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Short Communication

Determination of nadolol in serum by high-performance liquid chromatography with fluorimetric detection

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ABSTRACT

A sensitive high-performance liquid chromatographic method for a routine assay of nadolol in serum is described. Serum samples spiked with atenolol (internal standard) were extracted with diethyl ether. After centrifugation, the organic layer was evaporated to dryness. The residue was redissolved in the mobile phase and injected onto an octadecyl silica column (150 mm × 4.6 mm I.D.). The mobile phase was 0.05 *M* ammonium acetate (pH 4.5)–acetonitrile (85:15, v/v). Fluorometric detection (excitation 230 nm, emission 300 nm) was used. The minimum detectable level of nadolol in serum was 1 ng/ml.

INTRODUCTION

Nadolol, 2,3-*cis*-1,2,3,4-tetrahydro-5-[2-hydroxy-3-(*tert.*-butylamino)propoxy]-2,3-naphthalenediol, is a long-acting β-adrenoceptor blocking agent used clinically for the treatment of angina pectoris and hypertension. In order to measure serum nadolol levels in patients receiving nadolol once daily, a simple and sensitive assay procedure for nadolol is required. Various methods have been reported for nadolol assay in biological fluids. Gas chromatography (GC) with nitrogen–phosphorus detection as described by Yamaguchi *et al.* [1], has the required sensitivity, but needs extraction and derivatization steps. Ribick *et al.* [2] developed a very sensitive GC–mass spectrometric (MS) method, which successfully determined concentrations in the range 0.6–20 ng/ml. However, this GC–MS assay requires deuterated nadolol as the internal standard, and also a derivatization step. Because of low molecular extinction coefficient of nadolol, high-performance liquid chromatography (HPLC) with UV detection is not suitable for the determination of serum concentrations lower than 10 ng/ml. Gupta *et al.* [3] and Moncrieff [4] used HPLC with fluorometric detection, which yielded a higher sensitivity than that of UV detection, but the minimum detectable levels of nadolol in serum were 10 and 5 ng/ml, respectively. Piotrovskii *et al.* [5] used ion-exchange HPLC with fluorimetric detection and achieved a minimum detectable concentration of 1 ng/ml, but the method required two-stage extraction and a specific ion-exchange column.

This paper deals with a simple and sensitive method for a routine assay of nadolol in serum using HPLC with fluorimetric detection. The method includes one-stage extraction and is accurate to 1 ng/ml using 0.5 ml of serum.

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EXPERIMENTAL

Chemicals and reagents

Nadolol was a gift from the Squibb Institute (Princeton, NJ, USA). Atenolol (internal standard, I.S.) was purchased from Sigma (St. Louis, MO, USA). HPLC-grade solvents purchased from Wako (Osaka, Japan) were purified using a membrane filter (0.45 μm, Japan Millipore, Tokyo, Japan). Blank human serum was obtained from A Flow General Company (MacLean, VA, USA). Other chemical reagents were of analytical grade.

Apparatus

HPLC was carried out using a Model LC-6A system (Shimadzu, Kyoto, Japan) equipped with an RF-535 fluorescence detector (Shimadzu). An STR ODS-M column (Shimadzu, 5 μm, 150 mm × 4.6 mm I.D.) was employed.

HPLC conditions

The mobile phase was 0.05 *M* ammonium acetate (pH 4.5 adjusted with acetic acid)–acetonitrile (85:15, v/v). The flow-rate was 0.8 ml/min and the column temperature was 35°C. The fluorimetric detection wavelengths were 230 nm (excitation) and 300 nm (emission).

Standards

The stock solutions of nadolol (2.5 mg per 100 ml) in ethanol and of the I.S. (1 mg per 200 ml) in ethanol were stable for three months at 4°C. The working standard solutions of nadolol were made by dilution of the stock solution with ethanol. For the working solution of the I.S., 1 ml of the I.S. stock solution was diluted to 20 ml with ethanol when required.

Determination of nadolol in serum

To 0.5 ml of serum sample were added 80 μl of the working solution of the I.S., 0.5 ml of 10 *M* sodium hydroxide and 0.3 g of sodium chloride in a glass-stoppered 15-ml centrifuge tube. The tube was shaken with 5.0 ml of diethyl ether for 10 min and centrifuged for 5 min at 1500 *g*. The organic layer (4.0 ml) was transferred to another tube and evaporated to dryness under a nitrogen stream. The residue was redissolved in 200 μl of the mobile phase, and a 50-μl aliquot of the solution was injected into the column.

RESULTS AND DISCUSSION

Although nadolol is a mixture of two diastereomers (racemates A and B) that can be separated by HPLC [6], we analysed nadolol as a sharp single peak using a short ODS column (150 mm × 4.6 mm I.D.) in this study. Atenolol was selected as the I.S. because its fluorescence properties resemble those of nadolol. Several possible extraction solvents, such as butyl acetate, ethyl acetate, chloroform and diethyl ether, were examined. Of these diethyl ether was preferred because a much cleaner chromatogram was obtained, as shown in Fig. 1. The recovery of nadolol from the extraction procedure was 94% and that of atenolol was 62%. The lower recovery of the latter is due to its lower solubility in diethyl ether. The calibration curve that was obtained by plotting the peak-area ratio (nadolol/I.S.) *versus* concentration (ng per 0.5 ml of serum) was rectilinear over the range 1–250 ng/ml, with a correlation coefficient of 0.999. The inter-day precision was evaluated with serum samples spiked with known concentrations of nadolol. The coefficient of variation (C.V.) after five determinations was 2.4% at 10 ng/ml and 2.0% at 100 ng/ml (Table I). The intra-assay C.V. ranged from 1.0% (assay

