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Short communication

Sensitive determination of sulpiride in human plasma by high-performance liquid chromatography

H. Tokunaga, K. Kudo*, N. Jitsufuchi, Y. Ohtsuka, T. Imamura

Department of Forensic Medicine, Faculty of Medicine, Kyushu University 60, Fukuoka 812-82, Japan

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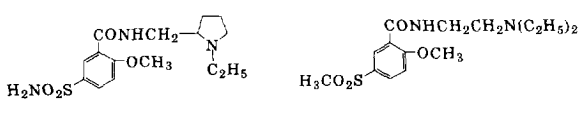
Abstract

We developed a simple, sensitive and reliable method for the determination of sulpiride, a specific antipsychotic drug, in human plasma using high-performance liquid chromatography. A structurally related benzamide, tiapride, was used as the internal standard. A Sep-Pak C_{18} cartridge was used to extract a sample from 1 ml of plasma. The extract was dissolved in methylene chloride, and then back-extracted with 0.01 *M* hydrochloric acid. The aqueous layer was put on a octadecylsilica column with a mobile phase of 50% acetonitrile in 0.01 *M* phosphate buffer (pH 3.0). A fluorescence detector with excitation at 300 nm and emission at 365 nm was used for detection. The calibration curve was linear in the concentration range of 10–1500 ng/ml, and the lower limit of detection was 1 ng/ml. We used this method to examine plasma levels of sulpiride in 14 inpatients being treated with sulpiride for 6 months. The determined plasma levels were 70.1–1121.2 ng/ml, and the correlation between daily dose and plasma concentration was positive. This simple, reliable method is expected to be put to good use in forensic and hospital laboratories.

Keywords: Sulpiride

1. Introduction

Sulpiride, 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide (Fig. 1), is one of the substituted benzamides classified as an anti-psychotic drug. Since this drug was clinically introduced in 1966, it has been widely prescribed because it has antipsychotic, antidepressive and antiulcer effects and the frequency of extrapyramidal side-effects is low.



sulpiride tiapride

Fig. 1. Structures of sulpiride and tiapride (I.S.).

A number of methods are available for analysis of sulpiride in biological fluids, including spectrofluorometric determination [1], gas chromatography (GC) [2,3], high-performance liquid chromatography (HPLC) with UV detection [4–7] and HPLC with fluorescence detection [8,9]. Spectrofluorometric determination [1] is not sensitive and specific enough for clinical studies and methylated derivatization is required for GC analysis to detect sulpiride as a

*Corresponding author.

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single peak on the gas chromatogram [2,3]. Although HPLC methods are routinely used, a large amount of sample or complicated extraction procedures are often necessary [4–9]. Such being the case, we devised a more sensitive and simpler method, using solid-phase extraction and HPLC with fluorescence detection.

2. Experimental

2.1. Reagents

Sulpiride and tiapride (Fig. 1) hydrochloride, used as the internal standard (I.S.), were provided by Fujisawa Pharmaceutical (Osaka, Japan) and Sep-Pak C_{18} cartridges (200 mg sorbent) were purchased from Waters (Milford, MA, USA). Methanol, acetonitrile and methylene chloride were of analytical-reagent grade, and were purified by distillation. Other chemicals used were of analytical-reagent grade. The mobile phase of HPLC was passed through a 0.45-μm filter prior to use.

2.2. Biological samples

Outdated plasma obtained from a blood bank was used as control samples. We tested plasma samples obtained from 14 male Japanese patients admitted to a psychiatric hospital, who were on sulpiride treatment for 6 months. The diagnosis according to DSM-III-R criteria was schizophrenia in 8, alcoholic dependence in 3, mental retardation in 2 and methamphetamine abuse in 1 patient. Ages ranged from 29 to 65 years and body weights were from 44 to 86 kg. Oral and written informed consent was obtained from each patient to participate in this study. Sulpiride was administered orally in tablet form after meals, 1–3 times a day. Blood samples were obtained once a month in the a.m. before ingestion of food or drugs. After centrifuging the blood, the plasma was stored at −20°C until analysis.

