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Rapid Determination of Atenolol in Human Plasma and Urine by High-Pressure Liquid Chromatography

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Received March 28, 1977, from the *Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri–Kansas City, Kansas City, MO 64108.* Accepted for publication November 2, 1977.

Abstract □ A rapid, specific, high-pressure liquid chromatographic determination of atenolol in plasma and urine was developed. This method employs the high sensitivity of fluorescence detection together with selective extraction and reversed-phase chromatography to measure concentrations as low as 20 ng of drug/ml of plasma with a coefficient of variation of 3.91%. The assay is specific enough to be valid in the presence of plasma and urine substances. The detection limit (*i.e.*, three times baseline noise) is 3 ng/ml.

Keyphrases □ Atenolol—high-pressure liquid chromatographic determination in biological fluids □ High-pressure liquid chromatography—analysis, atenolol in biological fluids □ Antiadrenergic agents—atenolol, high-pressure liquid chromatographic determination in biological fluids

Atenolol[1], 4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetamide (I), is a new β-adrenergic blocking agent. Studies in animals and humans suggest that this drug causes preferential blockade of cardiac β-adrenoreceptors, is free of intrinsic sympathomimetic activity, and is devoid of significant membrane-stabilizing activity (1–5). Initial clinical experience indicates that atenolol may be of value in the treatment of hypertension (6–9).

Previous procedures for the determination of atenolol in plasma and urine employed GLC with electron-capture detection (10, 11) or spectrophotofluorometry (12). The spectrophotofluorometric method is relatively simple but is of questionable specificity, and the detection limit is 50 ng/ml. Although the GLC methods are highly selective and have a detection limit of 20 ng/ml, they are relatively complex and rely on a demanding prechromatography derivatization.

The procedure reported here combines a selective two-step extraction with the separative capability of high-pressure liquid chromatography (HPLC) and the sensitivity of fluorescence detection. The method is rapid and specific, employs an internal standard, and is of sufficient sensitivity (3 ng/ml detection limit) for pharmacokinetic studies.

[1] Burdick and Jackson Laboratories, Muskegon, Mich.

EXPERIMENTAL

Materials—UV grade methanol[1], 1-butanol[1], and hexane[1] were used as obtained, as were reagent grade triethanolamine[2] and 1-heptane sulfonic acid in acetic acid[3]. Water was passed through an ion-exchange bed and then distilled. Atenolol[4] and procainamide[5] were used as obtained.

Apparatus—HPLC was performed in a system consisting of a universal injector[6], a high pressure pump[7], a bonded-phase column[8], and a fluorescence detector[9]. The fluorescence of atenolol was excited at 222 nm. A combination of a 7-54 and a UV 30 filter was used between the flowcell and the photomultiplier tube.

Chromatographic Parameters—The mobile phase was prepared by mixing 2.0 ml of 1-heptane sulfonic acid in acetic acid[3] with 100 ml of 0.1 *M* triethanolamine in distilled water and 1900 ml of methanol. The mobile phase was pumped at 2 ml/min and 20° through a stainless steel column (30.5 cm × 4 mm i.d.) packed with a high efficiency bonded-phase packing. Aliquots of 100–250 μl of the reextraction solution were injected directly on-column through the injector.

Analytical Procedure—*Plasma*—Two 1.0-ml aliquots of each plasma sample were placed in 15-ml screw-topped centrifuge tubes. To each tube were added 0.1 ml of 2.5 *N* NaOH and 5.0 ml of 10% 1-butanol in *n*-hexane. The tubes were vortexed for 30 sec and then centrifuged for 10 min at 2000 rpm. After centrifugation, the organic phase was removed and discarded.

Four milliliters of 50% 1-butanol in *n*-hexane was then added to each tube. This solution contained 50.0 ng of procainamide/ml as an internal standard. The tubes were again vortexed for 30 sec and centrifuged for 10 min at 2000 rpm. This second organic phase was transferred to a second centrifuge tube containing 300 μl of 0.1% acetic acid. After 30 sec of vortexing and 5 min of centrifugation at 2000 rpm, 100–250 μl of the lower (aqueous) phase was withdrawn with a syringe and injected into the injector loop.