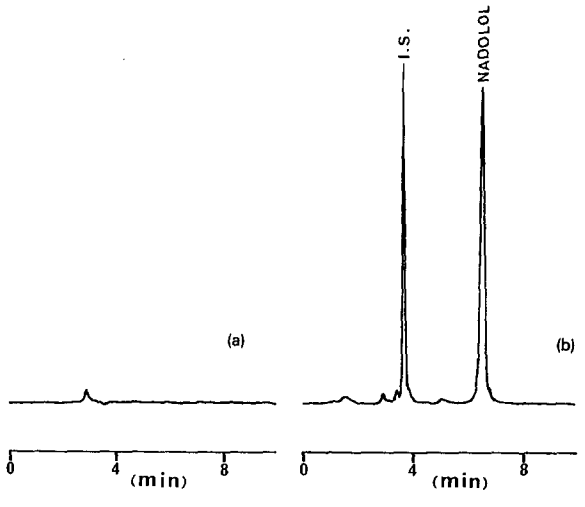

Fig. 1. Chromatograms of extracts from (a) blank human serum and (b) human serum spiked with 25 ng/ml nadolol and 20 ng/ml internal standard.

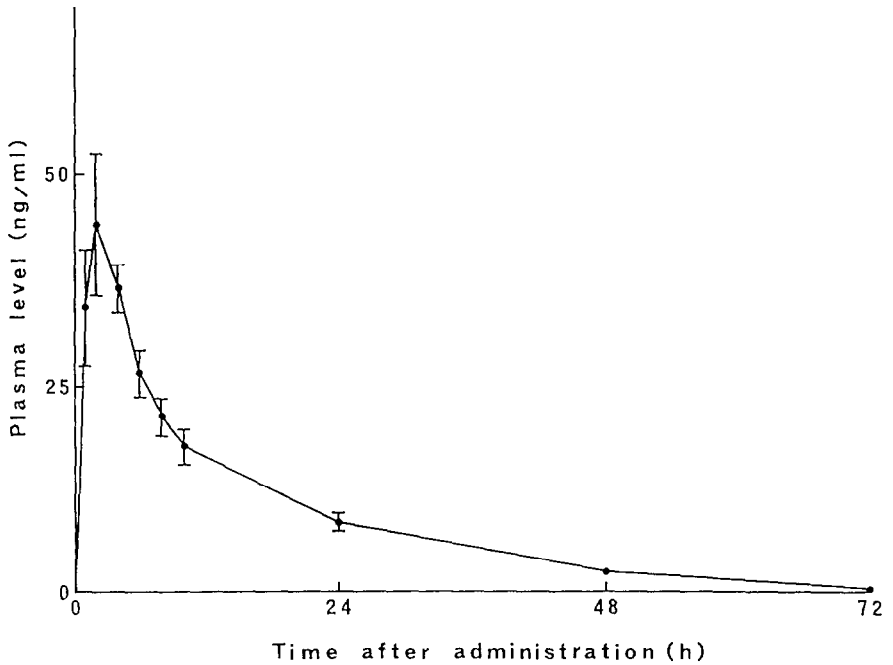

Fig. 2. Mean serum levels of nadolol in volunteers following a single oral administration of a tablet containing 30 mg of nadolol. Each point represents the mean ± standard error (n = 8).

TABLE I

INTER-DAY ACCURACY OF NADOLOL DETERMINATION

Theoretical concentration (ng/ml)	Determined concentration (mean ± S.D., n = 5) (ng/ml)	C.V. (%)
10.78	11.26 ± 0.26	2.4
107.8	107.1 ± 2.5	2.0

of 10 ng/ml, n = 5) to 1.2% (assay of 100 ng/ml, n = 5). These data show a very good reproducibility of the proposed method. The minimum detectable level of nadolol in serum was 1 ng/ml (signal-to-noise ratio 5:1).

The proposed method was applied to the determination of nadolol in plasma samples. Fig. 2 shows a typical plasma concentration–time profile after single oral administration of a 30-mg tablet to eight volunteers.

The method is thus simple, sensitive and readily adaptable to routine determination of therapeutic concentrations of nadolol in serum or plasma.

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REFERENCES

1 T. Yamaguchi, Y. Morimoto, Y. Sekine and M. Hashimoto, *J. Chromatogr.*, 239 (1982) 609.
2 M. Ribick, E. Ivashkiv, M. Jemal and A. I. Cohen, *J. Chromatogr.*, 381 (1986) 419.
3 R. N. Gupta, R. B. Haynes, A. G. Logan, L. A. Macdonald, R. Pickersgill and C. Achber, *Clin. Chem.*, 29 (1983) 1085.
4 J. Moncrieff, *J. Chromatogr.*, 342 (1985) 206.
5 V. K. Piotrovskii, V. G. Belolipetskaya, A. R. El'man and V. I. Metelista, *J. Chromatogr.*, 278 (1983) 469.
6 E. Matsutera, Y. Nobuhara and Y. Nakanishi, *J. Chromatogr.*, 216 (1981) 374.