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Note

Ion-exchange high-performance liquid chromatography in drug assay in biological fluids

III. Propranolol, nadolol and prazosin

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The successful application of cation exchangers for the high-performance liquid chromatographic (HPLC) determination of ethmozin [1] and verapamil [2] in biological fluids has been described previously. In the present work we use such sorbents to assay three other cardiovascular drugs: propranolol (a widely used β-adrenoceptor blocker), nadolol (a new β-adrenoceptor blocker) [3] and prazosin (a relatively new antihypertensive agent) [4]. There are several methods for propranolol assay in biological fluids using HPLC [5—13], mainly on reversed-phase columns. For nadolol several methods are known: fluorimetry [14], gas chromatography—mass spectrometry [15], HPLC with electrochemical detection [16] and thin-layer chromatography [17]. Some methods for prazosin determination in plasma based on HPLC have also been described [18—22].

We present here a new method applicable to all three drugs using nearly identical chromatographic conditions. With previously published methods for ethmozin [1] and verapamil [2] we now have a universal assay method for five cardiovascular drugs based on cation-exchange HPLC. With fluorescence detection our method provides sensitivity high enough for pharmacokinetic purposes. The extraction procedure does not consume much time.

EXPERIMENTAL

Apparatus and columns

Altex 110A pump with Model 210 injection valve (Altex Scientific,

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Berkeley, CA, U.S.A.) and Model FS-970 fluorescence detector (Schoeffel, Westwood, NJ, U.S.A.) were used. The column (250 × 4.6 mm I.D.) was packed with Partisil 10-SCX (10-μm particle size) from Altex Scientific. The precolumn (40 × 3.2 mm I.D.) was packed by us with the same sorbent. The recorder used was Omniscribe model B5217 (Houston Instrument, Austin, TX, U.S.A.) with 10 mV full scale deflection.

Reagents and standards

The mobile phase was prepared from acetonitrile (LiChrosolv®; E. Merck, Darmstadt, F.R.G.), double-distilled water, diethylamine (pure grade; E. Merck) and orthophosphoric acid (chemical pure grade; Reachim, U.S.S.R.). Amyl alcohol and diethyl ether of pure grade (Reachim) were washed with 0.1 mol/l sulphuric acid and distilled. Pentane of pure grade, potassium hydroxide, sulphuric acid of analytical grade (all from Reachim) were used as received. The glassware was siliconized with AquaSil (Pierce, Rockford, IL, U.A.A.).

A 5% solution of propranolol (Germed, G.D.R.) was used as a standard for this drug. Nadolol and prazosin standards were kindly supplied by Squibb (U.K.) and Orion Pharmaceutica (Finland), respectively. Glaucin (4,5,7,8-tetramethylaporphine hydrochloride; Tatchimpharmpreparaty, Kasan, U.S.S.R.) was used as an internal standard. All standard solutions were prepared in double-distilled water and stored at 4°C.

Extraction procedure

Prazosin. The sample of serum, saliva or urine (1 ml) was placed into the glass-stoppered Pyrex tube, and 0.1 ml of 1 μg/ml glaucin solution was added. The sample was alkalinized with 0.2 ml of 2 mol/l potassium hydroxide and was extracted with 5 ml of diethyl ether for 1 min using a vortex mixer. After centrifutation at 500 *g* for 10 min, the upper layer was transferred into a conical tube and extracted with 0.1 ml of 0.05 mol/l sulphuric acid for 1 min. After brief centrifugation an aliquot of a lower acidic layer was injected onto the column.

Propranolol and nadolol. To the 1-ml sample of biological fluid, 0.1 ml of glaucin solution (50 ng/ml in the case of serum and saliva, and 1 μg/ml in the case of urine) was added followed by 0.1 ml of 1 mol/l potassium hydroxide and 7 ml of pentane—amyl alcohol mixture, (19:1) and (4:1) for propranolol and nadolol, respectively. The tube was vortexed for 20 sec and centrifuged at 500 *g* for 2 min. The upper layer was transferred into a conical tube and extracted with 0.3 ml of 0.05 mol/l sulphuric acid for 20 sec. After centrifugation at 500 *g* for 2 min, an aliquot of the lower layer was injected onto the column.

