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Determination of timiperone in rat plasma by high-performance liquid chromatography with electrochemical detection

Toshiyuki Takayasu[a,*], Ikuhiro Kakubari[a], Akemi Fukamachi[a], Eiichi Mafune[b], Norio Takasugi[b], Kozo Takayama[c], Tsuneji Nagai[c]

[a]*Research Department, Saitama Daiichi Pharmaceutical Co., Ltd., 8-1, Minami sakae-cho, Kasukabe, Saitama 344, Japan*
[b]*Daiichi Pharmaceutical Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134, Japan*
[c]*Department of Pharmaceutics, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142, Japan*

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Abstract

We report a sensitive new method for the determination of timiperone in rat plasma by using high-performance liquid chromatography with electrochemical detection. The method involves extraction of plasma samples with heptane–isoamyl alcohol at pH>8, followed by back-extraction into dilute acetic acid. Separation was accomplished by reversed-phase high-performance liquid chromatography on an ODS column with the mobile phase consisting of 0.1 *M* phosphate buffer (pH 3.5)–acetonitrile–methanol (65:20:15, v/v). Recovery was greater than 80%. Calibration curve was linear over the concentration range 0.5–50.0 ng/ml. The limit of quantitation of timiperone was 0.5 ng/ml plasma.

Keywords: Timiperone

1. Introduction

Timiperone (Fig. 1A), 4′-fluoro-4-[4-(2-thioxo-2,3-dihydro-1-benzimidazolinyl)piperidino]butyrophenone [1], is a neuroleptic drug that is usually administered as an oral or injectable preparation.

To date only a few methods have been reported for the determination of the drug in serum or plasma. The metabolism and excretion of timiperone in rats, dogs and monkeys were investigated using ^{14}C-labeled timiperone, and a radioreceptor assay method has also been reported [2]. These methods are

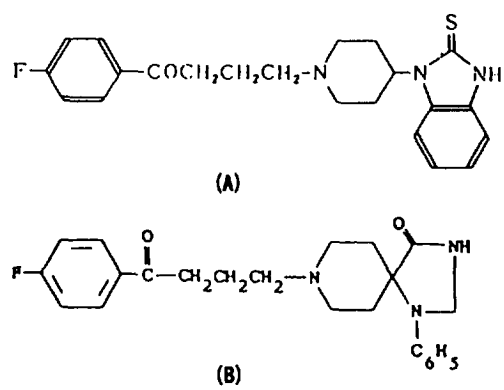

Fig. 1. Chemical structures of timiperone and spiperone.

*Corresponding author.

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sufficiently sensitive and selective for measurement of timiperone in the therapeutic dose range, but they require time-consuming sample clean-up procedures or ^{14}C-labeled timiperone.

Thus, for studying the bioavailability of timiperone preparations, a rapid, simple, sensitive and selective method for the measurement of timiperone in plasma has been sought.

Simultaneous determination of butyrophenone drugs similar to timiperone by high-performance liquid chromatography (HPLC) with electrochemical detection in rat plasma has been reported [3,4]. The present study aimed to establish such a method for the determination of timiperone in rat plasma, based on HPLC with electrochemical detection.

2. Experimental

2.1. Materials

Timiperone (lot 81) was provided by Daiichi Pharmaceutical (Tokyo, Japan). Spiperone (lot 31H4044, Fig. 1B) used as an internal standard was purchased from Sigma (St. Louis, MO, USA). Other chemicals and solvents employed were of analytical reagent or HPLC grade and were used without further purification.

2.2. Chromatographic conditions

The HPLC system consisted of a Model 7125 six-port injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop, a Model PU-980 pump (Japan Spectroscopic, Tokyo, Japan), an ECD-100 glassy carbon electrochemical detector (Eicom, Kyoto, Japan) and a Model Chromatocorder 21 (Sic System Instrument, Tokyo, Japan). Detector potential was set at +1000 mV versus a Ag/AgCl reference electrode. The analytical column consisted of an EicomPak MA-5ODS reversed-phase column (average particle size 5 μm; 150×4.6 mm I.D.).