2.3. Standard solution of sulpiride and tiapride

Sulpiride (10 mg) was dissolved in methanol to give a concentration of 1 μg/μl. This solution was further diluted to give concentrations of 100, 10, 1 and 0.1 ng/μl. A standard solution of I.S. was prepared by dissolving tiapride hydrochloride (11.11 mg) in methanol to give a concentration of 1 μg/μl, as free tiapride.

2.4. Extraction procedure

The method reported for the analysis of phenothiazines [10] was modified as follows: a Sep-Pak C_{18} cartridge was preactivated twice by passing through 3 ml of methylene chloride–acetonitrile (8:2, v/v), 10 ml of methanol and 30 ml of distilled water successively. One ml of plasma was mixed with 1 μl of I.S. solution (1 μg of tiapride), 16 ml of distilled water and 3 ml of 1 *M* sodium hydrogen carbonate. The preparation was vortex mixed and was loaded onto the Sep-Pak C_{18} cartridge at a flow-rate not greater than 5 ml/min. The cartridge was washed twice with 10 ml of distilled water, and the drugs were eluted with 3 ml of methylene chloride–acetonitrile (8:2, v/v). The small amount of aqueous layer (upper phase) of the eluate was discarded by aspiration with a Pasteur pipette and the organic layer (lower phase) was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 200 μl of methylene chloride, and the drugs were back-extracted with 200 μl of 0.01 *M* hydrochloric acid. A 100-μl aliquot of the aqueous layer was applied to the HPLC column.

2.5. Preparation of calibration curve

Plasma samples were prepared to contain sulpiride at the concentrations of 10, 50, 100, 500, 1000 and 1500 ng/ml, each containing 1 μg/ml of I.S. These samples were extracted in the same manner as described above (Section 2.4). The calibration curve was obtained by plotting the peak-area ratio of sulpiride to tiapride versus the amount of sulpiride in plasma.

2.6. HPLC conditions

The apparatus used was a Model LC100 high-performance liquid chromatograph (Yokogawa, Tokyo, Japan) with a Model F-1050 fluorescence detector (Hitachi, Tokyo, Japan). The system was

connected via a GP-IB data bus to a computer for system control and data integration. The analytical column was a Spherisorb-ODS-II-5 column, a 250× 4.6 mm I.D. stainless-steel tube packed with 5-μm particle size octadecylsilica (Phase Separation, UK). The column temperature was maintained at 35°C. The column was eluted with a solvent system consisting of acetonitrile–phosphate buffer (0.01 *M* monopotassium phosphate adjusted to pH 3.0 with phosphoric acid) (50:50, v/v) at a flow-rate of 1.0 ml/min. The detector was set at 300 nm for excitation and 365 nm for emission.

3. Results and discussion

3.1. HPLC conditions

With regard to detectors, a fluorescence detector gave a higher sensitivity than a UV detector for detection of sulpiride, thus the fluorescence detector was used.

3.2. Extraction procedure

As sulpiride was hardly soluble in organic solvents such as methylene chloride and *tert.*-butyl methyl ether, the recovery of sulpiride using these solvents was about 10%. When we used ethyl acetate as the extraction solvent, the recovery exceeded 50%, but another decomposed peak appeared on the chromatogram. This problem was overcome by using a solid-phase extraction procedure, and good recovery of sulpiride as well as I.S. was obtained. However, interfering peaks, presumably deriving from the Sep-Pak C_{18} cartridge appeared. These peaks were effectively removed by back-extraction with 0.01 *M* hydrochloric acid. The single use of Sep-Pak C_{18} cartridge is recommended because recovery of the drugs, sulpiride in particular, was significantly decreased with repeated use of the cartridge.

