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Short communication

Chiral determination of mirtazapine in human blood plasma by high-performance liquid chromatography

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Abstract

A method is described for the determination of the two enantiomers of mirtazapine in human blood plasma by high-performance liquid chromatography. Measurements were performed on drug free plasma spiked with mirtazapine and used to prepare and validate standard curves. Levels of enantiomers of mirtazapine were also measured in patients being treated for depression with racemic mirtazapine. Mirtazapine was separated from plasma by solid-phase extraction using CERTIFY columns. Chromatographic separation was achieved using a Chiralpak AD column and pre-column and compounds were detected by their absorption at 290 nm. Imipramine was used as an internal standard. The assay was validated for each analyte in the concentration range 10–100 ng/ml. The coefficient of variance was 16% and 5.5% for(+)-mirtazapine for 10 and 100 ng/ml control specimens respectively and 15% and 7.3% for mirtazapine for 10 and 100 ng/ml control specimens respectively. This assay is appropriate for use in the clinical range. The range of plasma mirtazapine concentrations from eleven patients taking daily doses of 30–45 mg of racemate was <5 to 69 ng/ml for (+)-mirtazapine and 13–88 ng/ml for (−)-mirtazapine for blood specimens collected 10–17.5 h after taking the dose.

Keywords: Enantiomer separatation; Mirtazapone

1. Introduction

The novel antidepressant mirtazapine (1,2,3,4,10,14 b-hexahydro-2-methylpyrazino[2,1-a] pyrido[2,3-c] benzazepine) has a unique pharmacological profile described as a noradrenergic and specific serotonergic antidepressant (NASSA) [1]. It is currently marketed as an antidepressant in Europe and the USA. It has a good record for safety and tolerability [2] and has demonstrated efficacy in randomised, double-blind clinical trials when compared to placebo, amitriptyline, clomipramine, doxepin and trazodone [3].

It is administered clinically as a 50:50 mixture of *S*(+) and *R*(−) enantiomers. The structure of mirtazapine enantiomers are given in Fig. 1. Mirtazapine is a basic, lipophilic drug. When given as a single oral dose it has a bioavailability of 50%, reaches maximum plasma concentration in 2 h and has an elimination half-life of 20–40 h. The drug is strongly bound to plasma protein with free concentrations ⩽15% [3]. It has two major metabolites, N-demethylmirtazapine and 8-hydroxymirtazapine. The

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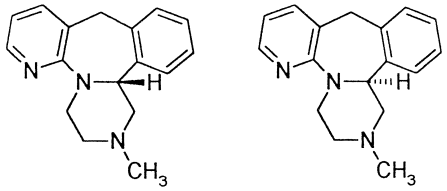

Fig. 1. Structure of the two enantiomers of mirtazapine.

parent compound demonstrates the major pharmacological activity. Some minor activity is shown by the demethyl metabolite while the hydroxy metabolite is not pharmacologically active. The receptor binding affinities of the enantiomers of mirtazapine are different, the (+) enantiomer being the more potent α_2-adrenoceptor antagonist of the two [4]. Separate quantitation of the enantiomers may be important in determining clinical response. Only a non-chiral determination of mirtazapine and demethylmirtazapine has been reported previously [5].

2. Experimental

2.1. Materials

Dichloromethane, hexane and methanol were purchased from Mallinckrodt (ChromAR HPLC grade, Kentucky, USA). Ammonium hydroxide was from BDH Chemicals (AnalaR, ammonia solution sp.gr. 0.91, Port Fairy, Australia) and potassium hydroxide was from BDH Chemicals (AnalaR, Kilsyth, Australia). Potassium dihydrogen phosphate was from Poulenc (AR grade, Clayton South, Australia). Glacial acetic acid was from Ajax Chemicals (Univar analytical reagent,Sydney, Australia). Isopropanol from Merck (Art. 9634 Darmstadt, Germany). Ethanol (denatured HPLC grade) and imipramine hydrochloride (purity >99.9%) were from Sigma Chemical Company (St. Louis, MO). Mirtazapine enantiomers (purity >98%) were a gift from N.V. Organon (Oss, NL). Bond Elute Certify solid-phase extraction columns (3cc/130 mg) were purchased from Varian sample preparation products (Harbor City, CA, USA).