The output of the detector was recorded on a chart recorder, and the areas of the atenolol and procainamide peaks were measured. The ratios of the peak areas were converted into plasma atenolol concentrations by comparison with a standard curve.

Urine—Urine, 100 μl, was added to 4.90 ml of 1.52 *M* phosphate buffer (pH 12.3) and mixed thoroughly. Then 1-ml aliquots of this diluted sample were placed in 15-ml centrifuge tubes and assayed by the same

[2] Fisher Scientific Co., St. Louis, Mo.
[3] B7 reagent, Waters Associates, Milford, Mass.
[4] Stuart Pharmaceutical Division of ICI United States, Wilmington, Del.
[5] E. R. Squibb & Sons, Princeton, N.J.
[6] U6K, Waters Associates, Milford, Mass.
[7] Model 6000A, Waters Associates, Milford, Mass.
[8] μBondapak CN, Waters Associates, Milford, Mass.
[9] Schoeffel, FS-970, Westwood, N.J.

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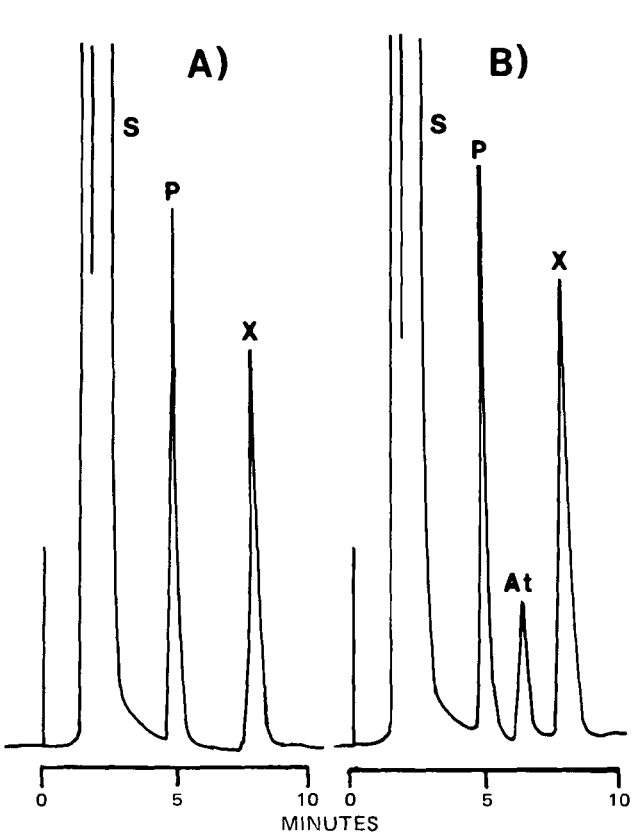

Figure 1—*High-pressure liquid chromatograms of extracts from plasma. Key: A, control plasma; B, plasma containing 40 ng of atenolol/ml; P, procainamide internal standard; S, solvent artifact; X, endogenous material; and At, atenolol.*