3.3. Determination of sulpiride by HPLC

Fig. 2A shows a chromatogram of the extract from plasma containing 50 ng/ml of sulpiride and 1 μg/ml of I.S. Sulpiride and I.S. were clearly separated, with retention times of 7.7 and 11.0 min, respectively. No interfering peaks were observed from the drug-free human plasma (Fig. 2B). The calibration curve was linear in the concentration range from 10 ng/ml to at least 1500 ng/ml, with a correlation coefficient of 0.999. The lower limit of detection, at a signal-to-noise ratio of 3, was 1 ng/ml, which was 1/10 times less than that described in the literature [1,3,5,7–9]. The reproducibility study was done using two different concentrations (50 and 500 ng/ml) by adding sulpiride to blank plasma. The coefficients of variation (C.V.) were 2.8 and 1.9% for the intra-day assay, and 3.3 and 3.6% for the inter-day assay (Table 1). The absolute recoveries of sulpiride at concentrations of

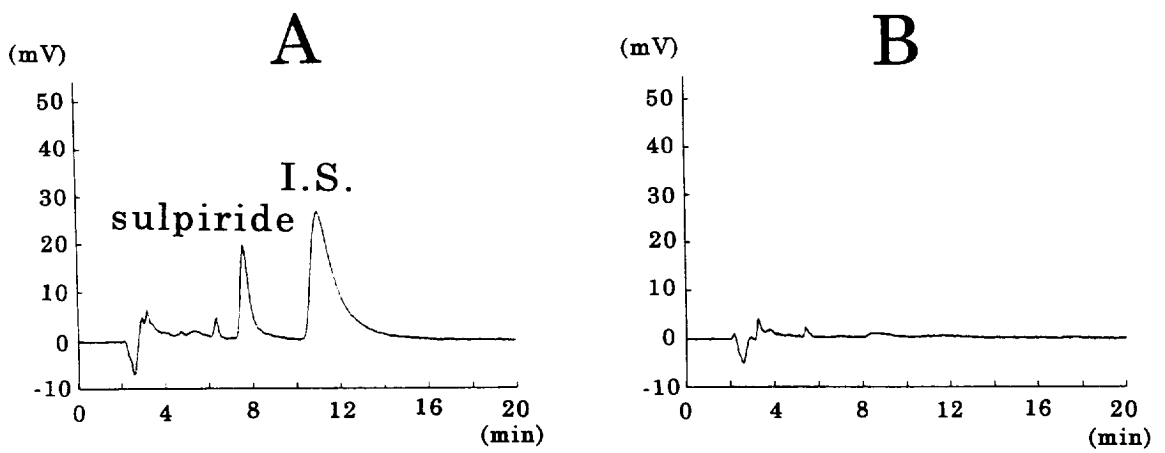

Fig. 2. Chromatograms of the extracts. (A) From plasma containing 50 ng/ml of sulpiride and 1 μg/ml of I.S.; (B) from blank plasma.

Table 1
Precision and accuracy of the sulpiride assay

Spiked concentration of sulpiride (ng/ml)	Intra-day (n=5)		Inter-day (n=5)	
	Detected concentration (mean±S.D.) (ng/ml)	C.V. (%)	Detected concentration (mean±S.D.) (ng/ml)	C.V. (%)
50	48.8±1.4	2.8	51.1±1.7	3.3
500	507±10	1.9	510±18	3.6

50 ng/ml and 500 ng/ml were 85.4±1.8% and 86.5±2.0%, respectively (mean±S.D., n=5).

These values are sufficiently sensitive and reliable for the drug monitoring of sulpiride in human plasma.

4. Practical application

The newly developed method we have described was used to determine plasma levels of sulpiride in 14 inpatients treated on a long term basis with sulpiride. The daily dose of the drug varied from 50 to 600 mg/day. Sulpiride was clearly detected with I.S. in all samples examined, and no interfering peaks were observed. Fig. 3 shows 70.1–1121.2 ng/ml concentrations of sulpiride in plasma determined with this method. The C.V. of monthly concentration of sulpiride in every patient ranged from 7.8 to 53.2% (average 31.0%). The correlation between daily dose and plasma concentration of sulpiride is shown in Fig. 4. Salminen et al. reported finding no correlation between the dose and the plasma level in this drug for depressed outpatients (n=42) [11]. Our study revealed a positive correlation with the coefficient (r) of 0.715, which was judged to be significant by two tailed t-test (n=74, p<0.05). This discrepancy is attributed to different

