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Short communication

Determination of maprotiline in plasma by high-performance liquid chromatography with chemiluminescence detection

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Abstract

A simple and sensitive high-performance liquid chromatographic method is described for the determination of maprotiline, an antidepressant, in plasma. After a single-step extraction from plasma (100 μl) with *n*-hexane–isoamylalcohol (19:1, v/v), the drug and desipramine (internal standard) are converted into their chemiluminescent derivatives by reaction with 6-isothiocyanatobenzo[*g*]phthalazine-1,4(2H,3H)-dione, a new chemiluminescence derivatization reagent for amines. The derivatives are separated within 60 min on a reversed-phase column, TSKgel ODS-80, using isocratic elution with acetonitrile–100 m*M* acetate buffer (pH 3.2), and produced chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in alkaline medium. The detection limit for maprotiline added to plasma is 0.36 pmol (0.1 ng)/ml plasma (1.5 fmol on column), at a signal-to-noise ratio of 3.

1. Introduction

Maprotiline, N-methyl-9,10-ethanoanthracene-9(10H)-propylamine), is an antidepressant which has been widely used for the treatment of depression. The first method, based on the double radioisotope derivative technique, was developed for the determination of maprotiline in plasma [1]. However, the method is not suitable for routine analysis. Recently, several methods including gas chromatography (GC) [2], GC–mass spectrometry (MS) [3,4] and high-performance liquid chromatography (HPLC) with UV [5] and fluorescence detection [6] have been reported. GC and HPLC methods have often been used for the monitoring of drug levels in plasma. These methods, however, have limited sensitivity and thus require a large amount of plasma (1 ml). Although GC–MS methods are fairly sensitive, they require a special, expensive instrument. Furthermore, all the methods reported need a tedious clean-up procedure such as several steps of purification by liquid–liquid extraction from plasma. Hence, a simple, reproducible and sensitive assay for plasma maprotiline is needed.

In recent years, the HPLC–chemiluminescence method has been successfully used for the sensitive determination of biogenic substances and drugs. We have previously developed a highly sensitive HPLC–chemiluminescence method [7] for the determination of primary and secondary amines. The method is based on the derivatization reaction of the amines with 6-

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isothiocyanatobenzo[*g*]phthalazine-1,4(2H,3H)-dione (IPO), a new chemiluminescent derivatization reagent for amines. The resulting thiocarbamoyl derivatives produce strong chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in alkaline medium. The method is highly sensitive, especially for secondary amines, and allows their determination at the fmol level.

It was found that maprotiline can be converted into the chemiluminescent IPO derivative and the resulting derivative produces chemiluminescence by the reaction mentioned above (Fig. 1). Our present objective is to develop a sensitive and selective HPLC method involving a simple pretreatment of plasma samples with one-step extraction followed by precolumn chemiluminescence derivatization with IPO for the quantification of maprotiline in plasma. Desipramine-(10,11 - dihydro - N - methyl - 5H - dibenz[*b*,*f*]-azepine-5-propanamine), which has a similar structure to that of maprotiline, was used as an internal standard (I.S.) for the precise determination of maprotiline. Maprotiline concentrations in plasma from two healthy male volunteers, after oral administration of the drug, were measured by the established method.

2. Experimental

2.1. Chemicals and solutions

All chemicals and solvents were of the highest purity available and were used as received. Distilled water, purified with a Milli-Q II system (Millipore, Milford, MA, USA), was used for all aqueous solutions. Hydrogen peroxide (31%, v/v) was purchased from Mitsubishi Gas Kagaku (Tokyo, Japan). IPO was prepared as described previously [7]. IPO solution (1.0 m*M*) was prepared in dimethylsulphoxide and used within 6 h. Triethylamine solution (75 m*M*) was prepared in 2-methoxyethanol. Maprotiline and desipramine solutions were prepared in 2-methoxyethanol containing 75 m*M* triethylamine. Hydrogen peroxide (20 m*M*) and potassium hexacyanoferrate(III) (40 m*M*) solutions were prepared in water and 1.5 *M* sodium hydroxide, respectively.

