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Letter to the Editor

Simple extraction of atenolol from urine and its determination by high-performance liquid chromatography

Sir,

We wish to report an extension to our previously published atenolol assay procedure [1] to include the determination of atenolol in urine. The solvent extraction of polar, highly water-soluble drugs such as atenolol can be difficult, whereas solid-phase extraction offers a viable alternative with a potential for enhanced selectivity. Other published methods for the determination of atenolol have been based on fluorescence spectrophotometry [2], gas chromatography with electron-capture detection [3–5] and high-performance liquid chromatography (HPLC) [6–10]. With one exception [8] all methods use one or more solvent extraction steps, making them time-consuming and tedious. Additionally not all of the methods have been applied to the analysis of urine samples. We have reported several examples of the benefits of solid-phase extraction techniques [11–14] in terms of speed and simplicity of extraction whilst maintaining high reproducibility and recovery for a range of diverse drugs and metabolites. This is now further demonstrated by the adaptation of our atenolol assay in plasma to that of atenolol in urine by changing the nature of the solid-phase extraction column from nitrile silica to a weak cation-exchange silica (CBA silica). The method we report here is both simple and rapid, with high recovery.

The extraction method is based on an ion-exchange silica CBA Bond-Elut™ column (carboxymethyl-modified silica, 3 ml capacity). Urine diluted 1:5 with water (1 ml) was passed through a previously activated column [washed with 2×1 ml acetonitrile–0.1 *M* hydrochloric acid (50:50) followed by 2×1 ml distilled water]. The column was then washed with 2×0.5 ml distilled water followed by 2×0.5 ml acetonitrile–water (10:90). After placing a collection tube under the column the atenolol was eluted off by 2×0.5 ml acetonitrile–0.1 *M* hydrochloric acid (35:65). A 20-μl aliquot of this extract was then injected onto a 25 cm×0.45 cm I.D. stainless-steel HPLC column packed with 5-μm Spherisorb nitrile HPLC packing. The HPLC column was eluted with a mobile phase consisting of acetonitrile–0.05 *M* phosphate, pH 7.0 (23:77) at a flow-rate of 1 ml/min, at room temperature. The atenolol was detected by a Schoeffel GM970

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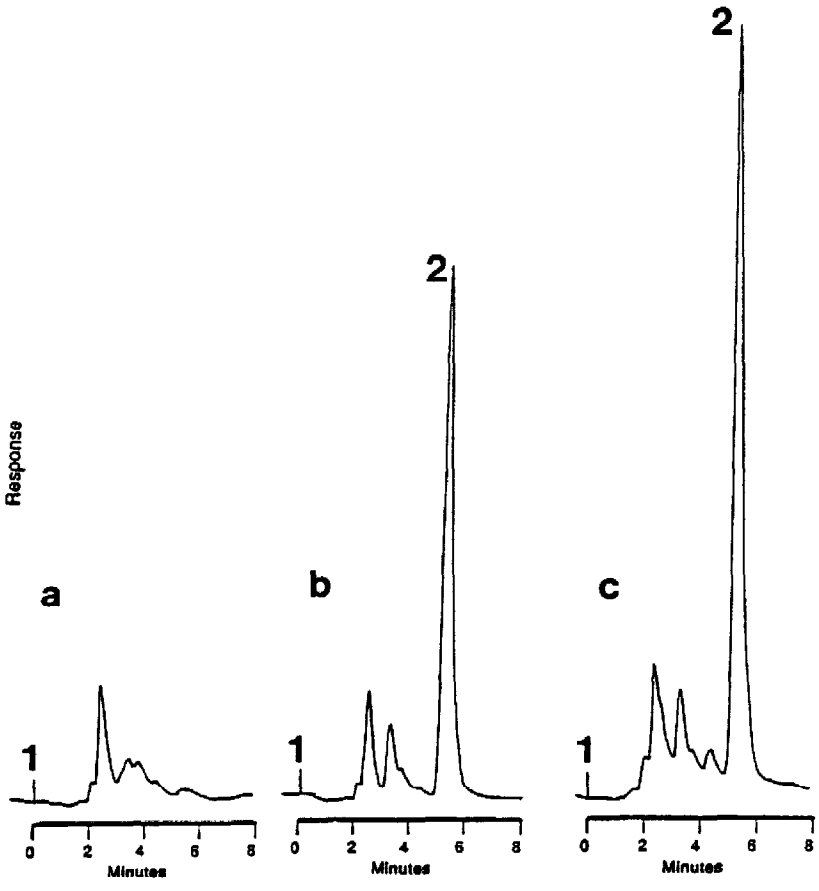