2.2. Apparatus

Solid-phase extraction from plasma was conducted using a ten port Vac-Elut rack (Analytichem International). All solutions for analytical determinations were prepared in volumetric flasks ('E-MIL' boroA, 25±0.03 ml at 20°C.

Analysis was conducted using a Shimadzu LC-10AT liquid chromatograph with an SIL-10 autoinjector and an SPD-10A UV–Vis detector. Data was collected using a CBM-10A communications bus module interfaced to a PC. Separation was achieved with a chiralpak AD 10 μm, 5 cm×4.6 mm I.D. precolumn and chiralpak AD 10 μm, 25 cm×4.6 mm I.D. column obtained from Daicel Chemical Industries (Tokyo, Japan).

2.3. Preparation of standard and quality control samples

Fresh frozen donor plasma used for producing standard curves and quality control samples was obtained from the Haematology Department of the Austin & Repatriation Medical Centre (Melbourne, Australia). Samples were prepared for producing standard curves by spiking human plasma with (+)-mirtazapine and with (−)-mirtazapine (100 μg/ml as free base in ethanol). Preparations for the standard curves were made of blank plasma and plasma spiked at 25, 50, 75 and 100 ng/ml with each enantiomer of mirtazapine. Measured volumes of mirtazapine solutions were pipetted separately into 25 ml volumetric flasks. Plasma was then added to bring the flasks to volume. The solutions were mixed, divided into 1.5 ml disposable tubes and stored at −20°C. Quality control samples at 10, 20 and 100 ng/ml were prepared by the same technique.

2.4. Collection of patient plasma samples

Human blood samples were obtained from seven men and four women being treated for depression with mirtazapine as part of a clinical trial. Subjects were receiving a daily dose of either 30 or 45 mg/day on retiring. Blood specimens were collected the following morning or early afternoon, 13.8±2 h (mean±SD) after the last dose. Blood was collected

into 10 ml lithium–heparin tubes and centrifuged for 15 min at 3360 *g*. The plasma was decanted into plain tubes and stored frozen at −20°C until analysed.

2.4.1. Solutions for extraction of mirtazapine from plasma

The following solutions were used in the extraction procedure:

1. Phosphate buffer: 0.1 *M* KH_2PO_4 adjusted to pH 6.0 with 1.0 *M* KOH. Stored at 4°C and used within 30 days.
2. 1.0 *M* Acetic acid prepared from glacial acetic acid and distilled water.
3. SPE eluant: 78:20:2 dichloromethane–isopropanol–ammonium hydroxide prepared fresh daily as required.

2.4.2. Extraction procedure

Thawed plasma specimens (patient samples, standards and QCs) (1.0 ml) were added to 0.1*M* phosphate buffer (2 ml) in polyethylene test tubes. Imipramine (100 μl of 1 μg/ml ethanol solution) was added as an internal standard and the tubes gently vortexed to ensure thorough mixing. SPE columns were conditioned by vacuum drawing in order, 3 ml of methanol, 3 ml of distilled water and 1 ml of phosphate buffer through the column. The column was not permitted to dry during this process. The prepared samples were applied to conditioned columns and allowed to slowly pass through the columns over a period of at least two min with gentle vacuum applied. Columns were then washed with 3 ml of water, 1 ml of 1.0 *M* acetic acid and 3 ml of methanol. Each column was allowed to dry by drawing air through it for 5 min using the vacuum pump. Drugs retained by the column were collected into a glass vial by allowing 3 ml of eluant to pass slowly through the column with low suction. The eluate was evaporated to dryness at 55°C under a stream of air. The dried extract was reconstituted in 120 μl of HPLC mobile phase and vortex mixed. An aliquot (80 μl) of the extract solution was injected onto the HPLC. Standards, QCs and patient samples were injected by batch schedule using an autoinjector.

2.5. Chromatographic conditions

The column and precolumn were washed with ethanol (0.5 ml/min for 4 h) prior to initial use. The mobile phase was 98:1:1 hexane–ethanol–isopropanol degassed by filtration. Mobile phase and column were maintained at room temperature. The flow-rate was 1.5 ml/min. Compounds were detected by UV absorption at 290 nm. Retention times were 3.8 min for imipramine, 12.5 min for (+)-mirtazapine and 15.0 min for (−)-mirtazapine (see Fig. 2). The resolution of the two enantiomers of mirtazapine was calculated as $R=1.603$.