2.2. Plasma samples

Heparinized human plasma samples were obtained from healthy volunteers. A 100-μl aliquot of plasma was pipetted into a screw-capped

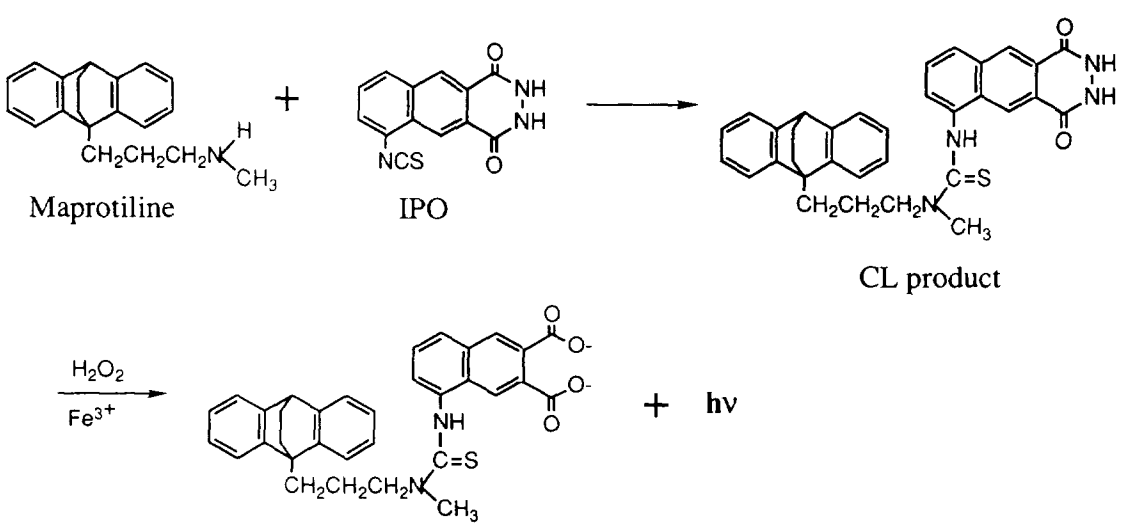

Fig. 1. Derivatization of maprotiline with IPO and chemiluminescence (CL) reaction of the derivative.

culture tube together with 100 μl of the I.S. (100 ng/ml) solution, 500 μl of 6 *M* sodium hydroxide and 3 ml of *n*-hexane–isoamylalcohol (19:1, v/v). The mixture was vortex-mixed for 5 min and centrifuged at 1000 *g* for 5 min. The organic layer (ca. 2.0 ml) was transferred into another screw-capped test tube (100 × 15 mm I.D.) and evaporated to dryness under a stream of nitrogen. The residue, dissolved in 100 μl of 2-methoxyethanol containing 75 m*M* triethylamine, was used as the test solution for analysis.

2.3. Derivatization procedure

To a 100-μl portion of a test solution of amines placed in a screw-capped test tube (100 × 15 mm I.D.) 100 μl of the IPO and 10 μl of the triethylamine solutions were added, respectively. The tube was tightly closed and heated at 80°C for 10 min. A 20-μl portion of the final reaction mixture was injected into the chromatograph.

The calibration graph was prepared as in the above procedure, except that 100 μl of the desipramine solution (I.S.) was replaced with desipramine solutions each containing 1–100 ng of maprotiline. The net peak-height ratios of the drug and desipramine were plotted against the concentrations of the drug added.

2.4. HPLC and chemiluminescence detection system

The HPLC system was essentially the same as that previously reported [7]. Chromatography was performed with a Hitachi L-6200 liquid chromatograph (Tokyo, Japan) equipped with a Rheodyne 7125 syringe-loading sample injector valve (20-μl loop). The IPO derivatives of maprotiline and I.S. were separated on a TSKgel ODS-80 (5 μm) reversed-phase column (150 × 4.6 mm I.D.; particle size, 5 μm; Tosoh, Tokyo, Japan) by isocratic elution with acetonitrile–100 m*M* acetate buffer (pH 3.2) (2:3, v/v) at flow-rate of 1.0 ml/min. The column temperature was ambient (23 ± 2°C).

The eluate from the HPLC column was first mixed with the hydrogen peroxide solution through a T-type mixing device and then with the potassium hexacyanoferrate(III) solution through a second T-type mixing device; delivery was by two Hitachi L-6000 pumps. The flow-rates of the hydrogen peroxide and potassium hexacyanoferrate(III) solutions were 1.0 and 2.0 ml/min, respectively. The generated chemiluminescence was monitored by an 825-CL chemiluminescence detector (Jasco, Tokyo, Japan) equipped with a 90-μl flow cell. Stainless-steel tubing (0.5 mm I.D.) was used for this system.

3. Results and discussion

3.1. HPLC conditions

The best separation of the IPO derivatives of maprotiline, desipramine (I.S.) and reagent blank components was achieved on a TSKgel ODS-80 reversed-phase column with acetonitrile–100 m*M* acetate buffer (pH 3.2) (2:3, v/v) as an eluent. Fig. 2 shows a typical chromatogram obtained with a standard solution. The individual drugs gave single peaks in the chromatogram and had retention times of 39.5 and 59.5 min, respectively. In this HPLC separation of the IPO derivatives, methanol can also be used instead of acetonitrile; methanol is a well-known quencher and cannot be used in the case of peroxalate chemiluminescence system.

