol were retained and recovered almost quantitatively down to pH 4 (Fig. 1A, C, F). However, hydrophobic interaction of these compounds with the precolumn matrix is not a main cause of their retention, as exemplified by propranolol and propafenone (Fig. 1A, C). The addition of methanol to the buffers shifted the curves to a slightly higher pH region, but quantitative retentions were still observed at around pH 5.

These β-blockers were not retained on the gel prepared by treating carboxymethylcellulose-coated glycidoxymethacrylate gel with tris(hydroxymethyl)aminomethane instead of *m*-aminophenyl boric acid. This indicates that their retention on the ISPBA precolumn is attributable to the specific interaction with internal-surface phenylboronic acid groups.

The difference in profile of the pH dependence curve between β-blockers could not be explained by differences in their pK_a and structure, whether they were classified as aryloxypropanolamines or arylethanolamines. However, the similarity of the profile between salbutamol (Fig. 1D) and catecholamines (Fig. 1H) may be explained by the fact that, in salbutamol, it is the 1,3-diol moiety, and not the hydroxyamino moiety (which may possibly be affected by adjacent bulky *tert.*-butyl groups), which forms the cyclic boronate.

To permit direct injection of biological samples, the ISPBA precolumn must be capable of removing endogenous components with high efficiency. The effects of the pH of the sample-loading solvent on the removal of urinary and plasma components from the precolumn, and on the analytical-column elution profile of the components which could not be removed from the precolumn, were examined. About 84% (plasma) and ≥90% (urine) of the UV-absorbing components were removed at pH 7 or above (measurements at 255 nm and 280 nm), and elution of the retained urine components from the analytical column was accelerated with an increase in pH. From these results and the pH dependency of β-blocker retention shown above, we decided to use the solvent with a pH of 9.2 (0.05 *M* disodium hydrogenphosphate).

A calibration graph for aqueous pindolol standards was constructed under the optimized column-switching HPLC conditions. Both peak area and peak height were proportional to the pindolol concentration in the range 0.5–100 nmol/ml: $r = 0.9998$, $y = 11703x - 5055$ (peak area). The slope of the equation was almost equal to that obtained by the conventional HPLC of pindolol ($y = 11697x - 1057$), indicating that it was retained on the precolumn quantitatively in this range.

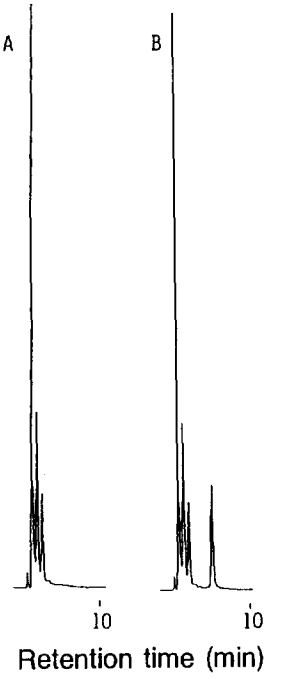

Fig. 2. Column-switching HPLC chromatograms of urine samples. A, Control urine; B, pindolol-spiked urine (40 nmol/ml). Detection 255 nm; solvent S2, methanol–0.05 *M* phosphate buffer (pH 2.0) (20:80, v/v).

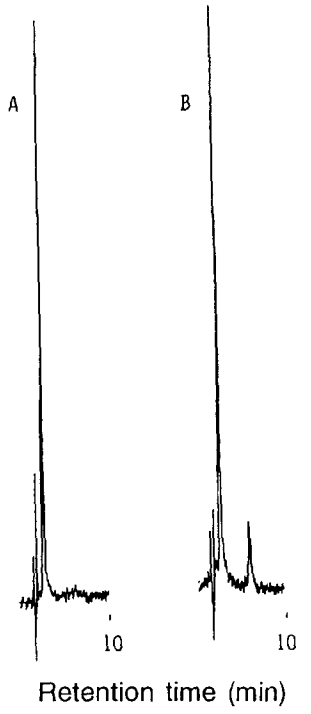

Fig. 3. Column-switching HPLC chromatograms of plasma samples. A, Control plasma; B, pindolol-spiked plasma (0.1 nmol/ml). Fluorescence detection, 255 nm (excitation), 315 nm (emission); solvent S2 as for Fig. 2.

The selectivity of the ISPBA precolumn to various compounds was examined under the optimized column-switching conditions. All the β-blockers were retained almost quantitatively, while no retention was observed for acetaminophen, caffeine, furosemide, hydrochlorothiazide, nalidixic acid, norfloxacin, pipemidic acid, phenylbutazone, salicylic acid, theophylline, tolubutamide and walfarin.

Fig. 2 shows the effectiveness of the ISPBA precolumn in the direct-injection determination of pindolol in urine. The interfering endogenous components were removed effectively, while pindolol was selectively retained. Plasma samples were also injected successfully without any pretreatment. Although the fluorescence detection was carried out at relatively short wavelength (excitation 255 nm, emission 315 nm) to monitor plasma levels, pindolol was detected without interference (Fig. 3). The recovery test for urine and plasma samples indicated that pindolol was recovered in good yield and the reproducibility was also satisfactory (Table 1).

The retention efficiency of the ISPBA precolumn was maintained without any gradual increase in back pressure after exposure to ≥4 ml each of urine and plasma samples (ca. 160 assays).

In conclusion, the ISPBA precolumn was used with satisfactory stability for the selective on-line extraction of β-blockers from biological samples. Various pretreatment methods such as liquid–liquid [18,19] solid-phase [16,20,21] and immunoaffinity [22] extractions have been developed for the HPLC determination of β-blockers in biological fluids. A solid-phase extraction method incorporating cyanopropyl-bonded silica has been used in combination with a column-switching system [23] and with an automatic system of sample pretreatment by extraction cartridges [24]. Like the other methods cited, the present

Table 1
Recovery of pindolol added to urine and plasma

Sample	Concentration added (nmol/ml)	Concentration assayed (nmol/ml)	R.S.D. ($n = 5$) (%)
Urine A	4.0	3.9	3.3
Urine B	40.0	38.5	2.8
Urine C	40.0	39.1	2.6
Plasma A	0.1	0.098	3.2[a]
	0.2	0.204	2.0[a]
Plasma B	0.1	0.102	1.7[a]

[a] $n = 4$.

method incorporating the ISPBA precolumn will probably prove useful for the direct-injection determination of β-blockers in biological fluids because of its simplicity and relatively high selectivity.

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