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Note

Determination of alprenolol and metoprolol in plasma by column liquid chromatography

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Alprenolol and metoprolol are β-adrenergic blockers often used in the treatment of hypertension, cardiac arrhythmias and angina pectoris. As the plasma concentrations are proportional to the degree of β-blockage, a sensitive method for assaying these β-blockers in plasma is required. Several methods for the determination of alprenolol in biological fluids, including gas chromatography (GC) [1,2], gas chromatography–mass spectrometry (GC–MS) [3,4] and high-performance liquid chromatography (HPLC) [5–7], have been reported. Similarly for metoprolol, GC [8–16], GC–MS [17,18] and HPLC [7,19–34] methods have been published. All these methods require extensive sample work-up. Here, we report an assay procedure that employs solid-phase extraction for sample preparation. It is a simple, rapid, sensitive, accurate and reproducible method for determining alprenolol and metoprolol using only 100 μl of plasma. This method obviates the commonly used solvent extraction steps and hence extensive sample work-up is not needed.

EXPERIMENTAL

Materials

Acetonitrile (LiChrosolv), dichloromethane for fluorescent spectroscopy and Extrelut-1 columns were obtained from E. Merck (Darmstadt, F.R.G.). Ortho-

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phosphoric acid (85%, density = 1.71 g/ml), potassium dihydrogenphosphate, sodium hydroxide and triethylamine were of analytical-reagent grade (Merck). Alprenolol hydrochloride (AH) and metoprolol tartrate (MT) were received as gifts from Hässle (Mölndal, Sweden) and Astra-IDL (Bangalore, India), respectively, and were used as received. The internal standard (I.S.), 1-[2-(2-carbamoyl-4-methylphenoxy)ethylamino]-3-(2-methylphenoxy)propan-2-ol (UK-6633) was supplied by Pfizer (Sandwich, U.K.) and used as received.

Preparation of mobile phase and standard solutions

The mobile phase was prepared by adding 85% orthophosphoric acid dropwise to 1 l of 0.03 *M* potassium dihydrogenphosphate so as to adjust the pH to 2.2. This phosphate buffer (500 ml) was mixed with acetonitrile (450 ml in the determination of alprenolol and 500 ml for that of metoprolol). Triethylamine (200 μl) was then added to 1 l of this solution and mixed well. The solution was filtered through a 0.5-μm Millipore filter and degassed in an ultrasonic bath before use. Stock solutions (100 μg/ml) of AH, MT and I.S. were prepared in distilled water; working standard solutions (500 ng/ml) were prepared daily.

Extraction procedure

Solid-phase extraction was employed using Extrelut-1 columns. A 100-μl volume of blank plasma, known amounts of drug (AH or MT) and 100 μl of internal standard solution were added successively to the Extrelut-1 column, then 1 *M* sodium hydroxide solution (ca. 1.0 ml) was added to make up the volume to 1.25 ml and the column was kept aside for impregnation (ca. 15 min). The drug was then eluted from the Extrelut-1 column with dichloromethane (3×3 ml). The organic phase was evaporated using an SC-3 solvent concentrator at 35 °C under a stream of nitrogen. The residue was dissolved in 120 μl of the mobile phase and 50 μl of the solution were injected into the chromatographic system.

Apparatus

The liquid chromatograph consisted of an Altex (Berkeley, CA, U.S.A.) Model 100A pump with a Schoeffel FS-970 fluorimetric detector (Kratos, Westwood, NJ, U.S.A.), operated at a time constant of 2 s, range 0.2/0.2, excitation wavelength 195 nm and a Cs-7-54 band filter (maximum ca. 320 nm) for emitted light. Chromatograms were recorded with a C-R1A Chromapac recording integrator (Shimadzu, Kyoto, Japan). A 20 mm×4.6 mm I.D. precolumn of Supelgel LC-18-DB, 5-μm, Supelcosil (Supelco, Bellefonte, PA, U.S.A.) was attached before a 15 cm×4.6 mm I.D. Spherisorb 3 ODS (particle size 3 μm) column (HPLC Technology, Macclesfield, U.K.). The mobile phase was pumped at a rate of 1 ml/min and the operating pressure was ca. 160 bar. The column was maintained at room temperature (ca. 20 °C).