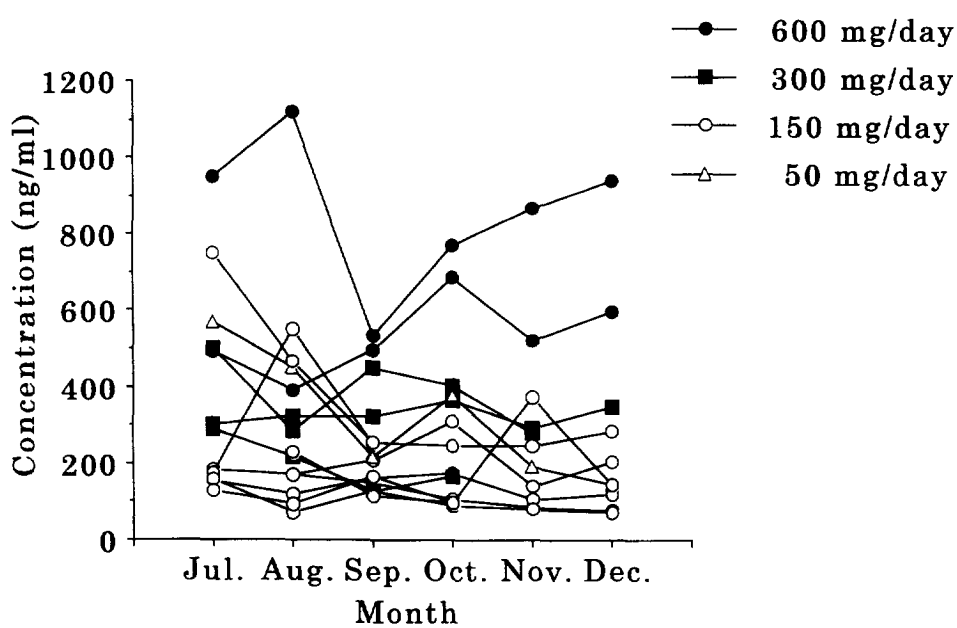

Fig. 3. Concentrations of sulpiride in the plasma obtained from 14 patients over a 6-months period.

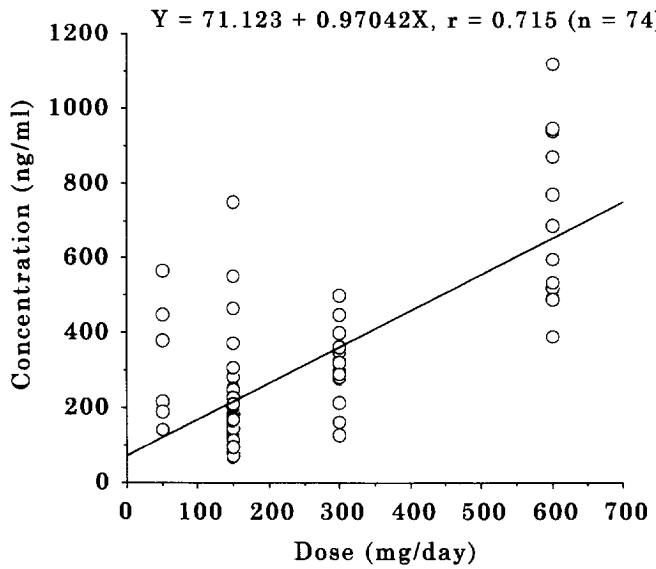

Fig. 4. Correlation between daily dose and plasma concentration of sulpiride.

conditions in sample number, analytical method, racial differences and life style between out and in-patients.

5. Conclusion

A simple, sensitive and reliable HPLC method was devised to determine sulpiride concentrations in human plasma. Our method is most practical to monitor plasma levels of this drug.

Acknowledgments

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