Other drugs, which have an amine residue in their molecules, also provided the corresponding chemiluminescent IPO derivatives. The retention times (min) for their derivatives are as follows: metoprolol (16.0), propranolol (47.9), amoxapine (23.8) and nortriptyline (51.0). Some drugs [synthetic corticosteroids (prednisolone and betamethasone), phenytoin, phenobarbital, triazoram and diazepam] have been commonly used in combination with maprotiline. However, they gave no chemiluminescent derivatives. Therefore, these drugs did not interfere with the sensitive determination of maprotiline.

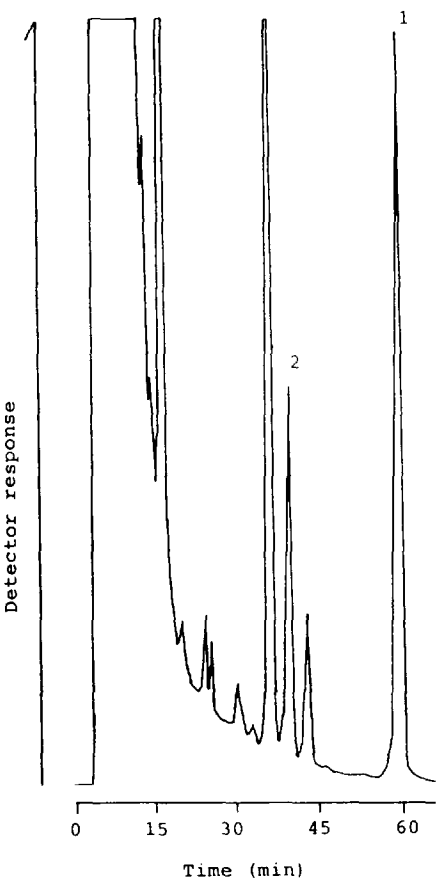

Fig. 2. Chromatogram of IPO derivatives of maprotiline and desipramine. A portion (100 μl) of a standard mixture (100 ng/ml each) was treated according to the procedure. Peaks: 1 = maprotiline; 2 = desipramine; others, reagent blank.

3.2. Chemiluminescence derivatization conditions

The chemiluminescence derivatization conditions for primary and secondary amines such as *n*-nonylamine, di-*n*-butylamine and dibenzylamine were described previously [7]. In this study, the conditions for maprotiline and desipramine were examined.

The derivatization reaction proceeds effectively in the presence of basic catalysts (triethylamine, pyridine, 4-pyrolidinopyridine, 4-pyperidinopyridine and 4-dimethylaminopyridine). Reproducible peak heights were not obtained without the catalysts. Triethylamine was the most effective reagent tested; 75 m*M* triethylamine was selected as optimum for the procedure. The IPO solution gave the most intense and constant peaks at concentrations >0.5 m*M*; 1.0 m*M* was adopted for the recommended procedure. The derivatization reaction of IPO with maprotiline and desipramine occurred more rapidly as reaction temperature was increased. The peak heights became maximum and constant after heating at 50°C for 30 min, while at 80 and 100°C the peak heights for the compounds did not reach those obtained at 50°C. Therefore, heating at 50°C for 40 min was selected in the recommended procedure.

The IPO derivatives in the final solution were stable and gave constant peak heights for at least 72 h in daylight at room temperature.

3.3. Chemiluminescence reaction

The chemiluminescence intensities were affected by the concentrations of hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide. The effects of the concentrations of the reagents on the chemiluminescence reaction were examined (Fig. 3). The concentrations of the reagents were varied one at a time to establish the maximum intensity obtainable. Based on these experiments, concentrations of 20 m*M* hydrogen peroxide, 40 m*M* potassium hexacyanoferrate(III) and 1.5 *M* sodium hydroxide were selected in the recommended procedure.

The chemiluminescence reaction occurred immediately after the eluate from the column was mixed with potassium hexacyanoferrate(III) in the second mixing device. Therefore, the length of tubing between the second mixing device and the detector affected the chemiluminescence response; 15 cm of tubing was employed as optimum (Fig. 4).

3.4. Determination of maprotiline in plasma

Fig. 5 shows chromatograms obtained with drug-free plasma (A) and with the same plasma (B) spiked with maprotiline and desipramine.