The mobile phase consisted of 0.1 *M* KH_2PO_4 (adjusted to pH 3.5 with 0.1 *M* phosphoric acid)–acetonitrile–methanol (65:20:15, v/v). After the mobile phase was prepared, 0.5 mg of Na_2EDTA was added. The mobile phase was set at a flow-rate of 1.0 ml/min for all separations.

2.3. Preparation of stock solutions and calibration samples

Stock solutions containing 100 μg/ml of timiperone and 100 μg/ml of spiperone as internal standard were prepared in methanol and stored under refrigeration. Standard solutions of timiperone were prepared by dilution of stock solution with 0.1 *M* phosphate buffer (pH 3.5). Internal standard solution was prepared at a concentration of 250 ng/ml by dilution of stock solution with 0.1 *M* phosphate buffer (pH 3.5). Plasma calibration samples were prepared by adding 100 μl of standard solution and internal standard solution to 0.5 ml of plasma. The amounts of timiperone added corresponded to concentrations ranging from 0.5 to 50.0 ng/ml in plasma.

2.4. Extraction procedure

Plasma (0.5 ml) was pipetted into a 10-ml glass centrifuge tube containing 100 μl of internal standard solution. The mixture was then made alkaline by the addition of 0.5 ml of 0.5 *M* phosphate buffer (pH 8.5) and extracted with 2.5 ml of heptane–isoamyl alcohol (98:2, v/v). After vigorous shaking for 5 min, the organic layer was separated by centrifugation at 1700 *g* for 10 min, and 2.0 ml of organic layer was transferred to a centrifuge tube containing 100 μl of 3 *M* acetic acid. After vigorous shaking for 20 min, the mixture was centrifuged at 1700 *g* for 10 min. The organic layer was aspirated and 20 μl of the aqueous layer was injected directly onto the HPLC column.

2.5. Percentage recovery determination

Timiperone or spiperone plasma standards were prepared as described in the extraction procedure. Each peak was compared to 3 *M* acetic acid standard solution of timiperone or spiperone. The percentage recovery was determined by the following equation:

$$\%\ \text{relative recovery of timiperone} = \frac{\text{timiperone or spiperone plasma standard}}{\text{timiperone or spiperone 3 } M \text{ acetic acid standard}}$$

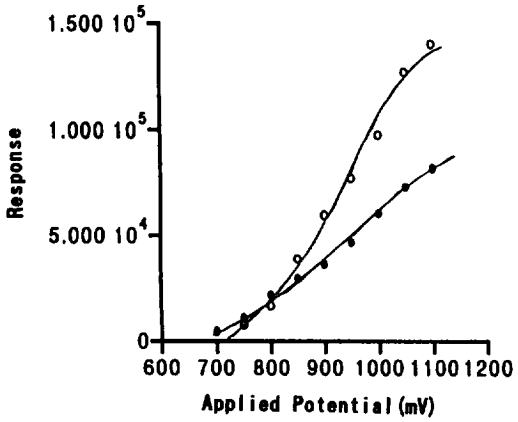

Fig. 2. Hydrodynamic voltammograms of timiperone (○) and spiperone (●). Concentration of timiperone and spiperone were both 100 ng/ml in 3 *M* acetic acid.

2.6. Assay precision

Intra- and inter-day assay precision were determined. Rat plasma spiked with timiperone was analyzed in four replicate samples and on four different days. The limit of quantitation was defined as a peak height equal to five times the baseline noise. Standard curve linearity was determined by peak height ratio versus concentration of timiperone and determination of the regression coefficient of the regression line.

