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Note

Rapid determination of metoprolol and α-hydroxymetoprolol in human plasma and urine by high-performance liquid chromatography

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Several methods are available for the analysis of the β-adrenoceptor antagonist, metoprolol, alone or together with its metabolites, in biological fluids. Although sensitive, gas—liquid chromatographic procedures involve a derivatisation step [1—3] and, in the case of gas chromatography—mass spectrometry [4] require expensive equipment not readily available in most laboratories. High-performance liquid chromatography (HPLC) overcomes these disadvantages, but published HPLC assays for metoprolol are associated with either a time-consuming extraction procedure [5, 6] or lengthy chromatographic retention times [7, 8].

This paper describes HPLC methods for the analysis of metoprolol in plasma and for the drug and its α-hydroxylated metabolite in urine. Both procedures are sensitive and selective and significantly more rapid than other HPLC assays.

Recent work in our laboratory, using debrisoquine as a probe for defective drug oxidation in man [9], has indicated that the metabolism of metoprolol exhibits genetic polymorphism [10]. To illustrate the application of the present assays the plasma and urine profiles of metoprolol and α-hydroxymetoprolol in a poor and in an extensive metaboliser of debrisoquine are presented.

MATERIALS AND METHODS

Chemicals and drugs

Metoprolol tartrate and α-hydroxymetoprolol *p*-hydroxybenzoate were gifts from Astra Pharmaceuticals (St. Albans, Great Britain) and Hässle (Mölndal, Sweden), respectively. The internal standard, 1-(4-butyramido-2-butyrylphenoxy)-2-hydroxy-3-isopropylaminopropane hydrochloride, was donated by May and Baker (Dagenham, Great Britain). HPLC-grade acetonitrile and

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methanol and glass-distilled dichloromethane were purchased from Rathburn Chemicals (Walkerburn, Great Britain). All other chemicals were of analytical grade.

HPLC instrumentation and conditions

The chromatograph consisted of a Model 6000A pump (Waters Assoc., Northwich, Great Britain), a Model 7125 Rheodyne injector (0.5-ml loop) (HPLC Technology, Macclesfield, Great Britain) a Model 970FS Schoeffel fluorimetric detector (Kratos, Manchester, Great Britain). The stainless-steel columns (10 cm × 5 mm I.D.) used were packed with either Hypersil 5-ODS (method I) or Spherisorb Phenyl (method II) reversed-phase materials (both 5-μm particle size) (HPLC Technology).

In method I, the mobile phase consisted of water—acetonitrile (80:20) containing 1% triethylamine and adjusted to pH 3 with orthophosphoric acid. Water—methanol (55:45) containing 5 m*M* sodium heptanesulphonate and 0.1% (w/v) acetic acid was the mobile phase in method II.

Chromatography was performed isocratically at a flow-rate of 2 ml/min and at ambient temperature. The detector excitation wavelength was set at 222 nm and a 320-nm emission filter was used.

Extraction procedures

Method I: metoprolol in plasma. Plasma (1.0 ml), internal standard (400 ng) and sodium hydroxide (100 μl, 0.1 *M*) were gently shaken with dichloromethane (2 ml) for 10 min. After centrifugation (900 *g*, 2 min) and removal of the upper aqueous layer the organic extract was transferred to a 10-ml conical tube and evaporated to dryness on a Buchler Vortex Evaporator (Baird and Tatlock, Romford, Great Britain). The residue was reconstituted in mobile phase (100 μl) and an aliquot (30—100 μl) was injected into the chromatograph.

Method II: metoprolol and α-hydroxymetoprolol in urine. Urine (1.0 ml), internal standard (20 μg) and sodium carbonate (250 μl, 0.5 *M*) were mixed and then extracted with dichloromethane in an identical manner to that described for metoprolol in plasma.

RESULTS

Under the chromatographic conditions of both methods I and II, metoprolol, α-hydroxymetoprolol and internal standard gave rapidly eluting, fully resolved and essentially symmetrical peaks (Fig. 1). In method I the retention times for metoprolol and internal standard were 2.3 and 5.9 min, respectively. Retention times for metoprolol, α-hydroxymetoprolol and internal standard in method II were 5.1, 3.0 and 7.7 min, respectively.

Although no endogenous compounds were found to co-elute with any of the drug peaks, a small, slowly eluting peak (retention time 11.2 min) was observed in chromatograms from several of the plasma extracts. By careful timing of injections, interference from this material could be easily avoided.

Samples from patients taking a variety of cardiovascular drugs were run through the assays. Of these drugs, only presumed metabolites of hydralazine

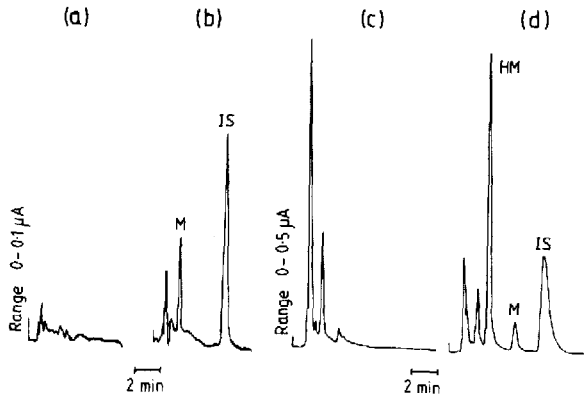

Fig. 1. Chromatograms of extracts of plasma and urine taken from a subject before and after oral administration of 200 mg metoprolol tartrate. Method I: (a) pre-dose plasma, (b) post-dose plasma containing 60 ng base per ml of metoprolol (M), and 400 ng per ml of internal standard (IS). Method II: (c) pre-dose urine, (d) post-dose urine containing 10.8 μg base per ml of α-hydroxymetoprolol (HM), 0.95 μg base per ml of metoprolol (M), and 20 μg per ml of internal standard (IS).

caused interference.