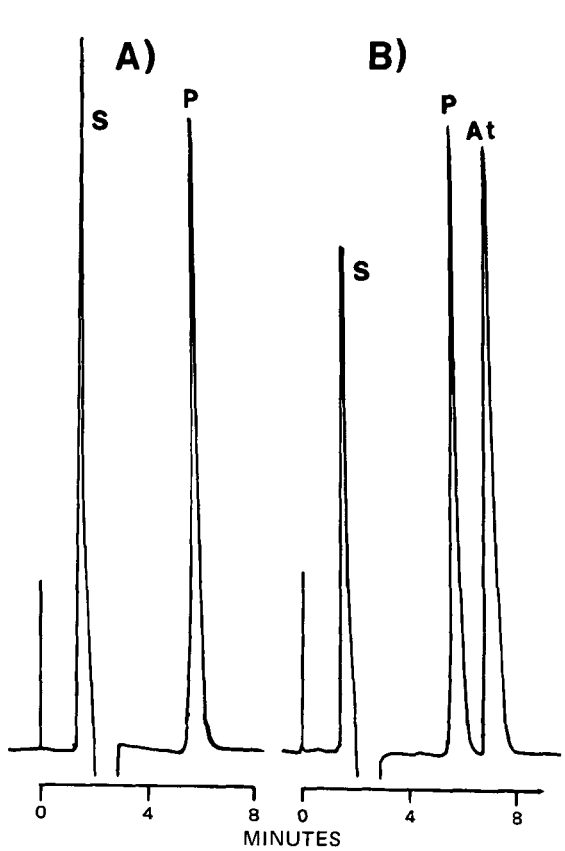

Figure 2—*High-pressure liquid chromatograms of extracts from urine. Key: A, control urine; B, urine containing 20 μg of atenolol/ml; P, procainamide internal standard; S, solvent artifact and endogenous material; and At, atenolol.*

procedure as described for plasma samples, except that the addition of 2.5 *N* NaOH was omitted.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of pooled plasma samples with and without atenolol. The atenolol peak was well separated from the procainamide internal standard peak and from artifacts. The retention times of procainamide and atenolol were 4.8 and 6.3 min, respectively. An unidentified compound, with a retention time of 7.8 min, was extracted from most plasma samples.

The ratios of the area under the atenolol peak to the area under the procainamide peak varied linearly with atenolol concentration. Analysis of six plasma samples at each concentration, 10, 30, 50, 70, and 100 ng/ml, gave a linear regression coefficient of 0.9967 for line $y = 0.00765x + 0.0057$, where y is the area ratio and x is the atenolol concentration in nanograms per milliliter of plasma. Analysis of six plasma samples of each concentration, 100, 300, 500, 700, and 1000 ng of atenolol/ml, gave a linear regression coefficient of 0.9976 for the line $y = 0.00889x + 0.0959$. The precision and accuracy of these values are given in Table I.

The analysis of 20 plasma samples to which 20 ng of atenolol/ml was added gave a mean of 20.2 ng/ml (range of 18.6–21.7) and a coefficient of variation of 3.91%. Concentrations down to 5 ng/ml can be quantitated with slightly less precision and accuracy. These figures on precision and accuracy relate to the entire analytical procedure, from pipetting the plasma to integrating the chromatogram.

Table I—Precision and Accuracy of Atenolol Measurement by HPLC

Atenolol Added to Plasma, ng/ml	*n*	Average of Calculated Atenolol Concentrations, ng/ml	*SD*	*CV*
1000	5	1000	40.4	4.04
700	6	702	14.2	2.02
500	6	496	25.1	5.07
300	6	303	6.82	2.25
100	6	100.8	2.20	2.88
70	6	67.9	3.35	4.93
50	6	51.5	2.79	5.43
30	6	29.9	1.03	3.44
10	6	10.0	0.90	9.00

The analysis of 25 samples[10] obtained from a clinical study of atenolol demonstrated the applicability of the method for samples from human subjects. The results were in close agreement with those obtained by GLC analysis[10]. In some samples, a small peak on the ascending portion of the atenolol peak was identified as the hydroxy metabolite of atenolol. Although it was not completely separated by the operating conditions, the separation was adequate to prevent interference with the quantitation of atenolol.

The analysis of plasma samples from 30 patients not taking atenolol resulted in no chromatographic peaks that interfered with atenolol or procainamide. In a pharmacokinetic study where the only drug permitted is atenolol, the described procedure is quite specific. Further study is required to determine if other drugs that may be used concomitantly in

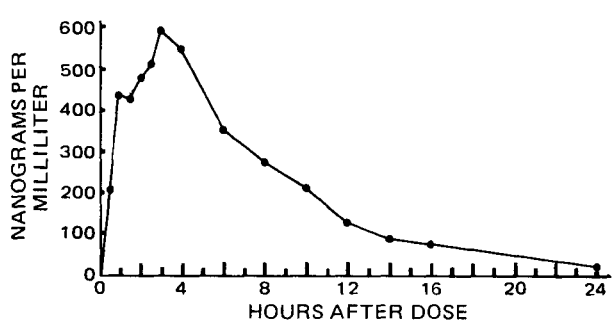

Figure 3—*Atenolol concentration* versus *time curve in a human volunteer following an oral dose of one 100-mg tablet.*

[10] Supplied by ICI United States.

therapy with atenolol, such as diuretics, might cause additional chromatographic peaks and/or interferences. These studies are currently underway.