Fig. 1. Chromatograms of (a) atenolol-free urine, (b) urine spiked with 75 μg/ml atenolol and (c) urine collected over 0–2 h from a patient dosed with 12.5 mg atenolol intravenously in lieu of a scheduled 25-mg oral dose, showing 106 μg/ml atenolol. 1 = Injection of sample onto column; 2 = atenolol peak.

fluorescence detector with the excitation wavelength set at 235 nm and no emission filter. The assay was calibrated by measuring atenolol peak height obtained by processing standards in an identical manner to the samples.

Fig. 1 shows chromatograms obtained from blank urine, an atenolol standard in urine and urine collected from a patient dosed intravenously with atenolol. The retention time for atenolol was 5.6 min and endogenous substances in urine caused no interference. Additionally, the assay was free from interference by other commonly co-prescribed drugs (verapamil, diltiazem, nifedipine, frusemide, chlorothiazide, captopril, digoxin, procainamide, isosorbide dinitrate, diazepam and chloral hydrate).

The extraction recovery of atenolol was determined by comparison with non-extracted standards of the same concentration prepared in the buffer used to elute atenolol from the Bond-Elut column. The recovery (mean ± S.D.) from eight replicates was 104.9 ± 1.7 and 99.0 ± 2.0% at 20 and 100 μg/ml atenolol, respec-

tively. Accuracy and precision were determined at 20 and 100 μg/ml and were (mean $\pm$S.D., $n=8$) 20.5 ± 0.3 and 103.2 ± 1.9 μg/ml, respectively, with coefficients of variation of 1.3 and 1.8%. The assay was linear over a concentration range of 0–100 μg/ml ($y=1.66x$, $r=0.996$), and the inter-assay variation of the slope was less than 10%.

In summary, the method we describe here offers a simple and rapid extraction of atenolol from urine followed by HPLC analysis. The recovery and reproducibility of this method is high and its freedom from interference by other commonly prescribed drugs would make it suitable for use in pharmacokinetic studies of patients.

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1 P.M Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr., 339 (1985) 429.
2 C.M. Kaye, Br. J. Clin. Pharmacol., 1 (1974) 84.
3 B. Scales and P.B. Copsey, J. Pharm. Pharmacol , 27 (1975) 430.
4 J.O. Malbica and K.R. Monson, J. Pharm. Sci., 64 (1975) 1992.
5 S.H. Wan, R.F. Maronde and S.B. Matin, J. Pharm. Sci., 67 (1978) 1340.
6 O.H. Weddle, E.N. Amick and W.D. Mason, J. Pharm. Sci , 67 (1978) 1033.
7 R.K. Bhamra, K.J. Thorley, J.A. Vale and D.W. Holt, Ther. Drug Monit., 5 (1983) 313.
8 C. Verghese, A. McLeod and D. Shand, J. Chromatogr., 275 (1983) 367.
9 L.G. Miller and D.J. Greenblatt, J. Chromatogr., 381 (1986) 201.
10 K.U. Bühring and A. Garbe, J. Chromatogr., 382 (1986) 215.
11 P.M. Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr , 339 (1985) 424.
12 P M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.
13 P.M. Harrison, A.M. Tonkin, S.T. Dixon and A.J. McLean, J. Chromatogr , 374 (1986) 204.
14 P M Harrison, A.M. Tonkin, S.T. Dixon and A.J. McLean, J. Chromatogr., 374 (1986) 223.

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