Journal of Chromatography, 339 (1985) 429—433
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2497

Note

Simple and rapid analysis of atenolol and metoprolol in plasma using solid-phase extraction and high-performance liquid chromatography

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(First received August 28th, 1984; revised manuscript received November 20th, 1984)

Metoprolol and atenolol are chemically diverse cardioselective β-adrenoceptor antagonists, with atenolol relatively hydrophilic and metoprolol relatively lipophilic. As a consequence of widespread use of these and other β-adrenoceptor antagonists in the treatment of hypertension and angina pectoris [1, 2], there are many published methods for their determination in plasma and urine. Early methods employed gas—liquid chromatography using either electron-capture [3—14] or mass spectrometric [11] detection. These methods, while specific and sensitive, involved a lengthy derivatization step. Recent methods based on high-performance liquid chromatography (HPLC) [15—23] have been shown to be selective and sensitive; however, extraction procedures used (differential, pH-dependent solvent extraction followed by evaporation and reconstitution with mobile phase) tend to be laborious, taking from 30 min to 1 h or more.

The procedure reported here for the extraction of metoprolol and atenolol from plasma is based on solid-phase extraction media used in Bond-Elut™ columns. The extraction is versatile, efficient, rapid (1 min for extraction) and avoids exposure to alkaline conditions. Extracts can be immediately subjected to sensitive and selective assay using HPLC with fluorometric detection. While a sample is being chromatographed the next can be extracted, or up to ten samples can be extracted at any one time. We are only aware of one previous report of this extraction technique being used [24], application being limited to the hydrophilic compound, atenolol.

0378-4347/85/$03.30

EXPERIMENTAL

Materials and reagents

Metoprolol was supplied by Ciba-Geigy (Switzerland) and atenolol by ICI (Australia). HPLC-grade acetonitrile was obtained from Waters Assoc. (Sydney, Australia), all other reagents were of analytical grade. Bond-Elut CN and C_{18} columns (1 ml capacity) and the Vac-Elut^{TM} manifold (Analytichem, Harbor City, CA, U.S.A.) were purchased from FSE Scientific (Melbourne, Australia).

The HPLC system consisted of a Constametric III pump (LDC, Riviera Beach, FL, U.S.A.), a Model 7010/7011 injection valve with a 20-μl loop for metoprolol and a 50-μl loop for atenolol (Rheodyne, Berkeley, CA, U.S.A.), a Model FS970 fluorescence detector (Schoeffel Instrument, Westwood, NJ, U.S.A.) and an Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.). For metoprolol determination a 30 cm × 0.46 cm I.D. stainless-steel column packed with C_{18} 10-μm μBondapak (Waters Assoc.) was used. For the atenolol determination a 25 cm × 0.3 cm I.D. stainless-steel glass-lined column packed with spherisorb 5-μm nitrile silica (SGE Scientific, Ringwood, Australia) was used.

Bond-Elut extraction procedure

Atenolol was extracted using a Bond-Elut column containing silica modified with covalently bound cyanopropyl groups (CN column) and metoprolol was extracted using a Bond-Elut column containing ODS-modified silica (C_{18} column). The Bond-Elut columns were placed in luer fittings in the top of the Vac-Elut cover, which has the capacity for ten columns. A vacuum of 25—50 cmHg was applied to the manifold to effect the various stages of the extraction. Both types of column were activated before use by washing with 2 × 1 ml of acetonitrile followed by 2 × 1 ml of distilled water.

To extract metoprolol from plasma, 1 ml of plasma was passed through the activated C_{18} Bond-Elut column which was then washed twice with 0.5-ml aliquots of distilled water—acetonitrile (90:10). The vacuum was released from the Vac-Elut and the stainless-steel needles of the Vac-Elut cover were wiped. Appropriately labelled tubes were placed under the column, which was then eluted with 0.5 ml of actonitrile—0.1 *M* hydrochloric acid (50:50), with the vacuum re-applied. The collected extract was then ready for injection onto the HPLC column.

A similar process was used to extract atenolol from plasma. Plasma (1 ml) was passed through an activated CN Bond-Elut column which was then successively washed with 0.5 ml distilled water and 0.5 ml acetonitrile. To elute the atenolol from the column two 0.25-ml aliquots of 0.05 *M* sodium dihydrogen orthophosphate—acetonitrile (70:30) containing 4 m*M* triethylamine adjusted to pH 4 with orthophosphoric acid were used.