Chromatographic conditions

The mobile phase was acetonitrile—water—diethylamine—85% orthophosphoric acid (20:80:0.2:0.15, v/v) and was degassed under vacuo before being used. The flow-rate was 2 ml/min, and the column temperature was ambient. Excitation wavelengths and emission filter data are presented in Table I. Detector sensitivity was set at 3.0, and the time constant was 0.5 sec.

TABLE I

RETENTION TIMES OF DRUGS AND THE INTERNAL STANDARD; EXCITATION AND EMISSION WAVELENGTHS FOR THE FLUORESCENCE DETECTION

Drug	Retention time (min)	Excitation wavelength (nm)	Emission filter
Propranolol	4.8	225	Without filter
Nadolol	6.0	205	Without filter
Prazosin	6.2	246	370 cut-off
Glaucin	9.5	—	—

Quantitation

The internal standard method was used for the determination of the drug concentrations in serum, saliva and urine. Peak height ratios of drug to glaucin were plotted versus the concentrations of drug added to the blank samples of biological fluids, and calibration graphs for propranolol and prazosin in serum, saliva and urine, and for nadolol in serum and urine, were thus obtained. The recovery was estimated as a ratio of the peak heights produced by the same amount of each drug after the analysis of the extract of the biological fluid spiked with it and of the standard solution.

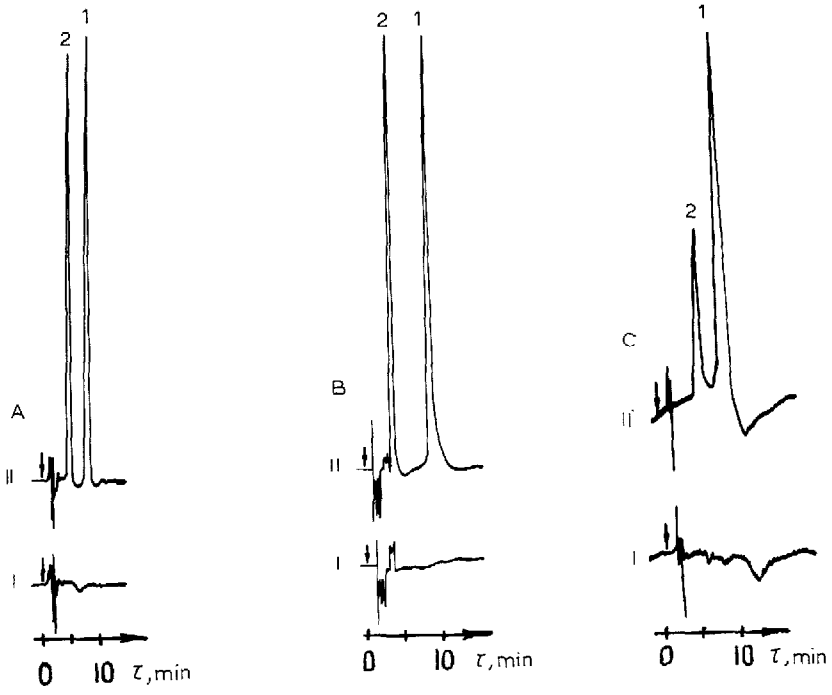

Fig. 1. Chromatograms of the extracts of biological fluid samples taken from patients before (I) and after (II) drug administration. A, Propranolol in serum. Peaks: 1 = glaucin (internal standard); 2 = propranolol (75 ng/ml found). Sensitivity range, 0.1 a.u.f.s. B, Nadolol in urine. Peaks: 1 = glaucin; 2 = nadolol (800 ng/ml found). Sensitivity range, 0.5 a.u.f.s. C, Prazosin in saliva. Peaks: 1 = glaucin; 2 = prazosin (10 ng/ml found). Sensitivity range, 0.05 a.u.f.s.

RESULTS AND DISCUSSION

The conditions described provided a rapid background separation of the drugs and internal standard. In Table I the retention time data are collected. Fig. 1 illustrates the determination of propranolol in serum (A), nadolol in urine (B) and prazosin in saliva (C) of patients treated with these drugs. In each case, the chromatogram marked I is a result of the analysis of the same biological fluid extract received before drug administration and shows the absence of interfering peaks.