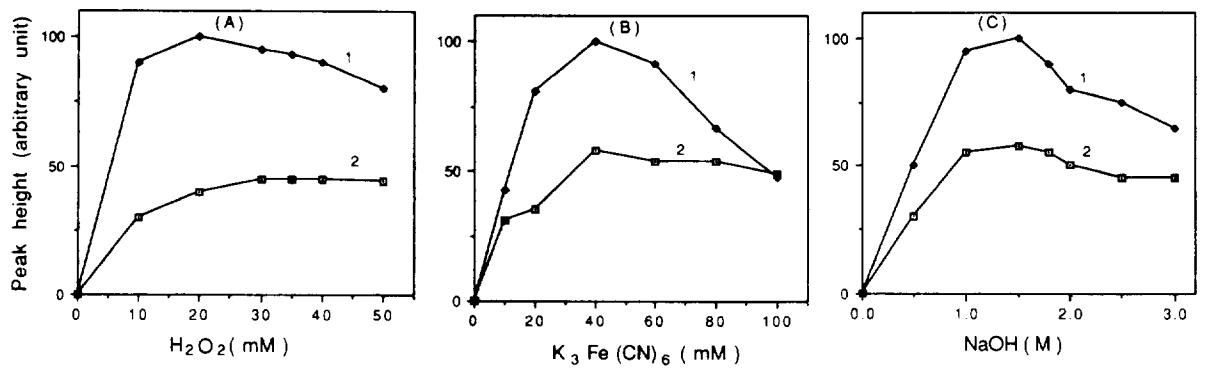

Fig. 3. Effects of the concentrations of hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide on the chemiluminescence reaction. Curves: 1 = maprotiline; 2 = desipramine.

No interfering peaks arose in drug-free plasma around the retention times of these compounds with only one-step extraction. This indicated that the derivatization was fairly selective for maprotiline and desipramine. Peaks 1 and 2 in Fig. 5B were identified as the IPO derivatives of maprotiline and desipramine, respectively, on the basis of their retention times compared with the standard compounds and also co-chromatography of the standard and the sample with 35–50% acetonitrile as mobile phase.

A linear relationship was observed between the ratios of the peak heights of maprotiline to that of desipramine (I.S.) and the amounts of maprotiline added to plasma up to at least 10 μg/ml plasma. The slope of the graph did not change with the plasma used. The linear regression equation [with linear correlation coefficients ($n = 6$)] of the graph for maprotiline was $y = 0.018x - 0.001$ ($r = 0.998$), where y and x are peak-height ratio and the concentration (ng/ml in plasma) of the drug, respectively. This result shows that the proposed method permits the determination of maprotiline in plasma over a wide range of concentrations.

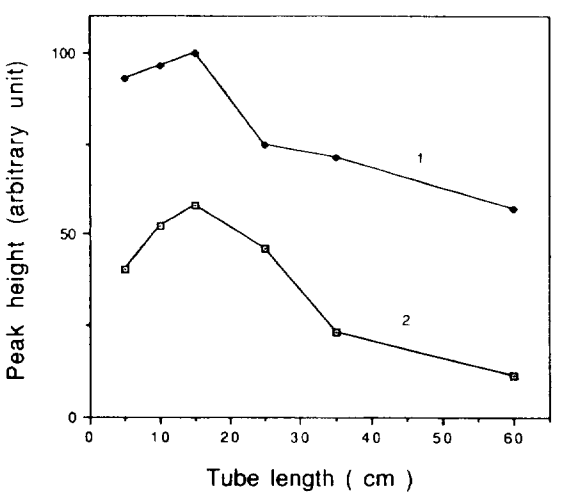

Fig. 4. Effect of the tube length between the second mixing device and chemiluminescence detector on the peak heights. Curves: 1 = maprotiline; 2 = desipramine.

The recovery of maprotiline added to pooled normal human plasma (100 ng/ml) was 99.4 ± 4.7% (mean ± S.D., $n = 9$). The within-day precision was established by repeated determination ($n = 10$) of maprotiline concentrations in plasma spiked with maprotiline (100 ng/ml); the relative standard deviation was 3.4%.

The detection limit (signal-to-noise ratio = 3) for maprotiline was 0.36 pmol (0.1 ng)/ml (1.5 fmol on column). This sensitivity is much higher (40–100 times) than those of the previous GC and HPLC methods [2,5,6] and comparable to those of GC–MS methods [3,4].