Preparation of standards

Stock solutions of metoprolol and atenolol at a concentration of 1 mg/ml were made in distilled water. Appropriate dilution of this solution with drug-free plasma gave a range of standards which could be used to standardize the extraction procedure and calibrate the HPLC determination. The amount of

drug in plasma samples was then determined from peak heights and a calibration line obtained with the standards.

Chromatography

For metoprolol determination the C_{18} HPLC column was eluted with acetonitrile—0.1% orthophosphoric acid (23:77) at a flow-rate of 1.0 ml/min. For atenolol determination the CN HPLC column was eluted with acetonitrile—0.05 *M* phosphate buffer (10:90) with pH adjusted to 7.0 with orthophosphoric acid at a flow-rate of 1.2 ml/min. For both assays the detector excitation wavelength was set at 193 nm with no emission filter. Sensitivity was set at 0.2 μA for metoprolol and 0.1 μA for atenolol.

Extraction recoveries

Recovery with these extraction procedures was estimated by comparing peak heights obtained by direct injection of solutions containing atenolol or metoprolol in the appropriate solvent with those obtained by extraction of plasma containing an equal concentration, an appropriate allowance being applied for the volume of the extract.

$$\text{Percentage recovery} = \frac{\text{peak height of extracted plasma sample} \times \text{extract volume} \times 100}{\text{peak height of non-extracted sample}}$$

Recoveries were determined from the mean of eight replicates taken for each drug.

Screening for interfering drugs

A range of drugs that are commonly co-administered with atenolol or metoprolol were screened for their possible interference in the assays. Plasma from a patient known to be taking the particular drugs was processed by the two methods and the HPLC chromatogram checked for any interfering peaks at the retention time for atenolol and metoprolol. This process eliminated any potential interference from the parent drug and its metabolites at the normal levels likely to be encountered in the clinical situation.

RESULTS

Representative chromatograms for atenolol and metoprolol are shown in Figs. 1 and 2, respectively. Each figure shows plasma spiked with a known amount of β-blocker (A), drug-free plasma (B), and plasma from a patient after a normal oral dose of β-blocker (atenolol or metoprolol, respectively) (C). The assay procedure was linear over the range 0—500 ng/ml for atenolol ($y = 0.078x$, $r = 0.993$) and 0—1000 ng/ml for metoprolol ($y = 0.161x$, $r = 1.000$). At atenolol concentrations above 500 ng/ml, the extraction capacity of the 1-ml CN Bond-Elut column appeared to decrease slightly causing a slight curvature in the calibration line. The recovery of atenolol at concentrations of 20 and 200 ng/ml was 74.5 ± 2.0% ($n = 8$) and 61.7 ± 1.5% ($n = 8$), respectively, while for metoprolol at concentrations of 400 ng/ml and 50 ng/ml it was 95.4 ± 2.7% ($n = 8$) and 99.8 ± 9.8% ($n = 8$), respectively. The sensitivity limit (three times baseline) for the atenolol assay was 10 ng/ml and for metoprolol 2 ng/ml.