In addition, the following drugs, when injected directly did not interfere with either assay: sotalol, nadolol, propranolol, timolol, metoprolol, oxprenolol, lignocaine, disopyramide, mexiletine, warfarin, canrenone, nifedipine, isosorbide dinitrate, frusemide, hydralazine and three of its metabolites: 3-hydroxymethyltriazolophthalazine, methyltriazolophthalazine and phthalazinone.

Calibration curves for metoprolol and α-hydroxymetoprolol in both methods I (range 10—400 ng base per ml) and II (range 0.5—40 μg base per ml) were linear and passed through the origin ($r^2 > 0.99$). Standards prepared by spiking control plasma or urine with known amounts of drug and metabolite were included in each analytical run. Intra-assay coefficients of variation are shown in Table I. The lowest measurable concentration of metoprolol in plasma was about 5 ng/ml and of metoprolol and α-hydroxymetoprolol in urine about 0.2 μg/ml.

TABLE I

INTRA-ASSAY COEFFICIENTS OF VARIATION (%) FOR THE PLASMA AND URINE ANALYSIS OF METOPROLOL AND α-HYDROXYMETOPROLOL (n = 6)

Compound	Plasma (method I)		Urine (method II)	
	50 ng/ml	200 ng/ml	1 μg/ml	20 μg/ml
Metoprolol	4.8	2.1	2.3	4.7
α-Hydroxymetoprolol	—	—	4.8	5.1

DISCUSSION

Enhancement of chromatographic peak shape and therefore resolution, of weak bases like metoprolol on reversed-phase HPLC is most often accomplished by the addition to the mobile phase of an ion-pair reagent of negative charge, e.g. heptanesulphonate. Another approach, namely the use of alkylamines as modifiers, can also give extremely good resolution thereby leading to rapid analysis times. For example, a baseline separation of four weakly basic local anaesthetics in less than 3 min has been obtained on reversed-phase HPLC by the addition of 1% triethylamine to the mobile phase [11]. There is some doubt as to whether the modifier acts by blocking residual silanol groups on the stationary phase, or through an ion-pairing mechanism [11, 12].

The rapid, sensitive and selective HPLC method for the plasma analysis of metoprolol described in this paper utilises triethylamine as a modifier. Because of the single extraction step and short chromatographic analysis times, as many as forty samples can be processed in one day.

Owing to interferences from endogenous compounds, difficulties were encountered in extending the plasma assay to the measurement of metoprolol and α-hydroxymetoprolol in urine. After evaluating various stationary and mobile phases, good resolution was obtained by substituting a phenyl for an octadecylsilane column and using a methanol—water mobile phase containing heptanesulphonate. The same internal standard was used and the

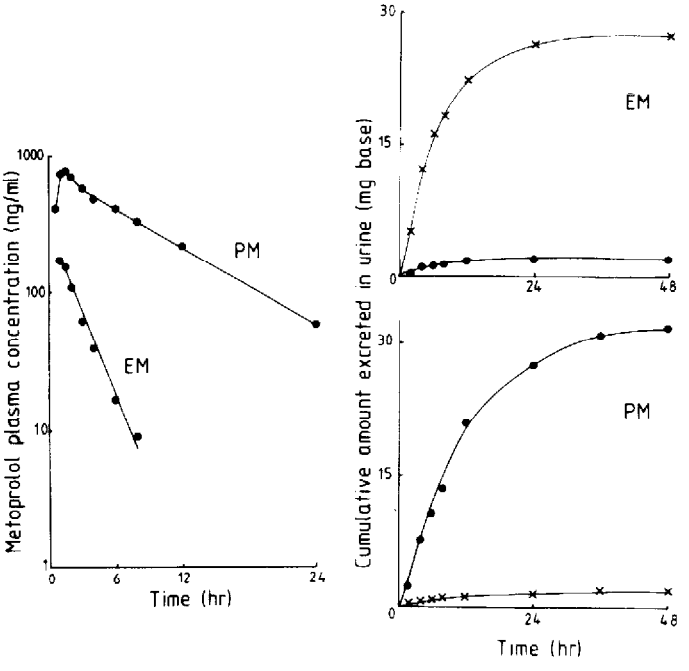

Fig. 2. Plasma concentrations of metoprolol and cumulative urinary excretion of metoprolol (•) and α-hydroxymetoprolol (×) in a poor (PM) and an extensive metaboliser (EM) of debrisoquine following a single oral dose of 200 mg metoprolol tartrate.

extraction procedure required only minor alteration. This method offers the advantage over that described by Pautler and Jusko [8] of having a much shorter analysis time (9 min instead of 28 min) making it better suited to routine use.

Large differences in the plasma and urine kinetics of metoprolol and α-hydroxymetoprolol were observed between the extensive and the poor metaboliser of debrisoquine (Fig. 2). The urine assay possessed sufficient sensitivity to detect the low drug and metabolite concentrations seen in the extensive and in the poor metaboliser, respectively.

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