The analysis of atenolol in urine involves the addition of one step to the analytical procedure: an initial sample dilution. Chromatograms (Fig. 2) of the urine extract were free of interfering peaks and had the same precision and accuracy as comparable concentrations in plasma.

The method was used in the analysis of 768 plasma and 384 urine samples in a pharmacokinetic study over 4 months. A plot of the plasma concentration *versus* time following a 100-mg dose to one volunteer is shown in Fig. 3. Over the 4 months, standard curves were prepared daily. When the same column was used, the day-to-day change in the slope of the standard curve never exceeded 7.2%; however, when new columns were installed, changes up to 15% were observed.

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Benzoyl Peroxide Assay Using High-Pressure Liquid Chromatography

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Received August 1, 1977, from the *G. S. Herbert Laboratories, Allergan Pharmaceuticals, Irvine, CA 92713.* Accepted for publication November 9, 1977.

Abstract □ A highly specific assay for benzoyl peroxide was developed using high-pressure liquid chromatography. A concentration curve was recorded from 0.1 to 3.0 μg with a correlation coefficient of 0.9969. The standard deviation for 10 individual analyses of a benzoyl peroxide preparation was ±0.547 (1.80% *RSD*).

Keyphrases □ Benzoyl peroxide—high-pressure liquid chromatographic analysis in bulk drug and commercial preparations □ High-pressure liquid chromatography—analysis, benzoyl peroxide in bulk drug and commercial preparations □ Keratolytic agents—benzoyl peroxide, high-pressure liquid chromatographic analysis in bulk drug and commercial preparations

Benzoyl peroxide is widely used in topical preparations for the treatment of acne. The methods currently available for benzoyl peroxide include titrimetry (1), spectrophotometry (2), and polarography (2). Only polarography is specific for benzoyl peroxide.

The visible absorption curves for benzoyl peroxide and analogous compounds, such as benzaldehyde and benzoic acid, obtained with the spectrophotometric method described in the USP (3) show marked similarity. If benzoyl peroxide is mixed with any of these analogous compounds, an accurate determination of benzoyl peroxide is impossible.

The sodium thiosulfate titration of liberated iodine is a back-titration, which is a limitation in itself. It also does not distinguish the difference between benzoyl peroxide and its chief degradation products, benzoic acid and benzaldehyde, because of the addition of iodine to aromatic double bonds.

The high-pressure liquid chromatographic (HPLC) method described in this paper is specific and provides accurate determinations of benzoyl peroxide, benzoic acid, and benzaldehyde, all with a single injection (Fig. 1).

EXPERIMENTAL

Apparatus—Absorbance measurements were recorded with a spectrophotometer[1] with matched 1-cm path length quartz cells[2]. A high-pressure liquid chromatograph[3] with a UV-visible detector[3] was used with an integrator[3] to separate and quantitate benzoyl peroxide.

Instrument Settings—All separations were run on an octadecylsilane 10-μm reversed-phase column utilizing isocratic elution (1.2 ml/min) with acetonitrile–water (50:50). A detector sensitivity of 0.16 aufs and a chart speed of 20 cm/hr were used.

Reagents—Acetonitrile[4] and deionized water were purified by filtration through a 0.22-μm solvent inert filter.

Procedure—Samples were prepared by taking aliquots of a benzoyl peroxide preparation containing approximately 30.0 mg of benzoyl peroxide and stirring in 75.0 ml of acetonitrile for 5 min. The samples were then filtered through a prewetted 0.22-μm solvent inert filter into a 100-ml volumetric flask and diluted to volume with acetonitrile. Samples were injected *via* a 10.0-μl fixed loop injector, and the UV absorption at 254 nm was used for detection and quantitation.

The concentration curve was prepared by accurately weighing 1.0, 2.0, 5.0, 10.0, 20.0, and 30.0 mg of benzoyl peroxide into 100-ml volumetric flasks and diluting to volume with acetonitrile.

[1] Beckman.
[2] Markson.
[3] Spectra Physics.
[4] Burdick & Jackson.

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