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Short communication

Solid-phase extraction on Styrosorb cartridges as a sample pretreatment method in the stereoselective analysis of propranolol in human serum

Dmitrii O. Rumiantsev*, Tatiana V. Ivanova

Laboratory of Pharmacokinetics, National Research Center for Preventive Medicine of the Public Health Ministry of Russia, Petroverigskii Lane 10, Moscow 101953, Russia

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Abstract

Sample pretreatment using solid-phase extraction (SPE) on cartridges filled with small-particle Styrosorb porous polystyrene-based sorbent has been used in the analysis of propranolol enantiomers in human serum by high-performance liquid chromatography (HPLC) with fluorescent detection. SPE on Sep-Pak C_{18} cartridges was used as a reference pretreatment method. The propranolol content of the samples was determined by achiral normal-phase HPLC and the enantiomeric ratio of propranolol (*S*/*R*) was then determined by chiral HPLC on a column with silica-bonded cellulose-tris(3,5-dimethylphenyl carbamate). Recoveries of propranolol from serum using SPE on Styrosorb and C_{18} phases were $97 \pm 5\%$ and $96 \pm 5\%$, respectively. Detection and quantification limits for propranolol enantiomers were 4 and 7 ng/ml, respectively.

1. Introduction

The accuracy and precision of a drug assay in biological material depend upon both sample preparation and instrument performance, but it is often the former that is the more laborious and less reliable part of the procedure. The classical method of sample preparation in drug assay in biological material is liquid–liquid extraction. A more advanced sample preparation technique is solid-phase extraction (SPE), which can be used in either manual or automatic mode. Most commercially available SPE cartridges are filled with bonded silica sorbents [1]. Less expensive cartridges filled with small-particle organic resins are also available [2].

Some years ago, a new type of porous hypercrosslinked sorbents derived from polystyrene, known as Styrosorb, were described [3]. The Styrosorbs were obtained by introducing a large number of rigid bridges between linear polystyrene chains. The crosslinking procedure results in a homogeneous network, which is highly porous. The inner surfaces of the sorbent beads are easily accessible to both small and large organic molecules due to a cooperative conformational rearrangement of the network under the influence of the sorbate molecules. Styrosorbs extract non-polar organic compounds efficiently from aqueous and polar organic media. It was shown that the sorption capacity of

* Corresponding author.

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Styrosorb is higher than that of well-known polystyrene-based sorbents of gel-like (e.g. Dowex-50) and macroporous (e.g. Amberlyte XAD-2, XAD-4) structure [3]. For this reason it seems reasonable to use Styrosorb for SPE. We employed cartridges for SPE filled with small-particle Styrosorb for the isolation of the β-adrenoblocker propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol hydrochloride] from human serum in stereoselective HPLC analysis. Propranolol was chosen as it is a typical highly lipophilic chiral drug that is used clinically as the racemic mixture of *R* and *S* enantiomers whose pharmacodynamic and pharmacokinetic properties differ substantially [4].

The most suitable columns for the direct stereoselective HPLC assay of propranolol and some other β-adrenoblockers are columns containing silica-bonded cellulose-tris(3,5-dimethylphenyl carbamate) [5–9], commercially available as Chiralcel-OD (Diacel Chemical Industries, Tokyo, Japan). Direct separation of propranolol enantiomers using ligand-exchange chiral stationary phases [10], Pirkle-type phases [11,12] or β-cyclodextrin phases [13] has the drawback of relatively low resolution. Columns on the basis of immobilized α-1-acid glycoprotein [14] are more efficient but lose resolution rapidly when biologically based samples are injected.

There are also several stereoselective HPLC methods which involve derivatization of propranolol enantiomers with chiral reagents [11, 15–20]. The diastereomers produced by these methods can be resolved by achiral HPLC, but sample preparation can be rather laborious. Further, a critical factor in these methods is the optical purity of the chiral reagent used.

In this paper we demonstrate the possibility of using SPE cartridges filled with Styrosorb for the isolation of propranolol from human serum in the stereoselective analysis of propranolol by HPLC on a Chiralcel-OD column with fluorimetric detection. The isolation of propranolol enantiomers from serum by SPE on Sep-Pak C_{18} cartridges described recently [20] was used as a reference sample pretreatment method.

2. Experimental

2.1. Reagents

Heptane, hexane, methanol, 2-propanol, diethylamine (all supplied by E. Merck, Darmstadt, Germany) were used as purchased. The water was purified by means of the Milli RO4/Milli Q system (Millipore, Bedford, MA, USA). Racemic propranolol hydrochloride was supplied by Zdorovie (Kharkov, Ukraine), racemic metoprolol by Slovakopharma (Bratislava, Slovakia). Samples of optically pure *R*-propranolol and *S*-propranolol hydrochlorides were a gift from Dr. W. Lindner (Karl-Franzens University of Graz, Graz, Austria). All glassware was silanized before use with Surfasil (Pierce, Rockford, IL, USA) diluted 1:10 with hexane.