3. Results

3.1. Validation of the method

Six standard curves were constructed using five calibration levels (0, 25, 50, 75 and 100 ng/ml) for (+) and for (−)-mirtazapine. Standard curves were produced by linear regression of concentration on the peak area ratio, where peak area ratio was calculated for each concentration as the analyte peak area relative to that of imipramine. For each set of standards regression coefficients exceeded 0.9. Quality control specimens were spiked at 10, 20 and 100 ng/ml with (+) and (−)-mirtazapine ($n=6$ for (+)- and (−)-mirtazapine at 10 ng/ml, $n=5$ for (+) and (−)-mirtazapine at 20 ng/ml, $n=8$ for (+)- and (−)-mirtazapine at 100 ng/ml). The inter-assay variability of the method was determined from the results of the assay of these control specimens and is shown in Table 1.

3.2. Recovery with solid-phase extraction

Recovery from plasma of the extracted mirtazapine was calculated from the ratio of peak areas between extracted (100 ng/ml, $n=6$) spiked plasma and non-extracted (100 ng, $n=6$) samples, where the non-extracted samples were mirtazapine solution in ethanol transferred into extraction tubes and evaporated to dryness, then reconstituted in mobile phase for injection into the HPLC. Recovery was 81±16% for (+)-mirtazapine, 80±20% for (−)-mirtazapine (mean±SD).

