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Simplified HPLC Method for the Determination of Mirtazapine in Human Plasma and Its Application to Single-dose Pharmacokinetics

Hye Sun GWAK[1], Na Young LEE, In Koo CHUN*

College of Pharmacy, Dongduk Women's University, 23-1 Wolgok-Dong Seongbuk-Gu, Seoul 136-714, Korea,
[1]College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong Seodaemun-Gu, Seoul 120-750, Korea

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Abstract - Mirtazapine is an antidepressant agent with dual action on both the noradrenergic and serotonergic neurotransmitter systems. A simple high performance liquid chromatographic method has been developed and validated for the quantitative determination of mirtazapine in human plasma. A reversed-phase C18 column was used for the determination of mirtazapine with a mobile phase composed of 0.01M ammonium acetate solution (pH 4.2) and acetonitrile (75 : 25, v/v%) at a flow rate of 1.2 mL/min. Terazosin hydrochloride was used as an internal standard. The fluorescence detector was set at excitation and emission wavelengths of 290 and 350 nm, respectively. Intra- and inter-day precision and accuracy were acceptable for all quality control samples including the lower limit of quantification of 3 ng/mL. Mirtazapine was stable in human plasma under various storage conditions. This method was used successfully for a pharmacokinetic study using plasma samples after oral administration of a single 30 mg dose as mirtazapine base to 8 healthy volunteers. The maximum plasma concentration of mirtazapine was 64.1 ± 28.0 ng/mL at 1.8 h, and the area under the curve and elimination half-life were calculated to be 674.1 ± 218.5 ng · h/mL and 23.4 ± 3.8 h, respectively.

Keywords □ Mirtazapine, terazosin hydrochloride, high performance liquid chromatography, pharmacokinetic study

INTRODUCTION

Mirtazapine, 1,2,3,4,10,14b-hexahydro-2-methylpiperazino [2,1a]pyrido[2,3-c]benzazepine, is a piperazinozepine derivative used in the treatment of depression. It has a unique pharmacological profile having dual action on both the noradrenergic and serotonergic neurotransmitter systems with a specific action on particular serotonergic receptor subtype (Boer *et al.*, 1994; Timmer *et al.*, 1997). It has been reported that mirtazapine was at least as effective as the tricyclic antidepressants (Bremner, 1995; Smith *et al.*, 1990; Zivkov and de Jongh, 1995; Mullin *et al.*, 1996; Zivkov *et al.*, 1995), clomipramine (Richou et al., 1995), doxepine (Marttila *et al.*, 1995) and trazodone (Halakis, 1995), and demonstrated a significantly earlier onset of action than selective serotonin reuptake inhibitors (Benkert et al., 2002).

Mirtazapine is a white to creamy white crystalline powder which is slightly soluble in water. The bioavailability at steady state was found to be 48 ± 7%, which was not significantly different from that of a single dosing (50 ± 8%). A long half-life of 21.7 ± 4.2 h after single-dosing and 22.1 ± 3.7 h after multiple-dosing was found in other study (Timmer *et al.*, 1997).

Since mirtazapine is often used to treat depressive patients, a simple, sensitive and fully validated analytical method is required for pharmacokinetic studies of mirtazapine. A validated capillary gas chromatographic method with nitrogen-sensitive detection for the monitoring of mirtazapine in plasma was reported earlier (Paanakker and Van Hal, 1987). Although the method is acceptable for the determination for mirtazapine, it is not recommended when there are many samples to measure in pharmacokinetic study. Maries *et al.* (1999) described an HPLC methodology with fluorescence detection for the assay of mirtazapine in human plasma. However, in that study, an isomer of mirtazapine was adopted as an internal standard, which is not commercially available.

In the present study, we developed a validated HPLC method to analyze mirtazapine from plasma samples by back-extraction technique using terazosin hydrochloride as an inter-

*Corresponding author
Tel: +82-2-940-4523, Fax: +82-2-941-7652
E-mail: ikchun@dongduk.ac.kr (I.K. Chun).

nal standard, and applied it to a pharmacokinetic study in Korean healthy volunteers.

MATERIALS AND METHODS

Equipment

The HPLC system consisted of a pump (PU-1580 Intelligent, Jasco, Japan) with a fluorescence detector (FP-2020 Plus, Jasco, Japan) and an integrator (Model 4290, Varian, Palo Alto, USA). A reversed-phase C18 column (10 μm, μBondapak C18, 3.9 × 300 mm, Waters, USA) equipped with a precolumn insert (μBondapak C18 10 μm 125 Å C18 Guard-Pak insert, Waters Corp., Milford, USA) was used.