2.2. Apparatus

The HPLC system consisted of a Model B-100-S2 pump (Eldex Labs., Menlo Park, CA, USA), a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model GM-970 fluorimetric detector (Kratos, Westwood, NJ, USA) and a Model 3390-A reporting integrator (Hewlett Packard, Avondale, PA, USA). The HPLC analytical columns were a Silasorb-NH_2 column, 250 × 4.0 mm, particle size 10 μm (Elsico, Moscow, Russia) with a 2-μm precolumn filter, and a Chiralcel-OD column, 250 × 4.6 mm, particle size 10 μm (Daicel Chemical Industries, Tokyo, Japan) with a precolumn (50 × 4.6 mm) packed with the same sorbent.

2.3. Cartridges

Cartridges filled with 15 mg Styrosorb (particle size 10–50 μm, average pore diameter 1.0–1.5 nm) were prepared by us (in cooperation with Biochrom, Moscow, Russia). The cartridges were made of polypropylene and were similar in design to those of Bond Elut cartridges. The Sep-Pak C_{18} cartridges were purchased from Waters Assoc. (Milford, MA, USA).

2.4. Samples

Stock solutions of propranolol and metoprolol were prepared in water at concentrations of 10 mg/ml and 25 mg/ml, respectively. Blood was drawn from the veins of healthy volunteers via an indwelling catheter. Blood was allowed to clot in glass tubes at room temperature for 1 h and then centrifuged at 1000 *g* for 20 min to separate the serum. Three pools of serum were gathered. Five spiked serum samples containing 25, 50, 200, 600 and 1000 ng/ml of racemic propranolol were prepared using serum from every pool. Metoprolol at a concentration of 2500 ng/ml was added to the samples as an internal standard. The samples were stored frozen at −20°C until analysis.

2.5. Procedures

Solid-phase extraction

The extraction cartridges (both Styrosorb and Sep-Pak C_{18}) were washed twice with 3 ml methanol and then twice with 3 ml water. In all instances the liquids were passed through the cartridges with the aid of centrifugation for 3 min at 500 *g*. The cartridges were not allowed to dry before the addition of the serum. The serum samples (1.0 ml) were passed through the cartridges and discarded. The serum interferences were removed by washing the cartridges twice with 3 ml water. Finally, the substances were eluted from the cartridges with 0.6 ml methanol containing 0.3% (w/w) diethylamine. The eluents were evaporated to dryness at 40°C under a gentle stream of air and reconstituted in 50 μl of mobile phase 1, and 20-μl aliquots were analysed by achiral HPLC.

Chromatography

The columns were kept at ambient temperature. In all cases the excitation wavelength of the fluorimetric detector was set at 220 nm and a 320 nm cut-off emission filter was employed.

Achiral chromatography was performed on a Silasorb-NH_2 column. Mobile phase 1 consisted of *n*-heptane–2-propanol–methanol (83:13:4, v/v) pumped at a flow-rate of 2.5 ml/min. The propranolol-containing eluates were collected, and after evaporation of the eluent were dissolved in 30 μl of mobile phase 2. An aliquot of the solution (10–20 μl) was injected onto the chiral column.

Chiral chromatography was performed on a Chiralcel-OD column. Mobile phase 2 consisted of *n*-heptane–2-propanol–methanol (77:8:15, v/v/v) pumped at a flow-rate of 1.3 ml/min. The elution order of *R*-propranolol and *S*-propranolol was confirmed via direct injection of the individual enantiomers. The enantiomeric ratios of *S*-propranolol to *R*-propranolol in the samples were determined on the basis of their peak areas on the chromatograms.

3. Results and discussion

Under the mobile phase conditions given, the respective retention times of racemic propranolol and the internal standard on the achiral column were 3.4 min and 4.1 min, while the retention times of *R*-propranolol and *S*-propranolol on the

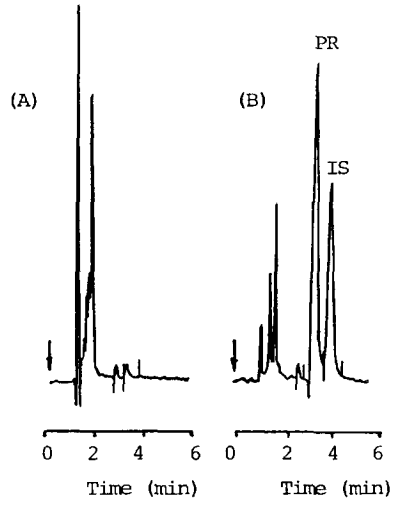

Fig. 1. Representative chromatograms on the Silasorb-NH_2 achiral column of (A) blank and (B) plasma sample spiked with propranolol (PR; 200 ng/ml) and internal standard (I.S.; 2500 ng/ml) after SPE on Styrosorb cartridges. The chromatographic conditions are described in the text.