In order to evaluate the proposed method for clinical use, it was applied to monitoring the drug concentrations in plasma samples obtained after a single oral dose of maprotiline (50 and 100 mg, respectively). Fig. 6 shows the time–concentration curves obtained by the plasma samples. The concentrations of maprotiline reached a maximum at 8–10 h after oral adminis-

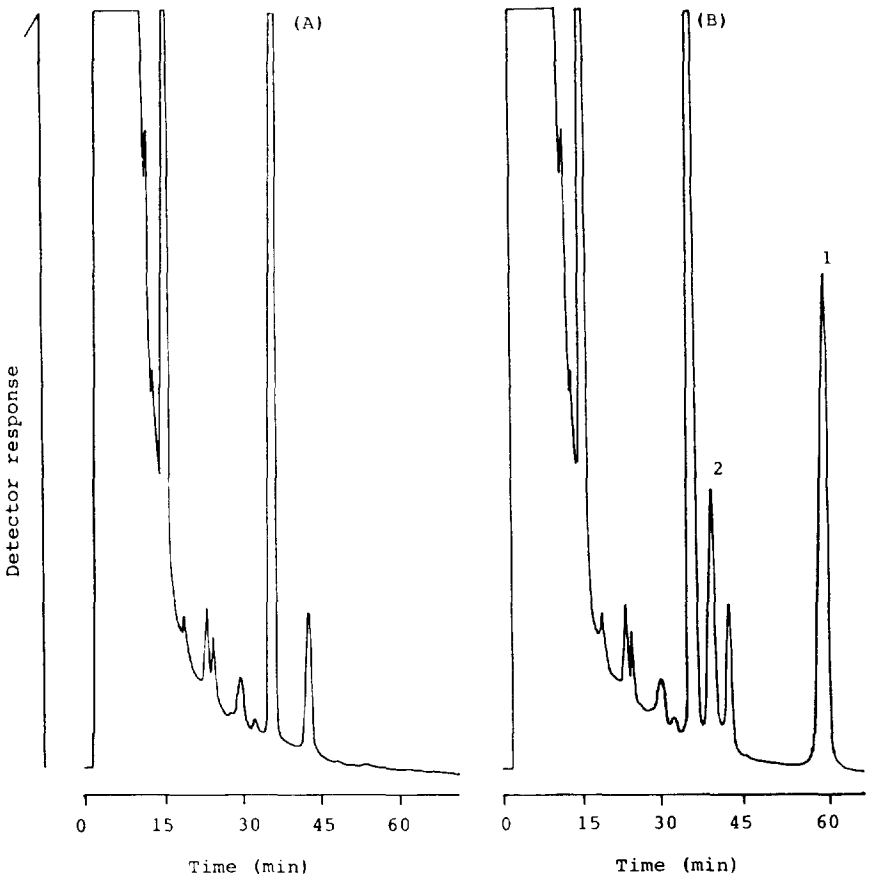

Fig. 5. Chromatograms obtained with drug-free plasma and plasma spiked with maprotiline (100 ng/ml) and I.S. Peaks: 1 = maprotiline; 2 = desipramine; others = reagent blank.

tration and then decreased gradually in both cases. The pattern of the curves was almost identical to those obtained by the radiometric assay [1].

This study has provided the first HPLC method with chemiluminescence detection for the measurement of maprotiline in human plasma. The proposed method only needs one-step extraction for the pretreatment of the plasma sample because of the selectivity of the derivatization with IPO. Moreover, it offers higher sensitivity to permit the quantification of maprotiline in 100 μl of human plasma after oral administration of the drug. The method can thus be useful in therapeutic and biomedical studies of maprotiline. In addition, the present derivatization method may be applicable for the determination of desipramine, metoprolol, propranolol, amoxapine and nortriptyline in biological materials.

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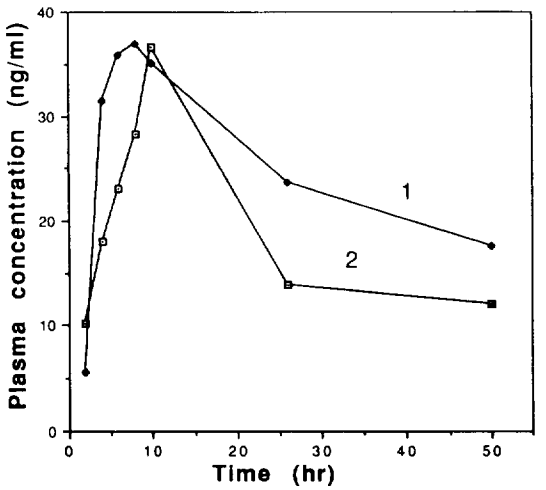

Fig. 6. Concentrations of maprotiline in plasma after oral administration of maprotiline to healthy male volunteers. Dose (mg/kg): 1 = 1.28; 2 = 0.76. Body weight and age: 1 = 78 kg, 25 years; 2 = 66 kg, 34 years.

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