Calibration and reproducibility

Calibration graphs were obtained by adding known concentrations of drug (equivalent to 22–438 ng/ml alprenolol base or 13–320 ng/ml metoprolol base) and I.S. to blank plasma. They were extracted and injected in the same manner

TABLE I

MEAN PEAK-AREA RATIOS OF DRUG TO INTERNAL STANDARD, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION WHEN DIFFERENT CONCENTRATIONS OF ALPRENOLOL OR METOPROLOL WERE SPIKED IN CONTROL PLASMA ON FIVE DIFFERENT DAYS

Concentration (ng/ml)	Amount of drug added to Extrelut-1 column (ng)	Mean peak-area ratio	Standard deviation	Coefficient of variation (%)
Alprenolol				
0	0	0	—	—
21.9	2.19	0.1800	0.0228	12.66
43.8	4.38	0.3425	0.0308	8.99
87.5	8.75	0.5556	0.0447	8.05
175.0	17.50	1.0191	0.0650	6.38
218.9	21.89	1.2328	0.1072	8.69
350.2	35.02	2.0321	0.1358	6.69
437.7	43.77	2.4760	0.1466	5.92
Metoprolol				
0	0	0	—	—
12.8	1.28	0.1576	0.0168	10.71
32.0	3.20	0.2636	0.0278	10.59
64.0	6.40	0.5005	0.0302	6.04
128.0	12.80	0.9684	0.0680	7.12
192.0	19.20	1.3976	0.0480	3.44
256.0	25.60	1.7542	0.0239	1.37
320.0	32.00	2.1807	0.0460	2.11

as described above. To check the reproducibility of the analytical procedure, five different plasma calibration graphs were constructed on five different days, and the mean peak-area ratios of drug to internal standard, standard deviations (S.D.) and coefficients of variation (C.V.) are given in Table I. The intra-day coefficient of variation was determined by extracting and injecting the same concentration of the drug several times on the same day.

Pharmacokinetic investigation in the dog

In order to check the utility of this technique in pharmacokinetic studies, rapid release (RR) and sustained release (SR) dosage forms containing 100 mg of AH or MT were administered separately to a dog together with food, and blood samples were drawn at regular intervals for a period of 24 h.

RESULTS AND DISCUSSION

Chromatograms obtained from blank plasma, plasma containing added drug and plasma from a dog that had received 100 mg of AH or MT orally are shown in Figs. 1 and 2. It is evident that no endogenous substances interfered with the