Table 1
Degree of extraction of propranolol and metoprolol from serum by SPE on Styrosorb and reversed-phase C_{18} cartridges

Substance	Concentration (ng/ml)	Extraction (%)	
		Styrosorb (mean ± S.D.)	Sep-Pak C_{18} (mean ± S.D.)
Propranolol	25	97 ± 6	98 ± 6
	50	98 ± 5	96 ± 5
	200	99 ± 4	94 ± 7
	600	96 ± 6	95 ± 5
	1000	94 ± 4	96 ± 4
Metoprolol	2500	98 ± 6	95 ± 4

$P < 0.05$; $n = 10$ for all studies.

chiral column were 6.8 min and 7.9 min, respectively. Representative chromatograms shown in Figs. 1 and 2 demonstrate that endogenous compounds did not interfere with any of the peaks of interest. The mean selectivity factor α for the propranolol enantiomers was 1.23 and the corresponding resolution factor R was 1.72. Under the conditions chosen, the R- and S-enantiomers of metoprolol can also be separated, retention times being 3.5 min and 4.6 min, respectively. More than 800 injections onto the chiral column were performed without any loss of resolution. In all cases the recovery of propranolol and internal standard from the serum by both extraction methods employed was ⩾94% (Table 1). The data confirming the stability of the isomeric ratio of propranolol during the extraction are presented in Table 2.

A linear correlation was found between the peak-area ratio of propranolol and the internal standard in the concentration range of 25–1000 ng/ml propranolol in serum. The correlation coefficients were 0.999 for SPE on Styrosorb and 0.998 for SPE on Sep-Pak C_{18}. Both extraction procedures were suitable for the analysis of propranolol enantiomers in serum down to concentrations of about 4 ng/ml (signal-to-noise ratio = 3:1). The quantification limit for propran-

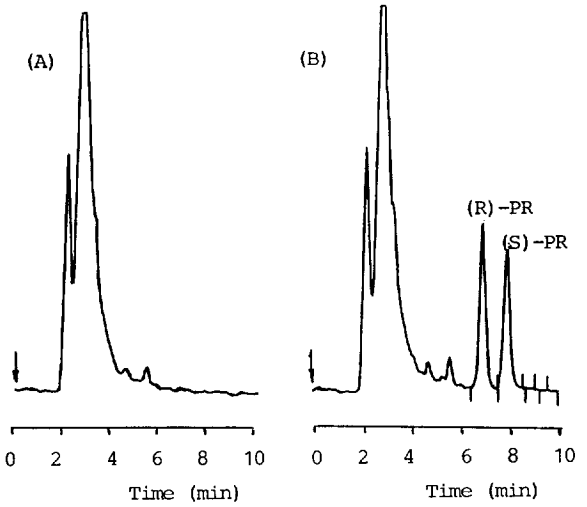

Fig. 2. Representative chromatograms of propranolol (PR) enantiomers on the Chiralcel-OD chiral column of (A) the blank and (B) plasma sample spiked with racemic propranolol (200 ng/ml) after SPE on the Styrosorb cartridges and quantitative analysis on the achiral column. The chromatographic conditions are described in the text.

Table 2
Enantiomeric ratios of propranolol (S/R) in samples after SPE on Styrosorb and reversed-phase C_{18} cartridges

Concentration (ng/ml)	Enantiomeric ratio	
	Styrosorb (mean ± S.D.)	Sep-Pak C_{18} (mean ± S.D.)
25	1.00 ± 0.05	1.01 ± 0.06
50	0.98 ± 0.06	0.99 ± 0.05
200	0.96 ± 0.05	1.00 ± 0.04
600	0.95 ± 0.02	0.97 ± 0.02
1000	0.95 ± 0.01	0.97 ± 0.02

$P < 0.05$; $n = 10$ for all studies.

Table 3
Reproducibility of the assay for propranolol in serum using SPE on Styrosorb and reversed-phase C_{18} cartridges

Concentration (ng/ml)	Inter-assay C.V. (%)		Intra-assay C.V. (%)	
	Styrosorb	C_{18}	Styrosorb	C_{18}
25	0.14	0.07	0.62	0.25
50	0.36	0.33	2.10	2.50
200	0.03	0.16	1.58	2.29
600	2.12	0.57	2.13	2.53
1000	0.04	1.20	2.92	1.60

Inter-assay C.V. = between-day coefficient of variation. Intra-assay C.V. = within-day coefficient of variation. $P < 0.05$; $n = 18$ for all concentrations.

olol enantiomers was found to be 7 ng/ml. A lower limit of sensitivity can be achieved by increasing the sample volume.

The reproducibility of the method was evaluated by calculating the intra-day and inter-day coefficients of variation at five propranolol concentrations ranging from 25 to 1000 ng/ml. The results are given in Table 3.

The sensitivity and the reproducibility of the assay on the basis of serum sample pretreatment using SPE on Styrosorb and on Sep-Pak C_{18} cartridges were comparable; the advantage of Styrosorb cartridges is that they are significantly less expensive. We used the method in the manual mode, but it could easily be automated using one of the achiral/chiral coupled-column systems described elsewhere [21,22]. The problems of column switching are minimal as both achiral and chiral columns operate in direct-phase chromatographic conditions and the mobile phase contents differ only in quantity. The possibility of using the Styrosorb cartridges for the isolation of several drugs from human serum is under investigation.

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