Materials and reagents

Working standard of mirtazapine was supplied by Janssen Korea Ltd. (Seoul, Korea). Terazosin hydrochloride (internal standard, IS) and *tert*-butylmethyl ether were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol (J.T. baker, Inc., Phillipsburg, NJ, USA) were of HPLC grade, and water (18 MÙ) was obtained by passing purified water through a Water Purification System (Elgastat UHQ PS, Elga Ltd., UK). Other reagents were of analytical grade.

Preparation of standard plasma samples

Working stock solutions of mirtazapine and terazosin hydrochloride were prepared in methanol at a concentration of 1 mg/mL. Prior to use, these two stock solutions were further diluted with water to obtain working solutions at the concentration of 1 μg/mL. An appropriate dilution of the working solution with drug free plasma from healthy volunteers gave a concentration range between 3 and 100 ng/mL of mirtazapine.

Six calibration samples (3, 5, 10, 20, 50 and 100 ng/mL) were prepared by spiking appropriate volumes of the working solutions to blank plasma. Quality control samples (3, 5, 10, 20, 50 and 100 ng/mL) and stability samples (10 and 50 ng/mL) were independently prepared in the same manner.

Sample preparation

To 0.5 mL of the prepared plasma were added 100 μL of IS at a 3 μg/mL solution in water and 200 μL of 1 mol/L sodium hydroxide solution. After a brief vortex mixing, 7 mL of *tert*-butylmethyl ether was added and vortexed for 7 min. The tubes were then centrifuged at 3000 *g* for 5 min, and the organic phase was transferred to another set of clean tubes to be back-extracted with 400 μL of 0.05% phosphoric acid (vortex mixing for 3 min). The tubes were then centrifuged at 3000 *g* for 5 min, and 30 μL of the aqueous phase was injected onto the analytical column.

Chromatographic conditions

The reversed-phase C18 column was eluted with a mixture of 0.01M ammonium acetate solution (pH 4.2) and acetonitrile (75 : 25, v/v) at a flow rate of 1.2 mL/min. The fluorescence detector was set at 290 and 350 nm for excitation and emission wavelengths, respectively. All analyses were performed at room temperature.

Method Validation

Specificity

The degree of interference by endogenous plasma constituents was evaluated by inspection of chromatogram of blank and spiked plasma samples, and also from processed blank samples injected during each analytical run.

Calibration curve

Calibration standards at the concentrations of 3, 5, 10, 20, 50 and 100 ng/mL were extracted and assayed as mentioned above. The calibration curve was constructed based on peak area ratio of the drug to IS.

Accuracy and precision

Intra-day accuracy and precision of the method were estimated by assaying five replicate plasma samples at six different concentrations, in five analytical runs. The overall mean precision was defined by the percentage of relative standard deviation (RSD) of five standards at six different concentrations analyzed on the same day. Inter-day variability was estimated from the analysis of the six standards on five separate days during method validation.

Recovery

Recovery of mirtazapine was determined by comparing the observed mirtazapine peak area in extracted plasma to that of non-processed standard solutions. The recovery was measured at three different concentrations (10, 50 and 100 ng/mL) over the concentration range used. Regarding the IS, recovery was only calculated at the working concentration (600 ng/mL).

Stability

The freeze-thaw stability of mirtazapine in plasma was evaluated over three freeze-thaw cycles. Stability control plasma samples in triplicate at the levels of 10 and 50 ng/mL were immediately frozen at -70°C and thawed at room temperature three consecutive times. After that, the samples were processed and assayed. The stability of mirtazapine in quality control

samples stored at room temperature for 24 h and at -70°C for 4 weeks was also assessed. The mean peak areas of mirtazapine were compared with the initial ones, which were assayed immediately after preparation of stability control plasma samples. The stability of extracts was examined for a storage period of 6 hrs at room temperature by comparing with freshly prepared extracts. The stability was expressed as a percentage of the initial value.

Single dose pharmacokinetic study

The assay procedure mentioned above was applied to a pharmacokinetic study of mirtazapine following 30 mg single dose. Eight healthy female volunteers aged between 20 and 23 years (21.0 ± 1.3 year), height between 158 and 169 cm (162.5 ± 4.6 cm) and weighing between 51 and 67 kg (54.5 ± 5.6 kg) were selected for the study. All subjects gave written informed consent, and the clinical protocol was approved by the Institutional Review Board. The volunteers were judged to be healthy and were not receiving any medication during the study period.