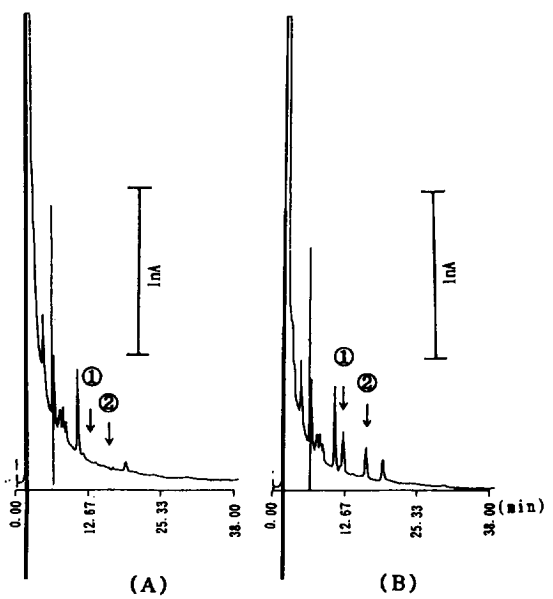

Fig. 3. Chromatograms of extracted rat plasma. (A) Blank plasma, (B) spiked plasma containing timiperone (peak 1) and spiperone (peak 2). Standard concentrations of timiperone and spiperone in rat plasma were 1.5 ng/ml and 2.5 ng/ml, respectively. The injection volume was 20 μl.

2.7. Animal study

Five male Wistar rats (average body weight 150 g; Nihon SLC, Tokyo, Japan) were anesthetized with ether before use. Each rat was administered a single 0.1 mg/kg intravenous or intramuscular dose of timiperone. Blood samples were collected into heparinized syringes via the abdominal vena cava at 5 min and 30 min after intravenous and intramuscular administration, respectively. Plasma samples were separated by centrifugation for 10 min at 1700 *g* and frozen at −20°C until assay.

3. Results and discussion

In electrochemical detection, the electrode response depends on the applied voltage. Although a higher potential yields a greater response, the background noise is also increased. The hydrodynamic voltammograms of timiperone and spiperone are illustrated in Fig. 2. Both compounds gave an initial electrochemical response at an applied potential of +700 mV. Response of both then increased steadily until the applied voltage reached +1100 mV. We decided to set an applied voltage of +1000 mV because this level gave the best signal-to-noise ratio. The retention times of timiperone and spiperone in a reversed-phase column are influenced by the concentration of organic solvents in the mobile phase.

Acetonitrile is known to promote reactions involving ionization; therefore, we used it here to shorten the retention times of hydrophobic compounds in the reversed-phase column. Furthermore, methanol was used for decreasing back ground noise. The effects of acetonitrile and methanol on the retention times of timiperone and spiperone were examined to determine the optimum concentration ratio. The concentrations of 20% (v/v) acetonitrile and 15% (v/v) methanol were optimum for separating the two compounds in one chromatographic run, and acetonitrile at this concentration did not interfere with the

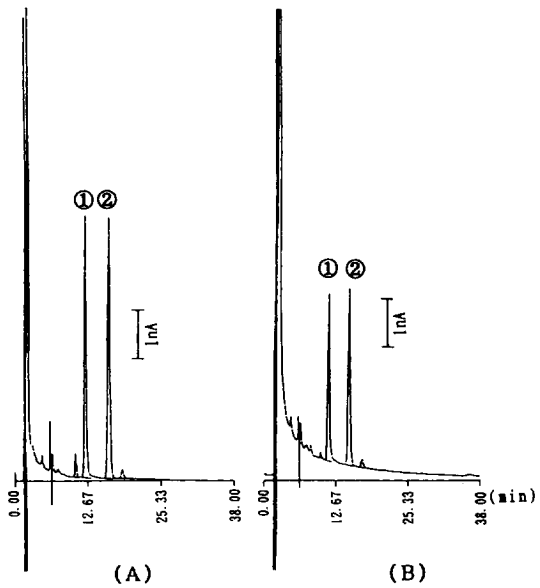

Fig. 4. Chromatograms of extracted plasma obtained from a rat 5 min after a 0.1 mg/kg intravenous dose of timiperone (A) and 30 min after a 0.1 mg/kg intramuscular dose of timiperone (B). Peak 1, timiperone; peak 2, spiperone. The injection volume was 20 μl.

electrochemical reactions. The peaks of timiperone and spiperone at a flow-rate of 1.0 ml/min were symmetrical and eluted at 11.3 and 14.9 min, respectively.