TABLE II

CALIBRATION GRAPH DATA

Drug	Biological fluid	Concentration range (ng/ml)	Calibration graphs equation*	r
Propranolol	Serum	5—200	$y = 2.24x + 0.03$	0.980
	Urine	100—1000	$y = 2.37x + 0.01$	0.999
	Saliva	10—200	$y = 2.12x + 0.02$	0.985
Nadolol	Serum	20—500	$y = 0.48x + 0.05$	0.981
	Urine	1000—10,000	$y = 0.45x + 0.02$	0.997
Prazosin	Serum	2—50	$y = 6.95x + 0.02$	0.999
	Urine	2—50	$y = 7.48x + 0.03$	0.999
	Saliva	2—50	$y = 6.20x + 0.07$	0.989

*y = peak height ratio of drug to standard; x = drug concentration (ng/ml).

TABLE III

ACCURACY OF THE DRUG DETERMINATION

Drug	Biological fluid	Concentration (ng/ml)	Coefficient of variation (%) (n = 5)
Propranolol	Serum	20	5.7
		100	3.2
	Urine	200	2.1
		700	5.4
	Saliva	20	3.5
		100	3.7
Nadolol	Serum	40	7.3
		300	12.1
	Urine	2000	2.6
		7000	8.1
Prazosin	Serum	2	3.2
		20	1.6
	Urine	2	4.6
		20	1.5
	Saliva	2	12.6
		20	4.6

The minimal detectable levels of drugs are not higher than 1 ng/ml (peak-to-noise ratio 5:1). The recoveries were about 85%, 75%, 60% and 80% for propranolol, nadolol, prazosin and glaucin, respectively. The recovery of prazosin can be made higher by adding the second ether extraction.

In Table II the calibration graphs data are presented. The graphs were linear for all drugs in each biological fluid as was confirmed by the high correlation coefficients. The y-axis intercepts in the regression equations do not significantly differ from zero.

TABLE IV

DRUGS WHICH DO NOT INTERFERE WITH THE DETERMINATION OF PROPRANOLOL, NADOLOL AND PRAZOSIN

Amobarbital sodium	Lidocaine
Ampicillin	Methyldopa
Aspirin	Mexiletin
Atenolol	Nitroglycerine
Caffeine	Oxazepam
Carbamazepine	Phenobarbital
Chlorpropamide	Procainamide
Diazepam	Quinidine
Digoxin	Reserpine
Ephedrine	Sulfamethoxazole
Furosemide	Theophylline
Hydrochlorthiazide	Verapamil*
Isosorbide dinitrate	

*The metabolites of this drug cause interference.

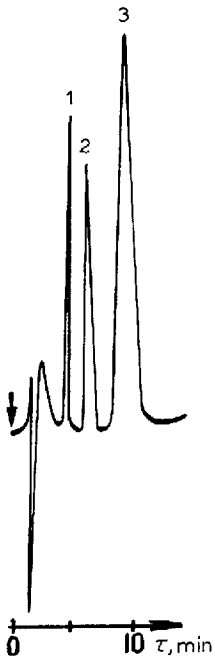

Fig. 2. Chromatogram of the propranolol (peak 1), prazosin (peak 2) and glaucin (peak 3) standard mixture.

The data showing the within-day accuracy of the assay are presented in Table III. They were obtained from five parallel determinations of each drug in each biological fluid at two different levels. The day-to-day variability was higher, so it is preferable to determine a calibration coefficient each day using blank samples of biological fluids.

We have also studied the interference on our assay method of some other commonly used drugs listed in Table IV. It was established that none of them interfered in the determination of propranolol, nadolol or prazosin. Only in the case of verapamil it was found that metabolites of this drug [norverapamil, 2-(3,4-dimethoxyphenyl)-2-propylamino-3-methyl butyronitrile and 2-(3,4-dimethoxyphenyl)-2-isopropyl-6-azaheptanitrile] when present in plasma and urine at high levels after oral administration of verapamil [2] interfered with the peaks of drugs studied here. Intact verapamil did not interfere with them.

Fig. 2 demonstrates the possibility of the co-determination of propranolol and prazosin. Their peaks and that of glacuin are completely separated. However, nadolol interferes with prazosin under these conditions.

Thus, the method presented can assay propranolol, nadolol and prazosin with high sensitivity and accuracy in serum, urine and saliva using the same mobile phase, cation-exchange column and internal standard. It is now being applied with success to the pharmacokinetic study of these drugs.

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