Remeron® tablet (mirtazapine 30 mg) was administered with 240 mL of water in the morning (8:30 A.M.) after at least 12 hr overnight fast. Food and drinks were withheld for at least 4 hr after dosing.

Seven milliliter of blood samples were collected in vacutainers (containing sodium heparin) via an in-dwelling cannula placed on the forearm before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 hr after the administration of the mirtazapine tablet. The blood samples were centrifuged at 3000 *g* for 15 min at room temperature, and the plasma was transferred to another tube, and stored at -70°C until analysis.

Pharmacokinetic parameters were calculated by non-compartmental analysis of plasma concentration-time curve data using WinNonlin software (Pharsight Corporation, California, USA). The peak concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined by individual plasma concentration-time profiles for mirtazapine. The area under the plasma concentration-time curve (AUC_{0-t}) was calculated by the linear trapezoidal rule from 0 to 120 hr. The terminal half-life ($t_{1/2}$) was calculated by least-squares regression on the individual log-linear terminal plasma levels.

RESULTS AND DISCUSSION

Specificity

Fig. 1 shows the well-resolved chromatographic peaks of mirtazapine and terazosin at 6.3 and 3.4 min, respectively. The blank plasma after extraction consistently contains no significant interfering peaks. Interference of mirtazapine with internal standard was not observed for the samples obtained at different time intervals from healthy volunteers orally given mirtazapine at a 30 mg dose and samples of the calibration curve. It appears that the proposed method was specific and selective for the determination of mirtazapine from the plasma samples.

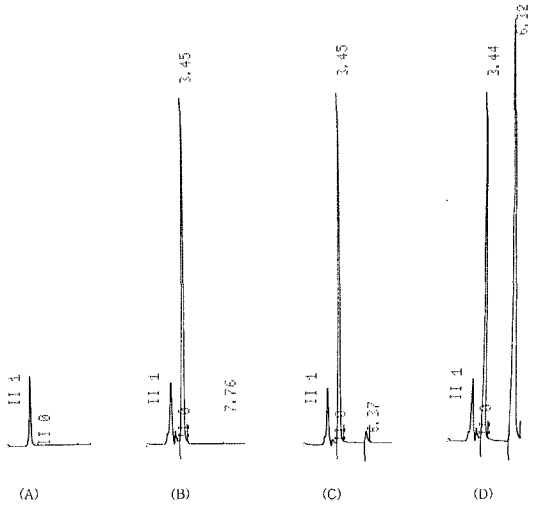

Fig. 1. Chromatograms for (A) control human plasma, (B) human plasma spiked with internal standard (IS, terazosin hydrochloride 600 ng/mL), (C) human plasma spiked with mirtazapine (3 ng/mL) and IS (600 ng/mL), and (D) typical chromatogram of samples obtained from a healthy volunteer "1 hr after a single oral dose of 30 mg mirtazapine tablet".

Calibration and linearity

The relation between mirtazapine concentrations and peak area ratio of mirtazapine to IS was linear over the range tested (3~100 ng/mL), including the limit of quantitation (LOQ). Linearity was assessed by a weighted least squares regression coefficient. The mean (± S.D.) regression equations from nine replicate calibration curves were found to have a correlation coefficient (r^2) of 0.9996, slope of 0.0153 ± 0.000272 and intercept of –(0.0073 ± 0.0011).

LOQ of mirtazapine was determined as the sample concentration of mirtazapine resulting in peak heights of 10 times S/N ratio. The LOQ was found to be 3 ng/mL. Based on 3 times peak height of baseline noise, the limit of detection was calculated to be 1 ng/mL.

Accuracy, precision and recovery

The intra- and inter-day precisions of the methods were determined by the assay of five samples of drug-free plasma

containing known concentrations of mirtazapine. As described in Table I, the intra- and inter-day RSD (%) was within 12.7%, which were acceptable for all quality control samples including the LOQ. The accuracy of mirtazapine ranged between 97.5 and 106.0%. For the criteria of intra- and inter-day accuracy and precision, accuracy values should be within 85~115% over the calibration range, except at the LOQ (80~120%). The RSD values should be not more than 15% over the calibration range, except at the LOQ, it should not exceed 20% (Kamas *et al.*, 1991; Food and Drug Administration, 2001). All the batches met the quality control acceptance criteria.