A typical chromatogram from blank plasma extracted with 2.5 ml of heptane–isoamyl alcohol is shown in Fig. 3. Typical chromatograms of extracted plasma obtained from rats after intravenous administration and intramuscular administration of timiperone are shown in Fig. 4.

In blank plasma, no interference by endogenous compounds was found.

The peak-height ratio of timiperone to the internal standard was plotted against plasma concentration of timiperone. The calibration curve was linear over the concentration range of 0.5 to 50.0 ng/ml. The least-squares linear regression equation was $y=0.04971x-0.05868$ (y=timiperone/spiperone peak-height ratio and x=concentration of timiperone). The correlation coefficient of timiperone was 0.9953 ($n=5$).

Mean recoveries of added timiperone and

Table 3
Plasma concentration of timiperone after 0.1 mg/kg intramuscular and intravenous administration in rats ($n=5$)

	Concentration (ng/ml)	
	Intramuscular (30 min)	Intravenous (5 min)
	20.5	40.0
	18.5	34.0
	16.0	37.9
	17.8	34.8
	18.2	29.5
Mean±S.D.	18.2±1.4	35.8±3.7

Table 1
Intra-day accuracy and reproducibility of HPLC analysis for timiperone (TMP) in rat plasma ($n=4$)

TMP added (ng/ml)	Recovery (mean±S.D.) (%)	TMP found (mean±S.D.) (ng/ml)	C.V. (%)
1.0	84.5±5.1	1.4±0.1	6.0
5.0	85.2±2.3	5.5±0.1	2.7
10.0	89.2±1.5	9.0±0.1	1.7
20.0	83.9±5.0	21.2±1.2	6.0

Table 2
Inter-day accuracy and reproducibility of HPLC analysis for timiperone (TMP) in rat plasma ($n=4$)

TMP added (ng/ml)	Recovery (mean±S.D.) (%)	TMP found (mean±S.D.) (ng/ml)	C.V. (%)
1.0	84.0±4.2	1.4±0.1	5.0
5.0	88.2±6.6	5.6±0.1	7.5
10.0	87.0±7.1	9.2±0.9	8.1
20.0	82.0±6.5	21.1±3.2	7.9

spiperone from plasma by this extraction procedure were 85.7% (range 83.9–89.2%) and 77.2% (range 75.2–80.7%), respectively.

The intra- and inter-day assay coefficients of variation for timiperone are presented in Table 1 and Table 2. In the intra-day assay, the ratios of the peak heights for timiperone and spiperone were compared for standards taken through the entire extraction procedure.

The mean intra-day coefficient of variation (C.V.) for timiperone in rat plasma was 4.1% (range 1.7–6.0%), while inter-day C.V. for the same plasma sample over 4 days was 7.1% (range 5.0–8.1%). These results demonstrate the high accuracy and reproducibility of this method. This made it possible to calculate the concentration of timiperone from a simple measurement of the ratio. The detection limit, defined as the amount of compound injected that caused a peak height five-times greater than the baseline noise, was found to be at 25 pg, equivalent to 0.5 ng/ml plasma.

The mean plasma concentration of timiperone at 5 min after intravenous administration was 35.9 ng/ml (range 29.5–40.0 ng/ml). Further, mean plasma level at 30 min after intramuscular administration was 18.2 ng/ml (16.0–20.5 ng/ml; Table 3).

Ohyabu et al. [5] reported that the plasma level at 5 min after intravenous injection of ^{14}C-labeled timiperone (0.1 mg/kg) was 37.7±4.9 ng/ml (equivalent timiperone) and 22.7±1.3 ng/ml (equivalent timiperone) at 30 min after intramuscular injection. Our results agree with these reported values.

In conclusion, the present electrochemical HPLC method provides a simple, highly sensitive, rapid, and accurate measure of timiperone in plasma. This assay can easily be adapted to the routine therapeutic monitoring of timiperone in both clinical and research settings.

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