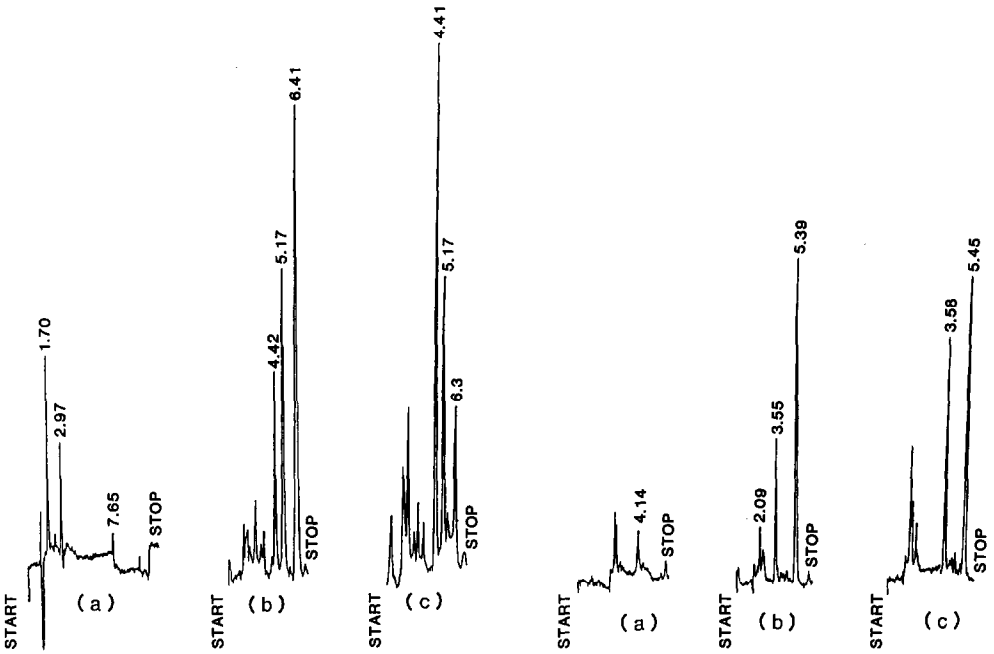

Fig. 1. Representative chromatograms of (a) drug-free plasma, (b) plasma containing 35 ng of alprenolol (peak with retention time of 6.41 min) and 50 ng of I.S. (peak with retention time of 5.17 min) and (c) dog plasma when 100 mg of alprenolol hydrochloride were administered orally (peaks with retention times of 6.3 and 5.17 min correspond to alprenolol and I.S., respectively). The amounts of alprenolol detected in b and c were 35 and 9 ng, respectively.

Fig. 2. Representative chromatograms of (a) drug-free plasma, (b) plasma containing 3.5 ng of metoprolol (peak with retention time of 3.55 min) and 50 ng of I.S. (peak with retention time of 5.39 min) and (c) dog plasma when 100 mg of metoprolol tartrate were administered orally (peaks with retention times of 3.58 and 5.45 min correspond to metoprolol and I.S., respectively). The amounts of metoprolol detected in b and c were 3.5 and 7 ng, respectively.

drug peaks. Chromatograms a and b in Figs. 1 and 2 were identical when human and dog plasma were used.

Under these conditions, the minimum detectable concentration for both the drugs is lower than 5 ± 0.1 ng/ml using 100 μl of plasma. The sensitivity of the method could be further increased proportionately by reducing the total volume of the extract to 75 μl. The calibration graphs are linear for both the drugs within the range studied. The slope, intercept and correlation coefficient (r^2) of the regression lines were 0.0557, 0.0499 and 0.9986 for AH and 0.0673, 0.0579 and 0.9978 for MT, respectively. The mean inter-day coefficient of variation was in the range 5.9–12.7% for AH and 1.4–10.7% for MT. The mean ($n=8$) intra-day coefficient of variation was 5 and 3% at a concentration of 218.9 ng/ml for alprenolol base and 192 ng/ml for metoprolol base, respectively. The extraction efficiency of the Extrelut-1 columns was assessed by comparison with direct injection of the solution of the respective compounds in the eluent. The mean absolute recoveries were $98 \pm 2\%$ for both drugs.

TABLE II

PLASMA CONCENTRATIONS OF ALPRENOLOL AND METOPROLOL AT DIFFERENT TIMES WHEN RAPID RELEASE (RR) OR SUSTAINED RELEASE (SR) DOSAGE FORMS CONTAINING 100 mg OF ALPRENOLOL HYDROCHLORIDE OR METOPROLOL TARTRATE WERE ADMINISTERED TO A DOG

Time (h)	Concentration (ng/ml)			
	Alprenolol		Metoprolol	
	RR	SR	RR	SR
0.0	0.0	0.0	0.0	0.0
0.5	64.34	77.16	86.14	16.19
1.0	955.29	78.94	995.98	88.40
1.5	400.79	75.37	741.37	85.06
2.0	284.86	199.21	520.62	174.16
2.5	223.63	284.91	603.60	181.03
3.0	131.66	330.91	275.96	350.98
4.0	178.64	221.10	190.91	372.13
6.0	94.76	201.20	67.00	194.92
8.0	74.66	180.80	23.86	87.20
12.0	32.31	120.93	15.56	69.98
24.0	5.65	40.41	11.43	50.95

There was no interference from diazepam, quinidine, hydroxybetaxolol and the metabolites of metoprolol, namely α-hydroxymetoprolol and O-demethylmetoprolol, indicating that the method is specific for these drugs.

This method has been successfully used to determine these drugs in dog plasma after administering rapid and sustained release formulations containing 100 mg of AH or MT as shown in Table II. Clinically it can also be used to determine alprenolol and metoprolol routinely in plasma from patients receiving these drugs, as the extraction procedure involved is very simple and reproducible with excellent recoveries. Moreover, it employs only 100 μl of plasma.

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