The extraction recoveries of mirtazapine at concentrations of 10, 50 and 100 ng/mL were 91.3 ± 0.5, 92.2 ± 0.8 and 92.6 ± 4.3% (n = 3), respectively, while it was 71.7 ± 0.7% (n = 3) for IS at concentration of 600 ng/mL. These results suggested that there was no difference in extraction recovery at different concentrations of mirtazapine.

Stability

It is well known that drugs may be degraded in plasma during storage or analytical run due to temperature, light, air and enzymes. Knowledge of the stability of the drug in test material is a prerequisite for obtaining valuable data. The stability of mirtazapine under various conditions is described in Table II. Under all conditions tested, mirtazapine was stable with detected concentrations of at least 94.7% of the initial concentration. Results of the study indicated that mirtazapine was stable in plasma samples when stored over one month period at -70°C, protected from light. Stability data obtained met acceptance criteria of stabilities that the deviation compared to the freshly prepared standard should be within ±15% (Food and Drug Administration, 2001).

Pharmacokinetics

This analytical method was applied to the quantitation of plasma mirtazapine concentrations in more than 120 samples from healthy volunteers in pharmacokinetic studies. The application of the method to determine the plasma level in humans is depicted in Fig. 2. After a single oral administration of mirtazapine tablets at 30 mg dose, C_{max}, T_{max} and AUC of mirtazapine were 64.1 ± 28.0 ng/mL, 1.8 h and 674.1 ± 218.5 ng · h/mL, respectively. The terminal half-life was calculated to be 23.4 ± 3.8 h, which was comparable with that in other study (21.7 ± 4.2 h) (Timmer et al., 1997).

In conclusion, the determination of mirtazapine using back-extraction technique together with HPLC has proven to be simple, rapid, sensitive, specific, accurate and reproducible. The intra- and inter-day precision and accuracy were acceptable in all quality control samples including the LOQ of 3 ng/mL. Recovery evaluations showed that mirtazapine was recovered at least 94.7%. Mirtazapine was stable in human plasma under

Table I. Intra-day and inter-day precision and accuracy of the determination of mirtazapine in plasma

Concentration (ng/ml)	RSD (%)		Accuracy (%)
	Intra (n = 5)	Inter (n = 5)	
3	7.7	6.6	100.1
5	11.9	2.8	103.0
10	12.1	8.2	106.0
20	12.7	8.8	97.5
50	11.3	4.4	97.7
100	9.8	6.4	100.9

RSD (Relative standard deviation, %) = S.D. / Mean × 100

Table II. Stability of mirtazapine in plasma

Concentration (ng/mL)	Treatment	Percentage of initial value
10	Three freeze-thaw cycles	94.7 ± 3.9
	Stored at room temperature for 24 h	96.7 ± 2.0
	Stored at -70°C for 4 weeks	97.9 ± 3.9
	Extracts at room temperature	100.7 ± 4.8
50	Three freeze-thaw cycles	103.7 ± 4.5
	Stored at room temperature for 24 h	98.2 ± 8.6
	Stored at -70°C for 4 weeks	96.0 ± 4.2
	Extracts at room temperature	97.7 ± 2.6

Each data represents the mean ± S.D. (n = 3).

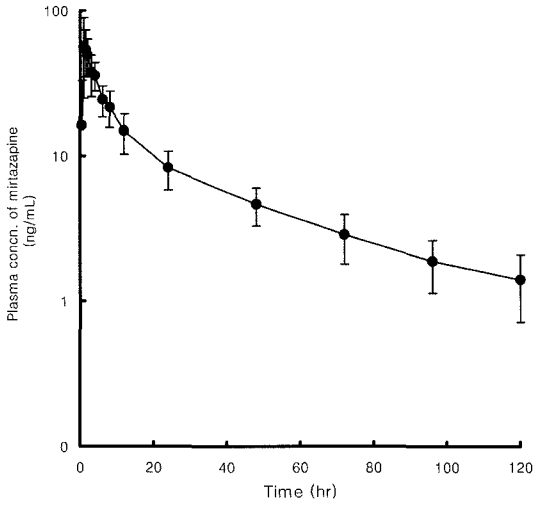

Fig. 2. Mean plasma concentration of mirtazapine vs. time "after a single oral dose of 30 mg mirtazapine tablet". Each point represents the mean ± S.D. of 8 healthy female subjects.

various storage conditions including three freeze-thaw cycles. The applicability of this method for pharmacokinetic and bioequivalence studies in human has also proved to be suitable. Therefore, this simple and validated assay could readily be used in any pharmacokinetic studies using humans.

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