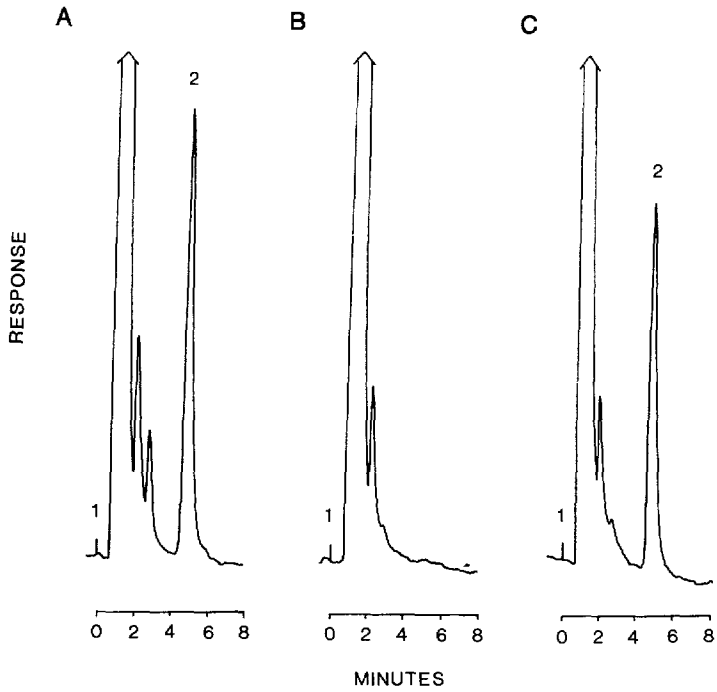

Fig. 1. HPLC profiles of (A) drug-free plasma spiked with 400 ng/ml atenolol, (B) drug-free plasma, (C) patient plasma sample collected 3 h after a dose of 50 mg atenolol. 1 = Injection site; 2 = atenolol.

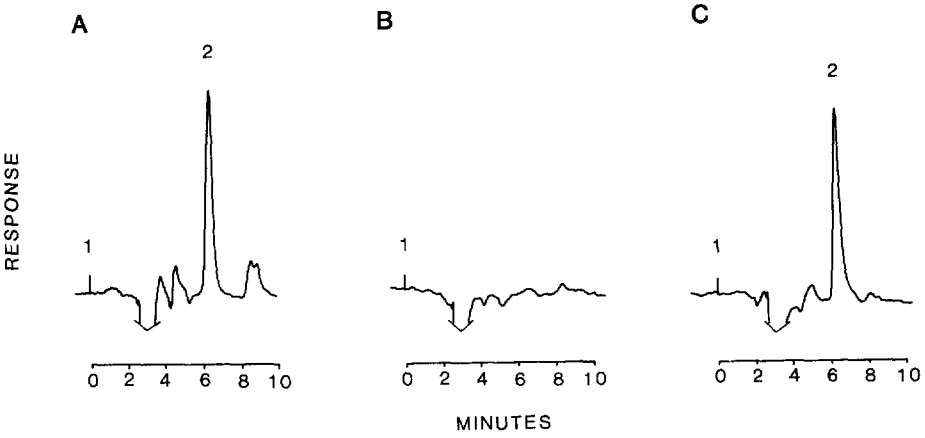

Fig. 2. HPLC profiles of (A) drug-free plasma spiked with 200 ng/ml metoprolol, (B) drug-free plasma, (C) patient plasma sample collected 2½ h after a dose of 100 mg metoprolol. 1 = Injection site; 2 = metoprolol.

The intra- and inter-day precision of the methods were determined by the assay of eight samples of drug-free plasma containing known concentrations of atenolol or metoprolol. The coefficient of variation of atenolol was 2.67% at 20 ng/ml and 3.06% at 400 ng/ml, while for metoprolol it was 6.89% at 20 ng/ml and 2.87% at 500 ng/ml.

Drugs which have been eliminated as causing potential interference in both assays are: chlorothiazide, prazosin, hydralazine, α-methyl-DOPA, verapamil,

frusemide, disopyramine and lignocaine. There was also chromatographic separation of other commonly used β-blockers, i.e. pindolol, propranolol, alprenolol, oxprenolol, practolol and timolol.

DISCUSSION

The determination of atenolol and metoprolol using solid-phase extraction techniques together with HPLC has proven to be simple, rapid, sensitive and specific. The assays are adequate to determine atenolol or metoprolol in plasma after normal oral doses of either drug as shown in Fig. 1C for atenolol and Fig. 2C for metoprolol.

A further advantage of solid-phase extraction systems is that they avoid the strongly alkaline conditions of the previous solvent extraction methods. This is important in the case of drugs which are subject to oxidation under such conditions or can bind to glassware (i.e. β-adrenoceptor antagonists).

The methods reported here do not suffer from interference from the drugs commonly co-administered with either atenolol or metoprolol and therefore they would be suitable for use in routine drug monitoring or pharmacokinetic studies.

In summary and conclusion we present a novel extraction procedure generally applicable to both hydrophilic and lipophilic β-blockers, illustrated in this communication with particular reference to metoprolol and atenolol. This method offers a major advance in ease of execution and speed without sacrifice of precision, sensitivity or selectivity.

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