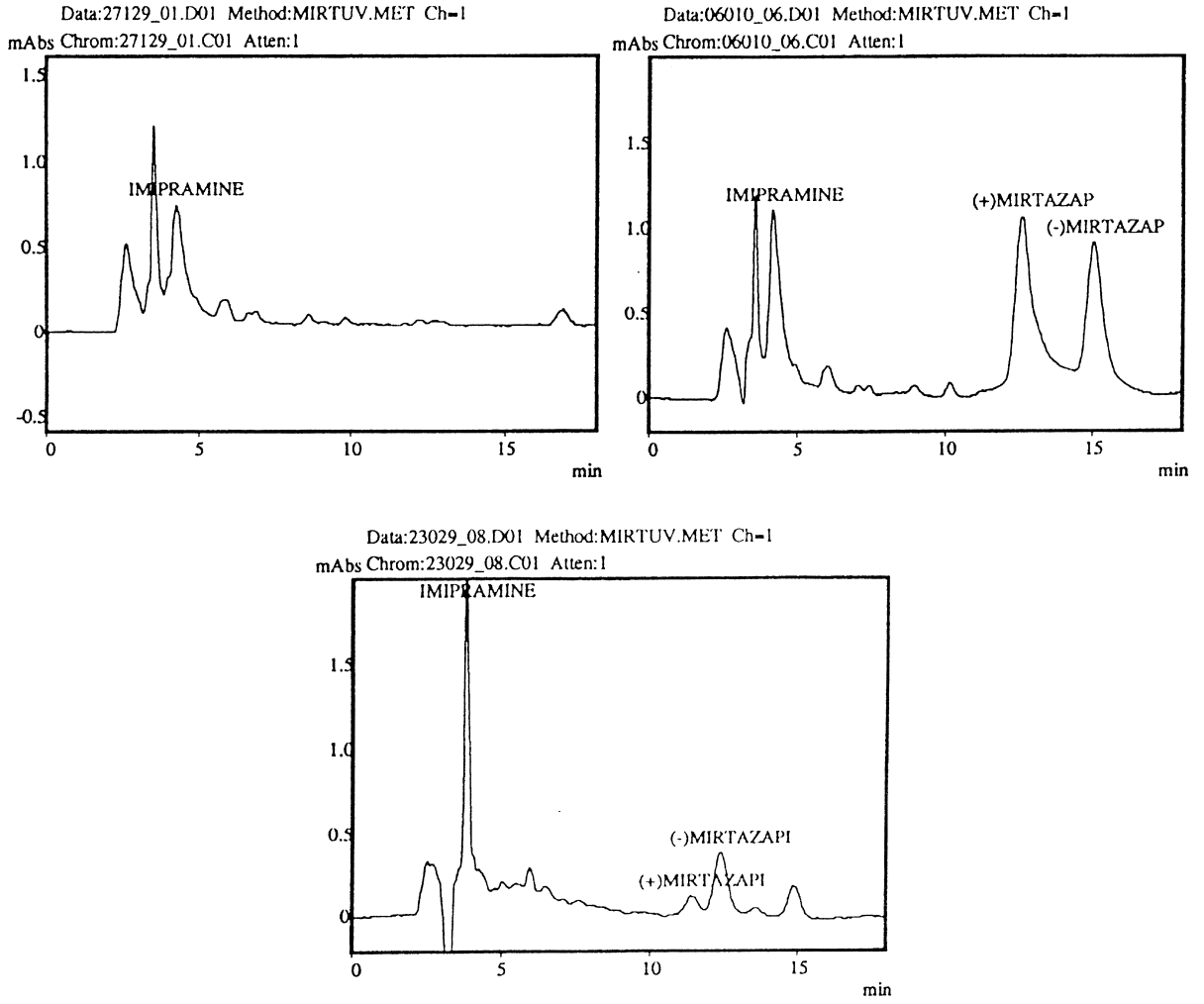

Fig. 2. Chromatograms of blank plasma spiked with imipramine (left), spiked with imipramine and 100 ng/ml (+)- and (−)-mirtazapine (centre) and a patient's specimen measuring 15.4 ng/ml (+)-mirtazapine and 63 ng/ml (−)-mirtazapine.

4. Discussion

A rapid, reliable method for the quantitative measurement by HPLC of the two enantiomers of mirtazapine has been presented. The metabolites of mirtazapine are not measured by this method. However, the parent compound is known to be the principal active agent in patients being treated with mirtazapine. The assay demonstrates a high degree of precision and adequate sensitivity for patients at steady-state plasma concentration levels being treated with a daily dose of 30 or 45 mg/day. The plasma mirtazapine concentration (mean standard deviation) for the eleven subjects measured at 14.1±15.3 ng/ml for (+)-mirtazapine and 33.3±13.4 ng/ml for (−)-mirtazapine for the 30 mg dose (n=20 plasma specimens) and 15.5±15.0 ng/ml for (+)-mirtazapine and 47.7±20.0 ng/ml for (−)-mirtazapine for the 45 mg dose (n=17 plasma specimens). Blood specimens were collected 13.8±2.0 h after the dose. Typically the trough level concentration of (+)-mirtazapine was only one third to half of the trough level concentration of (−)-mirtazapine, regardless of whether the oral dose was 30 or 45 mg/day. Therefore within this dose range, the recommended clinical dose range being 15–60

Table 1
Between day precision and accuracy for mirtazapine assayed in human plasma

Nominal plasma concentration	(+)-mirtazapine	(−)-mirtazapine
10 ng/ml		
mean±SD	9.7±1.6 ng/ml	12.2±1.9 ng/ml
%C.V.	16%	15%
%Accuracy	97%	78%
n	*n*=6	*n*=6
20 ng/ml		
mean±SD	21.3±3.6 ng/ml	21.6± 1.8 ng/ml
%C.V.	17%	8.3%
%Accuracy	94%	92%
n	*n*=5	*n*=5
100 ng/ml		
mean±SD	100.7±5.5 ng/ml	101.9±7.4 ng/ml
%C.V.	5.5%	7.3%
%Accuracy	99.3%	98.1%
n	*n*=8	*n*=8

mg/day, an oral dose which consists of a 50:50 mixture of enantiomers is producing a 25:75 mixture of racemate in the patients plasma. The probable cause of this change of isomeric ratio is enantiospecific preferences in the metabolism of mirtazapine by CYP450 enzymes, which has been demonstrated in-vitro [6] rather than enantiomeric interconversion. The hepatic liver enzyme CYP2D6 shows a strong preference for (+)-mirtazapine, while CYP3A4 shows a weak preference for (−)-mirtazapine. The differential effects of these two isozymes in-vivo may account for the observed enantiomeric differences at steady state. While the metabolites of mirtazapine are not relevant to the clinical action of the drug, it would be of interest to know the concentrations of the enantiomeric forms at steady state. The absence of pure samples of these compounds precludes such studies at this time. Subsequent to the development and use of this assay we have found that the tailing of the (+)-mirtazapine peak shown in Fig. 2 can be improved by adding the modifier butylamine to the mobile phase. Butylamine (0.5 ml) was added to the mobile phase (100 ml) and (+)-mirtazapine (100 ng) assayed before and after addition of the modifier. Peak width at the base improved from 65 to 55 s.

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